

LEONARDO FRANÇA DA SILVA
DENIS MEDINA GUEDES
JÉSSICA MANSUR SIQUEIRA FURTADO CRUSOÉ
(ORGANIZERS)



AGRICULTURAL SCIENCES UNVEILED:

EXPLORING THE DYNAMICS OF FARMING AND SUSTAINABILITY

Atena
Editora
2024

LEONARDO FRANÇA DA SILVA
DENIS MEDINA GUEDES
JÉSSICA MANSUR SIQUEIRA FURTADO CRUSOÉ
(ORGANIZERS)



AGRICULTURAL SCIENCES UNVEILED:

EXPLORING THE DYNAMICS OF FARMING AND SUSTAINABILITY

Atena
Editora
2024

Chief editor

Profª Drª Antonella Carvalho de Oliveira

Executive editor

Natalia Oliveira

Editorial assistant

Flávia Roberta Barão

Librarian

Janaina Ramos

Graphic project

Ellen Andressa Kubisty

Luiza Alves Batista

Nataly Evilin Gayde

Thamires Camili Gayde

Cover pictures

iStock

Art edition

Luiza Alves Batista

2024 by Atena Editora

Copyright © Atena Editora

Copyright of the text © 2024 The authors

Copyright of the edition © 2024 Atena Editora

Rights for this edition granted to Atena Editora by the authors.

Open access publication by Atena Editora



All content in this book is licensed under a Creative Commons Attribution License. Attribution-Non-Commercial-NonDerivatives 4.0 International (CC BY-NC-ND 4.0).

The content of the articles and their data in their form, correctness and reliability are the sole responsibility of the authors, and they do not necessarily represent the official position of Atena Editora. It is allowed to download the work and share it as long as credits are given to the authors, but without the possibility of altering it in any way or using it for commercial purposes.

All manuscripts were previously submitted to blind evaluation by peers, members of the Editorial Board of this Publisher, having been approved for publication based on criteria of academic neutrality and impartiality.

Atena Editora is committed to ensuring editorial integrity at all stages of the publication process, avoiding plagiarism, fraudulent data or results and preventing financial interests from compromising the publication's ethical standards. Suspected scientific misconduct situations will be investigated to the highest standard of academic and ethical rigor.

Editorial Board**Agricultural sciences**

Prof. Dr. Alexandre Igor Azevedo Pereira – Instituto Federal Goiano

Profª Drª Amanda Vasconcelos Guimarães – Universidade Federal de Lavras

Prof. Dr. Antonio Pasqualetto – Pontifícia Universidade Católica de Goiás

Profª Drª Ariadna Faria Vieira – Universidade Estadual do Piauí

Prof. Dr. Arinaldo Pereira da Silva – Universidade Federal do Sul e Sudeste do Pará

Profª Drª Carla Cristina Bauermann Brasil – Universidade Federal de Santa Maria
Prof. Dr. Cleberton Correia Santos – Universidade Federal da Grande Dourados
Profª Drª Diocléa Almeida Seabra Silva – Universidade Federal Rural da Amazônia
Prof. Dr. Écio Souza Diniz – Universidade Federal de Viçosa
Prof. Dr. Edevaldo de Castro Monteiro – Universidade Federal Rural do Rio de Janeiro
Prof. Dr. Fábio Steiner – Universidade Estadual de Mato Grosso do Sul
Prof. Dr. Fágner Cavalcante Patrocínio dos Santos – Universidade Federal do Ceará
Profª Drª Girlene Santos de Souza – Universidade Federal do Recôncavo da Bahia
Prof. Dr. Guilherme Renato Gomes – Universidade Norte do Paraná
Prof. Dr. Jael Soares Batista – Universidade Federal Rural do Semi-Árido
Prof. Dr. Jayme Augusto Peres – Universidade Estadual do Centro-Oeste
Profª Drª Jessica Mansur Siqueira Crusoé – Universidade Federal de Viçosa
Prof. Dr. Júlio César Ribeiro – Universidade Federal Rural do Rio de Janeiro
Profª Drª Lina Raquel Santos Araújo – Universidade Estadual do Ceará
Prof. Dr. Pedro Manuel Villa – Universidade Federal de Viçosa
Profª Drª Raissa Rachel Salustriano da Silva Matos – Universidade Federal do Maranhão
Prof. Dr. Renato Jaqueto Goes – Universidade Federal de Goiás
Prof. Dr. Ronilson Freitas de Souza – Universidade do Estado do Pará
Profª Drª Talita de Santos Matos – Universidade Federal Rural do Rio de Janeiro
Prof. Dr. Tiago da Silva Teófilo – Universidade Federal Rural do Semi-Árido
Prof. Dr. Valdemar Antonio Paffaro Junior – Universidade Federal de Alfenas

Agricultural sciences unveiled: exploring the dynamics of farming and sustainability

Diagramming: Ellen Andressa Kubisty
Correction: Maiara Ferreira
Indexing: Amanda Kelly da Costa Veiga
Review: The authors
Organizers: Leonardo França da Silva
Denis Medina Guedes
Jéssica Mansur Siqueira Furtado Crusoé

International Cataloging-in-Publication Data (CIP)

S586 Silva, Leonardo França da
Agricultural sciences unveiled: exploring the dynamics of farming and sustainability / Organizers Leonardo França da Silva, Denis Medina Guedes, Jéssica Mansur Siqueira Furtado Crusoé. – Ponta Grossa - PR: Atena, 2024.

Formato: PDF

Requisitos de sistema: Adobe Acrobat Reader

Modo de acesso: World Wide Web

Inclui bibliografia

ISBN 978-65-258-2396-6

DOI: <https://doi.org/10.22533/at.ed.966243004>

1. Agriculture. 2. Sustainability. I. Silva, Leonardo França da (Organizer). II. Guedes, Denis Medina (Organizer). III. Crusoé, Jéssica Mansur Siqueira Furtado (Organizer). IV. Title.

CDD 630

Prepared by Librarian Janaina Ramos – CRB-8/9166

Atena Editora

Ponta Grossa – Paraná – Brasil

Telephone: +55 (42) 3323-5493

www.atenaeditora.com.br

contato@atenaeditora.com.br

AUTHORS' DECLARATION

The authors of this work: 1. Attest that they do not have any commercial interest that constitutes a conflict of interest in relation to the published scientific article; 2. They declare that they actively participated in the construction of their manuscripts, preferably in: a) Study design, and/or data acquisition, and/or data analysis and interpretation; b) Elaboration of the article or revision in order to make the material intellectually relevant; c) Final approval of the manuscript for submission; 3. They certify that published scientific articles are completely free from fraudulent data and/or results; 4. Confirm correct citation and reference of all data and interpretations of data from other research; 5. They acknowledge having informed all sources of funding received for carrying out the research; 6. Authorize the publication of the work, which includes the catalog records, ISBN (Internacional Standard Serial Number), D.O.I. (Digital Object Identifier) and other indexes, visual design and cover creation, interior layout, as well as the release and dissemination according to Atena Editora's criteria.

PUBLISHER'S DECLARATION

Atena Editora declares, for all legal purposes, that: 1. This publication constitutes only a temporary transfer of copyright, right to publication, and does not constitute joint and several liability in the creation of published manuscripts, under the terms provided for in the Law on Rights copyright (Law 9610/98), in art. 184 of the Penal Code and in art. 927 of the Civil Code; 2. Authorizes and encourages authors to sign contracts with institutional repositories, with the exclusive purpose of disseminating the work, provided that with due acknowledgment of authorship and editing and without any commercial purpose; 3. All e-books are open access, so it does not sell them on its website, partner sites, e-commerce platforms, or any other virtual or physical means, therefore, it is exempt from copyright transfers to authors; 4. All members of the editorial board are PhDs and linked to public higher education institutions, as recommended by CAPES for obtaining the Qualis book; 5. It does not transfer, commercialize or authorize the use of the authors' names and e-mails, as well as any other data from them, for any purpose other than the scope of dissemination of this work.

Collection “Agricultural sciences unveiled: exploring the dynamics of farming and sustainability”: a work that has as its main focus a scientific discussion through various works that compose its chapters. The purpose of the Ebook is to explore different contents linked to environmental issues in 19 chapters, tracing a contemporary theme of sustainability and the direct action of human beings in the responsibility and creation of strategies for the development of the environment. The volume will address in a categorized and interdisciplinary way works, investigations, case reports and/or reviews that go through the various areas of the environment and sustainability.

It is worth mentioning that sustainability is tied to the growing demand for global progress, with the emergence of the need to expand studies that present alternative uses of the present resources in the environment in a responsible manner, without compromising the benefits and systems involved. Seeking to minimize impacts, develop environmental responsibility and strengthen sustainable growth. Thinking about development allied to sustainability involves economic, social, and cultural aspects.

In this way, the work “Agricultural sciences unveiled: exploring the dynamics of farming and sustainability” presents the foundation of the theory obtained in practice by the authors of this e-book, several professors, academics, and researchers who have arduously developed their work that will be presented here in a manner concise and didactic. The importance of this space for scientific dissemination is evidence or commitment and the structure of Atena Editors that provides us with a consolidated and trustworthy platform for researchers to expose and disseminate their results.

The authors hope to contribute with relevant content to provide technical, scientific, and constructive assistance to the reader, as well as demonstrate that sustainability is an important tool, becoming an ally of growth. From this perspective, Atena Editors is working to stimulate and encourage more and more researchers from Brazil and other countries to publish their work with a guarantee of quality and excellence in the form of books, book chapters and scientific articles.

Good reading!

Leonardo França da Silva

Denis Medina Guedes

Jéssica Mansur Siqueira Furtado Crusóé

CHAPTER 1..... 1**LOW-COST CLASS A EVAPOTRANSPIRATION PAN FOR REFERENCE EVAPOTRANSPIRATION ESTIMATION IN PROTECTED AND FIELD ENVIRONMENTS: A PRACTICAL APPROACH**

Silvio Roberto de Lucena Tavares

Natália Fernandes Rodrigues

Gabriela Martins Corrêa

Cristina Moll Hüther


 <https://doi.org/10.22533/at.ed.9662430041>**CHAPTER 2 10****SUPERAÇÃO DA DORMÊNCIA DE SEMENTES DE ARATICUM-DO-BREJO**

Walnice Maria Oliveira do Nascimento

Caio Xavier dos Santos

Lucas Rozendo de Lima Silva

Ester Costa Franco

 <https://doi.org/10.22533/at.ed.9662430042>**CHAPTER 3 19****STUDYING THE EFFECT OF THE INSECTICIDE USED IN AGRICULTURE (RUSTILE) ON THE LARVAE OF THE DOMESTIC MOSQUITO (*CULEX PIPIENS*)**

Habiba Gacem

Imene Khafallah

Hadia Rizzi

Amira Khedidja


Djeghader Nour El-Houda

Rabah Chaouch

Fayçal khelfaoui

Hicham Boughendjioua

Moussa Houhamdi

 <https://doi.org/10.22533/at.ed.9662430043>**CHAPTER 427****INSECTICIDAL EFFECT OF ESSENTIAL OILS OF ORIGANUM MAJORANA AGAINST *CULEX PIPIENS***

Nour El-Houda Djeghader

Khedidja Amira

Asma Fraia

Habiba Gacem


Fayçal Meziri

Rabah Chaouch

Hadia Delhoum


Hicham Boughendjioua

Hamid Boudjelida

 <https://doi.org/10.22533/at.ed.9662430044>


CHAPTER 534**ANTIOXIDANT ACTIVITY OF *THAPSIA GARGANICA***

Khedidja Amira
 Nour El-Houda Djeghader
 Habiba Gacem
 Hadia Delhoum
 Fayçal Meziri
 Rabah Chaouch
 Hicham Boughendjioua
 Hamid Boudjelida

 <https://doi.org/10.22533/at.ed.9662430045>


CHAPTER 6 41**SENESCENCE, TOTAL REFLECTANCE AND CHLOROPHYLL CONTENTS AT FULL HEADING, RELATIONSHIPS WITH AGRONOMIC TRAITS OF DURUM WHEAT (*TRITICUM DURUM* DESF.) GENOTYPES GROWING UNDER SEMI-ARID CONDITIONS**

Benalia Frih
 Ali Guendouz
 Abdelmalek Oulmi
 Sarah Benkadja
 Hocine Bendada

 <https://doi.org/10.22533/at.ed.9662430046>

CHAPTER 753**ESTUDIO DE LA CAPACIDAD DE PRODUCCIÓN DE SIDERÓFOROS EN CEPAS AISLADAS DESDE SUELO AGRÍCOLA PARA SU APLICACIÓN COMO BIOINSUMO**

Viviana Edith Calvente
 Julian Berca Espinosa
 Yesica Sabrina Lambrese
 Leonardo Gastón Navarta
 María Gabriela Sansone

 <https://doi.org/10.22533/at.ed.9662430047>


CHAPTER 867**THE ROLE OF INTESTINAL MICROBIOME IN THE HEALTH AND PRODUCTION OF COMMERCIAL POULTRY: A REVIEW**

Webert Aurino da Silva
 Júlio César dos Santos Nascimento
 Carlos Bôa-Viagem Rabello
 Lilian Francisco Arantes de Souza
 Apolônio Gomes Ribeiro
 Dayane Albuquerque da Silva
 Daniela Pinheiro de Oliveira
 Hilton Nobre da Costa
 Maria do Carmo Mohaupt Marques Ludke
 Elisabete Albuquerque dos Santos Benvenuto
 Adrielle Carneiro de Araújo Santos
 Elton Francisco de Oliveira

 <https://doi.org/10.22533/at.ed.9662430048>


CHAPTER 979**AGREGADOS BIOGÊNICOS E FISIOGÊNICOS EM SISTEMAS AGROFLORESTAIS SINTRÓPICOS DIFERENTES IDADES E MATA SECUNDÁRIA**

Amanda Vanessa Araújo dos Santos
 Anaila Alencar de Amaral
 Breno dos Santos Barros
 Sheila dos Santos Tavares
 Maria Eduarda da Conceição Lourinho
 Adylson Pereira Campos Junior

 <https://doi.org/10.22533/at.ed.9662430049>


CHAPTER 1088**EFEITOS DA NUTRIÇÃO DE MATRIZES GESTANTES NO IMPRINTING FETAL EM SUÍNOS: REVISÃO**

Jessica Mansur Siqueira Crusóé
 Leonardo França da Silva
 Genelício Crusóé Rocha
 Leonardo Fonseca Faria
 Victor Crespo de Oliveira
 Matheus Faria Souza
 Diego de Ávila Martins Braga
 Denis Medina Guedes
 Michael Bergsten Eliphelete Rodrigues Barbosa
 Cauã Godinho Gomes Fraga
 Pedro Arthur Rezende Aguiar Moura
 Juarez Lopes Donzele

 <https://doi.org/10.22533/at.ed.96624300410>


CHAPTER 11110**PRODUCTION OF CACTUS PEAR IRRIGATED WITH BRACKISH WATER AND ORGANIC MATTER**

Daniel Bezerra do Nascimento
 André Luiz Rodrigues Magalhães
 Glayciane Costa Gois
 Tuany Nathan Epaminondas de Amaral
 Túlio Vandeilton de Oliveira Albuquerque
 Priscila Barreto da Silva

 <https://doi.org/10.22533/at.ed.96624300411>

CHAPTER 12123**EFEITO DA ADUBAÇÃO DE NITROGÊNIO NA CULTURA DO MILHO (ZEA MAYS) COMPARADO ENTRE OS DOIS HÍBRIDOS**

Kentaro Tomita

 <https://doi.org/10.22533/at.ed.96624300412>

CHAPTER 13..... 138**¿SE JUSTIFICA LA OVARIECTOMÍA EN VACAS? PRINCIPALES RIESGOS Y BENEFICIOS**


Gabriel Gerardo Aguirre Espíndola

Felicitas Vázquez Flores

Néstor Méndez Palacios


Sandra Ortiz González

José Alfredo Galicia Domínguez

 <https://doi.org/10.22533/at.ed.96624300413>**CHAPTER 14..... 144****GASTROINTESTINAL HELMINTHS OF SHEEP BREED IN SPREAD BELGRADE AREA**

Ivan Pavlovic

Aleksandra Tasic

 <https://doi.org/10.22533/at.ed.96624300414>**CHAPTER 15..... 172****TRENDS IN CELL-BASED SEAFOOD: THE USE OF BIOTECHNOLOGY FOR NUTRITION AND SUSTAINABILITY**

Narcilo Quadros Cardoso

Carla Eliana Davico

Michael dos Anjos dos Santos

Rayanne Antunes Fernandes Sales

Isabel Virgínia Gomes e Silva

Letícia de Almeida Sant'Anna Trindade

Dayana Muniz Maia Montalvão


Carolina Siqueira Safra Terra Melo

Tailah Bernardo de Almeida

Guilherme Búrgio Zanette

Ricardo Coutinho

Giselle Pinto de Faria Lopes

 <https://doi.org/10.22533/at.ed.96624300415>**CHAPTER 16..... 195****COMPÓSITO REFORÇADO COM FIBRA DO PECÍOLO DA PALMEIRA DE DENDÊ: FABRICAÇÃO DE MATERIAL SUSTENTÁVEL**


Roberto Yuri Costa Dias

Rafael Vilhena Soares

Maria de Fátima Mendes Leal


Pedro Victor de Mendonça Maia

Roberto Tetsuo Fujiyama

 <https://doi.org/10.22533/at.ed.96624300416>


CHAPTER 17.....205**SISTEMA CARDIOVASCULAR BOVINO: CONSERVAÇÃO E MONTAGEM**

Giovana Balbinot
 Naiane Dierings
 Géssica Gutierres
 Eliz Roberta Kuhn
 Marciane Parizotto
 Kleberon Leonardo Schafer
 Eduardo Rebelato Sakis
 Gabriela Tonello
 Bárbara Thaisi Zago

 <https://doi.org/10.22533/at.ed.96624300417>


CHAPTER 18.....222**GYPSUM IN AGRICULTURE**

Alceu Linares Pádua Junior
 Hamilton César de Oliveira Charlo
 Jose Luiz Rodrigues Torres
 Ernane Miranda Lemes

 <https://doi.org/10.22533/at.ed.96624300418>

CHAPTER 19.....276**PHENOTYPIC CORRELATIONS BETWEEN LINEAR TRAITS AND GROWTH CURVES OF HOLSTEIN CALVES**

Leidiane Bora Cardoso
 Welington Hartmann

 <https://doi.org/10.22533/at.ed.96624300419>

ABOUT THE ORGANIZERS285**INDEX.....286**

CHAPTER 1

LOW-COST CLASS A EVAPOTRANSPIRATION PAN FOR REFERENCE EVAPOTRANSPIRATION ESTIMATION IN PROTECTED AND FIELD ENVIRONMENTS: A PRACTICAL APPROACH

Acceptance date: 02/05/2024

Silvio Roberto de Lucena Tavares

Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA SOLOS, Jardim Botânico- RJ

Natália Fernandes Rodrigues

Doutoranda no Programa de Pós-Graduação em Agronomia, Seropédica, - UFRRJ, Rio de Janeiro, Brasil

Gabriela Martins Corrêa

Mestranda no Programa de Pós-Graduação em Engenharia de Biossistemas - UFF, Niterói-RJ

Cristina Moll Hüther

Professora no Departamento de Engenharia Agrícola e Ambiental e no Programa de Pós-Graduação em Engenharia de Biossistemas – UFF, Niterói-RJ

ABSTRACT: Currently, there are various methods for determining the Class A pan coefficient (K_p) in estimating reference evapotranspiration (E_{To}), which is of utmost importance for water management in agriculture. This study aimed to estimate E_{To} inside and outside agricultural greenhouses using a low-cost constructed

Class A pan (TCA-c) method. To estimate E_{To} , it is necessary to establish the K_p , as E_{To} is the product of ECA multiplied by K_p . This study employed the calibration method, comparing E_{To} determined by Penman-Monteith (E_{ToPM}) with Class A pan evaporation (ECA) to determine K_p . E_{ToPM} served as the standard for correlations with reference evapotranspiration using the TCA-c method inside and outside an agricultural greenhouse, avoiding the installation of a “Class A” pan inside the greenhouse. The experiment was conducted at UFF’s Gragoatá campus in Niterói - RJ. Four TCA-c pans were installed (three inside and one outside an agricultural greenhouse) and managed for one year. Principal Component Analyses (PCA) revealed significant differences in E_{To} throughout the seasons. Adjusted K_p s were established for all pans. It was observed that E_{To} inside the greenhouse was lower than that estimated outside. It is recommended to install the TCA-c pan inside the greenhouse for E_{To} estimation, utilizing different K_p s throughout the seasons.

KEYWORDS: Reference evapotranspiration; Pan coefficient; Class A Pan.

INTRODUCTION

Brazil is the second-largest producer of crops in protected environments in Latin America, with an approximate area of 30,000 hectares in 2019, trailing only Mexico, which had 41,000 hectares. This type of production is entirely conducted under irrigation. What is observed throughout the country is a complete absence of soil moisture monitoring instruments to determine the necessary irrigation for the crops. This irrigation requirement should be calculated based on the evapotranspiration rate removed from the system. Evapotranspiration is a complex process involving the evaporation of water from the soil and vegetated surfaces, as well as plant transpiration. To measure evapotranspiration, various techniques and instruments can be employed, utilizing both direct and indirect methods. One way to assess the accuracy of reference evapotranspiration (ET_o) estimation methods is by comparing them with the Penman-Monteith method, which has been recommended by the FAO as the standard method for ET_o estimation (Allen et al., 1998).

There is currently a trend towards the use of automated meteorological stations that assist in determining reference evapotranspiration, thereby reducing errors in the water depth to be applied to crops. When programmed, these stations can employ the Penman-Monteith method to calculate ET_o. However, most farmers use alternative methods and lack access to such equipment, preventing them from determining ET_o using the standard method. Therefore, correction equations in relation to the Penman-Monteith method (the FAO standard method) are desirable to minimize errors in ET_o calculation.

Reference evapotranspiration can be estimated through various methods, and the Class A Pan method has been one of the most widely employed methods worldwide, owing to its simplicity, relatively low cost, and its ability to provide daily estimates of evapotranspiration. However, its use within greenhouses remains a subject of controversy. Research results regarding which Class A Pan Coefficient (K_p) should be used inside the greenhouse are inconclusive. Furthermore, some producers consider it unfeasible to allocate approximately 10 m² of unproductive space for the Class A pan container inside the greenhouse.

Vetiver grass (*Vetiveria zizanioides* L. Nash) is used by part of Asia, mainly India, to make handicraft products, manufacture perfumes, medicines, and insect repellent (Gomes et al., 2020). It has been widely used in several countries because it has a deep and abundant root system, and because it is very resistant to climatic variations and tolerates contaminants (Ucker & Almeida, 2013). Vetiver grass is easy to adapt and is used in sediment control, phytoremediation, affluent treatment, and slope stabilization (Medeiros et al, 2020).

MATERIALS AND METHODS

The study was conducted at the UFF Gragoatá campus. Two automated weather stations of the brand/model E5000 by IRRIPLUS® were installed: one in an outdoor area with *Paspalum maritimum* vegetative cover, having a 10-meter border, and the other inside an Agricultural Greenhouse (AG). In these areas, four TCA-c pans were also installed (one outdoors and three inside the AG). The meteorological data required for reference evapotranspiration (ET_o) calculation (global radiation, air temperature, relative humidity, and wind speed) were collected from the aforementioned stations. The equation used for calculating reference ET_o using the Penman-Monteith Method was as follows the Equation 1:

$$ET_o \text{ (PM)} = 0.409(R_n - G) + (900 / (T + 273)) * W * (e_s - e) / (1 + 0.34 * V) \text{ (Eq. 1)}$$

Where:

- ET_o (PM) = reference evapotranspiration using the PM method, in millimeters per day (mm d⁻¹).
- R_n = net radiation, MJ m⁻² d⁻¹; G = soil heat flux, MJ m⁻² d⁻¹;
- T = mean air temperature, °C;
- W = mean wind speed at 2m height, m s⁻¹;
- (e_s - e) = vapor pressure deficit, kPa;
- and 900 = conversion factor.

The development and construction of the low-cost evapotranspiration pan followed all dimensions and installation protocols recommended by USWB/USA and FAO. The difference is that it was manufactured by adapting a 200-liter iron drum, cut to a height of 25.4 cm (10") and an internal diameter of 120.6 mm. These TCA-c pans were painted in light gray, and wooden slats (from discarded pallets) were made to the prescribed measurements. A mechanism with graduated rulers in millimeters was installed on the internal walls of the TCA-c pans for reading purposes. Water levels inside the pans fluctuated between 5-7.5 mm from the edge of the pans.

Daily readings from the pans were obtained at the same time during the period from 01/09/2021 to 01/09/2022 (one year), and hourly data from the weather stations (EMs) were collected. The evaporation from the Class A pans (ECA) was measured by calculating the difference between daily readings. The Class A Pan Coefficient (K_p) was established based on the season and the reference ET_o determined by the EMs.

The collected data were assessed for normality, subjected to multiple comparisons (Tukey, 0.05 significance level), and analyzed using Principal Component Analysis (PCA) and clustering techniques with R software.

RESULTS AND DISCUSSION

Table 1 presents the average values of meteorological data provided by the weather stations installed inside and outside the agricultural greenhouse. In Table 2, the Class A Pan Coefficients (Kp) are shown, based on the correlation between Penman-Monteith reference evapotranspiration (EToPM) and Class A Pan evaporation (ECA), for both the protected environment and field conditions, across the four seasons of the year.

Seasons	T In	UR In	W In	R In	T Out	UR Out	W Out	R Out	P Out
	°C	%	m s ⁻¹	MJ	°C	%	m s ⁻¹	MJ	mm
Spring	24.24	81.85	0	3.93	23.58	81.03	0.48	16.84	5.11
Summer	27.93	78.74	0	4.61	26.46	77.09	0.35	20.17	4.23
Fall	24.24	81.76	0	2.39	23.32	83.46	0.13	13.28	3.08
Winter	22.59	82.08	0	2.25	22.17	85.56	0.21	12.92	1.46

T: Average Temperature; UR: Average Relative Humidity; W: Average Wind Speed; R: Average Solar Radiation; P: Average Precipitation; In: Inside; Out: Outside.

Table 1. Weather data collected from weather stations inside and outside the greenhouse.

Seasons	ECA In	EToPM In	Kp In	ECA Out	EToPM Out	Kp Out
	----mm----			----mm----		
Spring	1.93 b	1.14 b	0.62	5.45 a	3.42 b	0.89
Summer	2.61 a	1.28 a	0.55	5.68 a	4.31 a	0.96
Fall	1.75 b	0.80 c	0.60	3.98 b	2.41 c	0.82
Winter	1.59 b	0.79 c	0.60	3.49 b	2.30 c	0.81
CV (%)	56.79	27.45	78.15	61.97	36.21	106.65

ECA: Evapotranspiration of the class A pan; EToPM: Penman-Monteith Evapotranspiration; Kp: Pan coefficient; In: Inside; Out: Outside. Consecutive means with the same letters in the column do not differ by Tukey's test at 5%.

Table 2. Evapotranspiration of the class-A pan and Penman-Monteith and Pan coefficients inside and outside the greenhouse.

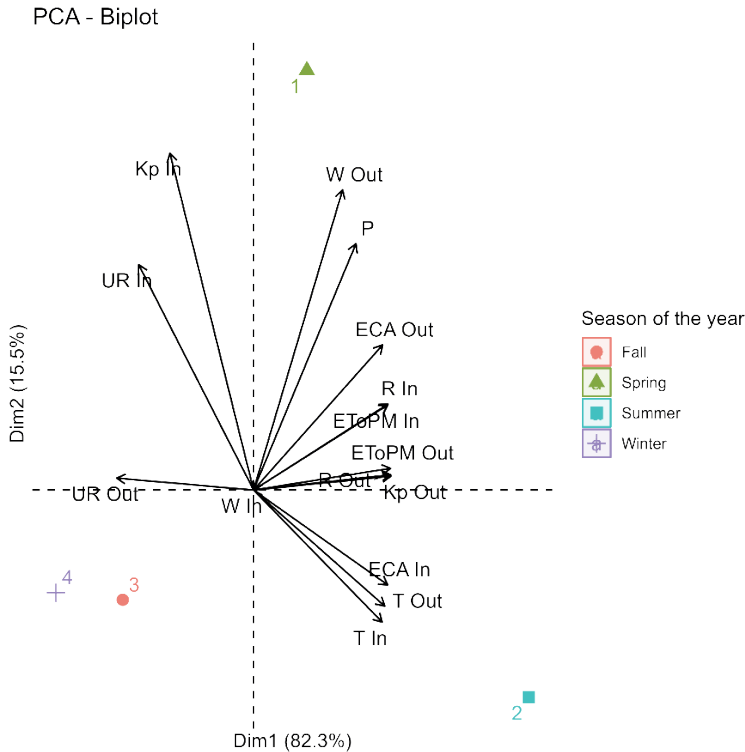
The protected environment condition exhibited a lower Kp, with evaporation ranging from 54 to 64% less, resulting in an evapotranspiration 65 to 70% lower across the analyzed seasons. Kp variations in the protected environment ranged from 0.55 to 0.62, while in the field, they varied from 0.81 to 0.96. There was greater data variability in field conditions. These same trends were also observed by Cunha (2011) when determining TCA pan Kps using different methods: Doorenbos and Pruitt (1977), Cuenca (1989), Snyder (1992), Pereira et al. (1995), Allen et al. (1998), and the correlation between Penman-Monteith reference evapotranspiration (EToPM) and Class A Pan evaporation (ECA) in both protected and field environments in the Botucatu-SP region.

The author concluded that, in a protected environment, the methods of Allen et al. (1998) and Snyder (1992) are the most recommended for dry months, while the correlations between EToPM and ECA and Cuenca (1989) are suitable for rainy months. In field conditions, the methods of Allen et al. (1998) and the correlations between EToPM and ECA are suitable for dry months, and Allen et al. (1998) and Cuenca (1989) for rainy months. The Allen et al. (1998) method proved to be the most efficient, regardless of the environment or the analyzed months.

The plastic covering used in the experimental agricultural greenhouse, combined with the use of a 70% shade net, significantly alters the radiation balance compared to the external environment. This is due to the attenuation (absorption and reflection) of incident solar radiation, resulting in a reduction of the internal radiation balance and, consequently, affecting evapotranspiration (Sentelhas, 2001). The difference between internal and external evapotranspiration varies with meteorological conditions. In this study, it was observed that daily radiations in the external environment were, on average, 76.6% higher than internal radiations throughout the year. Autumn/winter showed the highest radiation variations (82.0 and 82.6%), due to the variation in solar incidence within the greenhouse, which was oriented southeast/northwest.

The proximity of a forest to the east contributed to this difference, impacting the average data of the three internal TCA pans distributed longitudinally in the greenhouse. This resulted in varying amounts of radiation received, leading to differences in pan evaporation.

Figure 1 displays the Principal Component Analysis in a biplot graph. Inside the AG, a positive relationship between air humidity and K_{pin} was observed. The pan evaporation values closely align with the EMs' evapotranspiration values, which increases K_p values under high humidity conditions. In the external environment, the opposite phenomenon occurs. High air humidity within the AG hinders evaporation due to the lower energy differential between the two environments.



ECA: Evaporation of the class A pan; EToPM: Penman-Monteith Evapotranspiration; Kp: Pan coefficient; T: Average Temperature; UR: Average Relative Humidity; W: Average Wind Speed; R: Average Solar Radiation; P: Average Precipitation; In: Inside; Out: Outside. 1: spring; 2: summer, 3: fall, and 4: winter.

Figure 1. Principal Component Analysis (PCA) of the parameters across different seasons of the year.

The wind speed and precipitation only impact the external environment since these meteorological phenomena are not present inside the agricultural greenhouse. It was observed that these parameters had a greater impact on the spring during the study period. This is because, in addition to magnitude, the direction of the coefficients of the original variables exhibited higher absolute values.

In summer, the internal temperature in the AG is negatively related to KpIn because the internal Class A Pans evaporate more water, necessitating larger corrections in the calculation of evapotranspiration.

The parameter most strongly related to Kpout is solar radiation, as it is the primary energy source for the planet and can transform liquid water into vapor. Thus, the evapotranspiration process is determined by the amount of energy available to vaporize water. In the case of WSout, ECAout closely approximates the values of EToPM.

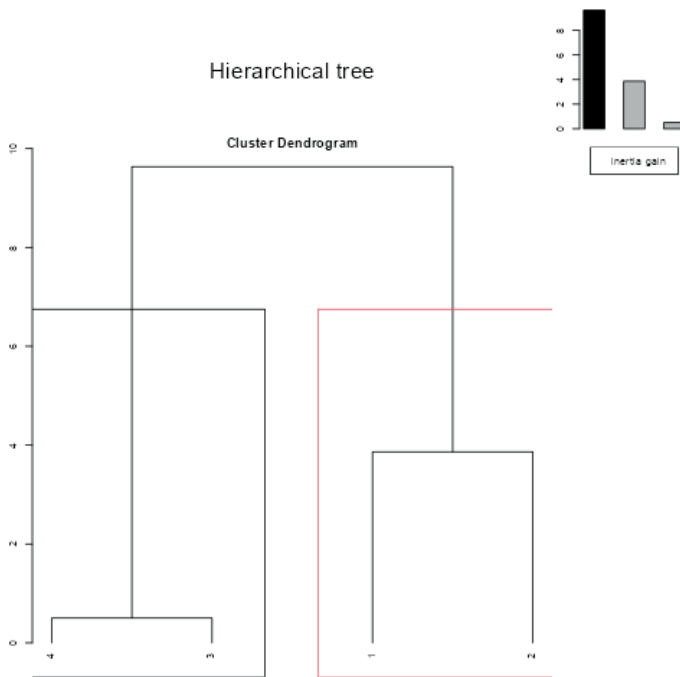


Figure 2: Cluster analysis in which 1 represents spring, 2 summer, 3 autumn, and 4 winter.

Cluster analysis hierarchically separated the seasons into two groups: fall/winter and spring/summer (Fig. 2). Thus, it is evident that a single Kp value cannot be used in both protected and open environments, as there are various variations in climatological parameters throughout the year.

Lopes Filho (2000) estimated ETo inside an AG and estimated the evaporation occurring in three types of Class A Pans (TCAUSWB, Mini metallic pan, and Mini plastic pan). ETo was estimated using the PenmanMonteith-FAO method based on meteorological data also collected from an automatic weather station installed inside the AG. The Kp values ranged from 0.25 to 0.93 for TCAUSWB, 0.23 to 0.85 for the mini metallic pan, and 0.30 to 0.95 for the mini plastic pan.

A variety of machine learning (ML) algorithms, such as random forest (Lu et al., 2018), artificial neural networks (Goyal et al., 2014), support vector machines (Kisi, 2015), among others, have been extensively utilized to predict TCAC. These ML methods offer robust, nonparametric models that don't require extensive knowledge of internal variables, allowing them to handle complex, nonlinear functions and diverse data more effectively than traditional models (Wang et al., 2023). Compared to traditional statistical models, ML models are known for their superior predictive performance, adaptability across various scenarios, and flexibility in using different input variables (Schmidt et al., 2020).

This research will continue to establish the TCAC coefficient more accurately, incorporating other measurement equipment such as TCAUSWB, atmometer, and automatic reading Class A Pan with pressure transducer.

CONCLUSION

The results obtained and the experimental conditions lead to the following conclusions: A single K_p value cannot be used for both protected and open environments due to variations in climatological parameters throughout the year. The protected environment exhibits a lower K_p compared to the external environment. K_p variations in the protected environment ranged from 0.55 to 0.62, while in the field, they varied from 0.81 to 0.96. Field conditions exhibited greater data variability. Evapotranspiration in the external environment is greater than in the protected environment. To more accurately account for microclimatic variations in the protected environment, it is recommended to use a longer series of data for K_p calculation, allowing for monthly and seasonal analysis, depending on the agricultural crop.

REFERENCES

- ALLEN, R. G.; PEREIRA, L. S.; RAES, D.; SMITH, M. Crop evapotranspiration: guidelines for computing crop water requirements. Roma: FAO, 1998. 300 p. (Irrigation and Drainage, n.56).
- CUENCA, R. H. Irrigation system design: an engineering approach. New Jersey: Prentice-Hall, Englewood Cliffs. 1989. 133 p. DOORENBOS, J.; PRUITT, W. O. Crop water requirements. Rome: FAO, 1977. 179 p. (FAO. Irrigation and Drainage Paper, 24).
- GOYAL, M. K.; BHARTI, B.; QUILTY, J.; ADAMOWSKI, J.; PANDEY, A. (2014). Modeling of daily pan evaporation in sub tropical climates using ANN, LS-SVR, Fuzzy Logic, and ANFIS. Expert systems with applications, v.41, n.11, p.5267-5276, 2014.
- KISI, O. Pan evaporation modeling using least square support vector machine, multivariate adaptive regression splines and M5 model tree. Journal of Hydrology, v.528, p.312-320, 2015.
- LOPES FILHO, R. P. Utilização de diferentes tanques evaporimétricos em ambiente protegido. Lavras: UFLA. 2000.79p.: il.
- LU, X.; JU, Y.; WU, L.; Fan, J.; Zhang, F.; Li, Z. Daily pan evaporation modeling from local and cross-station data using three tree-based machine learning models. Journal of Hydrology, v.566, p.668-684, 2018.
- PEREIRA, A. R.; VILLA NOVA, N.; PEREIRA, A. S.; BARBIERI, V. A model for the class A pan coefficient. Agricultural and Forest Meteorology, Amsterdam, v. 76, n. 2, p. 75-82, 1995.
- SCHIMDT, L.; HEBE, F.; ATTINGER, S.; KUMAR, R. Challenges in applying machine learning models for hydrological inference: a case study for flooding events across Germany. Water resources research, v.56, p.5, e2019WR025924, 2020.

SENTELHAS, P. C.; FOLEGATTI, M. V. Class-A pan coefficients (K_p) to estimate daily reference evapotranspiration (ET_o). *Revista Brasileira de Engenharia Agrícola e Ambiental*, Campina Grande, v. 7, n. 10, p. 111-115, 2003.

SNYDER, R. L. Equation for evaporation pan to evapotranspiration conversion. *Journal of Irrigation and Drainage Engineering of ASCE*, New York, v. 118, n. 6, p. 977-980, 1992.

WANG, H.; SUN, F.; LIU, F.; WANG, T.; LIU, W.; FENG, Y. Reconstruction of the pan evaporation based on meteorological factors with machine learning method over China, *Agricultural Water Management*, v. 287, 108416, 2023.

SUPERAÇÃO DA DORMÊNCIA DE SEMENTES DE ARATICUM-DO-BREJO

Acceptance date: 02/05/2024

Walnice Maria Oliveira do Nascimento

Pesquisador da Embrapa Amazônia Oriental, Belém, PA

Caio Xavier dos Santos

Acadêmico do curso de Agronomia da UFRA
Universidade Federal Rural da Amazônia

Lucas Rozendo de Lima Silva

Acadêmico do curso de Agronomia da UFRA
Universidade Federal Rural da Amazônia

Ester Costa Franco

Acadêmica do curso de Agronomia da UFRA
Universidade Federal Rural da Amazônia

RESUMO: *Annona glabra* L. é espécie originária da América Tropical, com ampla distribuição geográfica no território brasileiro. Apresenta frutos com sabor pouco agradável, tem importância na fruticultura pelo fato de se constituir em porta-enxerto para a gravioleira (*Annona muricata* L.) e outras anonáceas produtoras de frutos comestíveis. A produção de porta-enxertos de araticunzeiro-do-brejo é efetuada por via sexuada, tendo como principal problema a germinação lenta e

desuniforme, pois as sementes apresentam dormência. O trabalho teve como objetivo verificar o efeito da escarificação do tegumento e da embebição em soluções de ácido giberélico sobre a germinação de sementes de araticum-do-brejo. Os tratamentos consistiram da combinação de sementes escarificadas e não escarificadas com a embebição, durante 24 horas, em soluções de ácido giberélico (AG₃), nas seguintes concentrações: 0, 250, 500, 750 e 1.000 mg.L⁻¹. O experimento foi conduzido em delineamento inteiramente casualizado com quatro repetições, em esquema fatorial 2 x 5 (condição da semente x concentração de ácido giberélico). Cada parcela foi constituída por 50 sementes. Observou-se que o efeito do ácido giberélico na germinação de sementes de araticum-do-brejo foi mais pronunciado que a escarificação. As sementes germinaram em menor tempo quando embebidas em soluções de AG₃ nas concentrações de 750 e 1.000 mg.L⁻¹. A escarificação do tegumento e a embebição em soluções de ácido giberélico, nas concentrações de 500, 750 ou 1.000 mg.L⁻¹, constitui-se em método eficiente para superação da dormência de sementes de araticum-do-brejo.

PALAVRAS-CHAVE: Annonaceae, propagação, porta-enxerto

OVERCOMING POND APPLE SEED DORMANCY

ABSTRACT: The *Annona glabra* is originally species of Tropical America, with wide geographical distribution in the Brazilian territory. It presents fruits with little flavor, but has importance in the horticulture for the fact of constituting in rootstock for soursop (*Annona muricata*) and other annonas producing of eatable fruits. The production of pond apple tree rootstock is made by sexuada, as main problem the slow and desuniforme germination, because the seeds present dormancy. The work had as objective to verify the effect of the scarification of the tegument and of the soaking in solution of giberelic acid about the germination of pond apple seeds. The treatments consisted of the combination of seeds scarification and non scarification with soaking, for 24 hours, in solution of giberelic acid (AG_3), in the following concentrations: 0, 250, 500, 750 e 1,000 $mg.L^{-1}$. The experiment was carried out completely randomized design, with four replicates, of 50 seeds, in factorial scheme 2 x 5 (condition of the seed x concentration of giberelic acid). It was observed that the effect giberelic acid in the germination of pond apple seeds was more pronounced than the scarification. The seeds germinated in smaller time when soaked in solution of AG_3 in the concentration of 750 and 1,000 $mg.L^{-1}$. The scarification of the tegument and the soaking in solution of giberelic acid, in the concentrations of 500 or 1,000 $mg.L^{-1}$, is constituted in efficient method for overcoming pond apple seeds dormancy.

KEYWORDS: Annonas, propagation, rootstock

INTRODUÇÃO

O araticunzeiro-do-brejo (*Annona glabra* L.) é espécie originária da América Tropical, com ampla distribuição geográfica no território brasileiro, ocorrendo desde a Amazônia até o Estado de Santa de Catarina. É encontrado, com maior freqüência, em áreas periodicamente inundadas, advindo dessa particularidade o seu nome comum (LUCENA et al., 2011).

Essa espécie, conquanto apresente frutos com sabor pouco agradável, em decorrência do baixo teor de açúcares, tem importância na fruticultura pelo fato de se constituir em porta-enxerto passível de ser utilizado em outras anonáceas produtoras de frutos comestíveis, em particular para a gravioleira (*Annona muricata* L.). Quando utilizada como porta-enxerto para essa espécie, na Amazônia Oriental Brasileira, além da boa porcentagem de enxertos pegos, possibilitou a obtenção de produtividades entre 54,2 kg e 62,5 kg de frutos por planta, 27 meses após o plantio (CARVALHO et al., 2003).

A produção de porta-enxertos de araticunzeiro-do-brejo é efetuada por via sexuada, tendo como principal problema a germinação lenta e com acentuada desuniforme, pois as sementes apresentam dormência, requerendo, em média, 90 dias para atingirem 95% de germinação (CARVALHO et al., 2001), enquanto, sementes de graviola atingem esta mesma porcentagem aos 36,3 dias após a sementeira (CARVALHO et al., 1998).

A germinação lenta e desuniforme de sementes de anonáceas está, geralmente, associada à impermeabilidade do tegumento à água como no caso de *Annona squamosa* L. (PAWSHE et al., 1997) ou à ocorrência de dormência morfofisiológica como em *Annona*

spraguei Saff., *Xylopia aromatica* (Lam.) Mart. e *Xylopia frutescens* Aubl. ou, ainda, a dormência morfofisiológica combinada com a impermeabilidade do tegumento à água, como em *A. cherimolla* Mill. (SMET et al., 1999). Nas sementes de araticum (*Annona montana* Macf.), graviola (*Annona muricata* L.) e biribá (*Rollinia mucosa* (Jacq.) Bail.) é provável que a dormência por impermeabilidade do tegumento à água só se manifeste após a secagem, haja vista a obtenção de porcentagens de germinação superiores a 94% em espaço de tempo inferior a 50 dias, quando as sementes são semeadas imediatamente após serem extraídas dos frutos, ocasião em que apresentam teor de água em torno de 30% (CARVALHO et al., 1998).

Diversos tratamentos pré-germinativos têm sido indicados para a superação da dormência de sementes de anonáceas, tais como: pré-embebição em água, imersão em água quente, escarificação mecânica ou química e pré-embebição em ácido giberélico (PINTO, 1976; HERNANÉZ, 1993). Em sementes de ata (*Annona squamosa* L.), resultados bastante expressivos, em termos de aumentar e acelerar a germinação, foram obtidos com a escarificação do tegumento e a pré-embebição em solução de ácido giberélico com concentrações entre 50 e 100 mg.L⁻¹ (STENZEL et al., 2003). Por outro lado, em sementes de araticum-do-brejo a associação de escarificação com pré-embebição em ácido giberélico em concentrações variando entre 50 e 250 mg.L⁻¹ não teve efeito sobre a porcentagem de germinação, embora tenha reduzido o tempo requerido para a germinação (SOUZA NETO et al., 2006).

Este trabalho teve como objetivo, verificar os efeitos da escarificação do tegumento e da pré-embebição em soluções de ácido giberélico sobre a germinação de sementes de araticum-do-brejo.

MATERIAL E MÉTODOS

Foram utilizadas sementes oriundas de seis plantas estabelecidas na coleção de fruteiras nativas da Embrapa Amazônia Oriental, em Belém, PA. As sementes foram extraídas manualmente de frutos completamente maduros, caracterizados pela cor amarela do epicarpo e pela consistência mole da polpa. Após a extração, as sementes foram lavadas em água corrente, enxugadas superficialmente com papel absorvente e submetidas à secagem, em dessecador contendo sílica gel, até que o grau de umidade atingisse valor em torno de 7%. Em seguida, efetuou-se seleção manual, com o objetivo de eliminar as sementes chochas e as atacadas por insetos, em particular pela broca-das-sementes (*Bephratelloides pomorum* Fabricius). O tempo decorrido entre a extração das sementes e a aplicação dos tratamentos foi de 20 dias.

Os tratamentos consistiram da combinação de sementes escarificadas e não-escarificadas com a pré-embebição, durante 24 horas, em soluções de ácido giberélico (AG₃), nas seguintes concentrações: 0, 250, 500, 750 e 1.000 mg.L⁻¹.

A escarificação foi efetuada removendo-se pequena porção do tegumento na extremidade apical de cada semente. Essa operação foi efetuada com um alicate de cortar unhas, tendo-se o cuidado de não afetar as estruturas internas da semente.

Após a aplicação dos tratamentos pré-germinativos, efetuou-se a semeadura em recipientes de plástico com dimensões de 25 cm de diâmetro e 18 cm de altura, contendo como substrato a mistura de areia com pó de serragem, na proporção volumétrica de 1:1. O substrato foi previamente esterilizado em água fervente durante duas horas.

Os testes de germinação foram conduzidos em ambiente sem controle de temperatura e umidade relativa do ar, nas condições de ambiente natural de Belém (temperatura média de 26,8°C). O número de sementes germinadas, em cada parcela, foi controlado diariamente, para fins de estimativa do tempo médio de germinação e para elaboração das curvas de germinação. Foram consideradas germinadas apenas sementes que originaram plântulas normais, ou seja, com todas as estruturas essenciais perfeitamente desenvolvidas e sadias.

As seguintes variáveis foram consideradas na avaliação dos tratamentos: comprimento e diâmetro do hipocótilo em plântulas originadas de sementes submetidas a escarificação, porcentagens de germinação em intervalos regulares de 20 dias, a partir da data de semeadura, até 220 dias, início, término e tempo médio de germinação, o qual foi determinado por meio do critério estabelecido por Silva e Nakagawa (1995). Esse índice representa a média ponderada do tempo necessário para a germinação, tendo como fator de ponderação a germinação diária, calculado pela equação (1):

$$TMG = \frac{N_1T_1 + N_2T_2 + \dots + N_nT_n}{N_1 + N_2 + \dots + N_n}$$

Em que:

T_m = é o tempo médio em dias, necessário para atingir a germinação máxima;

G_1 , G_2 e G_n é o número de sementes germinadas nos tempos T_1 , T_2 e T_n , respectivamente.

O experimento foi conduzido em delineamento inteiramente casualizado com quatro repetições, em esquema fatorial 2 x 5 (condição da semente x concentração de AG_3). Cada parcela foi constituída por 50 sementes. As médias foram comparadas pelo teste de Tukey, a 5% de probabilidade. Os dados expressos em porcentagem foram transformados de acordo com a equação $y = \sqrt{x/100}$ e os em dia foram transformados em raiz quadrada de $x + \mu$, os valores apresentados na tabela e figuras são os originais.

RESULTADOS E DISCUSSÃO

O efeito da pré-embebição em soluções de ácido giberélico sobre a germinação das sementes e desenvolvimento do hipocótilo em plântulas de araticum-do-brejo foi mais pronunciado que o da escarificação. A porcentagem de germinação foi superior a 90% em todos os tratamentos com escarificação associada a embebição das sementes em soluções de ácido giberélico (GA_3). Entretanto, no período avaliado não houve diferença significativa entre os tratamentos aplicados às sementes escarificadas ou não.

Com relação à avaliação do crescimento do hipocótilo, o qual foi verificado apenas nas sementes escarificadas, não houve diferenças significativas entre as diferentes concentrações de ácido giberélico, tanto para o comprimento, quanto para o diâmetro (Tabela 1).

AG 3 (mg.L ⁻¹)	Germinação		Crescimento do hipocótilo	
	Não escarificada (%)	Escarificada (%)	Comprimento (cm)	Diâmetro (cm)
0	85,7 Aa	93,0 Aa	8,5 A	0,31A
250	94,0 Aa	95,5 Aa	8,4 A	0,31 A
500	91,0 Aa	95,0 Aa	9,3 A	0,29 A
750	94,5 Aa	96,5 Aa	9,1 A	0,29 A
1000	92,5 Aa	96,0 Aa	9,5 A	0,28 A
C.V. (%)				

Médias seguidas pela mesma letra maiúscula na coluna e minúscula na linha, não diferem entre si pelo teste de Tukey a 5%.

TABELA 1. Porcentagem de germinação e crescimento do hipocótilo em plântulas de araticum-do-brejo, submetidas a diferentes tratamentos para superação de dormência. Belém, 2013.

No entanto, observou-se que a escarificação e a embebição em ácido giberélico reduziu o tempo requerido para o início da germinação. Nas sementes que não foram escarificadas, e nem embebidas em ácido giberélico, o início de germinação verificou-se, em média, 45 dias após a sementeira. Enquanto, nas sementes embebidas em solução de ácido giberélico na concentração de 250 mg.L⁻¹ o início de germinação foi mais rápido verificando-se, em média 23,5 dias após a sementeira. Reduções mais acentuadas foram obtidas em sementes submetidas as concentrações entre 500 e 1.000 mg.L⁻¹ (Figura 1). Resultados semelhantes foram encontrado por Stenzel et al. (2003), com a aumento de 50 para 100 mg.L⁻¹ na concentração de GA_3 , o que proporcionou elevação no índice de velocidade de germinação de sementes de *Annona squamosa*.

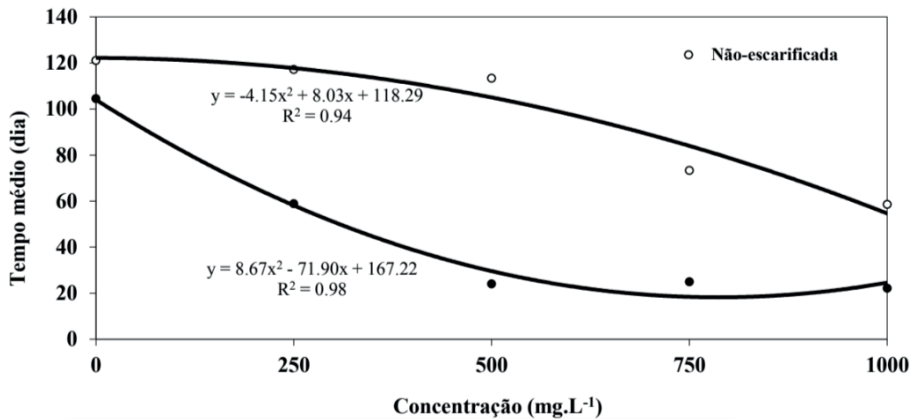


FIGURA 1. Tempo médio de germinação de sementes de araticum-do-brejo, escarificadas e não-escarificadas, em função da concentração de ácido giberélico.

Diferenças significativas pronunciadas em função da escarificação e da embebição em ácido giberélico foram observadas em relação ao tempo médio de germinação. Sementes não escarificadas e sem pré-embebição em ácido giberélico, o tempo médio de germinação foi de 211 dias. Nas sementes que não foram escarificadas a redução do tempo médio de germinação somente foi observada quando as sementes foram embebição em solução de ácido giberélico nas concentrações de 750 e 1.000 mg.L⁻¹. A concentração de 1000 mg.L⁻¹ de AG₃, acelerou o processo germinativo, nessa condição o tempo médio de germinação foi de 58,5 dias (Figura 2).

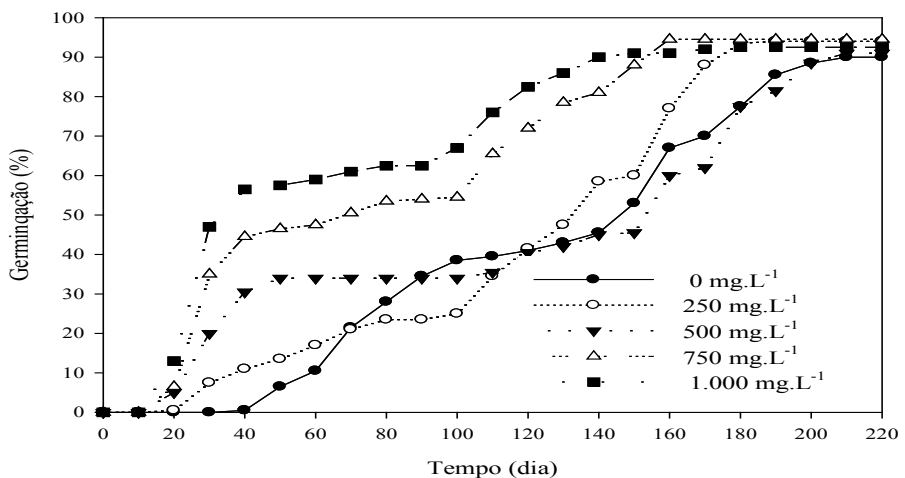


FIGURA 2. Porcentagem de germinação de sementes de araticum-dobrejo não submetido à escarificação e pré-embebidas em soluções de ácido giberélico de diferentes concentrações, em função do tempo.

Em todas as concentrações de ácido giberélico testadas, o tempo médio de germinação foi menor para as sementes escarificadas (Figura 3). A redução no tempo médio de germinação se verificou a partir de 250 mg.L⁻¹, sendo particularmente acentuada quando foram embebidas em soluções com concentrações de 500, 750 e 1.000 mg.L⁻¹. Sementes escarificadas e embebidas em soluções de ácido giberélico, nessas concentrações, apresentaram tempo médio de germinação inferior a 25 dias, valor este bem menor que o tempo requerido para a germinação de sementes de graviola que, em média, é de 36,3 dias (CARVALHO et al., 1998).

O efeito do ácido giberélico na aceleração da germinação de sementes de araticum-do-brejo foi mais pronunciado que a escarificação. Tal fato ocorreu devido ao estímulo da giberelina, que atua sobre a síntese de enzimas como a α -amilase que digerem as reservas armazenadas no endosperma das sementes, formando açúcares, aminoácidos e ácidos nucleicos, que são absorvidos e transportados para regiões de crescimento do embrião, estimulando o alongamento celular, fazendo com que a raiz rompa o tegumento da semente, acelerando a germinação com maior uniformidade (HOPKINS, 1999).

Com relação à concentração de ácido giberélico, constatou-se que quando não se efetuou a escarificação das sementes, estas germinaram mais rapidamente quando embebidas em soluções mais altas nas concentrações 750 e 1.000 mg.L⁻¹ (Figura 3). Resultados semelhantes foram encontrados por Ferreira et al. (2002) em sementes de *Annona squamosa*, utilizando concentrações de acima da 100 mg.L⁻¹, os quais verificaram diferenças significativas na porcentagem e velocidade na germinação das sementes.

Para as sementes escarificadas e embebidas em soluções de 500, 750 e 1.000 mg.L⁻¹ a germinação foi bastante rápida e uniforme, não sendo detectadas diferenças significativas, na porcentagem de germinação final computada aos 220 dias após a semeadura (Figura 3).

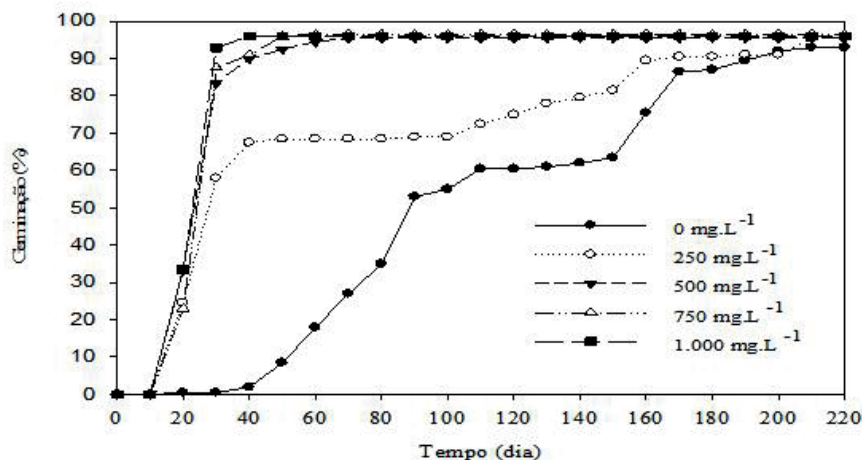


FIGURA 3. Porcentagem de germinação de sementes de araticum-do-brejo escarificadas e pré-embebidas em soluções de ácido giberélico de diferentes concentrações, em função do tempo.

Nessas concentrações, as sementes apresentaram 30 dias após a semeadura, porcentagens de germinação de 83,5, 87,5 e 93%, respectivamente. Enquanto que, para as sementes não escarificadas as porcentagens de germinação, ao final desse período, foram bem menores, sendo registrados valores de 20, 35 e 47%, respectivamente. Ressalte-se que, 30 dias após a semeadura, nenhuma semente não escarificada e sem embebição em ácido giberélico germinou. Enquanto, no tratamento que envolveu escarificação sem embebição em ácido giberélico a porcentagem de germinação foi inferior a 1% (Figura 3).

CONCLUSÕES

A escarificação do tegumento e a embebição em soluções de ácido giberélico, nas concentrações de 500, 750 ou 1.000 mg.L⁻¹, constitui-se em método eficiente para superação da dormência de sementes de araticum-do-brejo.

REFERÊNCIAS

CARVALHO, J. E. U. de; MÜLLER, C. H.; NASCIMENTO, W. M. O. do; FRAZÃO, D. A. C. Sistemas de propagação e técnicas de cultivo de espécies frutíferas tropicais na Amazônia Oriental. In: SEMINÁRIO TÉCNICO BRASIL - JAPÃO: PROJETO "DESENVOLVIMENTO TECNOLÓGICO PARA AGRICULTURA SUSTENTÁVEL NA AMAZÔNIA ORIENTAL, 1.,2003, Belém. **Anais...**Belém: Embrapa Amazônia Oriental, 2003, p. 57-62.

CARVALHO, J.E.U. de; NASCIMENTO, W.M.O. do; MÜLLER, C.H. **Características físicas e de germinação de sementes de espécies frutíferas nativas da Amazônia**. Belém: Embrapa-CPATU, 1998. 18p. (Embrapa-CPATU. Boletim de Pesquisa, 203).

CARVALHO, J.E.U. de; NASCIMENTO, W.M.O. do; MÜLLER, C.H. Tolerância de sementes de araticum-do-brejo (*Annona glabra* L.) ao dessecamento e ao congelamento. **Revista Brasileira de Fruticultura**, Jaboticabal, v.23, n.1, p.179-182, 2001.

FERREIRA, G.; ERIG, P.R.; MORO, E. Uso de ácido giberélico em sementes de fruta-do-conde (*Annona squamosa* L.) visando à produção de mudas em diferentes embalagens. **Revista Brasileira de Fruticultura**, Jaboticabal, v. 24, n. 1, p. 178-182, 2002.

HERNANDEZ, L. V. **La reproducción sexual y multiplicación vegetativa de la annonaceas**. Xalapa: Universidade Veracruzana, 1993. 35p.

HOPKINS, W. G. The role of hormones in plant development. In: **Introduction to plant physiology**. 2 ed. New York: John Wiley & Sons, 1999.

LUCENA, E.M.P. de; MAJOR, I.; BONILLA, O.H. **Frutas do litoral cearense**. Fortaleza: Ed. UECE. 2011. 112p.

PAULA, J. E. de; ALVES, J. L. de H. **Madeiras nativas: anatomia, dendrologia, dendrometria, produção e uso**. Brasília: Empresa Gráfica Gutenberg, 1997. 543p.

PAWSHE, Y.H.; PATIL, B.N., PATIL, L.P. Effect of pregermination seed treatment on the germination and vigour of seedlings in custard apple (*Annona squamosa* L.). **Annual of Plant Physiology**, v.11, n.2, p.150-154, 1997.

PINTO, A. C. Q. Influência de hormônio sobre o poder germinativo de sementes de graviola. CONGRESSO BRASILEIRO DE FRUTICULTURA, 3., 1975, Rio de Janeiro. **Anais...** Rio de Janeiro: SBF, 1976. p. 415-420.

SILVA, J.B.C.; NAKAGAWA, J. Estudo de fórmulas para cálculo de velocidade de germinação. Informativo ABRATES. Brasília, v.5, n.1, p.62-73, 1995.

SMET, S. DE; DAMME, P. VAN; SCHELDEMAN, X.; ROMERO, J. Seed structure and germination of cherimoya (*Annona cherimolla* Mill.). **Acta Horticulturae**, Wageningen, n. 497, p. 269-278, 1999.

SOUZA NETO; CARVALHO, J.E.U de; MÜLLER, C.H. **Germinação de sementes de araticum-do-brejo (*Annona glabra* L.) submetidas à pré-embebição em ácido giberélico.** In: FRAZÃO, D.A.C.; HOMMA, A.K.O; VIÉGAS, I. de J.M.(Ed.). Contribuição ao desenvolvimento da fruticultura na Amazônia. Belém: Embrapa Amazônia Oriental, 2006. p. 129-134.

STENZEL, N. M. C.; MURATA, I. M.; NEVES, C. S. V. J. Superação de dormência em sementes de atemóia e fruta-do-conde. **Revista Brasileira de Fruticultura**, Jaboticabal, v. 25, n.2, p.305-308, 2003.

CHAPTER 3

STUDYING THE EFFECT OF THE INSECTICIDE USED IN AGRICULTURE (RUSTILE) ON THE LARVAE OF THE DOMESTIC MOSQUITO (*CULEX PIPPIENS*)

Acceptance date: 02/05/2024

Habiba Gacem

Higher Normal School of Technological Education, Skikda, Algeria
Laboratory of Biology, Water and Environment, University of 08 May 1945, Guelma, Algeria
Laboratory of physical, Chemistry and Biology of Materials, Skikda, Algeria

Imene Khafallah

Higher Normal School of Technological Education, Skikda, Algeria
Laboratory of physical, Chemistry and Biology of Materials, Skikda, Algeria

Hadia Rizi

Département de Biologie, Faculté des Sciences et de la Nature, Université Chadli Bendjedid El-Tarf, Algérie

Amira Khedidja

Higher Normal School of Technological Education, Skikda, Algeria
Laboratory of physical, Chemistry and Biology of Materials, Skikda, Algeria

Djehader Nour El-Houda

Laboratory of physical, Chemistry and Biology of Materials, Skikda, Algeria
Department of Biology, Faculty of Sciences, University of BADJI Mokhtar, Annaba, Algeria

Rabah Chaouch

Higher Normal School of Technological Education, skikda, Algeria
Laboratory of physical, Chemistry and Biology of Materials, Skikda, Algeria

Faycal khelfaoui

Higher Normal School of Technological Education, skikda, Algeria
Laboratory of Biology, Water and Environment, University of 08 May 1945, Guelma, Algeria

Hicham Boughendjioua

Higher Normal School of Technological Education, skikda, Algeria
Laboratory of physical, Chemistry and Biology of Materials, Skikda, Algeria

Moussa Houhamdi

Laboratory of Biology, Water and Environment, University of 08 May 1945, Guelma, Algeria

ABSTRACT: Mosquitoes are the first cause of transmission of viral diseases for both humans and animals, as they can transmit some viral diseases. These insects have attracted the attention of specialists, especially in the field of control. Extensive

use of pesticides in agriculture and public health programs leads to numerous environmental problems and toxic effects on aquatic animals, especially non-target organisms. Thus, this method has many negative effects. Our study aims to know the effect of the insecticide used in agriculture Rustile to eliminate four stages of the life cycle of mosquitoes without causing damage to the ecosystem. So that we concluded through our work that the effect of these pesticides varies according to the concentration used, as shown by the sharp differences in the proportion and rate of mosquito mortality. The use of a 2.5 ml dose of rustic ide had a greater effect compared to both the 0.5 ml and 4.5 ml doses on the third day. If it turns out that using a dose of 4.5 ml of the pesticide was more effective in the third and fourth stages (L_3 , L_4) compared to doses of 0.5 ml and 2.5 ml of the same pesticide. However, this concentration is not recommended as it is not effective in the first larval instars (I and II) and thus allows them to develop into later instars (III and IV) which require a higher concentration in order to eliminate them. In addition to causing a lot of pollution, especially water pollution and its accumulation, thus affecting the balance of the water environment used there. Therefore, the use of a rusticide with the active substance ACETAMIPRID 20% at a specific concentration can be considered very effective in eliminating mosquito larvae and thus getting rid of harmful mosquitoes without harming the environment or non-target organisms.

KEYWORDS: Acetamiprid, *Culex pipiens*, Ecotoxicity, Rustile.

ESTUDAR O EFEITO DO INSETICIDA UTILIZADO NA AGRICULTURA (RUSTILE) SOBRE AS LARVAE DO MOSQUITO DOMÉSTICO (*CULEX PAPIENS*)

RESUMO: Os mosquitos são a primeira causa de transmissão de doenças virais tanto para humanos quanto para animais, pois podem transmitir algumas doenças virais. Esses insetos têm atraído a atenção de especialistas, principalmente na área de controle. O uso extensivo de pesticidas na agricultura e em programas de saúde pública leva a numerosos problemas ambientais e efeitos tóxicos em animais aquáticos, especialmente organismos não-alvo. Assim, este método tem muitos efeitos negativos. Nosso estudo tem como objetivo conhecer o efeito do inseticida utilizado na agricultura Rustile em eliminar quatro etapas do ciclo de vida dos mosquitos sem causar danos ao ecossistema. De modo que concluímos através do nosso trabalho que o efeito desses pesticidas varia de acordo com a concentração utilizada, como mostram as diferenças acentuadas na proporção e taxa de mortalidade dos mosquitos. O uso da dose de 2,5 ml de ide rústico teve efeito maior em comparação às doses de 0,5 ml e 4,5 ml no terceiro dia. Acontece que o uso de uma dose de 4,5 ml do pesticida foi mais eficaz na terceira e quarta etapas (L_3 , L_4) em comparação com doses de 0,5 ml e 2,5 ml do mesmo pesticida. No entanto, esta concentração não é recomendada porque não é eficaz nos primeiros instares larvais (I e II) e assim permite que evoluam para instares posteriores (III e IV) que requerem uma concentração mais elevada para serem eliminados. Além de causar muita poluição, principalmente a poluição das águas e seu acúmulo, afetando assim o equilíbrio do meio hídrico ali utilizado. Portanto, o uso de um rusticida com a substância ativa ACETAMIPRID 20% em concentração específica pode ser considerado muito eficaz na eliminação de larvas de mosquitos e, assim, livrar-se dos mosquitos nocivos sem agredir o meio ambiente ou organismos não-alvo.

PALAVRAS-CHAVE: Acetamipride, *Culex pipiens*, Ecotoxicidade, Rustile.

INTRODUCTION

Concerned experts and specialists agree that environmental sciences currently occupy an important space between basic, applied and human sciences. And people have started to view these developments as global problems that countries can only bring together, put in place appropriate frameworks and solutions. It emerged as an objective need to research living organisms and their environmental habitats (Hanoush, 2004) and to study the relationships between living and non-living elements. Insects are among the living organisms that have aroused great interest among environmental scientists, as they are among the most important classes of the arthropod phylum, both in terms of number and biological diversity, and in terms of economic importance. Among the insects that have been the subject of much scientific research are the mosquito families, because they are the first enemy of humans and animals. They are vectors of the pathogenic agents of many diseases, including malaria, yellow fever, elephantiasis, filariasis or filariasis, etc. Biodiversity can be understood as the study of difference, that is, what distinguishes and makes at the same time two neighboring entities in space or time. Conservation of biodiversity necessarily requires a complete knowledge of the distribution of fauna and flora (Couturier et al. 1985). Arthropods are abundant in all habitats from deserts to tropical forests. Insecticides are insecticides intended to kill insects. Pesticides are widely used in agriculture and community health and are also present in the local environment and are mostly responsible for health effects (Morsli et al. 2015) affecting insects as well as their toxic effects on humans (Lechekhab. 2018). Mosquitoes are considered harmful insects because they are the main vector of viral diseases to humans and animals. We conducted a comparative chemical study using the chemical pesticide «Rustile» used in agriculture on the larvae of domestic mosquitoes.

MATERIALS AND METHODS

Presentation of the study area Garaate Djamel

Garaate Djamel is located in the commune of El Chat, daira of Ben Mhidi on the left side of the wilaya road number 109 connect the wilaya of Annaba with El- Kala (Gacem, 2013).

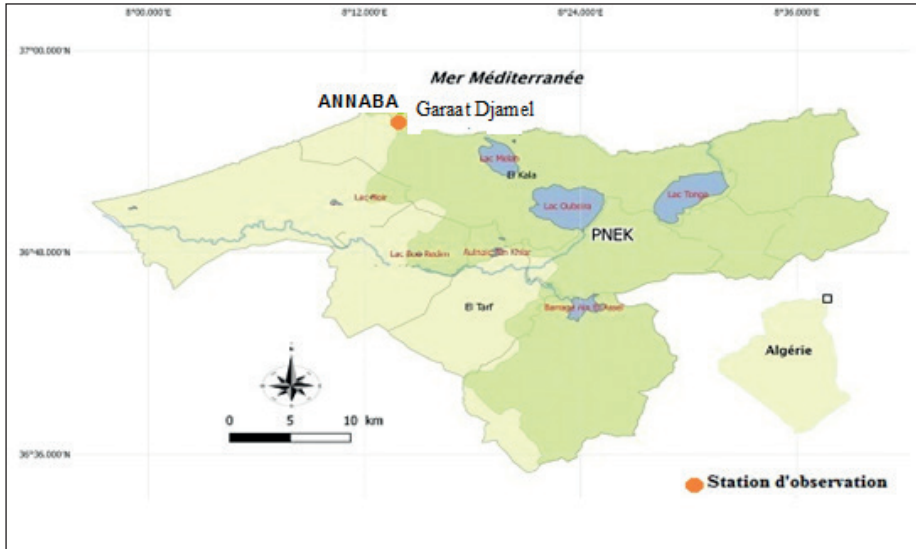


Figure 1: Location of observation station (Garaat Djamel) (Gacem *et al.*, 2023).

Insecticide Rustile

They are active substances or phytosanitary preparations that have the property of killing insects and/or their larvae and/or eggs. They are part of the family of pesticides, themselves included in the family of chemical pesticides, subject to regulation in Europe through specific directives. The generic term “insecticide” also includes pesticides intended to control arthropods that are not insects (such as spiders or mites such as ticks) as well as sometimes repellents. A distinction is made between contact products. Insecticides are chemical pesticides intended to destroy insects: widely used in agriculture and community health (disease vector control), they are also present in the home environment, although interesting from the point of view of operator safety, they have been subject to significant limitations in use due to Presumed adverse effects on non-target groups (Habiba, 2022). Both of these are great. The insecticide families are reviewed respectively, toxicokinetics, mode of action at the biochemical level and described toxic effects in humans in the main exposure contexts: household accidents, suicidal poisoning by high doses (Gacem, 2023).

Les Culicidae *Culex pipiens* Linnaeus, 1758

Mosquitoes are Arthropods. They are grouped in the Culicidae family, presenting sucker-biting type mouthparts. The duration of larval stages is closely linked to certain components of the environment, in particular temperature, humidity and photoperiod (Himmi, 1998). The life cycle of mosquitoes shows many variations depending on the species. The egg, larva and nymph stages are aquatic, while the adult is aerial.

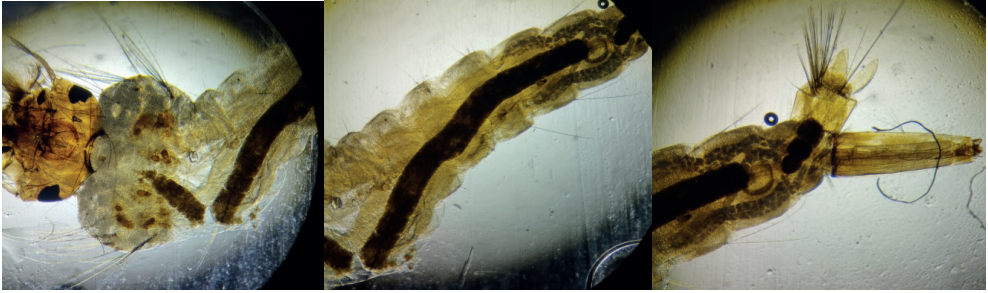


Figure 2: The anterior parts of the *Culex pipiens* larva Gr. x150 (Personal photos).

ANALYSIS AND STATISTICS

The results obtained are represented by the mean, the standard deviation and the percentage of mortality; we used the EXEL 2007 and EXEL 2010.

RESULTS

Figure (3) shows us the effect of the insecticide when placed with the larvae of the mosquito, *Culex pipiens pipiens*. A significant value of the death rate of the larvae of the first stage was estimated at 40%, followed by the death rate of the larvae of the second stage, which was estimated at 26.66%, to decrease the death rate. The larvae of the third instar compared to the death rate of the larvae of the first and second instars, which is 20%, to record the lowest mortality rate in the box containing the larvae of the fourth instar, which was estimated at 6.66%. These results are due to the small size of the larvae of the first and second instars and their effect on the pesticide and the inability to resist, while the larvae of the third and fourth instars are larger and more resistant to the insecticide.

Through figure (4) it is clear to us that when mosquito larvae, *Culex pipiens pipiens*, were placed with a little insecticide and left after 48 hours. The same death rate was recorded for each of the first, second and fourth instar larvae, except that it decreased by a large percentage compared to the first day, when we recorded 6.66%. As for the mortality rate for the third stage, it was completely absent.

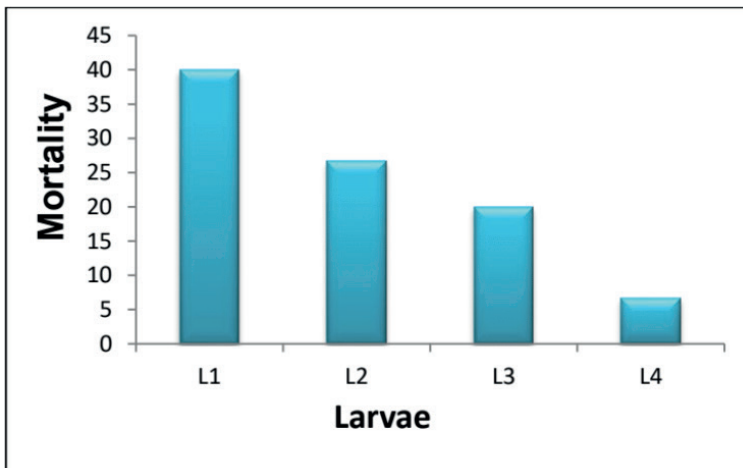


Figure 3 : Effect of the insecticide on *Culex pipiens pipiens* mosquito larvae within 24 hours.

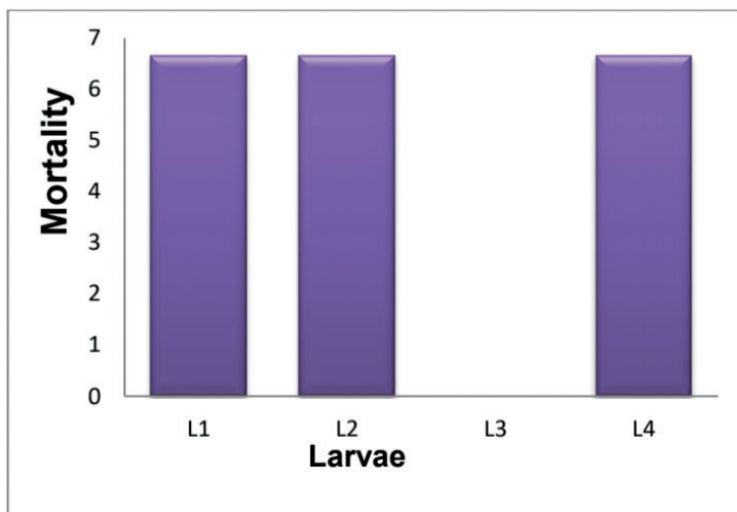


Figure 4: The ratio of the death rate of the four instars of the *Culex pipiens pipiens* larva within 48 hours.

DISCUSSION

Insects are among the most widespread arthropods on earth. In terms of epidemiological significance and human nuisance, mosquitoes are the first pathogen vectors, followed by Acariens mites, ticks, ticks, and finally Puces fleas (Lecointre, 2001). In order to combat these vector factors, chemical control was the main method for controlling disease vectors, but it has many disadvantages with regard to the high cost of pesticides, and it negatively affects the environment and the health of non-target groups such as humans, in addition to the emergence of resistance to some pesticides (Zaidi & Soltani, 2011). These

species are highly responsive to pesticides in the world and Algeria (Bendali-Saoudi *et al*, 2013). It has succeeded in developing systems that are resistant to conventional insecticide. As a result, the use of biological control has become a necessary alternative, thanks to its effectiveness against a wide range of pests (Soltani, 2010). Biological control is a traditional and alternative method to chemical control, non-selective and specific (Chaouch, 2024).

Insects are among the most widespread arthropods on earth. In terms of epidemiological significance and human nuisance, mosquitoes are the first pathogen vectors, followed by Acariens mites, ticks, ticks, and finally Puces fleas (Lecointre, 2001). In order to combat these vector factors, chemical control was the main method for controlling disease vectors, but it has many disadvantages with regard to the high cost of pesticides, and it negatively affects the environment and the health of non-target groups such as humans, in addition to the emergence of resistance to some pesticides (Habiba, 2022). These species are highly responsive to pesticides in the world and Algeria (Bendali-Saoudi *et al*, 2013). It has succeeded in developing systems that are resistant to conventional insecticide. As a result, the use of biological control has become a necessary alternative, thanks to its effectiveness against a wide range of pests (Soltani, 2010). Biological control is a traditional and alternative method to chemical control, non-selective and specific.

CONCLUSION

Our experiments dealt with a study of the mortality rate of *Culex pipiens pipiens* mosquito larvae treated with an insecticide Rustile during four stages of its life cycle. Through our research, we concluded that this pesticide has a toxic effect on mosquito larvae, *Culex pipiens pipiens*, where the effect is on the first small larval instars, the first larval instar L_1 , then the second larval instar L_2 , compared to the third instar L_3 and the fourth L_4 . Hence, farmers must use this insecticide used in agriculture, and this confirms the toxicity of the insecticide, that is, the chemical control of mosquito larvae L_1 , L_2 , L_3 and L_4 , and therefore biological control has more effectiveness in eliminating predators without environmental or health damage, which made it more effective. Widely used of chemical control.

REFERENCES

- Hanoush, A. H. A. 2004. The Iraqi Environment. *Problems and Prospects, Ministry of Environment, Dar Al-Araji for Printing and Publishing, Baghdad*, 75.
- Bendali-Saoudi, F., Oudainia, W., Benmalek, L., Tahar, A., & Soltani, N. 2013. Morphometry of *Culex pipiens pipiens* Linneus, 1758 (Dipterae: Culicidae) principal vector of West Nile Virus, harvested from 2 zones, humid, semiarid (East of Algeria). *Annals of biological research*, 4(10), 79-86.
- Soltani, N., Larhem, A. B., & Boudjelida, H. 2010. Lutte chimique contre le moustique: évaluation d'un insecticide sélectif à l'égard des larves de *Culex pipiens*. *Travaux de l'Institut Scientifique, Série Zoologie, Rabat*, (47), 177-182.

Couturier, G., Boussinguet, J., Dosso. 1985. *Pnue-let-Orstom-Rab-Unesco* Mab, 152 p.

Gacem, H., Bendali-saoudi, F., Serradj, N., Houmani, M., & Soltani, N. 2023. Risk assessment of the neonicotinoid insecticide acetamiprid on two non-target species, *daphnia magna* straus, 1820 (crustacea, cladocera) and *plea minutissima* leach, 1817 (insecta, heteroptera). *Applied Ecology & Environmental Research*, 21(2).

Habiba, G., Imen, K., Rabeh, C., & Lina, L. 2022. A comparative study between biological and chemical control against domestic mosquito larvae. *Biodiversitas Journal of Biological Diversity*, 23(12).

Gacem, H., Bendali-Saoudi, F., & Soltani, N. 2013. Ecological study of some species of water mites (Acari; Hydrachnidia) newly identified harvested at the extreme North-eastern Algeria. *Annals of Biological Research*, 4(6), 230-235.

Himmi, O., Trari, B., El Agbani, M. A., & Dakki, M. 1998. Contribution à la connaissance de la cinétique et des cycles biologiques des moustiques (Diptera, Culicidae) dans la région de Rabat-Kénitra (Maroc). *Bulletin de l'Institut scientifique, Rabat*, 21, 71-79.

Chaouch, R., Gacem, H., Bouaziz, A., & Saoudi, B. 2024. Study of the Toxic Effect of Chemical Pesticide Actellic 50EC Against *Culiseta Longiareolata* Mosquito Larvae. *Indian Journal of Entomology*, 1-6.

Lechekhab H., Soltani N. 2018. *Fresenius Environ Bull*, 27 (3): 1862-1867.

Lecointre, G., Le Guyader, H., & Visset, D. 2001. *Classification phylogénétique du vivant* (Vol. 2). Paris: Belin.

Morsli SM., Merad I., Khebbab MEH., Soltani N. 2015. *Adv Environ Biol*, 9 (3): 518-525.

Zaidi, N., & Soltani, N. 2011. Environmental risks of two chitin synthesis inhibitors on *Gambusia affinis*: chronic effects on growth and recovery of biological responses. *Biological Control*, 59(2), 106-113.

INSECTICIDAL EFFECT OF ESSENTIAL OILS OF ORIGANUM MAJORANA AGAINST CULEX PIPIENS

Acceptance date: 02/05/2024

Nour El-Houda Djeghader

Department of Biology, Faculty of
Sciences, University of Badji Mokhtar,
Annaba, Algeria
Laboratory of physical, Chemistry and
Biology of Materials, Skikda, Algeria

Khedidja Amira

Laboratory of physical, Chemistry and
Biology of Materials, Skikda, Algeria
Higher Normal School of Technological
Education, Skikda, Algeria

Asma Fraia

Department of Biology, Faculty of
Sciences, University of Badji Mokhtar,
Annaba, Algeria

Habiba Gacem

Higher Normal School of Technological
Education, Skikda, Algeria
Laboratory of Biology, Water and
Environment, University of 08 May 1945,
Guelma, Algeria

Fayçal Meziri

Laboratory of physical, Chemistry and
Biology of Materials, Skikda, Algeria
Higher Normal School of Technological
Education, Skikda, Algeria

Rabah Chaouch

Laboratory of physical, Chemistry and
Biology of Materials, Skikda, Algeria
Higher Normal School of Technological
Education, Skikda, Algeria

Hadia Delhoum

Department of Biology, Faculty of
Sciences, Djilali Bounaama University,
Khemis Miliana, Algeria

Hicham Boughendjioua

Laboratory of physical, Chemistry and
Biology of Materials, Skikda, Algeria
Higher Normal School of Technological
Education, Skikda, Algeria

Hamid Boudjelida

Department of Biology, Faculty of
Sciences, University of Badji Mokhtar,
Annaba, Algeria

ABSTRACT: The use of conventional pesticides is one of the most widespread methods of combatting harmful insects and disease vectors. However, the side effects of these chemicals on the environment as well as on human health have encouraged the development of several alternative methods to chemical control, such as the

use of biopesticides. The objective of this work is to test the effectiveness of essential oils from the leaves of *Origanum majorana*, cultivated on the region of Ain Defla, against the disease vector *Culex pipiens*. Toxicological tests were conducted with a series of essential oil solutions (5, 10 and 20 μ l) prepared in tubes containing 1 ml of ethanol. Three repetitions were performed in containers holding 15 newly ecdysed L3 stage larvae and 150ml of rearing water. The results revealed a toxic effect with a concentration-response. A very significant mortality rate was observed after treatment with *Origanum majorana*, especially the mortality rate reached with the highest concentration and with the lowest concentration. The LC₅₀ and LC₉₀ are respectively: 5.37 μ l/ml and 15.48 μ l/ml.

KEYWORDS : biopesticide, essential oils, *Origanum majorana*, *Culex pipiens*.

EFFET INSECTICIDE DE L'HUILE ESSENTIELLE DE L'*ORIGANUM MAJORANA* CONTRE *CULEX PIPENS*

RÉSUMÉ: L'utilisation des pesticides conventionnels est l'un des types de lutte les plus répandus contre les insectes nuisibles et vecteurs de maladies. Cependant, les effets secondaires de ces produits chimiques sur l'environnement ainsi que sur la santé humaine, ont encouragés le développement de plusieurs méthodes alternatives à la lutte chimique comme l'utilisation des biopesticides. L'objectif de ce travail est de tester l'efficacité des huiles essentielles des feuilles d'*Origanum majorana*, cultivée dans la région d'Aïn Defla, à l'égard du vecteur de maladie *Culex pipiens*. Les essais toxicologiques ont été réalisés avec une série de solutions des huiles essentielles (5, 10 et 20 μ l) préparé dans des tubes contenant 1 ml d'éthanol. Trois répétitions ont été effectués dans des récipients contenant 15 larves du stade L3 nouvellement exuviées et 150ml d'eau d'élevage. Les résultats ont révélé un effet toxique avec concentration-réponse. Un taux de mortalité très important a été constaté après le traitement avec l'*Origanum majorana*, notamment le taux de mortalité atteint avec la concentration la plus forte et avec la concentration la plus faible. Les CL₅₀ et CL₉₀ sont respectivement : 5.37 μ l/ml and 15.48 μ l/ml.

MOTSCLÉS: biopesticide, huiles essentielles, *Origanum majoran*, *Culex pipiens*.

INTRODUCTION

Origanum majorana, belonging to the Lamiaceae family, is an herbaceous plant found in Southern Europe and the Mediterranean region, known as marjoram, which can reach up to 60 cm. *O. majorana* is widely used as a garnish in food preparation, as well as being a medicinal plant used for various purposes in the traditional medicine of different regions.

Studies on this plant have identified a large number of its active compounds (Nasser, 2018); the most important of these bioactive constituents, which are mainly secondary metabolites, include alkaloids, flavonoids, tannins, phenolic compounds, and essential oils, which explain its biological properties. According to Fournier (2003), aqueous extracts, powders, and essential oils from plants contain some types of secondary metabolites have an insecticidal property.

For several years the fight against harmful insects and disease vectors has been mainly done using chemical pesticides. However, the repeated and continuous use of these products has encountered several difficulties, such as resistance phenomenon, ecosystem imbalances, lack of specificity, and persistent effects in non-biodegradable insecticides, which are the most common issues (WHO, 2018; Richards et al, 2020). Therefore, resorting to natural insecticidal molecules turns out to be an alternative approach. Currently, insecticides based on essential oils are under study to take the place of chemical insecticides in the field of phytoprotection.

The objective of this study would be to test the insecticidal activity of *Origanum majorana* essential oils, on the L3 larvae of *Culex pipiens*; known as a significant vector of diseases.

MATERIALS AND METHODS

Plant material

The sampling method used involves randomly collecting leaves, cultivated in the region of Ain Defla) 36.2634° N, 1.9679° E(. The collected sample is placed in a bag and then transported to the laboratory.

Mosquito rearing

The larvae of *Culex pipiens* (Diptera: Culicidae) were obtained from a stock colony of the laboratory. Each 25 larvae were kept in Pyrex storage jar containing 150 ml of stored tap water and maintained at temperature between 25-27°C and a photoperiod of 14L:10D. Larvae were daily fed with fresh food consisting of a mixture of Biscuit Petit Regal-dried yeast (75:25 by weight), and water was replaced every four days (Bendali et al, 2001).

Extraction and toxicity of essential oils of *Origanum majorana*

The extraction of essential oil from oregano is carried out by hydrodistillation using a Clevenger-type extraction. The process involves introducing 140g of dried plant material into a 1-liter flask, to which a quantity of distilled water equivalent to 2/3 of the flask's volume is added. The extraction operation is carried out for three hours from the start of boiling. Finally, the obtained oil is stored and conserved at a temperature of 4°C. The toxicity tests were conducted in accordance with the protocol recommended by the World Health Organization, adopted for testing the sensibility of larvae to insecticides used in control campaigns (WHO, 2005). A preliminary test (1 ml of 96° ethanol and 99 ml of rearing water) is carried out to demonstrate that the solvent (ethanol) has no larvicidal effect. The solvent acts as a dispersing agent for the essential oil in the water. The treated series of newly exuviated L3 of *Cx. pipiens* were exposed to the product for 24 h and the control series

were exposed to water only. After the exposure time, the larvae were removed and placed in untreated water. The tests were carried out with three replicates containing 15 larvae for each.

Mortality was registered daily until the end of the treated stage. The percentage of recorded mortality was corrected (Abott, 1925) and toxicity data were studied by probit analysis (Fisher & Yates, 1957) and LC_{50} & LC_{90} were calculated by the method of (Finney, 1971).

Yield

The yield of essential oil is the ratio between the weight of the extracted oil and the weight of the dry matter of the plant (AFNOR, 1987). It is expressed as a percentage and is calculated using the following formula:

$Y=(WA/WB) \times 100$, where:

- Y: Yield of oil in %
- WA: is the Weight of the oil in g.
- WB: is the Weight of the dry matter of the plant in g.

RESULTS

Yield

The weight of the essential oil after extraction was 0.336g, therefore the yield is calculated as follows:

$$Y:(0.336g/140g) \times 100=0.24\%.$$

The essential oil from *Origanum majorana* is yellow, clear with a pleasant smell, and with a yield of 0.24% of the dry matter of the leave part of the plant.

Toxicity of *Origanum majorana* on *Culex pipiens* larve

Fig.01 illustrates the variation in the mortality rate of *Culex pipiens* larvae according to the 4 concentrations used ($p < 0.005$), after 24h of exposure. Indeed, the treatment of L3 with the essential oils show average mortalities correlated to the doses used. The mortality reaches (96%) for the high concentration 20 μ l/ml compared to (58%) for the low concentration 5 μ l/ml.

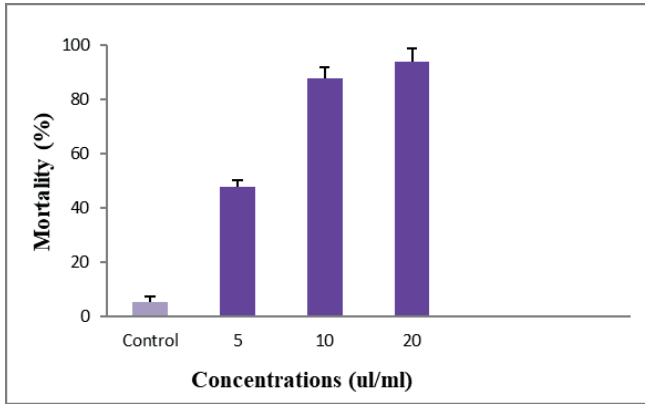


Figure 01: Concentration-response relationship for treatment of *Origanum majorana* on 3th instar larvae of *Culex pipiens* for 24h.

The regression line of larval mortality after treatment with *Origanum majorana* is presented in Fig.02. The corresponding LC_{50} and LC_{90} values are $5.37\mu\text{l/ml}$ and $15.48\mu\text{l/ml}$ after 24h of contact.

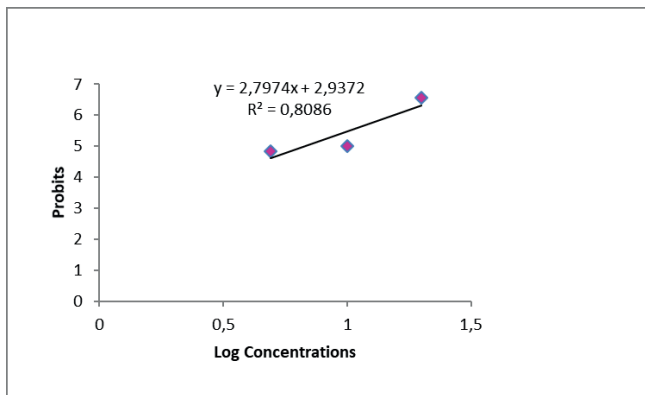


Figure 02: Probit transformed responses with equation regression and coefficient of determination R^2 for essential oils extracted from *Origanum majorana* and tested on 3th instar larvae of *Culex pipiens* for 24h.

DISCUSSION

Due to their chemical nature, pesticides are pollutants and toxic, and several harmful effects resulting from their use have been documented. The use of natural substances of plant origin, namely essential oils, constitutes a promising alternative (Ayed et al, 2017). In this study, we attempted to evaluate the bio-insecticidal effect of essential oils from *Origanum majorana* on the larvae of stage L3 of *Cx. pipiens*, depending on studied concentrations.

The yields of essential oil extracted from *Origanum majorana* was 0.24%. In general, this yield varies from one plant to another; it is 0.5% for *Artemisia meslantica* and 0.2% for *Artemisia campestris* (Khebri, 2011), 0.5% and 0.9% for *Kaempferia galanga L.* (Manat & Weerachai, 2022). The insecticidal properties of certain essential oils have been specifically tested on mosquito larvae. In this context, the work of Pitarokili et al, (2011), demonstrates that oils extracted from three species of *Mentha*: *M. pulegium*, *M. piperita*, and *M. spicata*, possess larvicidal activity against *C. pipiens* with LC₅₀ values of 46.4, 40.28 and 27.23 ppm, respectively. Furthermore, Singh et al, 2003, revealed a larvicidal activity of essential oils (EOs) extracted from *Ocimum canum* with lethal concentrations (LC50=301, 340 and 234 ppm, respectively) that are higher than those found in our experimentation and this against three species of mosquitoes: *A. aegypti*, *C. quinquefasciatus* and *A. stephensi*. These extracts contain on average 20 to 60 compounds, most of which are relatively simple molecules. Their mechanism of action is unknown, and relatively few studies have been conducted on this subject (Isman, 2000). It is considered that these mechanisms are unique, and essential oil-based biopesticides can be tools of choice in pest resistance management programs. With these specific mechanisms of action, these biopesticides can be used alone and repeatedly without potentially inducing the development of resistance in pests (Windley et al, 2012).

The results obtained in this work show that the essential oils of *Origanum majorana* could constitute very interesting raw materials for the formulation of bio-insecticides to combat the mosquito larvae. However, field trials will be necessary to confirm the practical interest of these results.

CONCLUSION

The very encouraging results obtained in this experiment deserve to be exploited through further research on these botanical species. In fact, this test was conducted only at the laboratory, and additional work would be necessary to be able to establish a means of biological control based on essential oils from plants that would be effective, economical, and environmentally friendly.

REFERENCES

AYED F., BEN HAMOUDA A., HAFSI C., LAARIF A. & CHAIEB I. 2017. **Caractérisation chimique de l'huile essentielle de *Mentha arvensis* et évaluation de ses potentialités antifongiques et insecticides.** Premier symposium maghrébin sur la protection intégrée des plantes. Tunisie. pp79.

Abbott W.S. 1925. **A method of computing the effectiveness of an insecticide.** *J. Econ. Entomol.*, 18 : 265- 267.

- Bendali F., Djebbar F. & Soltani N. 2001. **Efficacité comparée de quelques espèces de poissons à l'égard de divers stades de *Culex pipiens* L. dans des conditions de laboratoire.** *Parasitica*. 57 (4): 255-265.
- Fisher R.A. & Yates. 1957. **Statistical tables for biological agricultural and medical research** .5ème édition, Oliviner et Boyd. London. pp 64-66.
- Finney D.J. 1971. **Probit analysis**. (3ème edn) Cambridge University Press, London: p 38.
- Fournier J. 2003. **Insecticides**. In: **Chimie des Pesticides**. Ed. Des trois Moutiers, Vienne. pp235-320.
- Isman, M. B. 2000. **Plant essential oils for pest and disease management**. *Crop protection*, 19(8-10): 603-608.
- Khebri, S. 2011. **Etude chimique et biologique des huiles essentielles de trois *Artemisia*** (Doctoral dissertation, Batna, Université El Hadj Lakhder. Faculté des Sciences).
- Manat H. & Weerachai Ph. 2022. **Optimisation, rendement et composition chimique de l'huile essentielle de *Kaempferia galanga* L. Rhizome : étude comparative avec l'extraction assistée par micro-ondes et l'hydrodistillation.** *Journal of Essential Oil Bearing Plants*. 25(23) :444-445.
- Nasser H. R. A. A. 2018. **Anti-Colon Cancer Effect of *Origanum majorana* Essential Oil**. Memoire de Master, United Arab Emirates University, Emirates, 28P. Pitarokili, D., Michaelakis, A., Koliopoulos, G., Giatropoulos, A., & Tzakou, O. 2011. **Chemical composition, larvicidal evaluation, and adult repellency of endemic Greek *Thymus* essential oils against the mosquito vector of West Nile virus.** *Parasitology research*, 109(2): 425-430.
- Richards S.L., Byrd B.D., Reiskind M.H. & White A.V. 2020. **Assessing Insecticide Resistance in Adult Mosquitoes: Perspectives on Current Methods.** *Environ. Health Insights*. 14:1–7.
- Singh, N.P, Kumari, V. & Chauhan, D. 2003. **Mosquito larvicidal properties of the leaf extract of an herbaceous plant, *Ocimum canum* (Family: Labitae).** *Journal of Communicable Diseases*. 35 :43-45.
- WHO. 2005. **Lignes directrices pour les essais en laboratoire sur le terrain des larvicides contre les moustiques.**
- WHO. 2018. **Global Report on Insecticide Resistance in Malaria Vectors: 2010–2016.** Geneva, Switzerland: 2018.
- Windley, M.J., Herzig, V., Dziemborowicz, S.A., Hardy, M.C., King, G.F. & Nicholson, G.M. 2012. **Spider-venom peptides as bioinsecticides.** *Toxins*. 4: 191-227.

ANTIOXIDANT ACTIVITY OF *THAPSIA GARGANICA*

Acceptance date: 02/05/2024

Khedidja Amira

Higher Normal School of Technological Education (HNSTE), Skikda, Algeria
Laboratory of physical, Chemistry and Biology of Materials, HNSTE, Skikda, Algeria

Nour El-Houda Djeghader

Laboratory of physical, Chemistry and Biology of Materials, HNSTE, Skikda, Algeria
Department of Biology, Faculty of Sciences, University of BADJI Mokhtar, Annaba, Algeria

Habiba Gacem

Higher Normal School of Technological Education (HNSTE), Skikda, Algeria
Laboratory of Biology, Water and Environment, University of 08 May 1945, Guelma, Algeria

Hadia Delhoum

Department of Biology, Faculty of Sciences, Djilali Bounaama University, Khemis Miliana, Algeria

Fayçal Meziri

Higher Normal School of Technological Education (HNSTE), Skikda, Algeria
Laboratory of physical, Chemistry and Biology of Materials, HNSTE, Skikda, Algeria

Rabah Chaouch

Higher Normal School of Technological Education (HNSTE), Skikda, Algeria
Laboratory of physical, Chemistry and Biology of Materials, HNSTE, Skikda, Algeria

Hicham Boughendjioua

Higher Normal School of Technological Education (HNSTE), Skikda, Algeria
Laboratory of physical, Chemistry and Biology of Materials, HNSTE, Skikda, Algeria

Hamid Boudjelida

Department of Biology, Faculty of Sciences, University of BADJI Mokhtar, Annaba, Algeria

ABSTRACT: *Thapsia garganica* is a medicinal plant belonging to the Apiaceae family, commonly known in Algeria as Derias. This plant contains several bioactive compounds, some of which may have antioxidant properties. The Antioxidant activity of essential oils of *Thapsia garganica*, using the powder of aerial parts can be evaluated with DPPH (2,2-diphenyl-1-picrylhydrazyl) test. DPPH is a free radical that has a dark purple color in solution.

When it reacts with antioxidants, it loses an electron, and its purple color disappears, indicating its antioxidant power. It was compared with Ascorbic acid or vitamin C as a reference. The findings from the antioxidant activity analysis revealed that the plant being studied demonstrated a high activity, showing an inhibition percentage (I) of 78.3% when compared to Ascorbic acid as control, which exhibited 82.17% inhibition at the highest concentration, which means a good antioxidant activity of this plant.

KEYWORDS: *Thapsia garganica*, Antioxidant activity, DPPH, Inhibition.

ACTIVITE ANTIOXYDANTE DE *THAPSIA GARGANICA*

RÉSUMÉ: *Thapsia garganica* est une plante médicinale appartenant à la famille des Apiacées, communément connue en Algérie sous le nom de Derias. Cette plante contient plusieurs composés bioactifs, dont certains peuvent avoir des propriétés antioxydantes. L'activité antioxydante des huiles essentielles de *Thapsia garganica*, en utilisant la poudre de parties aériennes, peut être évaluée avec le test du DPPH (2,2-diphényl-1-picrylhydrazyl). Le DPPH est un radical libre qui a une couleur pourpre foncé en solution. Lorsqu'il réagit avec des antioxydants, il perd un électron, et sa couleur pourpre disparaît, indiquant son pouvoir antioxydant. Il a été comparé à l'acide ascorbique ou vitamine C comme référence. Les résultats de l'analyse de l'activité antioxydante ont révélé que la plante étudiée présentait une activité élevée, montrant un pourcentage d'inhibition (I) de 78,3% par rapport à l'acide ascorbique comme témoin, qui a présenté une inhibition de 82,17% à la concentration la plus élevée, ce qui signifie une bonne activité antioxydante de cette plante.

MOTSCLÉS: *Thapsia garganica*, Activité antioxydante, DPPH, Inhibition.

INTRODUCTION

There has been a growing need for natural antioxidants as alternatives to synthetic ones due to the health risks associated with the latter. However, the antioxidant effectiveness may be linked to the concentration of phenolic compounds in a species. Phenolic compounds are recognized for their capacity to neutralize toxic reactive oxygen species owing to their high redox potentials, acting as reducing agents particularly for copper (Ben Haj Koubaier, 2014; Lahmadi et al, 2021).

A considerable array of medicinal plants and their refined components have displayed promising therapeutic potentials. Various herbs and spices have been noted for their antioxidant activity (Khalaf et al, 2008).

The objective of this study is to extract essential oils from the dried aerial parts of *Thapsia garganica* using hydrodistillation. Subsequently, the antioxidant activity is assessed using the DPPH method from the same plant, with ascorbic acid (Vitamin C) as a reference.

MATERIALS AND METHODS

Presentation of the study area

The plant (*T. garganica*) was harvested in April from Kheiri, Oued Adjoul in Jijel. A coastal province located in the northeast of Algeria, Its coastline has a length of 121.2 kilometers and with an area of 62.38 square kilometers.



Figure 1: Location of the study area (Jijel, Algeria).

Source: https://fr.wikipedia.org/wiki/Khe%C3%AFri_Oued_Adjoul.

Plant material

The genus *Thapsia* consists of flowering plants in the Apiaceae family, encompassing many original species from the Mediterranean region with medicinal properties (Ladjel et al, 2011; Athmouni et al, 2015).

Among them, *Thapsia garganica*, stands out, containing a molecule called Thapsigargarine, which acts as a potent non-competitive inhibitor of ubiquitary enzymes (Calcium/ATPases). By doing so, it increases the calcium concentration in the endoplasmic reticulum and induces cellular apoptosis (Makunga et al, 2006; López et al, 2018).

After harvesting, the aerial parts of the plant were cleaned, washed, dried for 15 days at room temperature, in a dry place protected from sunlight to maintain the integrity of the molecules to the greatest extent possible, then ground into a fine powder using an electric grinder.

Antioxidant activity

The obtained powder of the cited plant was placed in a distillation container (Hydrodistillation by Clevenger) to obtain the essential oils (Clevenger, 1928). The antioxidant activity of the essential oils of *Thapsia garganica*, with different concentrations

(2, 4, 6 and 8 $\mu\text{g/ml}$) and also vitamin C (Ascorbic acid) as control can be evaluated by the DPPH test (2,2-diphényl-1-picrylhydrazyl) (Athmouni et al, 2015). It is a free radical that appears dark violet in solution; due to scavenging of stable free DPPH radicals, its violet color changes to yellow, indicating its antioxidant capacity (Khadri et al, 2020), which is representing by inhibition percentage as follows (Chibani et al, 2014):

$$\text{Inhibition I} = (\text{Ao} - \text{Ae} / \text{Ao}) * 100$$

Ao: Absorbance of DPPH in solution

Ae: Absorbance of the studied sample

The change in color is measured by the decrease in absorbance values at a wavelength of 517 nanometers. A decrease in absorbance indicates an increase in free radical scavenging activity, and vice versa.

RESULTS

The first graph (Figure 2) represents the inhibition activity of *Thapsia garganica* against the DPPH radical at different concentrations. It can be observed that as the concentration increases, the inhibition activity against the DPPH radical becomes stronger. It was 71.94% with the lowest concentration and 78.3% with the highest concentration.

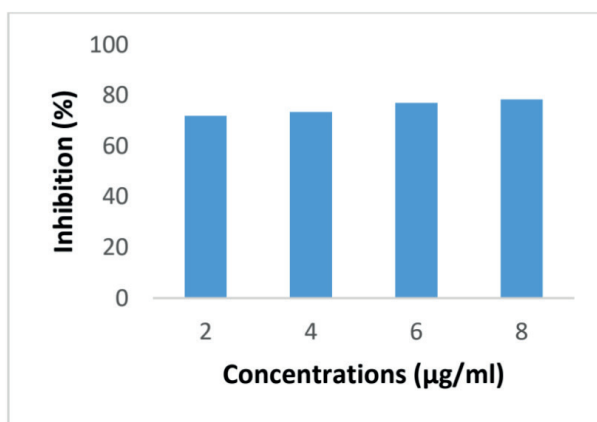


Figure 2: Inhibition (%) of *T. garganica* essential oils.

The second graph (Figure 3) represents the inhibition activity of vitamin C (Ascorbic acid) against the DPPH radical at different concentrations. It can be observed that as the concentration increases, the inhibition activity against the DPPH radical becomes stronger. It was 75.08% with the lowest concentration and 82.17% with the highest concentration.

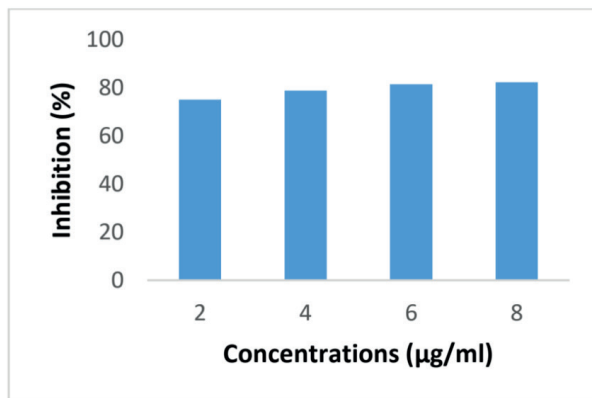


Figure 3: Inhibition (%) of Vitamin C.

The antioxidant activity against Ascorbic acid is greater than the antioxidant activity against *Thapsia garganica* oils. However, despite this, the oils of this plant is considered to have high antioxidant activity.

DISCUSSION

Based on the findings of the study on the antioxidant activity of *T. garganica* oils, the inhibition percentage at the different concentrations was high which indicates a good level of inhibition. These results are consistent with what was found by (Chibani et al, 2014) using the same plant and the same method (DPPH) with an inhibition of 28.3 to 68.7% and 28.5 to 73.2% the lowest and highest concentration (1 and 200 $\mu\text{g/ml}$) for leaves and flowers respectively.

This difference between the cited studies can be attributed to variations in environmental conditions such as soil pH, light, geographical location, prevailing climate, as well as the extent of exposure to different stresses, which play a significant role in altering their physiology. This leads to changes in the nature, type, and quantity of compounds produced, as well as the plant's age stage.

The antioxidant activity of *Thapsia garganica* essential oils is attributed to the presence of flavonoid compounds (Chibani et al, 2014; Aici and Benmehdi, 2021; Meratate et al, 2022) that act by inhibiting enzymes that produce free radicals, neutralizing them, or regenerating antioxidant systems. This has been confirmed by several studies on the same plant. In addition to its antioxidant activity, the essential oils from various parts of *Thapsia garganica* exhibits other notable biological properties like antiacetylcholinesterase, antimicrobial, anticancer and insecticidal (Casiglia et al, 2016; Khadri et al, 2020; Aici and Benmehdi, 2021; Khemis et al, 2023; Cao et al, 2024), suggesting promising therapeutic prospects.

CONCLUSION

We can conclude that the studied plant has a satisfactory antioxidant activity, although it is lower than that of Vitamin C and this is what gives it a significant therapeutic importance.

REFERENCES

- Agarwal N., Chandra A., & Tyagi L. K. 2011. **Herbal medicine: Alternative treatment for cancer therapy**. *International Journal of Pharmaceutical Sciences and Research*, 2(9), 2249-2258.
- Aici D., Benmehdi H. 2021. **Phytochemical Content, Antioxidant and Antimicrobial Effects of *Thapsia garganica* L. Leaves and Roots Grown Wild in Northwest Algeria**. *Indian Journal of Agricultural Research*, 55(5), 519-526.
- Athmouni K., BELGHITH T., Bellassouad K., El Feki A., Ayadi H. 2015. **Effect of solvent polarity on the content of biomolecules and antioxidant activity of *Thapsia garganica* (Apiaceae)**. *Algerian Journal of Natural Products*, 3(3), 194-208.
- Ben Haj Koubaier, H., Snoussi, A., Essaidi, I., Chaabouni, M. M., Thonart, P., & Bouzouita, N. (2014). **Betalain and phenolic compositions, antioxidant activity of Tunisian red beet (*Beta vulgaris* L. *conditiva*) roots and stems extracts**. *International journal of food properties*, 17(9), 1934-1945.
- Cao F., Zhang H.L., Guo C., Xu X.L., Yuan Q. 2024. **Targeting oxidative stress with natural products: A novel strategy for esophageal cancer therapy**. *World Journal of Gastrointestinal Oncology*. 15; 16(2), 287-299.
- Casiglia S., Riccobono L., Bruno M., Rosselli S., Senatore F. & Senatore F. 2016. **Chemical composition of the essential oil from *Thapsia garganica* L. (Apiaceae) grown wild in Sicily and its antimicrobial activity**. *Natural Product Research*, 30(9), 1042-1052,
- Chibani S., Al-Dabbas M., Abuhamdah S., Aburjai T., Bencheraiet R., Kabouche A., Jay M., & Kabouche Z. 2014. **Flavonoids and antioxidant activity of *Thapsia garganica* from Algeria**. *Chemistry of Natural Compounds*, 50(2),357-359.
- Clevenger JF. 1928. **Apparatus for the Determination of Volatile Oil**. *Journal of the American Pharmaceutical Association*, 17(4), 345-349. https://fr.wikipedia.org/wiki/Khe%C3%AFri_Oued_Adjoul
- Khadri M., Neffati S., Smiti S., Falé P., Lino A.R.L., Serralheiro M.L.M. & Araújo M.E.M. 2020. **Antioxidant, antiacetylcholinesterase and antimicrobial activities of *Cymbopogon schoenanthus* L, Spreng (lemon grass) from Tunisia**. *Food Science and Technology*, 43: 331-336.
- Khalaf N. A., Shakya A. K., Al-Othman A., El-Agbar Z., & Farah H. 2008. **Antioxidant activity of some common plants**. *Turkish Journal of Biology*, 32(1), 51-55.
- Khemis E., Mustapha M. B., Chaieb I., Ascrizzi R., Flamini G., Harrath A. H., & Zardi-Bergaoui A. 2023. **Chemical Composition and Insecticidal Activity against *Tribolium Castaneum* of *Thapsia garganica* L. Seed Essential Oil**. *Chemistry & Biodiversity*, 20(2), e202200646.
- Ladjel S., Zellagui A., Gherraf N. 2011. **Reinvestigation of essential oil content of *Thapsia garganica* grown in the east of Algeria**. *Journal of Fundamental and Applied Sciences*, 3(2), 165-168.

Lahmadi, S., Belhamra, M., Karoune, S., Imad, K., Bensouici, C., Kechebar, M. S. A., Halis Y. & Ksouri, R. 2020. **Phenolic constituents and antioxidant activity of *Euphorbia retusa* Forssk.** *Natural product research*, 34(24), 3545-3547.

López C. Q., Corral P., LorrainLorrette B., MartinezSwatson K., Michoux F. and Simonsen H. T. 2018. **Use of a temporary immersion bioreactor system for the sustainable production of thapsigargin in shoot cultures of *Thapsia garganica*.** *Plant Methods*, 14(79), 1-17.

Makunga N. P., Jager A. K., Staden J. V. 2006. **Improved in vitro rooting and hyperhydricity in regenerating tissues of *Thapsia garganica* L.** *Plant Cell Tissue and Organ Culture*, 86,77–86.

Meratate F., Lalaoui A., Rebbas K., Aiche G., Benseddik H., Meratate H. & Demiratas, I. 2022. **Phytochemical study of medicinal plant «*Thapsia garganica*».** *Journal of EcoAgriTourism*, 18(1), 56- 61.

CHAPTER 6

SENESCENCE, TOTAL REFLECTANCE AND CHLOROPHYLL CONTENTS AT FULL HEADING RELATIONSHIPS WITH AGRONOMIC TRAITS OF DURUM WHEAT (*TRITICUM DURUM* DESF.) GENOTYPES GROWING UNDER SEMI-ARID CONDITIONS

Acceptance date: 02/05/2024

Benalia Frih

Department of Biology and Plant ecology,
VRBN Lab, Farhat Abbas University Setif
1, Algeria

Ali Guendouz

National Institute of Agronomic Research
of Algeria (INRAA), Sétif Unit, Algeria

Abdelmalek Oulmi

Department of Biology and Plant ecology,
VRBN Lab, Farhat Abbas University Setif
1, Algeria

Sarah Benkadja

Department of Agronomy, VRBN Lab,
Farhat Abbas University Setif 1, Algeria.

Hocine Bendada

Department of Agricultural Sciences,
Relizane University , Algeria.

ABSTRACT: This study was conducted during the 2020/2021 cropping season at Setif Agricultural Experimental Station; it aims to assess the efficiency of using Numerical Image Analysis (NIA) in the selection of durum wheat genotypes in semi-arid areas. The genetic materials used in this study consist of 11 advanced

lines and 4 genotypes of which 3 are local landraces used as control to evaluate their performance, the genotypes tested were sown in a randomized block design (RDB) with three replications. Each plot consisted of 6 lines of 10 m long spaced of 0.2 m width makes 12 m² as plot area. Analysis of variance showed that all the parameters measured numerically (senescence and total reflectance) had a very high genotypic significance. The chlorophyll content at full heading showed a very highly significant genotypic effect. Thousand kernels weight, number of spikes per meter square, number of days to heading and plant height had a significant genotypic effect. The correlation study showed that all senescence parameters were significantly correlated. A significant and negative correlation was observed between chlorophyll contents; average of velocity and total reflectance. Grain yield was highly and significantly correlated with thousand kernels weight and number of spikes per meter square. Number of spikes per meter square was significantly and positively correlated with average of velocity and negatively correlated with sum of temperatures at mid-senescence. Number of days to heading was significantly and negatively correlated with senescence

average and maximum of senescence average. A significant correlation was observed between plant height and sum of temperatures at mid-senescence.

KEYWORDS: Durum wheat, grain yield, senescence, reflectance, chlorophyll contents, semi-arid.

INTRODUCTION

Durum wheat [*Triticumturgidum* L. ssp. *durum* (Desf.) Husn.] is the 10th most important crop worldwide with an annual production of over 40 million tons (Sall *et al.*, 2019). The largest producer is the European Union, with 9 million tonnes in 2018, followed by Canada, Turkey, United States, Algeria, Mexico, Kazakhstan, Syria, and India (Tedone *et al.*, 2018). Durum wheat [*Triticumturgidum* L. ssp. *durum* (Desf.) Husn.] is one of the most cultivated cereals in the Mediterranean basin, where drought is a limiting factor for its production. (Royo *et al.*, 1998), it's mostly grown under rain-fed conditions, where drought and heat stress usually constrain yield potential during the grain filling period (Simane *et al.*, 1993). Senescence is a universal phenomenon in living organisms, and the word senescence has been used by scientists working on a variety of systems, such as yeast, fruit fly, worm, human being and plants. However, the meaning of the word senescence to scientists working on different organisms can be different, and the difference can be subtle in some cases and very obvious in some other cases (Hafsi and Guendouz, 2012). Senescence is subject to strong environmental and genetic regulation, and prior to visual yellowing and chlorosis up to 50 % of leaf chlorophyll may be lost (Buchanan-Wollaston *et al.*, 2005; Borrill *et al.*, 2019). Photosynthesis is the primary source of dry matter production and grain yield in crop plants, the improvements of leaf photosynthesis have occurred with the advance of breeding high-yielding cultivars (Jiang *et al.*, 2002). The breeding of new cereal grain varieties requires methods that are rapid and preferably non-destructive, to assess the quality of grain in early generations. Near infrared (NIR) spectroscopy has great potential to meet these requirements for the durum wheat breeder (Sisons *et al.*, 2006). Our study we aim to test the efficiency of the use of flag leaf senescence parameters, total reflectance and the chlorophyll contents as selection criteria for durum wheat genotypes growing under semi-arid conditions.

MATERIALS & METHODS

The study site

This study was conducted during the 2020/2021 cropping season at Setif Agricultural Experimental Station (ITGC-AES, 36 ° 12'N and 05 ° 24'E and 1.081 m asl, Algeria).

Plant material

The genetic material used in this study consists of 11 advanced lines and 4 genotypes of which 3 are local landraces and used as control to evaluate the performance of the experimental material. (table 1).

Genotype	Pedigrees
G1	RASCON_37/GREEN_2/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/...
G2	MINIMUS_6/PLATA_16//IMMER/3/SOOTY_9/RASCON_37/9/...
G3	CMH77.774/CORM//SOOTY-9/RASCON-37/3/SOMAT-4
G4	CNDO/PRIMADUR//HAI-OU-17/3/SNITAN/4/SOMAT-3/
G5	CNDO/VEE//CELTA/3/PATA_2/6/ARAM_7//CREX/ALLA/5/ENTE/ ...
G6	SILVER 14/MOEWE//BISU_I/PATKA_3/3/PORRON_4/YUAN_I/9/...
G7	GUANAY /HU ALITA / 10/PLATA _10/6/MQUE/4/USDA573/...
G8	BCRIS/BICUM//LLARETA INIA/3/DUKEM_ 12/2* RASCON 21/5/R
G9	Simeto/3/Sora/2*Plata_12//SRN_3/Nigris_4/5/Toska_26/...
G10	Ossl1/StjS5/5/Bicrcderaal/4/BEZAIZSHF//SD19539/Waha/3/St
G11	Stj3//Bcr/Lks4/3/Ter-3/4/Mgnl3/Aghrass2
G12	Jupare C 2001
G13	Boussellem
G14	Boutaleb
G15	Oued Bared

Table 1. Varieties and their pedigrees

EXPERIMENTAL DEVICE

The genotypes tested were sown at November 19 with sowing density adjusted to 300 g m⁻² in a Randomized Block Design (RBD) with three replications. Each plot consisted of 6 lines of 10 m long spaced of 0.2 m width which makes 12 m² as plot area.

PARAMETERS MEASURED

The following parameters were measured:

Physiologic traits

1. *Sénescence parameters* : ($Sa\%$; Vsa ; $Vmax$; $\sum T_{50s}$)

We have followed the evolution of leaf senescence during 13 dates of assessment from flowering until 100% of leaf senescence (S1-S13) by Numerical Image Analysis (NIA)

according to Guendouz and Maamri (2011), Leaves were photographed on black surface, between 11:00 and 12:00 solar time with a color digital camera (Canon, Power Shot A460, AiAF, CHINA). Images were stored in a JPEG (Joint Photographic Expert Group) prior to downloading onto a PC computer and analyzed using IPP (Image Pro Plus, Version 4, Media Cybernetics, Silver Spring, MA, USA) software. The percentage of senescence (S) was calculated foreach date of observation and for each genotype studied using Digimizer software (Fig 1) by calculating the percentage of senescent zone from the total of leaf surface $S (\%) = (\text{senescent area of flag leaf} / \text{flag leaf area}) * 100 (S1-S13)$. The 13 dates of assessment will be expressed subsequently in cumulative temperatures $\sum T^{\circ}C (\sum 1-\sum 13)$. In the base of these values the following parameters were calculated:

- a. *Average of senescence Sa (%)* : is the average of the following percentage of senescence in the following dates

$$Sa \% = (S1+S2+\dots+S_n) / n$$

n= number of dates

- b. *Average of velocity Vsa (% / $^{\circ}C$)* :

- The velocity of senescence (Vs) was calculated every date of senescence values

$$Vs = (S_{n+1} - S_n) / (\sum TC_{n+1} - \sum TC_n)$$

$\sum TC$ = sum of temperatures in the date of senescence value

- Average of velocity Vsa is the average of the following velocity of the following dates

$$Vsa = (Vs1+Vs2+\dots+Vsn) / n$$

- c. *Maximum of the senescence velocity (Vmax)*: is the highest velocity unregistered.

- d. *Sum of temperatures at mid- senescence ($\sum T_{50s}$)*: estimated from the curve :

$S = f (\sum T^{\circ}C_s)$ (Fig 3) as the sum of temperatures corresponding to S=50%.

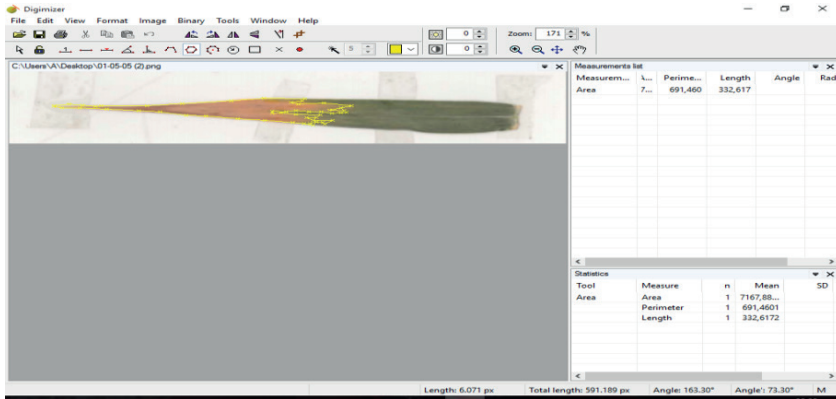


Figure 1. Estimation of senescence by Digitizer software

2. Total reflectance: $R(t)$ (at full heading)

With the same images (using to calculate senescence), we calculate reflectance using Mesurim_pro_02 software (Guendouz and Maamri, 2011), this software can measure the reflectance at Red, Blue, Green band and total reflectance. We used the total reflectance for our work (Fig 2).

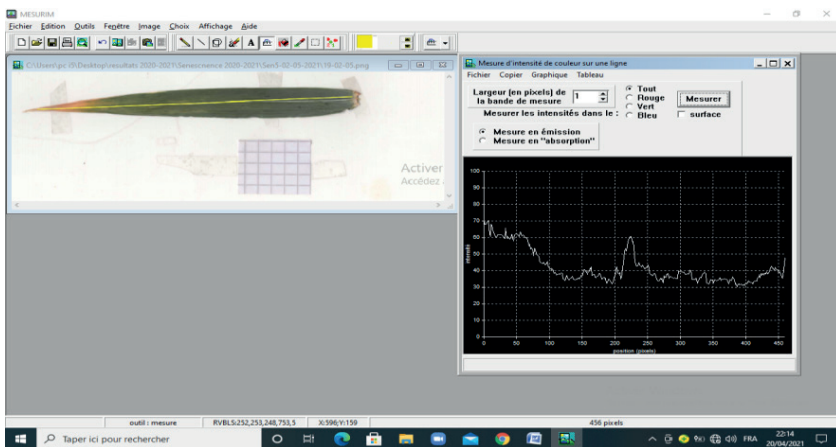


Figure 2. Reflectance calculating in total band using Mesurim_pro_02 software.

3. Chlorophyll contents CC (at full heading)

Chlorophyll contents (CC) of the flag leaf was measured using digital chlorophyll meter (CCM) with (cci) units, this device allows measuring the absorbance of light in the leaf.

Agronomic traits (at maturity)

- **Grain yield (GY):**The cereal yield performances of the different cultivars were measured at maturity in quintals per hectare (Qs. ha⁻¹) by measuring the grain yield in one linear meter and converting it into quintals per hectare.
- **Thousand kernels weight(TKW) (g).**
- **Number of spikes per meter square (NSm²).**
- **Number of days to heading (DH) (days):**calculated from sown date 19/11/2020.
- **Plant height (PH) cm.**

DATA ANALYSIS

All statistical analyses will be performed by Costat 6.400 (1998) software.

RESULTAS & DISCUSSION

Analysis of variance (ANOVA)

1. Agronomic traits

ANOVA (table 2) showed that genotypic effect was significant ($p < 0.05$; 0.001) with Thousand Kernels Weight (TKW), number of spikes per meter square, days to heading and Plant Height (PH). Grain Yield average ranged from 2.87 Q.ha⁻¹ for G11 to 13.59 Q.ha⁻¹ for Boutaleb with genotypic mean of 6.34 Q.ha⁻¹. Thousand kernels weight arranged from 30.91 g for G8 to 46.69 g for G9 with 39.40 g as genotypic mean. Comparing to general mean, high values of TKW was observed with the local landraces Boutaleb (44.96 g) .NSm²ranged from 178.33 s.m⁻² for G4 to 320s.m⁻² for Boutaleb with a genotypic mean of 255.77s.m⁻². The number of days to heading ranged from 136 days for advanced lines G1, G2, G8, G10 and Jupare C 2001 to 147 for Boutaleb local landrace with 140.6 as genotypic mean. Plant height ranged from 56.11 cm for G4 to 67.38 cm for G10 with a mean of 62.76, local landrace Boutaleb registered a high plant height (66.16 cm).

Genotypes	Agronomic traits				
	GY (Qs.ha ⁻¹)	TKW (g)	NSm ²	DH (days)	PH (cm)
G1	7.00 (bc)	41.98 (bc)	236.66 (bcde)	136 (d)	62.72 (cd)
G2	3.96 (bc)	35.62 (ef)	213.33 (de)	136 (d)	61.50 (d)
G3	7.05 (bc)	40.83 (bcd)	220.00 (cde)	142 (c)	61.88 (d)
G4	2.88 (c)	36.80 (de)	178.33 (e)	142 (c)	56.11 (e)

G5	4.70 (bc)	36.10 (e)	220.00 (cde)	142 (c)	61.27 (d)
G6	6.16 (bc)	31.88 (fg)	240.00 (bcde)	142 (c)	61.27 (d)
G7	6.89 (bc)	34.36 (efg)	253.33 (abcde)	142 (c)	57.66 (e)
G8	4.45 (bc)	30.91 (g)	268.33 (abcd)	136 (d)	61.16 (d)
G9	6.61 (bc)	46.69 (a)	310.00 (ab)	142 (c)	65.55 (abc)
G10	8.69 (ab)	41.98 (bc)	268.33 (abcd)	136 (d)	67.38 (a)
G11	2.95 (c)	44.47 (abc)	285.00 (abcd)	142 (c)	67.22 (a)
Jupare C 2001	7.89 (bc)	41.98 (bc)	255.00 (abcd)	136 (d)	63.61 (bcd)
Boussellem	4.75 (bc)	40.39 (cd)	291.66 (abc)	142 (c)	63.94 (bcd)
Boutaleb	13.59 (a)	44.96 (ab)	320.00 (a)	147 (a)	66.16 (ab)
Oued El Bared	7.59 (bc)	43.27 (abc)	276.66 (abcd)	146 (b)	63.88 (bcd)
Mean	6.34	39.40	255.77	140.6	62.76
Min	2.87	30.91	178.33	136	56.11
Max	13.59	46.69	320	147	67.38
Génotype effect	ns	***	*	***	***
LSD _(5%)	5.672	4.162	76.53	1.526	3.167
CV %	53.45	6.30	17.89	6.4901e-8	3.017

ns : none significant ;*: significant (P<0.05) ;very highly significant (P<0.001).

Table 2. Analysis of variance of agronomic traits.

1.2 physiologic traits

For all genotypes studied, the senescence function with sums of temperatures after flowering was of sigmoid type. $\Sigma T50_s$ the sums of temperatures corresponding to S= 50% (mid-senescence) differed markedly amongst genotypes, as shown in (Fig.2).ANOVA showed that genotype effect was highly significant (P<0.001) with all senescence parameters calculated Sa; Vsa; Vmax and $\Sigma T50_s$, total reflectance R (t) and chlorophyll contents at full heading (table 3). Senescence average (Sa) ranged from 29.68 % for advanced line G4 to 44.76 % for advanced line G2 with genotypic mean of 38.76 %. Average of velocity (Vsa) arranged from 0.319 %/°C for G3 to 0.429 % /°C for Boussellem with a genotypic mean of 0.379 % / °C .Vmaxranged from 0.792 %/°C for Boutaleb to 1.593 %/°C for G2 with genotypic mean of 1.139 %/°C. $\Sigma T50_s$ arranged from 276.44°C for Boussellem to 337.13°C for G4 with genotypic mean of 302.25 °C. The local landrace Boutaleb witch was the best yielding genotype registered the low Sa% (32.49%), a low value of Vs % parameter (0.347 %/°C), a low value of Vmax (0.792%/°C) and a high value of $\Sigma T50_s$ (311.91°C) comparing to genotypic means. The genotypes with lowest values of senescence average (Sa%) and highest values of sum of temperatures at mid-senescence were the most tolerant and adapted genotypes (Hafsi and Guendouz, 2020). At full heading, R (t) was ranged from 32.74% for G7 to 40.77% for G3 with genotypic mean of 36.37%, Boutaleb registered a low value of R(t) (35.85%) comparing to mean . Chlorophyll content was ranged from 26.65 cci

for Oued El Bared to 40.81 cci for G4 with genotypic mean of 33.05 cci. The diversity of the yield is a consequence of the variation of the duration rather than the variation of the rate of the photosynthetic activity; the delay of the senescence is a good parameter of selection of the cereals which must be followed (Hafsi et Guendouz, 2020).

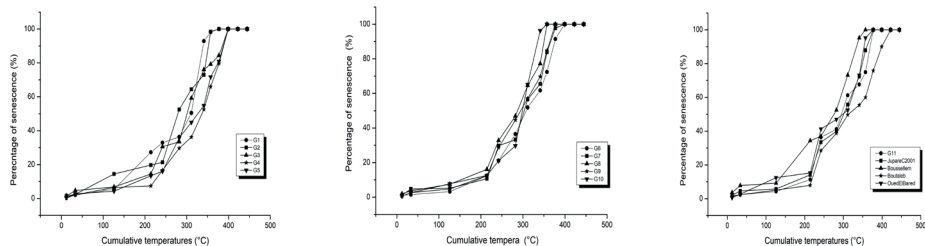


Figure 3. Senescence functions with sums of temperatures after flowering of the 15 genotypes studied.

Genotypes	Sénescence parameters				T. Reflectance	Chl. contents
	Sa (%)	Vsa (%/°C)	Vmax (%/°C)	$\Sigma T50_s$ (°C)	R(t) (%)	CC (cci)
G1	44.66 (b)	0.327 (m)	1.412 (b)	310.37 (c)	36.87 (c)	29.00 (de)
G2	44.76 (a)	0.382 (h)	1.593 (a)	278.97 (i)	38.73 (b)	30.18 (de)
G3	39.03 (g)	0.319 (n)	0.887 (i)	301.72 (ef)	40.77 (a)	30.21 (de)
G4	29.68 (l)	0.335 (l)	0.837 (j)	337.73 (a)	36.84 (c)	40.81 (a)
G5	32.52 (k)	0.348 (k)	1.059 (f)	327.69 (b)	36.45 (cd)	29.71 (de)
G6	35.32 (j)	0.370 (i)	0.954 (gh)	307.4 (d)	35.63 (def)	31.40 (cde)
G7	38.83 (h)	0.405 (f)	1.149 (e)	302.72 (e)	32.74 (g)	37.73 (ab)
G8	38.95 (g)	0.403 (g)	1.425 (b)	288.47 (h)	35.98 (cde)	32.15 (cde)
G9	40.53 (e)	0.415 (e)	0.941 (h)	295.14 (g)	36.17 (cde)	36.12 (abc)
G10	37.31 (i)	0.357 (j)	1.041 (f)	299.81 (f)	34.93 (f)	33.83 (bcd)
G11	40.06 (f)	0.420 (c)	1.254 (d)	295.14 (g)	35.71 (def)	35.90 (abc)
Jupare C 2001	41.59 (d)	0.417 (d)	0.975 (g)	299.81 (f)	35.61 (def)	38.16 (ab)
Boussellem	41.65 (d)	0.429 (a)	1.380 (c)	276.44 (j)	35.25 (ef)	34.16 (bcd)
Boutaleb	32.49 (k)	0.347 (k)	0.792 (k)	311.91 (c)	35.85 (def)	29.66 (de)
Oued El Bared	44.02 (c)	0.422 (b)	1.380 (c)	300.42 (f)	37.98 (b)	26.65 (e)
Mean	38.76	0.379	1.139	302.25	36.37	33.05
Min	29.68	0.319	0.792	276.44	32.74	26.65
Max	44.76	0.429	1.593	337.73	40.77	40.81
Génotype effect	***	***	***	***	***	***
LSD (5%)	0.093	0.002	0.024	2.189	0.925	5.533
CV %	0.14	0.29	1.30	0.43	1.52	10.01

***: very highly significant ($P < 0.001$).

Table 3. Analysis of variance of physiologic traits

Correlations among traits

2.1 Correlations among agronomic traits

The study of the correlations among agronomic traits (table 4) showed that grain yield was high significantly ($P < 0.01$; 0.001) and positively correlated with thousand kernels weight and number of spikes per meter square ($r = 0.38^{**}$; 0.61^{***}). A high and significant ($P < 0.01$; 0.001) and positive correlation was observed between thousand kernels weight and number of spikes per meter square and plant height ($r = 0.39^{**}$; 0.61^{***}). Number of spikes per meter square was significantly ($P < 0.05$) and positively correlated with plant height ($r = 0.34^*$). Several works have proven the high correlation between Grain Yield and some agronomic traits (Guendouz et al., 2012; Aissaoui and Fenni, 2021 and Mansouri et al., 2018).

2.2 Correlations among physiologic traits

The study of the correlations among senescence parameters (table 3) showed that senescence average (Sa) was highly and significantly ($p < 0.01$; 0.001) correlated with average of Velocity of senescence (Vsa) and maximum of Velocity (Vmax) ($r = 0.47^{**}$; 0.73^{***} respectively) and very highly and negatively correlated with sum of temperatures at 50% of senescence ($\sum T50s$) ($r = -0.74^{***}$). Velocity of senescence average (Vsa) was significantly ($p < 0.01$; 0.001) correlated with maximum of velocity ($r = 0.40^{**}$) and negatively correlated with sum of temperatures at mid-senescence and Total reflectance R (t) ($r = -0.63^{***}$; -0.39^{**}). Reflectance at total band R (t) was significantly ($P < 0.05$) and negatively correlated with chlorophyll contents ($r = -0.35^*$), the decrease of leaf reflectance increase the photosynthetic capacity of leaves by increasing radiations absorbed by chlorophyll pigments (Guendouz and Hafsi, 2016). Chlorophyll tends to decline more rapidly than carotenoids when plants are under stress or during leaf senescence (Gitelson and Merzlyak, 1994).

2.3 Correlation among physiologic and agronomic traits

Days to heading was high significantly ($p < 0.01$) and negatively correlated with senescence average and maximum of velocity ($r = -0.38^{**}$, $r = -0.41^{**}$) this results imply that precocity increases senescence rate, velocity average had a positive significance ($P < 0.05$) correlation with number of spikes per meter square ($r = 0.35^*$), Sum of temperatures a mid-senescence was significantly ($P < 0.05$) and negatively correlated with number of spikes per meter square and plant height ($r = -0.30^*$; -0.34^*) (table 4). these results are relatively adequate with those of Guendouz et al., (2013) who suggested more or less similar

correlations with some agronomic traits.

	Sa	Vsa	Vmax	ΣT50s	R(t)	CC	GY	TKW	NSm ²	DH	PH
Sa	1										
Vsa	0.47**	1									
Vmax	0.73***	0.40**	1								
ΣT50s	-0.74***	-0.63***	-0.61***	1							
R(t)	0.19	-0.39**	0.07	-0.01	1						
CC	-0.22	0.19	-0.28	0.11	-0.35*	1					
GY	-0.05	-0.13	-0.28	0.05	-0.04	-0.02	1				
TKW	0.19	0.10	-0.20	-0.07	0.14	-0.08	0.38**	1			
NSm ²	0.14	0.35*	-0.01	-0.30*	-0.27	0.07	0.61***	0.39**	1		
DH	-0.38**	0.04	-0.41**	0.29	0.02	-0.12	0.18	0.28	0.20	1	
PH	0.24	0.21	0.02	-0.034*	0.01	-0.29	0.27	0.61***	0.35*	0.03	1

* significant (P<0.05);** highly significant (P<0.01); *** very highly significant (P<0.001)

Table 4. Correlations among physiologic and agronomic traits

CONCLUSION

This study confirms the efficiency of the use of flag leaf senescence, total reflectance and chlorophyll contents at full heading as selection criteria for durum wheat in semi-arid areas using Numerical Image Analysis (NIA). ANOVA showed that genotype effect was significant for thousand kernels weight, number of spikes per meter square, number of days to heading and plant height. Genotype effect were also significant for all senescence parameters, total reflectance and chlorophyll contents at full heading. The study of the correlations showed that grain yield was high significantly and positively correlated with thousand kernels weight and number of spikes per meter square, in addition all senescence parameters were significantly correlated. The negative correlation between chlorophyll contents and total reflectance at full heading suggest that the decrease of leaf reflectance increase the photosynthetic capacity of leaves by increasing radiations absorbed by chlorophyll pigments. The negative correlation between number of days to heading and senescence average and maximum of velocity implies that precocity increases senescence rate.

CONFLICT OF INTERES

The authors declare that they have no conflict of interest

REFERENCES

- Aissaoui, M.R. and Fenni, M. 2021. Effect of supplemental irrigation on bread wheat genotypes yield under Mediterranean semi-arid conditions of north-eastern Algeria. *Revista Facultad Nacional de Agronomía Medellín*, 74(1), 9431-9440.
- Borrill, P., Harrington S.A., Simmonds, J. and Uauy, C. 2019. 'Identification of Transcription Factors Regulating Senescence in Wheat through Gene Regulatory Network Modelling'. *Plant Physiology*, 180 (3): 1740–1755. <http://dx.doi.org/10.1104/pp.19.00380>
- Buchanan-Wollaston, V., Page, T., Harrison, E., Breeze, E., Oklim, P., GilNam, H., Lin, J.F., Wu, S.H., Swidzinski, J., Ishizaki, K. and Leaver, C.J. 2005. Comparative transcriptome analysis reveals significant differences in gene expression and signaling pathways between developmental and dark/starvation-induced senescence in *Arabidopsis*. *Plant Journal*, 42(4): 567–585. <http://dx.doi.org/10.1111/j.1365-3113.2005.02399.x>.
- Costat 6.400, 1998. Copyright©1998-2008, CoHort Software 798 Lighthouse Ave BMP 320, Monterey, CA 93940, USA, Email: info@cohort.com, URL <http://www.cohort.com>.
- Gitelson, A.A. and Merzlyak, M.N. 1994. Spectral reflectance changes associate with autumn senescence of *Aesculus hippocastanum* L. and *Acer platanoides* L. leaves. Spectral features and relation to chlorophyll estimation. *Journal of Plant Physiology*, 143, 286 – 292.
- Guendouz, A. and Maamri, K. 2011. Research Note Evaluating durum wheat performance and efficiency of senescence parameter usage in screening under Mediterranean conditions. *Electronic Journal of Plant Breeding*, 2(3), 400-404.
- Guendouz A, Guessoum S, Maamri k and Hafsi M. 2012. The effect of supplementary irrigation on grain yield, yield components and some morphological traits of durum wheat (*Triticum durum* Desf.) cultivars. *Advance in Environmental Biology* 6(2), 564-572.
- Guendouz, A., Guessoum, S., Maamri, K., Benidir, M. and Hafsi, M. 2013. Performance of ten durum wheat (*triticum durum* Desf.) cultivars under semi arid conditions (North Africa -Algeria-). *Indian Journal of Agricultural Research*, 47 (4), 317 – 322.
- Guendouz, A. and Hafsi, M. (2016). Effect of supplementary irrigation on reflectance and some physiological properties of durum wheat (*Triticum durum* Desf.). *Revue Agriculture*, 12, 86 – 99.
- Hafsi, M. and Guendouz, A. 2012. Some Aspects of Leaf Senescence, Senescence, Dr. Tetsuji Nagata (Ed.), ISBN: 978-953-51-0144-4, InTech, Available from: <http://www.intechopen.com/books/senescence/someaspects-of-leaf-senescence>
- Hafsi, M. and Guendouz, A. 2020. Leaf Senescence in Wheat: A Drought Tolerance Measure, *Plant Science - Structure, Anatomy and Physiology in Plants Cultured in Vivo and in Vitro*, Ana Gonzalez, María Rodriguez and Nihal Gören Sağlam, IntechOpen, <http://doi.org/10.5772/intechopen.89500>
- Jiang, H., Wang, XH., Deng, QY, Yuan, LP and Xu, DQ. 2002. Comparison of some photosynthetic characters between two hybrid rice combinations differing in yield potential. *Photosynthetica*, 40, 133-137.
- Mansour, A., Oudjehih, B., Benbelkacem, A., Fellahi, Z. and Bouzerzour, H. 2018. Variation and Relationships among Agronomic Traits in Durum Wheat [*Triticum turgidum* (L.) Thell. ssp. *Turgidum* conv. *Durum* (Desf.) Mackey] under South Mediterranean Growth. *International Journal of Agronomy* Volume 2018, Article ID 8191749, 11 pages <https://doi.org/10.1155/2018/8191749>.

Royo, C., Michelena, A., Carrillo, J.M., Garcí a, P., Juan -Aracil, J and Soler, C. 1998. Spanish durum wheat breeding program. In: Nachit MM, Baum M, Porceddu, Monneveux, Picard E (eds). SEWANA (South Europe, West Asia and North Africa) durum research network. Proceedings of the SEWANA durum network workshop, 20 –23 March 1995. ICARDA: Aleppo, Syria, pp 80 –87.

Sall, A., Chiari, T., Legesse, W., Seid-Ahmed, K., Ortiz, R., van Ginkel, M. 2019. Durum wheat (*Triticum durum* Desf.): origin, cultivation and potential expansion in Sub-Saharan Africa. *Agronomy* 9:263. <https://doi.org/10.3390/agronomy9050263>.

Simane, B., Struik, P.C. , Nachit, M. M. and Peacock, J. M. 1993. Ontogenetic analysis of yield components and yield stability of durum wheat in water -limited environments. *Euphytica.*, 71:211 -219.

Sissons, M., Osborne, B., Sissons, S. 2006. Application of near Infrared Reflectance Spectroscopy to a Durum Wheat Breeding Programme. *Journal of Near Infrared Spectroscopy* 14(1),17-25. <https://doi.org/10.1255%2Fjnirs.582>

Tedone, L., Alhajj Ali, S., De Mastro, G. 2018. Optimization of nitrogen in durum wheat in the Mediterranean climate: The agronomical aspect and greenhouse gas (GHG) emissions. In *Nitrogen in Agriculture—Updates*; Amanullah, K., Fahad, S., Eds.; InTech: London, UK ; Volume 8, pp. 131–162.

ESTUDIO DE LA CAPACIDAD DE PRODUCCIÓN DE SIDERÓFOROS EN CEPAS AISLADAS DESDE SUELO AGRÍCOLA PARA SU APLICACIÓN COMO BIOINSUMO

Acceptance date: 02/05/2024

Viviana Edith Calvente

Laboratorio de Microbiología Industrial,
Facultad de Química, Bioquímica y
Farmacia, Universidad Nacional de San
Luis
<https://orcid.org/0000-0003-1079-1531>

Julian Berca Espinosa

Laboratorio de Microbiología Industrial,
Facultad de Química, Bioquímica y
Farmacia, Universidad Nacional de San
Luis
<http://orcid.org/0009-0003-4660-7158>

Yesica Sabrina Lambrese

Facultad de Ingeniería y Ciencias
Agropecuarias, Universidad Nacional de
San Luis
<https://orcid.org/0009-0004-7861-6398>

Leonardo Gastón Navarta

Laboratorio de Microbiología Industrial,
Facultad de Química, Bioquímica y
Farmacia, Universidad Nacional de San
Luis
<https://orcid.org/0009-0007-7754-4603>

María Gabriela Sansone

Laboratorio de Microbiología Industrial,
Facultad de Química, Bioquímica y
Farmacia, Universidad Nacional de San
Luis
<https://orcid.org/0009-0004-2776-6273>

RESUMEN: El hierro es un micronutriente esencial involucrado en muchos procesos bioquímicos en los microorganismos. Apesar de que el hierro es bastante abundante en la naturaleza, la insolubilidad del ión férrico (III) hace su adquisición dificultosa para ellos. Por esto muchos microorganismos han desarrollado mecanismos específicos para su adquisición, produciendo un agente quelante de bajo peso molecular, denominado “sideróforo”, que excreta al medio, el cual tiene gran afinidad por el hierro. La raíz de la planta también secreta sideróforos para mantener el nivel de hierro para sus actividades metabólicas y fisiológicas en suelos degradados y estresados por hierro, pero muchas veces no es suficiente. Según estudios recientes, el uso de sideróforos como biofertilizantes ha demostrado ser efectivo en la mejora del crecimiento de las plantas y en la absorción de nutrientes. Además los sideróforos se han utilizado en biocontrol de hongos fitopatógenos. La utilización del sideróforo, ácido *rhodotorúlico*, producido por levaduras rosadas y del sideróforo *enteroquelina* producido por la bacteria *Kosakonia radicincitans* redujeron el crecimiento de hongos patógenos. El objetivo del presente trabajo fue seleccionar a partir de

microorganismos aislados de suelos agrícolas, aquellos con capacidad de producir sideróforos de tipo hidroxamato y/o catecolato, para luego aplicarlos en el desarrollo de un biofertilizante. Los resultados mostraron que de las 94 cepas ensayadas, 38 produjeron hidroxamatos y 20 catecolatos; destacándose 12 cepas que produjeron ambos tipos de sideróforos. Por lo tanto 46 cepas presentaron producción de uno o ambos sideróforos, es decir más del 43 % del total fueron positivas para producción de estos quelantes de hierro en las condiciones del ensayo. Los microorganismos estudiados podrían mediante su producción de sideróforos, no solo aportar hierro a la planta, mejorando su crecimiento, sino también actuar como agentes de control biológico; lo cual los hace excelentes candidatos para el desarrollo de un bioinsumo.

PALABRAS-CLAVE: sideróforo, catecolato, hidroxamato, microorganismos, bioinsumo

STUDY OF THE SIDEROPHORES PRODUCTION CAPACITY IN ISOLATED STRAINS FROM AGRICULTURAL SOIL FOR APPLICATION AS BIOINPUTS

ABSTRACT: Iron is an essential micronutrient involved in many biochemical processes in microorganisms. Although it is quite abundant in nature, it is mostly found as the ferric ion (III), which is not soluble for plants and microorganisms, making it a nutrient that is hard to absorb. Due to this fact, many microorganisms have developed a specific mechanism for its acquisition, by producing a low molecular weight chelating agent, called “siderophore”, which they excrete into the environment and has a high affinity for iron. The plant’s root also secretes siderophores to maintain the iron levels for its metabolic and physiological activities in degraded and low-iron soils. Still, many times this is not enough to reach the optimum level. According to recent studies, the use of siderophores as biofertilizers has proven to be effective in improving plant growth and nutrient absorption. Additionally, siderophores have been used for biological control against phytopathogenic fungi. It has been shown that the use of the siderophore, rhodotorulic acid, produced by pink yeasts, and the siderophore enterochelin, produced by the bacteria *Kosakonia radicincitans*, reduced the growth of some pathogenic fungi. In the present work, the goal was set to select from microorganisms isolated from agricultural soils, those with the capacity to produce siderophores of the hydroxamate and/or catecholate type, and then apply them in the development of a biofertilizer. The results showed that of the 94 strains tested, 38 produced hydroxamates and 20 catecholates; highlighting 12 strains that produced both types of siderophores. Therefore, 46 strains showed the production of one or both siderophores, that is, more than 43% of the total were positive for the production of these iron-chelating compounds under the test conditions. Thus, these microorganisms, through their production of siderophores, can not only provide iron to the plant, improving its growth, but also act as biological control agents; which makes them excellent candidates for the development of a bioinput.

KEYWORDS: siderophore, catecholate, hydroxamate, microorganisms, bioinput

INTRODUCCIÓN

El hierro es un micronutriente esencial involucrado en muchos procesos bioquímicos en los microorganismos. La existencia de dos estados de oxidación le da al hierro su capacidad para actuar como transportador de electrones. De esta manera el hierro está presente en los citocromos y en transportadores de electrones, siendo esencial para el

crecimiento y la supervivencia de muchas especies microbianas. A pesar de que el hierro es bastante abundante en la naturaleza, la insolubilidad del ión férrico (III) hace su adquisición dificultosa para los microorganismos. Debido a esta baja disponibilidad de hierro, muchos microorganismos han desarrollado mecanismos específicos para su adquisición. El mecanismo es esencialmente el siguiente: el microorganismo produce un agente quelante de bajo peso molecular, denominado “sideróforo”, que excreta al medio, el cual tiene gran afinidad por el hierro. Este fenómeno también se puede reproducir en medios de cultivo de laboratorio, particularmente cuando el medio posee bajo contenido de hierro. La función de los sideróforos es solubilizar tanto hierro como esté disponible en el ambiente externo y transportarlo al interior de la célula. Cuanto menor es el hierro disponible, mayor es la cantidad de sideróforo producido. Los sideróforos se producen como ligandos libres (en la forma desferri) y luego complejados con el hierro disponible en el medio para dar el ferro-complejo. Este complejo es luego utilizado en la captación de hierro por la célula. Dado que los sideróforos aumentan la disponibilidad de hierro para un microorganismo, ellos pueden funcionar como factores de virulencia ayudando al microorganismo patógeno a crecer en la célula huésped. La especificidad del complejo férrico-sideróforo a veces previene que el hierro sea complejado y utilizado por otros microorganismos diferentes a la célula productora dando así a este organismo productor una ventaja en la competición con otros microorganismos (MESSENGER and RATLEDGE, 1985). Los sideróforos son moléculas especiales, muchos contienen aminoácidos modificados y se ha visto una gran variación en la estructura de los sideróforos desde una especie microbiana a otra. En general se clasifican en tres grupos estructurales: catecolatos o fenolatos, mayoritariamente producidos por las bacterias; hidroxamatos que los producen principalmente hongos y bacterias; y sideróforos mezcla, los cuales presentan grupos hidroxamato, catecolato y carboxilato en una misma estructura (TIMOFEEVA *et al.*, 2022). La mayoría de los microorganismos aerobios y anaerobios facultativos producen al menos un sideróforo, cuando crecen en condiciones de carencia del mencionado metal (NEILAND, 1995). Desde los años 80 se demostró la presencia de sideróforos en suelo y también en ambientes acuáticos (POWELL *et al.*, 1980). Los sideróforos ejercen un efecto positivo sobre las plantas, en un estudio la Crisobactina producido por *Erwinia chrysanthemi* activó la vía de señalización mediada por el ácido salicílico induciendo la expresión del gen PR1 en *Arabidopsis* (DELLAGI *et al.*, 2009). La raíz de la planta también secreta sideróforos para mantener el nivel de hierro para su actividades metabólicas y fisiológicas en suelos degradados y estresados por hierro, pero muchas veces esto no es suficiente para llegar al nivel perfecto (HERLIHY *et al.*, 2020). La deficiencia de hierro es un fenómeno observado en suelos desérticos ricos en CaCO_3 a pH alto y se demostró deficiencia de hierro debido a una menor solubilidad del hierro en niveles altos de pH. Por otro lado el riego por inundación y aumento de la concentración de nitratos y fosfatos (uso exógeno de fertilizantes sintéticos) en el suelo reduce la solubilidad del hierro, altera la translocación del hierro e induce su deficiencia en la planta (SINGH *et al.*, 2022). En agricultura, los sideróforos tienen aplicaciones en la promoción del crecimiento de las plantas, se observó que el uso de microorganismos productores de sideróforos,

pueden mejorar la vida de las plantas en suelos estresados por baja concentración de hierro (SAHA *et al.*, 2016). Según estudios recientes, el uso de sideróforos como biofertilizantes ha demostrado ser efectivo en la mejora del crecimiento de las plantas y en la absorción de nutrientes (AL-ANI *et al.*, 2021). La bacteria *Pseudomonas putida* secreta sideróforos tipo hidroxamatos, que juegan un rol en la promoción de crecimiento y en la regulación de mecanismos de defensa de plantas (ASHAJYOTH *et al.*, 2023). Además los sideróforos se han utilizado en el control biológico de hongos fitopatógenos. La utilización del sideróforo, ácido *rhodotorúlico*, producido por levaduras rosadas y del sideróforo *enteroquelina* producido por la bacteria *Kosakonia radicincitans* redujeron el crecimiento de hongos patógenos (CALVENTE *et al.*, 2001a; CALVENTE *et al.*, 2001b; LAMBRESE *et al.*, 2018).

Por todo lo expuesto es que en el presente trabajo se planteó el objetivo de seleccionar a partir de microorganismos aislados de suelos agrícolas, aquellos con capacidad de producir sideróforos de tipo hidroxamato y/o catecolato, para luego aplicarlos en el desarrollo de un biofertilizante.

MATERIALES Y MÉTODOS

Microorganismos utilizados

Los microorganismos ensayados fueron aislados de muestras de suelo provenientes de 10 fincas con cultivo de vid, de la provincia de Mendoza (Argentina). Los aislamientos se realizaron mediante el método de diluciones sucesivas, en tres medios de cultivo seleccionados con la finalidad de detectar y poder aislar microorganismos con potencial capacidad de promoción de crecimiento de plantas, los cuales fueron: medio base con extracto de suelo, medio Sabouraud para hongos y levaduras y medio para identificar microorganismos solubilizadores de fosfato. Se trabajó con muestras de suelo extraídas en dos temporadas de cultivo (2021/2022 y 2022/2023); y a su vez en cada temporada en dos etapas del ciclo fenológico de la vid (brotación y envero). Finalmente de las más de 150 cepas de interés que se aislaron, se logró probar para producción de sideróforos 94 de ellas, entre bacterias y levaduras.

Observaciones macro y microscópicas

Además de realizar las determinaciones de producción de distintos tipos de sideróforos, se realizaron observaciones macro y microscópicas de cada una de las cepas. Las observaciones macroscópicas consistieron en una caracterización de las colonias según: color, brillo, textura, bordes de las colonias, etc. Las observaciones microscópicas se realizaron mediante preparados en fresco y consistieron en observar: forma, tamaño, movimiento, si se encontraban encadenados o agregados entre sí o cualquier otra característica de importancia.

Cultivo y producción de sideróforos

A partir de estas cepas ya aisladas, se repicaron cada una en un medio de bajo contenido en hierro, Vitamin Free (VF) conteniendo: Glucosa 10 g, $(\text{NH}_4)_2\text{SO}_4$ 3.5 g, Asparagina 1.5 g, Histidina 0.010 g, Metionina 0.020 g, Triptofano 0.020 g, KH_2PO_4 1 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g, NaCl 0.5 g, Agar-agar 20 g, Agua destilada 1000 ml, pH 7. Este medio se lo formuló tanto de manera sólida (con agar-agar) como para medio líquido (sin agar). Todos los microorganismos fueron repicados dos veces en medio sólido VF, con el fin de lograr una estimulación de la producción de sideróforos a partir de la falta del hierro. Aquellos microorganismos que sobrevivieron a los dos repiques, se los cultivó en el medio VF líquido y se los llevó a agitación a 120 rpm en cuarto estufa a 25 ± 1 °C, por 120 horas. Transcurrido este tiempo, se tomaron alícuotas de 10 mL de cada una de las muestras y se las centrifugó a 10000 rpm durante 10 minutos.

Determinación de sideróforos

Luego de la centrifugación, se utilizó el sobrenadante para determinar hidroxamatos y/o catecolatos mediante ensayos colorimétricos: método de Arnow para catecolatos y la prueba de perclorato férrico para hidroxamatos. Método de Arnow: consistió en el agregado secuencial de 1 mL del sobrenadante, luego 1 mL de HCl 0.5 N, 1 mL del reactivo de Arnow y por último 1 mL de NaOH 1N. Esta prueba se considera positiva en el caso de que, luego del agregado del último reactivo la muestra vire del amarillo al color rojo. La determinación se realizó a 510 nm en espectrofotómetro. Método del Perclorato Férrico: consistió en el agregado de 0.5 mL de sobrenadante junto con 2.5 mL de perclorato férrico 0.5 M en ácido perclórico. La prueba se consideró positiva si la muestra presentó viraje hacia los tonos naranjas o rojos por formación del quelato. En este caso se midió a 480 nm en espectrofotómetro. (PAYNE, 1994)

RESULTADOS Y DISCUSIÓN

Los resultados de las observaciones macro y microscópicas evidenciaron una amplia mayoría de bacterias y en menor proporción levaduras. Los mismos se presentan en las **Tabla 1**, **Tabla 2**, **Tabla 3** y **Tabla 4**, junto con los resultados de las pruebas de Arnow y Perclorato férrico. Los resultados de las determinaciones de sideróforos se expresaron en forma semicuantitativa ya que como se explicó anteriormente los mismos presentan un amplio rango de estructuras, las cuales no se conocían al momento de realizar este trabajo, lo que impidió contar con el patrón adecuado para cuantificar cada determinación. Por tanto, los resultados de las reacciones colorimétricas se indican en la tabla según su densidad óptica (DO) como: (-) sin producción, de 0 a 0,030; (+/-) dudoso o variable de 0,031 a 0,100; (+) positivo débil, de 0,101 a 0,200; (++) positivo moderado de 0,201 a 0,500 y (+++) positivo alto, desde 0,501 o más.

CEPAS	Observación MACROSCÓPICA	Observación MICROSCÓPICA	SIDERÓFOROS	
			CATECOLATOS	HIDROXAMATOS
ChM2a	Colonia naranja cremosa con brillo	Levaduras con inclusiones	(-)	(-)
ChM2b	Colonia naranja cremosa con brillo.	Levaduras con inclusiones	(-)	(-)
ChM4	Colonia amarillenta de aspecto seco.	Bacilos pequeños.	(-)	(+)
ChM17b	Colonia beige cremosa con brillo.	Levadura sin inclusiones	(-)	(-)
ChM17a	Colonia beige cremosa con brillo	Levadura sin inclusiones	(-)	(-)
ChM20b	Colonias muy pequeñas blancas.	Bacilos chicos a medianos.	(-)	(+/-)
ChM21a	Colonias aisladas y blancas	Bacilos chicos a medianos.	(-)	(-)
ChM22a	Colonia blanco azulado, opaca.	Bacilo pequeño.	(-)	(-)
ChM23b	Colonia naranja rugosa sin relieve.	Bacilos chicos a medianos móviles.	(-)	(+)
ChM27b	Colonia beige claro, opaca.	Diplococos y coco bacilos.	(-)	(-)
ChM28c	Colonia azul verdosa poco de brillo.	Bacilos largos y grandes.	(-)	(+/-)
ChM30b	Colonia blanco tiza, textura cremosa.	Bacilos medianos a grandes.	(-)	(++)
ChM34a	Colonias blancas.	Bacilos chicos con movilidad	(-)	(-)
ChM34b	Colonia, color crema, brillo y textura.	Bacilos muy finos y largos.	(-)	(-)
ChM37b	Colonia seca, color amarillo naranja.	Bacilos muy pequeños.	(+/-)	(-)

(-) Negativo, (+/-) Dudoso, (+) Positivo débil, (++) Positivo medio, (+++) Positivo alto

Tabla 1 : características de los microorganismos aislados en período de brotación temporada 2021/2022 y su producción de sideróforos.

CEPAS	Observación MACROSCÓPICA	Observación MICROSCÓPICA	SIDERÓFOROS	
			CATECOLATOS	HIDROXAMATOS
ChM41	Colonia blanca y cremosa.	Bacilo mediano sin movimiento.	(+)	(+/-)
ChM44	Colonias cremosas, color naranja.	Bacilos, movimiento browniano.	(-)	(++)
ChM52c	Colonia color crema claro, opaca.	Bacilos medianos, esporulado.	(-)	(-)
ChM54a	Colonia naranja opaco, duras.	Bacilo mediano	(-)	(-)
ChM58a	Colonia amarilla, con textura, opaca y dura.	Filamentos largos y finos con ramificaciones.	(-)	(+/-)
ChM58b	Colonia naranja claro, pocas colonias.	Bacilos medianos con movimiento.	(-)	(++)
ChM62a	Colonia crema, brillante y cremosa.	Bacilos chicos amedanos, agrupados.	(+)	(-)
ChM63a	Blanca cremosa y con halo	Bacilo mediano sin movilidad	(-)	(++)
ChM63d	Colonia opaca, blanca / amarillenta.	Bacilos con una especie de ramificacion.	(-)	(-)
ChM64a	Colonia poco cremosa, color crema.	Bacilo chico con movimiento.	(-)	(-)
ChM64c	Colonia blanco / crema con poco brillo.	Bacilo largo, movimiento browniano.	(-)	(-)
ChM64d	Colonia naranja a rosa.	Bacterias, cadena de cocos.	(++)	(+/-)
ChM64f	Colonia seca, amarillo translúcido.	Bacteria, cocos en cadena.	(-)	(-)
ChM65c	Colonia blanca cremosa y brillante.	Bacilos grandes y alargados.	(-)	(-)
ChM65e	Colonia rosa claro, cremosa y brillante.	Cocos en cadena, chicos.	(-)	(-)
ChM72a	Colonias rosa-naranja.	Levaduras ovals, brotes polares.	(-)	(+)
ChM72b	Colonias rosadas	Levaduras ovals, brotes polares e inclusiones.	(-)	(-)
ChM73	Colonia amarilla a beige, cremosa.	Bacilos chicos.	(-)	(+/-)
ChM74a	Colonia beige a marrón, con textura.	Bacilos chicos.	(-)	(-)
ChM74b	Colonia color crema.	Levadura redondeada, brotes polares.	(-)	(+/-)
ChM76b	Colonia color beige/ amarillento.	Bacilos medianos.	(+)	(-)

ChM76c	Colonia rosa clarito a crema.	Bacilos chicos móviles	(-)	(-)
ChM78a	Colonia casi transparente y gelatinosa.	Bacilos medianos, agrupados.	(+)	(-)
ChM78c	Colonia cremosa	Bacilos medianos no móviles	(++)	(+/-)
ChM79	Colonia rosa claro, poco crecimiento.	Levaduras ovals, múltiples brotes.	(-)	(-)
ChM80	Colonia casi transparente.	Bacilos medianos sin movilidad.	(+)	(-)

(-) Negativo, (+/-) Dudoso, (+) Positivo débil, (++) Positivo medio, (+++) Positivo alto

Tabla 2: características de los microorganismos aislados en período de enero, temporada 2021/2022 y su producción de sideróforos.

CEPAS	Observación MACROSCÓPICA	Observación MICROSCÓPICA	SIDERÓFOROS	
			CATECOLATOS	HIDROXAMATOS
ChM85	Colonia crema seca.	Bacteria filamentosas	(-)	(-)
ChM86	Colonia blanco-crema, cremosa con brillo	Bacilos chicos y algunos agrupados.	(-)	(-)
ChM87	Colonia beige-crema, cremosa y brillante.	Bacilo chico (cocobacilo), agrupados.	(++)	(+/-)
ChM89	Colonia naranja intenso, con brillo.	Bacilo muy pequeño.	(-)	(++)
ChM90a	Colonia naranja, produce un pigmento.	Bacilo chico.	(+/-)	(+/-)
ChM90b	Colonia beige/crema. Crecimiento definido.	Bacilo chico, con movimiento browniano.	(+/-)	(++)
ChM90c	Colonia crema/beige, cremosa, dispersa.	Bacilo pequeño y redondeado.	(+++)	(+/-)
ChM95b	Colonia blanco amarillento, brillante.	Bacilos chicos, agrupados en pares.	(-)	(+)
ChM96e	Colonia amarilla, crecimiento definido.	Cocobacilos medianos/pequeños.	(-)	(+)
ChM96f	Colonia beige oscuro, con brillo.	Bacilos medianos con movimiento.	(-)	(+/-)
ChM96g	Colonia beige, con brillo.	Bacilo pequeño, agrupados en cadenas.	(-)	(+/-)
ChM97b	Colonia amarillo-mostaza, cremosa, brillo.	Cocobacilos, sin movimiento.	(-)	(+)
ChM97c	Colonia blanca, con brillo.	Bacilos chicos/medianos. Agrupados.	(-)	(-)
ChM97d	Colonia beige viscosa, con brillo.	Bacilos medianos con movimiento.	(-)	(-)

ChM97e	Colonia verde musgo, cremosa, brillante.	Bacilos chicos, en cadenas de a dos o más.	(-)	(+/-)
ChM99	Colonia verde oscuro, crecimiento definido.	Bacilos medianos, agregados en pares.	(-)	(+)
ChM99a	Colonia amarillo fuerte, brillante.	Bacilos pequeños muy agregados.	(-)	(-)
ChM99d	Colonia amarillo/beige, cremosa.	Bacilos medianos a grandes, largos.	(-)	(+/-)
ChM100a	Colonia blanca, centro amarillo, cremosa.	Bacilos chicos, agregado de a 2 o más.	(-)	(-)

(-) Negativo, (+/-) Dudoso, (+) Positivo débil, (++) Positivo medio, (+++) Positivo alto

Tabla 3: características de los microorganismos aislados en período de brotación temporada 2022/2023 y su producción de sideróforos.

CEPAS	Observación MACROSCÓPICA	Observación MICROSCÓPICA	SIDERÓFOROS	
			CATECOLATOS	HIDROXAMATOS
ChM104	Colonia blanca , filamentosa	Bacilo pequeño, formando cadenas.	(-)	(-)
ChM105	Colonia blanca, brillante.	Levaduras con brotes en los polos.	(-)	(-)
ChM105a	Colonia beige, brillante.	Levaduras ovaladas.	(-)	(+/-)
ChM105e	Colonias beige a blancas, secas.	Bacilos muy chicos, agregados.	(+/-)	(-)
ChM106a	Color beige. Opaca y cremosa.	Levaduras redondas en germinación.	(+/-)	(-)
ChM107a	Colonias amarillo fuerte, secas.	Cocobacilo pequeño, agregados.	(+++)	(+)
ChM107b	Colonias blanco rosadas, gelatinosa.	Bacilos grandes unidos de a dos.	(-)	(-)
ChM107c	Colonias amarillo-naranja, viscosas.	Bacilos grandes y largos, en cadena.	(-)	(-)
ChM107d	Color beige, brillante y cremosa.	Bacilo pequeño, algunos agregados.	(-)	(+)
ChM108a	Colonia beige claro, cremosa.	Bacilos chicos, agregados de a dos.	(-)	(-)
ChM108b	Colonia beige, viscosa, brillante.	Bacilo pequeño, agregados entre si.	(-)	(-)
ChM109b	Colonia amarilla. Brillante y cremosa.	Bacilo chico sin movimiento	(-)	(-)
ChM110a	Colonia blanca, brillante, cremosa.	Bacilos chicos agregados, filamentosos.	(-)	(-)
ChM110b	Colonia amarilla, aspecto seco.	Bacilos muy pequeños.	(++)	(+/-)

ChM111d	Colonia beige, cremosa y brillante.	Bacilo pequeño, algunos agregados.	(-)	(-)
ChM111y	Colonia amarilla bordes irregulares.	Bacilo mediano, algunos agregados.	(-)	(+/-)
ChM111z	Colonia beige cremosa	Bacilo chico a mediano, agregados.	(-)	(-)
ChM112b	Colonia blanco/beige, brillo, cremosa.	Bacilos muy chicos, algunos agregados.	(+/-)	(-)
ChM113f	Colonia blanco/beige, brillo, cremosa.	Bacilos chicos, agregados de a 2.	(-)	(-)
ChM114a	Color rojo, aspecto seco, brillante.	Bacilo mediano algunos agregados.	(-)	(-)
ChM114c	Color blanco, cremosa y brillante.	Bacilos pequeños sin movimiento.	(+)	(+/-)
ChM114d	Colonia amarillo, translúcida.	Bacilos alargados agregados.	(++)	(+/-)
ChM114e	Colonia blanca cremosa.	Bacilos medianos.	(-)	(-)
ChM115	Colonia beige claro, cremosa.	Bacilos chicos y alargados, esporulado.	(-)	(-)
ChM116e	Colonia amarilla	Bacilo pequeño, agregados entre sí.	(-)	(-)
ChM117b	Colonia casi transparente	Bacilo pequeño, movilidad, agregados.	(-)	(-)
ChM118a	Colonia amarillo, seco, con brillo.	Bacilos pequeños, agregados.	(-)	(-)
ChM118e	Colonia cremosa, amarillo borde blanco.	Bacilo mediano y alargado, agregados.	(-)	(-)
ChM119	Colonia beige, brillante y cremosa.	Bacilo chico, algunos agregados.	(-)	(-)
ChM119b	Colonia beige cremosa	Bacilo mediano.	(-)	(-)
ChM119c	Colonia rosada oscura	Bacilo grande, esporulado.	(-)	(+)
ChM119e	Colonia beige claro	Bacilo pequeño, agregados entre si.	(-)	(+/-)
ChM120	Colonia beige cremosa	Bacilo mediano, agregados entre si.	(-)	(-)
ChM120y	Colonia rosado claro.	Bacilo grande, esporulado.	(+++)	(++)

(-) Negativo, (+/-) Dudoso, (+) Positivo débil, (++) Positivo medio, (+++) Positivo alto

Tabla 4: características de los microorganismos aislados en período de enero, temporada 2022/2023 y su producción de sideróforos.

Como se observa en los resultados de las 94 cepas ensayadas, 38 produjeron hidroxamatos y 20 catecolatos; destacándose 12 cepas que produjeron ambos tipos de sideróforos. Por lo tanto 46 cepas presentaron producción de uno o ambos sideróforos, es decir más del 43 % del total fueron positivas para producción de sideróforos en las condiciones del ensayo. En la **Figura 1** se muestran las reacciones colorimétricas

correspondientes al ensayo de Arnow y el del Perclorato férrico en un grupo de pruebas realizadas. Observándose en el caso del ensayo de Arnow la prueba positiva de color rojo violáceo y los negativos de color amarillo ya que no se ha producido el viraje final que indica la presencia del catecolato. En el caso de los ensayos de Perclorato férrico se observan con diferentes intensidades de color rojo las pruebas positivas e incoloras las negativas.

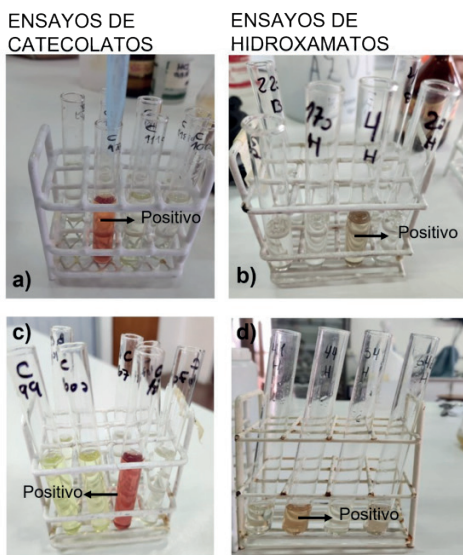


Figura 1: Prueba de Arnow para determinación de catecolatos (a y c) y Prueba del perclorato férrico para determinación de hidroxamatos (b y d)

En la **Figura 2** se representan los porcentajes de pruebas que dieron negativo, dudoso, positivo débil, moderado o alto para catecolatos y lo mismo para hidroxamatos.

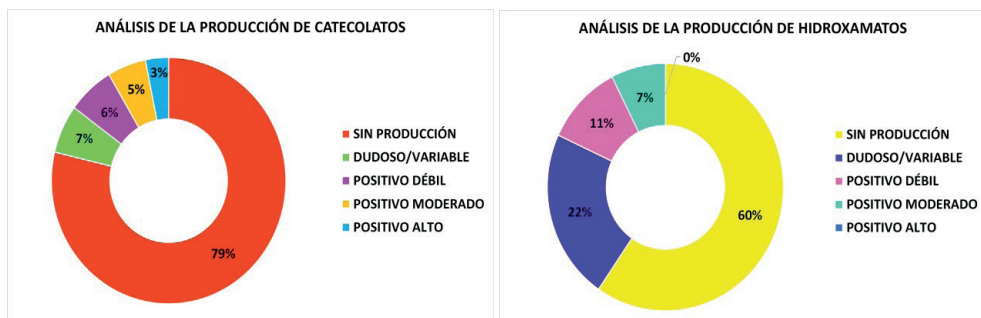


Figura 2: Porcentajes de pruebas que dieron producciones negativas, dudosas, débil, moderadas o altas para catecolatos (A) y para hidroxamatos (B)

Si bien la proporción de cepas productoras fueron mayoritariamente medias y bajas, se debe tener en cuenta que esta es una selección preliminar de microorganismos productores de sideróforos y no se ha intentado aún producirlos en medios de cultivo específicos para catecolatos o hidroxamatos, ni tampoco optimizar dicha producción, por ejemplo manipulando condiciones del cultivo como cambio de fuentes de C y N, pH, temperatura de crecimiento, agitación, etc. (CALVENTE *et al.*, 2001b). No obstante, estos resultados son prometedores ya que nos indican que los microorganismos de estos suelos presentan la capacidad de producir sideróforos lo que puede ayudar a las plantas en su crecimiento como fue demostrado por numerosos autores.

CONCLUSIONES

Si tenemos en cuenta que la población mundial está en constante crecimiento y la superficie de tierra cultivable va disminuyendo o baja sus rendimientos debido a malos manejos, existe una fuerte necesidad de introducir nuevas tecnologías agrícolas. Es posible garantizar una alta demanda de alimentos de alto valor nutricional en los siguientes años; para satisfacerla se deberán utilizar soluciones agronómicas de bajo impacto para aumentar la resistencia de las plantas a los efectos adversos y condiciones de suelos deteriorados (TIMOFEEVA *et al.*, 2022). En mayo de 2020, la Unión Europea anunció el programa Farm to Fork estrategia (F2F), destinado a reducir la dependencia de pesticidas, antimicrobianos y el uso excesivo de fertilizantes de síntesis química, es por esto que durante los últimos años, la investigación se ha centrado en nuevas tecnologías agroecológicas, enfoques dirigidos a la gestión de la agrobiodiversidad (CASTIGLIONE *et al.*, 2021). Los bioestimulantes vegetales son productos de próxima generación que probablemente sean eficaces para la sostenibilidad agrícola. Estos bioestimulantes vegetales pueden combinar fertilizantes químicos con microorganismos y, por lo tanto, se clasifican como bioestimulantes vegetales microbianos. Actualmente, hay numerosos estudios dirigidos a seleccionar microorganismos con actividad de crecimiento específica para mejorar la asimilación de nutrientes en el caso de su baja disponibilidad, así como en la aplicación de microorganismos promotores de crecimiento de plantas, aislados de regiones afectadas por la salinización y la desertificación (CASTIGLIONE *et al.*, 2021). Como ya se mencionó anteriormente los microorganismos productores de sideróforos, pueden producir numerosos compuestos quelantes del hierro, que pueden mejorar la vida de las plantas en suelos estresados por hierro. Estos microorganismos productores de sideróforos, tienen actividades de biofertilizantes y biocontrol de la planta (SINGH *et al.*, 2022). En el caso del presente trabajo muchos de los microorganismos que presentaron producciones medias y altas de sideróforos también mostraron alguna otra característica de promoción de crecimiento de plantas (solubilización de P, fijación de nitrógeno atmosférico y/o producción de fitohormonas). Por sus características de aportar nutrientes y/o

fitohormonas podrían favorecer el crecimiento de las plantas; además por su producción de sideróforos no solo pueden aportar hierro al vegetal, sino también actuar como agentes de control biológico; lo cual los hace excelentes candidatos para el desarrollo de un bioinsumo. Por lo tanto en estudios futuros se intentará estimular la producción de los sideróforos y probar estos compuestos como también sus organismos productores, en el control de hongos fitopatógenos como *Botrytis cinerea*. Desde este punto de vista se alcanzó el objetivo propuesto que era seleccionar microorganismos productores de sideróforos para desarrollar un posible bioinsumo.

AGRADECIMIENTOS

Los autores agradecen el apoyo y financiamiento de la Universidad Nacional de San Luis. PROICO 2–0520 “Desarrollo de bioinsumos destinados al sector agroalimentario”. También se agradece el apoyo y soporte técnico de la Sra. Flavia Silvina Guíñez.

REFERENCIAS

- AGUADO-SANTACRUZ, G., MORENO-GÓMEZ, B., JIMÉNEZ-FRANCISCO, B., GARCÍA-MOYA, E.; PRECIADO-ORTIZ, R. Impacto de los sideróforos microbianos y fitosideróforos en la asimilación de hierro por las plantas: una síntesis. **Revista Fitotecnia Mexicana**, 35 (1), 9-21. 2012.
- AL-ANI, L.; AGUILAR-MARCELINO, L.; BECERRA, A.; SALAZAR-VIDAL, V. Fe-Chelating Compounds Producing Fungal Communities and Their Applications. In **Recent Trends in Mycological Research, Fungal Biology**, YADAV, A. N(Ed.), Vol 1. Cap 6 . (p.135-158). Springer Nature Switzerland. 2021.
- ASHAJYOTHI, M.; VELMURUGAN, S.; KUNDU, A.; BALAMURUGAN, A.; CHOUHAN, V.; KUMAR, A. Hydroxamate siderophores secreted by plant endophytic *Pseudomonas putida* elicit defense against blast disease in rice incited by *Magnaporthe oryzae*. **Letters in Applied Microbiology**, 76, 1–10. 2023.
- CALVENTE, V.; ORELLANO, M.; SANSONE, G.; BENUZZI D, SANZ DE TOSETTI, MI. A simple agar plate assay for screening siderophore producer yeasts. **Journal of Microbiological Methods**, 47, 273-279. 2001a.
- CALVENTE, V.; ORELLANO, E.; SANSONE, G.; BENUZZI, D.; SANZ DE TOSETTI, MI. Effect of nitrogen source and pH on siderophore production by *Rhodotorula* strains and its application to biocontrol of phytopathogenic moulds. **Journal of Industrial Microbiology and Biotechnology**, 26, 226-229. 2001b.
- CASTIGLIONE, A.M.; MANNINO, G.; CONTARTESE, V.; BERTEA, C.M.; ERTANI, A. Microbial Biostimulants as Response to Modern Agriculture Needs: Composition, Role and Application of These Innovative Products. **Plants** 10, 1533, 1-23. 2021.
- LAMBRESE, Y.; GUÍÑEZ, M.; CALVENTE, V.; SANSONE, G.; CERUTTI, S.; RABA, J., *et al.* Production of siderophores by the bacterium *Kosakonia radcincitans* and its application to control of phytopathogenic fungi. **Bioresource Technology Reports**, 3, 82–87. 2018.

MESSENGER, A and RATLEDGE C. Siderophores. In **Comprehensive Biotechnology. The principles, applications and regulations of biotechnology in industry agriculture and medicine.** MURRAY MOO-YOUNG (Ed. in chief). 1° ed. Vol 3. Cap 13. (p.275-295) Pergamon Press, U.K. 1985.

NEILANDS, J.B. Siderophores: structure and function of microbial iron transport compounds. **The Journal of Biological Chemistry**, 270 (45), 26723-26726.1995.

PAYNE, S. Detection, isolation and characterization of siderophores. In: **Methods in Enzimology**, 235 (p.329-344). Academic Press, Inc. NY. 1994.

POWELL, P.; CLINE, G.; REID, C.; SZANISZLO, P. Occurrence of hydroxamate siderophores iron chelators in soils. **Nature**, 287, 833-834. 1980.

SAHA, M.; SARKAR, S.; SARKAR, B.; SHARMA, B., BHATTACHARJEE, S.; TRIBEDI, P. Microbial siderophores and their potential applications: a review. **Environ Sci Pollut Res**, 23, 3984–99. 2016.

SINGH, P.; CHAUHAN, P.; UPADHYAY, S.; SINGH, R.; DWIVEDI, P.; JING, WANG, J., *et al.* Mechanistic Insights and Potential Use of Siderophores Producing Microbes in Rhizosphere for Mitigation of Stress in Plants Grown in Degraded Land. **Frontiers Microbiology**, 13, 1-17. 2022.

TIMOFEEVA, A.; GALYAMOVA, M. SEDYKH, S. Bacterial Siderophores: Classification, Biosynthesis, Perspectives of Use in Agriculture. **Plants**, 11,(3065),1-23. 2022.

CHAPTER 8

THE ROLE OF INTESTINAL MICROBIOME IN THE HEALTH AND PRODUCTION OF COMMERCIAL POULTRY: A REVIEW

Data de submissão: 27/03/2024

Acceptance date: 02/05/2024

Webert Aurino da Silva

Universidade Federal Rural de Pernambuco, Departamento de Zootecnia Recife-PE
<https://orcid.org/0000-0003-0802-1773>

Júlio César dos Santos Nascimento

Universidade Federal Rural de Pernambuco, Departamento de Zootecnia Recife-PE
<https://orcid.org/0000-0003-3107-5876>

Carlos Bôa-Viagem Rabello

Universidade Federal Rural de Pernambuco, Departamento de Zootecnia Recife-PE
<https://orcid.org/0000-0002-5912-162X>

Lilian Francisco Arantes de Souza

Universidade Federal Rural de Pernambuco, Departamento de Zootecnia Recife-PE
<https://orcid.org/0000-0002-0142-664X>

Apolônio Gomes Ribeiro

Universidade Federal da Paraíba, Departamento de Zootecnia Areia-PB
<https://orcid.org/0000-0001-6730-0209>

Dayane Albuquerque da Silva

Universidade Federal Rural de Pernambuco, Departamento de Zootecnia Recife-PE
<https://orcid.org/0000-0001-6243-3969>

Daniela Pinheiro de Oliveira

Universidade Federal Rural de Pernambuco, Departamento de Zootecnia Recife-PE
<https://orcid.org/0000-0001-7955-3780>

Hilton Nobre da Costa

Universidade Federal Rural de Pernambuco, Departamento de Entomologia Recife-PE
<https://orcid.org/0000-0002-3485-3162>

Maria do Carmo Mohaupt Marques Ludke

Universidade Federal Rural de Pernambuco, Departamento de Zootecnia Recife-PE
<https://orcid.org/0000-0003-4895-2599>

Elisabete Albuquerque dos Santos Benvenuto

Universidade Federal Rural de Pernambuco, Departamento de Agronomia Recife-PE
<https://orcid.org/0000-0002-6625-4797>

Aдриelle Carneiro de Araújo Santos

Universidade Federal Rural de Pernambuco, Departamento de Zootecnia Recife-PE
<https://orcid.org/0000-0002-4793-5167>

ABSTRACT: The gastrointestinal tract (GIT) and the community of intestinal microorganisms play a crucial role in the health and functioning of birds, influencing nutrient absorption, strengthening the immune system, and protecting against diseases. Changes in the composition of this microbial community can negatively impact food efficiency, productivity, and bird welfare. The gastrointestinal compartments of birds are inhabited by complex bacterial communities, which recent studies have recognized as having fundamental roles in bird nutrition, physiology, and intestinal development. The intestinal microbiota can act as a protective barrier, adhering to the intestinal epithelial walls and reducing colonization by pathogenic bacteria. These bacteria produce beneficial compounds such as vitamins, short-chain fatty acids, and antimicrobial compounds, which provide nutrition and protection to the animal. However, the intestinal microbiome can also serve as a source of bacterial pathogens, posing a threat to public health. A healthy intestinal microbiota brings significant benefits, including effective competition against pathogens, immune system development, and the creation of protective barriers against undesirable microorganisms. On the other hand, the commensal microbiota also presents challenges, such as competition for energy and proteins in the proximal intestine and the generation of harmful metabolites. Despite the benefits, these processes increase the demand for energy and protein from the host, impacting bird growth performance. In summary, understanding and managing the intestinal microbiota is fundamental to sustainably promoting birds health and productivity. This study aims to analyze the influence of the intestinal microbiome on commercial poultry health and production, focusing on its effects on the immune system, digestion, and disease resistance.

KEYWORDS: intestinal health, symbiotic, probiotic

O PAPEL DO MICROBIOMA INTESTINAL NA SAÚDE E PRODUÇÃO DE AVES COMERCIAIS: UMA REVISÃO

RESUMO: O trato gastrointestinal (TGI) e a comunidade de microorganismos intestinais desempenham um papel crucial na saúde e funcionamento das aves, influenciando a absorção de nutrientes, fortalecimento do sistema imunológico e proteção contra doenças. Alterações na composição dessa comunidade microbiana podem impactar negativamente a eficiência alimentar, produtividade e bem-estar das aves. Os compartimentos gastrointestinais das aves são habitados por complexas comunidades bacterianas, que recentes estudos têm reconhecido como tendo papéis fundamentais na nutrição, fisiologia e desenvolvimento intestinal das aves. A microbiota intestinal pode agir como uma barreira protetora, fixando-se às paredes epiteliais do intestino e reduzindo a colonização por bactérias patogênicas. Essas bactérias produzem compostos benéficos, como vitaminas, ácidos graxos de cadeia curta e compostos antimicrobianos, que fornecem nutrição e proteção ao animal. No entanto, o microbioma intestinal também pode servir como fonte de patógenos bacterianos,

representando uma ameaça à saúde pública. Uma microbiota intestinal saudável traz benefícios significativos, incluindo competição eficaz contra patógenos, desenvolvimento do sistema imunológico e criação de barreiras protetoras contra microrganismos indesejáveis. Por outro lado, a microbiota comensal também apresenta desafios, como competição por energia e proteínas no intestino proximal, e geração de metabólitos prejudiciais. Apesar dos benefícios, esses processos aumentam a demanda por energia e proteína do hospedeiro, impactando o desempenho de crescimento das aves. Em suma, entender e gerenciar a microbiota intestinal é fundamental para promover a saúde e produtividade das aves de forma sustentável. Este estudo tem como objetivo analisar a influência do microbioma intestinal na saúde e na produção de aves comerciais, explorando seu impacto no sistema imunológico, na digestão e na resistência a doenças.

PALAVRAS-CHAVE: saúde intestinal, simbiótico, probiótico

INTRODUCTION

The health and proper functioning of the gastrointestinal tract (GIT) and the intestinal microorganism community are crucial for efficient nutrient absorption, strengthening the immune system, and protection against diseases. Changes in the composition of the microbial community in the GIT can impair feed efficiency, productivity, and poultry welfare (GASKINS; COLLIER; ANDERSON, 2002; KOHL, 2012). The gastrointestinal compartments of chickens are densely populated by complex microbial communities dominated by bacteria (FARKAS et al., 2022). Recent studies on interactions between the host and the intestinal bacterial microbiome of commercial poultry have been conducted, emphasizing the importance of the microbiome in the nutrition, physiology, and intestinal development of birds (FENG et al., 2023; NEMATHAGA et al., 2023).

The intestinal microbiota can form a protective barrier by attaching to the epithelial walls of enterocytes, thus reducing the opportunity for colonization by pathogenic bacteria (KHAN et al., 2021). These bacteria produce vitamins, short-chain fatty acids, organic acids, antimicrobial compounds, and triglycerides, and induce non-pathogenic immune responses, providing nutrition and protection to the animal (PAN; YU, 2014). Conversely, the intestinal microbiome can also be a source of bacterial pathogens such as *Salmonella* and *Campylobacter*, which can spread to humans or act as a reservoir for antibiotic resistance and transmission, posing a serious threat to public health (JUN-XION, 2016).

A healthy intestinal microbiota brings both benefits and costs to the host. Primary benefits offered by commensal microbiota include effective competition against pathogens or non-native microorganisms, as well as contributions to host nutrition (DIBNER; RICHARDS, 2005). Additionally, commensal microbiota can promote the development of the immune system, including different components such as the mucosal layer, intestinal epithelial cells, intestinal immune cells, and lamina propria (SHAKOURI; IJI; MIKKELSEN; COWIESON, 2009). These tissues play a crucial role in creating barriers between the host and microbes, as well as combating undesirable intestinal microorganisms (OAKLEY et al., 2014).

On the other hand, commensal microbiota also presents challenges for the host. In proximal intestinal segments such as the gizzard and small intestine, microorganisms compete with the host for energy and proteins. In both proximal and distal intestines, microbes generate potentially harmful metabolites, such as amino acid catabolites, and degrade bile acids, which can negatively affect bird growth and fat digestibility, respectively (GASKINS; COLLIER; ANDERSON, 2002).

In the presence of microbiota, the intestinal mucus layer increases mucin production and the rate of epithelial cell turnover, ensuring lubrication of the gastrointestinal tract and preventing the invasion of microorganisms into the host intestinal epithelial cells (ARIYADI, B.; HARIMURTI, 2015). Furthermore, the intestinal immune system is more robust and secretes immunoglobulin A (IgA), which specifically binds to bacterial components, aiding in regulating bacterial composition in the gut (GUTZEIT; MAGRI; CERUTTI, 2014). Despite its benefits, these processes increase the host's demand for energy and protein, which can influence bird growth performance (SUZUKI; NAKAJIMA, 2014).

Considering these factors, this study aims to analyze the impact of the intestinal microbiome on the health and production of commercial poultry, exploring its influence on the immune system, digestion, and disease resistance.

THE FUNCTION OF THE GASTROINTESTINAL MICROBIOTA

The entire gastrointestinal tract (GIT) of chickens is colonized by complex microbial communities, composed of viruses, protozoa, fungi, bacteria, and archaea (WEI; MORRISON; YU, 2013). The set of interactions between the avian microbiome and the host has been extensively studied by many researchers (KUMAR et al., 2018; KOGUT, 2017; YITBAREK, A. et al 2018; WARD, T.L. et al. 2019), given that such factors play important roles in the physiology, intestinal morphology, and nutrition of birds (KOGUT et al., 2018).

A balanced intestinal microbiota brings benefits and costs to the host. The main attributions given by the balanced microbiota include the reduction of pathogenic or non-native bacteria through competitive exclusion mechanisms, stimulation of the birds' immune system, and contributions to the host's nutrient metabolism (DIBNER; RICHARDS, 2005). Previous studies have established that conventionally raised animals are less susceptible to pathogenic microorganisms compared to germ-free animals (AL-ASMAKH; ZADJALI, 2015).

Additionally, symbiotic microbiota can stimulate the development of the immune system, including stimulation of intestinal mucosa, intestinal immune cells (T cells, immunoglobulin-producing cells, and phagocytic cells), and lamina propria. These tissues constitute barriers between bacteria and the host, assisting in combating undesirable intestinal microorganisms (OAKLEY et al., 2014). In the distal portion of the avian intestine, the microbiota also produces energy and nutrients such as amino acids, short-chain fatty

acids (SCFAs), vitamins, and amino acids from undigested food, which are eventually available to the host (DIBNER; RICHARDS, 2005). These SCFAs have bacteriostatic components capable of eliminating pathogenic bacteria such as *Salmonella spp* and *Clostridium perfringens* (RICKE, 2003). SCFAs are also a source of energy for animals and can increase stimulation for the proliferation of intestinal epithelial cells, thus increasing the gastrointestinal absorption area (DIBNER; RICHARDS, 2005).

On the other hand, beneficial microbiota incurs costs for the host. In the small intestine and gizzard, microorganisms compete with the host for energy and protein (BARKO et al., 2017). In both the proximal and distal intestines, bacteria produce toxic metabolites and catabolize bile acids, which can reduce bird growth and decrease fat digestibility (GASKINS; COLLIER; ANDERSON, 2002). In the presence of diverse microbiota, the intestinal mucus layer increases mucin secretion and turnover of epithelial cells, thereby keeping the gastrointestinal tract lubricated while preventing microorganisms from invading avian intestinal epithelial cells (SHIRA; FRIEDMAN, 2018).

The more developed intestinal immune system secretes IgA, which specifically binds to bacterial antigenic determinants, helping to regulate the bacterial composition in the intestine (SUZUKI; NAKAJIMA, 2014).

An imbalanced intestinal microbiota is often referred to as dysbiosis, which can be conceptualized as a qualitative and quantitative imbalance of the normal microbiota in the small intestine, which may lead to adverse reactions in the GI tract, including decreased intestinal barrier function (such as reduction of intestinal villi) and poor nutrient digestion, thus increasing the risk of colonization by pathogenic bacteria that may cause an inflammatory response. Both infectious and non-infectious stress conditions can lead to dysbiosis. Infectious causes include bacterial, viral, coccidial challenges, or toxic metabolites such as toxins produced by bacteria like *Clostridium perfringens*. Non-infectious agents include factors of nutritional imbalances, environmental stress, mycotoxins, and host genetic or enzymatic dysfunction (TEIRLYNCK et al., 2011).

The gastrointestinal microbiota can also be classified as mucosal microbiota and luminal microbiota. The composition of mucosa-related microbiota is influenced by various host factors such as expression of specific adhesion sites on the enterocyte membrane, immunoglobulin secretion, and mucin production ratio. The composition of luminal microbiota is established by available nutrients, food passage rate, and the presence of antimicrobial substances. Mucosal microbiota and luminal-associated microbiota also mutually influence each other; therefore, it is of paramount importance to consider that diet can modify both mucosal and luminal microbiota and consequently influence intestinal health (JEURISSEN SHI et al., 2002).

Studies have already compared the taxonomic composition of these two partitions in birds and highlighted how variability changes according to the food or additive in bird diets (AWAD et al., 2016; METZLER-ZEBELI et al., 2019; PARASKEUAS; MOUNTZOURIS,

2019). However, studies on the metabolic functions of these two microbial habitats have not yet been evidenced. Nevertheless, the ongoing study of variations between luminal and mucosal bacterial communities and studies on metabolic functions of the microbiota of each partition are of great importance for a better understanding of the modulation of the intestinal microbiota in birds. Additionally, the study of the bacterial community linked to the mucosa is fundamental to understanding the host mucosal responses, as all changes in mucosal immunity may have implications for the health and productive performance of birds (BORDA-MOLINA et al., 2016).

COMPOSITION OF THE GASTROINTESTINAL MICROBIOTA

The small intestine (duodenum, jejunum, and ileum) of birds primarily functions in the digestion and absorption of nutrients from food (SOUSA et al., 2015). Alongside these microenvironments of the small intestine, other areas such as the esophagus, crop, proventriculus, and gizzard harbor microbial colonies that perform important functions in the growth, health, and productive performance of the animal (CHRISTOFOLI et al., 2020).

The composition of the gastrointestinal tract microbiota in laying hens differs among segments, and its composition reflects the physicochemical changes of different microenvironments (Figure 1). The pH of the compartment, growth substrates, redox potential, antibacterial secretions, and host and microbiota metabolites directly influence the colonization efficiency of microorganisms in intestinal segments. Segments of the proximal part of the intestine are characterized by low pH, which selects for acid-tolerant bacteria and eliminates most pathogenic microorganisms (THOMPSON; HINTON, 1997).

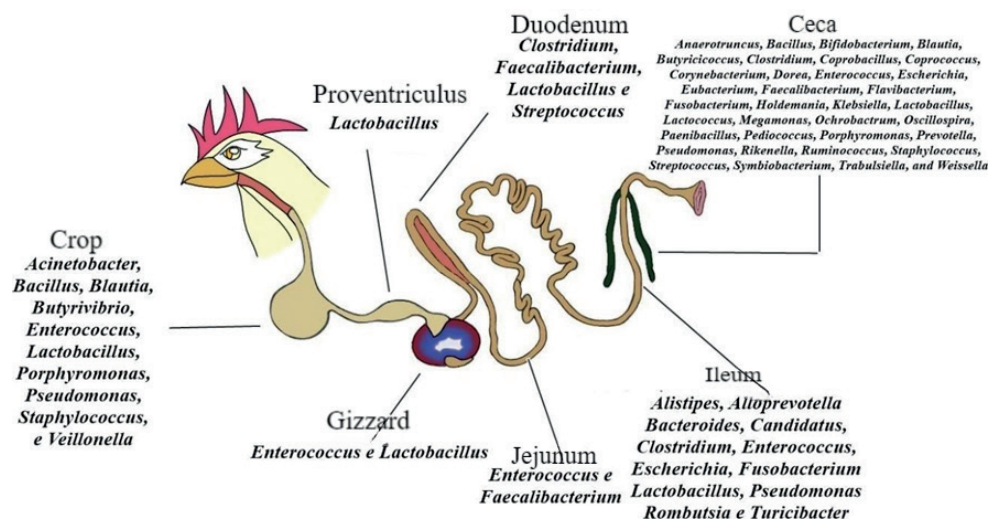


Figure 1. The composition of predominant microorganisms and the variation in diversity of the gastrointestinal microbiota in different segments of birds. Adapted from (KHAN et al., 2020).

The crop is responsible for storing food during periods without food and for moistening the food before enzymatic digestion, harboring approximately 10^8 to 10^9 bacteria per gram of crop contents (KIEROŃCZYK et al., 2016; YEOMAN et al., 2012). The crop microbiota is predominantly dominated by *Bacillus*, *Blautia*, *Enterococcus*, *Lactobacillus*, *Pseudomonas*, and *Staphylococcus* (SAXENA et al., 2016). Lactic acid-producing bacteria, such as *Lactobacillus*, can almost entirely colonize this segment, reducing their proportion along the GI tract (CHRISTOFOLI et al., 2020). The proventriculus is considered the true stomach of birds. The proventriculus is the part of the GI tract that secretes hydrochloric acid and pepsin, aiding in chemical digestion and chyme production, while the gizzard acts as a mechanical function on food (BEDFORD, 2006).

Together, such organs harbor fewer bacteria due to the acidity of the environment (REHMAN et al., 2007). The gizzard has approximately 10^7 to 10^8 bacteria per gram of chyme. Predominant bacterial populations in the chicken gizzard include *Enterobacteriaceae* and *Lactobacillus* (YEOMAN et al., 2012). Within what has been reported, in the proventriculus, the microorganism population is 10^4 to 10^6 CFU/g of bacteria. The proventricular microbiota of birds is dominated by *Lactobacillus*, similar to the gizzard, but unlike the crop, *Lactobacillus* does not adhere to the proventricular epithelium (FULLER; TURVEY, 1971; OAKLEY et al., 2014).

The small intestine of birds is divided into three different segments: the duodenum, jejunum, and ileum. The duodenum has a short transit time with a low pH, which functions to activate enzymes. Pancreatic and biliary secretions aid in digestion, ultimately diluting the chyme and limiting the number of bacteria capable of colonizing the GI tract (REHMAN et al., 2007). It is observed that the duodenal microbiota of birds consists mainly of *Clostridia*, *Streptococcus*, *Enterobacteria*, and *Lactobacillus* (WAITE; TAYLOR, 2015).

Therefore, as the chyme enters the jejunum and ileum, there is a decrease in digestive enzyme activities, and bile acids are unconjugated, thereby facilitating bacterial colonization with increased pH in the following portions (REHMAN et al., 2007), with the jejunum inhabited by *Lactobacillus*, *Streptococcus*, and *Proteobacteria*, and the ileum by *Lactobacillus*, *Enterococcus*, *Bifidobacterium*, *Bacteroides*, *Streptococcus*, *Clostridium*, *Fusobacterium*, and *Coliforms*. The jejunum and ileum have approximately 10^8 to 10^9 bacteria per gram of digestive matter, while the duodenum has 10^3 to 10^5 (STANLEY; HUGHES; MOORE, 2014).

The ceca contributes to various functions in avian physiology, such as nitrogen recycling, water absorption, and electrolytes. The cecal microbiota is capable of food fermentation and synthesizing metabolites such as SCFAs, which can then be used by the host. The intestinal segment has the highest bacterial density in birds; this segment has a cecal content pH ranging from 6.0 to 7.0 and a bacterial concentration of 10^{10} to 10^{12} CFU/g and can be colonized by *Lactobacillus*, *Bacteroides*, *Proteobacteria*, *Bacillus*, *Clostridium*, *Bifidobacterium*, *Fecalibacterium*, *Ruminococcus*, *Eubacterium*, and *Fusobacterium* (STANLEY; HUGHES; MOORE, 2014; SVIHUS, 2014).

MICROBIAL COMPOSITION ACCORDING TO AGE

The structure of the microbiota undergoes several changes throughout the life of birds due to modifications in metabolic function and cell density (SHANG et al., 2018). Differences in microbiota structure change with age, which, in turn, alters modulation and mechanisms in the intestine. Structure refers to total population density, the main community colonizing the microenvironment, the native population, and how these microorganisms interact with each other. In a favorable scenario, this balanced population structure can exclude pathogenic bacterial populations. Intestinal stability reduces the possibility of dysbiosis, resulting in a healthy microbiota (FEYE et al., 2020). The intestinal microbiota rapidly increases from the 1st to the 3rd day, and around the 7th day, most microorganisms inhabiting the mature microbiota are already present; therefore, the number of colonies varies weeks before stabilizing. Two weeks after hatching, *Oscillopira* and *Ruminococcus* bacteria significantly increase their population, whereas the number of *Enterococcus* is reduced (BALLOU et al., 2016). When comparing chickens at 8 and 30 weeks of age, it is observed that bacteria of the *Firmicutes* and *Bacteroidetes* genera become more abundant in the GI tract (CUI et al., 2017). Evaluating the effect of age on laying hens from 1 to 60 weeks old on the composition of the intestinal microbiota, it was observed that *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* formed the vast majority of the microbiota population at all evaluated stages (VIDENSKA et al., 2014). These data show that gram-negative bacteria dominate the intestine in younger birds, while *Firmicutes* become more prevalent in the later stage of the laying hen cycle (KHAN et al., 2020).

As chickens age, the intestinal barrier is compromised due to changes in the composition of the intestinal microbiota, and studies indicate that reducing microbiota variation may trigger dysbiosis in older birds, thus requiring the use of intestinal microbiota balancers (BALLOU et al., 2016; MALIK et al., 2019; XU et al., 2016).

As chickens age, the intestinal barrier is compromised due to changes in the composition of the intestinal microbiota, and studies indicate that reducing microbiota variation may trigger dysbiosis in older birds, thus requiring the use of intestinal microbiota balancers (BALLOU et al., 2016; MALIK et al., 2019; XU et al., 2016).

CONCLUSION

In recent years, significant advances have been made in understanding the taxonomic composition of the gastrointestinal microbiome and its impact on intestinal health. However, there is a growing recognition of the need to expand our knowledge about the crucial role that the microbiome plays in the nutrition, health, disease, and productivity of birds. To advance in this direction, future studies need to adopt integrative approaches, combining data from genomics, transcriptomics, proteomics, and metabolomics. This holistic approach will allow for a more comprehensive understanding of the interactions between intestinal microorganisms and their hosts.

By better understanding the complex interactions between the microbiome and its host, we will be able to develop more targeted and effective interventions to promote intestinal health in birds. This includes modifying the metabolic pathways associated with the microbiome, which may open up new opportunities for innovative therapeutic and preventive interventions.

REFERENCES

- AL-ASMAKH, M., & ZADJALI, F. **Use of Germ-Free Animal Models in Microbiota-Related Research.** *Journal Of Microbiology And Biotechnology*, [S.L.], v. 25, n. 10, p. 1583-1588, 2015. Disponível em: <https://doi.org/10.1016/j.anifeedsci.2014.07.008>.
- ARIYADI, B., & HARIMURTI, S. **Effect of indigenous probiotics lactic acid bacteria on the small intestinal histology structure and the expression of mucins in the ileum of broiler chickens.** *International Journal of Poultry Science*, v. 14, p. 276-278, 2015. Disponível em: <https://doi.org/10.3923/IJPS.2015.276.278>.
- AWAD, W. A. et al. **Age-Related Differences in the Luminal and Mucosa-Associated Gut Microbiome of Broiler Chickens and Shifts Associated with Campylobacter Jejuni Infection.** *Frontiers in Cellular and Infection Microbiology*, v. 6, 22 Nov. 2016. Disponível em: [https://doi.org/10.1011](https://doi.org/10.1011/jvim.14875).
- BALLOU, A. L. et al. **Development of the Chick Microbiome: How Early Exposure Influences Future Microbial Diversity.** *Frontiers in Veterinary Science*, v. 3, 20 Jan. 2016. Disponível em: <https://doi.org/10.1016/j.anifeedsci.2014.07.008>.
- BARKO, P., MCMICHAEL, M., SWANSON, K., & WILLIAMS, D. **The Gastrointestinal Microbiome: A Review.** *Journal of Veterinary Internal Medicine*, v. 32, p. 9-25, 2017. Disponível em: <https://doi.org/10.1111/jvim.14875>.
- BEDFORD, M. R. **Effect of non-starch polysaccharidases on avian gastrointestinal function.** In: *Avian gut function in health and disease*. Wallingford: CABI, 2006. p. 159–170. Disponível em: <https://doi.org/10.1093/jas/skac334>.
- BORDA-MOLINA, D. et al. **Insights into Broilers' Gut Microbiota Fed with Phosphorus, Calcium, and Phytase Supplemented Diets.** *Frontiers in Microbiology*, v. 7, 19 dez. 2016. Disponível em: <https://doi.org/10.1016/j.anifeedsci.2014.07.008>.
- CHRISTOFOLI, M. et al. **Microbiota intestinal benéfica e prejudicial na avicultura: Revisão.** *Research, Society and Development*, v. 9, n. 7, p. e43973667, 27 abr. 2020. Disponível em: <https://doi.org/10.1016/j.anifeedsci.2014.07.008>.
- CUI, Y. et al. **Age-Related Variations in Intestinal Microflora of Free-Range and Caged Hens.** *Frontiers in Microbiology*, v. 8, 11 Jul. 2017. Disponível em: <https://doi.org/10.1016/j.anifeedsci.2014.07.008>.
- DIBNER, J.J.; RICHARDS, J.D. **Antibiotic growth promoters in agriculture: history and mode of action.** *Poultry Science*, [S.L.], v. 84, n. 4, p. 634-643, apr. 2005. Disponível em: <http://dx.doi.org/10.1093/ps/84.4.634>.

- FARKAS, V. et al. **Microbiota Composition of Mucosa and Interactions between the Microbes of the Different Gut Segments Could Be a Factor to Modulate the Growth Rate of Broiler Chickens.** *Animals: an Open Access Journal from MDPI*, v. 12, 2022. Disponível em: <https://doi.org/10.3390/ani12101296>.
- FEYE, K. M. et al. **Influential factors on the composition of the conventionally raised broiler gastrointestinal microbiomes.** *Poultry Science*, v. 99, n. 2, p. 653–659, Feb. 2020. Disponível em: <https://doi.org/10.1016/j.anifeedsci.2014.07.008>.
- GASKINS, H. R.; COLLIER, C. T.; ANDERSON, D. B.. **ANTIBIOTICS AS GROWTH PROMOTANTS: mode of action.** *Animal Biotechnology*, [S.L.], v. 13, n. 1, p. 29-42, jul. 2002. Disponível em: <http://dx.doi.org/10.1081/abio-120005768>.
- GERRITSEN, J. et al. **Intestinal microbiota in human health and disease: the impact of probiotics.** *Genes & Nutrition*, v. 6, n. 3, p. 209–240, 27 ago. 2011. Disponível em: <http://dx.doi.org/10.1007/s12263-011-0229-7>.
- GUTZEIT, Cindy; MAGRI, Giuliana; CERUTTI, Andrea. **Intestinal IgA production and its role in host-microbe interaction.** *Immunological Reviews*, [S.L.], v. 260, n. 1, p. 76-85, 19 jun. 2014. Disponível em: <http://dx.doi.org/10.1111/imr.12189>.
- JEURISSEN SHI et al. **Parameters and techniques to determine intestinal health of poultry as constituted by immunity, integrity, and functionality.** *Current Issues in Intestinal Microbiology*, v. 1, p. 1–14, mar. 2002.
- KHAN, S. et al. **The Gut Microbiota of Laying Hens and Its Manipulation with Prebiotics and Probiotics To Enhance Gut Health and Food Safety.** *Applied and Environmental Microbiology*, v. 86, n. 13, 17 jun. 2020. Disponível em: <https://doi.org/10.1128/AEM.00600-20>.
- KHAN, I. et al. **Mechanism of the Gut Microbiota Colonization Resistance and Enteric Pathogen Infection.** *Frontiers in Cellular and Infection Microbiology*, v. 11, 2021. <https://doi.org/10.3389/fcimb.2021.716299>.
- KOHL, Kevin D. **Diversity and function of the avian gut microbiota.** *Journal Of Comparative Physiology B*, [S.L.], v. 182, n. 5, p. 591-602, 14 jan. 2012. <http://dx.doi.org/10.1007/s00360-012-0645-z.3>
- KOGUT, M.H. 2017. **Issues and consequences of using nutrition to modulate the avian immune response.** *Journal Of Applied Poultry Research*, [S.L.], v. 26, n. 4, p. 605-612, dez. Disponível em: <https://doi.org/10.3382/japr/pfx028>.
- KOGUT, Michael H. **The effect of microbiome modulation on the intestinal health of poultry.** *Animal Feed Science And Technology*, [S.L.], v. 250, p. 32-40, apr. 2019. Disponível em: <http://dx.doi.org/10.1016/j.anifeedsci.2018.10.008>.
- KUMAR, S. et al. **Effect of antibiotic withdrawal in feed on chicken gut microbial dynamics, immunity, growth performance and prevalence of foodborne pathogens.** *PLOS ONE*, v. 13, n. 2, p. e0192450, 14 feb. 2018. Disponível em: <https://doi.org/10.1371/journal.pone.0192450>.
- NEMATHAGA, Mishumo; SMITH, Rae M.; MALATJI, Dikeledi P. **Interactions between the helminth and intestinal microbiome in smallholder chicken farming systems.** *Frontiers in Veterinary Science*, v. 10, 2023. Disponível em: <https://doi.org/10.3389/fvets.2023.1309151>.

MALIK, J. K. et al. **Synbiotics in Animal Health and Production**. *Nutraceuticals in Veterinary Medicine*, p. 287–301, 2019. Disponível em: https://doi.org/10.1007/978-3-030-04624-8_20.

METZLER-ZEBELI, B. U. et al. **Feed Restriction Modifies Intestinal Microbiota-Host Mucosal Networking in Chickens Divergent in Residual Feed Intake**. *mSystems*, v. 4, n. 1, 26 fev. 2019. Disponível em: <https://doi.org/10.1128/msystems.00261-18>.

OAKLEY et al. **The chicken gastrointestinal microbiome**. *Fems Microbiology Letters*, [S.L.], v. 360, n. 2, p. 100-112, 13 out. 2014. <http://dx.doi.org/10.1111/1574-6968.12608>.

PAN, D., & YU, Z. **Intestinal microbiome of poultry and its interaction with host and diet**. *Gut Microbes*, v. 5, p. 108 - 119, 2014. <https://doi.org/10.4161/gmic.26945>.

PARASKEUAS, V.; MOUNTZOURIS, K. C. **Broiler gut microbiota and expressions of gut barrier genes affected by cereal type and phytogetic inclusion**. *Animal Nutrition*, v. 5, n. 1, p. 22–31, mar. 2019. Disponível em: <https://doi.org/10.1016/j.aninu.2018.11.002>.

REHMAN, H. U. et al. **Indigenous bacteria and bacterial metabolic products in the gastrointestinal tract of broiler chickens**. *Archives of Animal Nutrition*, v. 61, n. 5, p. 319–335, 31 out. 2007. Disponível em: <https://doi.org/10.1080/17450390701556817>.

RICKE, S. **Perspectives on the use of organic acids and short chain fatty acids as antimicrobials**. *Poultry Science*, v. 82, n. 4, p. 632–639, apr. 2003. Disponível em: <https://doi.org/10.1093/ps/82.4.632>.

SHANG, Y. et al. **Chicken Gut Microbiota: Importance and Detection Technology**. *Frontiers in Veterinary Science*, v. 5, 23 out. 2018. Disponível em: <https://doi.org/10.3389/fvets.2018.00254>.

SHAKOURI, M. D.; IJI, P. A.; MIKKELSEN, L. L.; COWIESON, A. J.. **Intestinal function and gut microflora of broiler chickens as influenced by cereal grains and microbial enzyme supplementation**. *Journal Of Animal Physiology And Animal Nutrition*, [S.L.], v. 93, n. 5, p. 647-658, 10 set. 2009. Disponível em: <http://dx.doi.org/10.1111/j.1439-0396.2008.00852.x>.

SHIRA, ENAV BAR; FRIEDMAN, AHARON. **Innate immune functions of avian intestinal epithelial cells: response to bacterial stimuli and localization of responding cells in the developing avian digestive tract**. *Plos One*, [S.L.], v. 13, n. 7, p. 13-28, 6 jul. 2018. Disponível em: <http://dx.doi.org/10.1371/journal.pone.0200393>.

SOUSA, D. C. et al. **Caracterização morfológica do trato gastrointestinal de frangos de corte dalinhagem Cobb 500®**. *Pesquisa Veterinária Brasileira*, v. 35, n. suppl 1, p. 61–68, dez. 2015. Disponível em: <https://doi.org/10.1590/S0100-736X2015001300011>.

STANLEY, D.; HUGHES, R. J.; MOORE, R. J. **Microbiota of the chicken gastrointestinal tract: influence on health, productivity and disease**. *Applied Microbiology and Biotechnology*, v. 98, n. 10, p. 4301–4310, 19 maio 2014. Disponível em: <https://doi.org/10.1007/s00253-014-5646-2>.

SUZUKI, Keiichiro; NAKAJIMA, Akira. **New aspects of IgA synthesis in the gut**. *International Immunology*, [S.L.], v. 26, n. 9, p. 489-494, 28 maio 2014. Disponível em: <http://dx.doi.org/10.1093/intimm/dxu059>.

SVIHUS, B. **Function of the digestive system**. *Journal of Applied Poultry Research*, v. 23, n. 2, p. 306–314, jun. 2014. Disponível em: <https://doi.org/10.3382/japr.2014-00937>.

TEIRLYNCK, E. et al. **Morphometric evaluation of “dysbacteriosis” in broilers.** Avian Pathology, v. 40, n. 2, p. 139–144, abr. 2011. Disponível em: <https://doi.org/10.1080/03079457.2010.543414>.

VIDENSKA, P. et al. **Succession and Replacement of Bacterial Populations in the Caecum of Egg Laying Hens over Their Whole Life.** PLoS ONE, v. 9, n. 12, p. e115142, 12 dez. 2014. Disponível em: <https://doi.org/10.1371/journal.pone.0115142>.

YEOMAN, C. J. et al. **The microbiome of the chicken gastrointestinal tract.** Animal health research reviews / Conference of Research Workers in Animal Diseases, v. 13, n. 1, p. 89-99, 2012. Disponível em: <https://doi.org/10.1017/S1466252312000138>.

YITBAREK, A. et al. **Gut microbiota modulates type I interferon and antibody-mediated immune responses in chickens infected with influenza virus subtype H9N2.** Beneficial Microbes, [S.L.], v. 9, n. 3, p. 417-427, 25 abr. 2018. Disponível em: <https://doi.org/10.3920/BM2017.0088>.

WAITE, D. W.; TAYLOR, M. W. **Exploring the avian gut microbiota: current trends and future directions.** Frontiers in Microbiology, v. 6, 3 jul. 2015. Disponível em: <https://doi.org/10.3389/fmicb.2015.00673>.

WARD, T.L. et al. **Antibiotics and Host-Tailored Probiotics Similarly Modulate Effects on the Developing Avian Microbiome, Mycobiome, and Host Gene Expression.** Mbio, [S.L.], v. 10, n. 5, p. 1-17, 29 out. 2019. Disponível em: <https://doi.org/10.1128/mbio.02171-19>.

WEI, S.; MORRISON, M.; YU, Z. **Bacterial census of poultry intestinal microbiome.** Poultry Science, v. 92, n. 3, p. 671–683, mar. 2013. Disponível em: <https://doi.org/10.3382/ps.2012-02822>.

XU, Y. et al. **High-throughput sequencing technology to reveal the composition and function of cecal microbiota in Dagu chicken.** BMC Microbiology, v. 16, n. 1, p. 259, 4 dez. 2016. Disponível em: <https://doi.org/10.1186/s12866-016-0877-2>.

AGREGADOS BIOGÊNICOS E FISIOGÊNICOS EM SISTEMAS AGROFLORESTAIS SINTRÓPICOS DIFERENTES IDADES E MATA SECUNDÁRIA

Data de submissão: 08/03/2024

Acceptance date: 02/05/2024

Amanda Vanessa Araújo dos Santos

Universidade Federal Rural da Amazônia,
Belém - Pará
<https://lattes.cnpq.br/7193972176982764>

Anaila Alencar de Amaral

Universidade Federal Rural da Amazônia
Belém - Pará
<https://lattes.cnpq.br/4720087705371791>

Breno dos Santos Barros

Universidade Federal Rural da Amazônia,
Belém - Pará
<http://lattes.cnpq.br/6970888605995875>

Sheila dos Santos Tavares

Universidade Federal Rural da Amazônia
Belém - Pará
<https://lattes.cnpq.br/6566527218158130>

Maria Eduarda da Conceição Lourinho

Universidade Federal Rural da Amazônia
Belém - Pará
<https://lattes.cnpq.br/4441423867757633>

Adylson Pereira Campos Junior

Universidade Federal Rural da Amazônia
Belém - Pará
<http://lattes.cnpq.br/3193759906128201>

RESUMO: O estudo foi realizado na propriedade rural Ecovila landê, em Santa Bárbara do Pará, com o objetivo de avaliar a influência do tempo e do tipo de manejo em Sistemas Agroflorestais (SAFs) na formação de agregados biogênicos e fisiogênicos, utilizando uma mata secundária como referência. As áreas de estudo, compostas por SAFs de 5, 4 e 2 anos, além da mata secundária, foram localizadas em uma biossequência com o mesmo tipo de solo. Foram realizadas coletas em cinco pontos diferentes, com amostragem em três profundidades (0-5, 5-10 e 10-20 cm). A análise dos agregados foi feita por meio de peneiramento das amostras e caracterização morfológica. Após a coleta dos dados, foram realizadas análises estatísticas para avaliar a normalidade e homogeneidade dos dados, seguidas por análise de variância. Os resultados indicaram diferenças significativas na distribuição de agregados biogênicos nas camadas de 5-10 cm e 10-20 cm, sendo que o SAFs de 2 anos apresentou a maior média percentual de agregados biogênicos. Além disso, esse sistema também teve sucesso na redução da fração de terra solta nas camadas de 0-5 cm e 5-10 cm. O estudo sugere que o SAFs mais recente exerce

uma influência maior na formação de agregados em comparação com os sistemas mais antigos e a mata secundária. Estudos futuros são necessários para melhor compreensão dessa relação entre cobertura vegetal e formação de agregados biogênicos em áreas de SAFs.

PALAVRAS-CHAVE: manejo sustentável; análise do solo; propriedade física, atividade biogênica

BIOGENIC AND PHYSIOGENIC AGGREGATES IN SYNTROPIC AGROFORESTRY SYSTEMS OF DIFFERENT AGES AND SECONDARY FOREST

ABSTRACT: The study was carried out on the Ecovila landê rural property in Santa Bárbara do Pará, with the objective of evaluating the influence of time and type of management in Agroforestry Systems (SAFs) on the formation of biogenic and physiogenic aggregates, using a secondary forest as a reference. The study areas, composed of 5-, 4- and 2-year SAFs, in addition to secondary forest, were located in a biosequence with the same soil type. Collections were carried out at five different points, with sampling at three depths (0-5, 5-10 and 10-20 cm). The aggregates were analyzed by sieving the samples and morphological characterization. After data collection, statistical analyzes were performed to assess the normality and homogeneity of the data, followed by analysis of variance. The results indicated significant differences in the distribution of biogenic aggregates in the 5-10 cm and 10-20 cm layers, with the 2-year SAFs presenting the highest average percentage of biogenic aggregates. Furthermore, this system was also successful in reducing the fraction of loose soil in the 0-5 cm and 5-10 cm layers. The study suggests that the more recent SAFs exert a greater influence on aggregate formation compared to older systems and secondary forest. Future studies are needed to better understand this relationship between vegetation cover and the formation of biogenic aggregates in SAF areas.

KEYWORDS: sustainable management; soil analysis; physical property, biogenic activit

INTRODUÇÃO

Com a evolução da agricultura no mercado e demandas cada vez maiores por alimentos têm instigado produções mais aceleradas e em larga escala, porém as práticas de manejo insustentáveis afetam diretamente o meio ambiente. Desse modo, ações e propostas de plantios e manejo sustentáveis devem ser implementadas para que a agricultura e a conservação ambiental sejam praticadas em complementaridade (YOUNGBERG; DEMUTH, 2013). Assim, surgiu a Agricultura Sintrópica, uma nova proposta de cultivo elaborada pelo pesquisador e agricultor suíço Ernst Götsche, que se tornou mais avançada pela sua nova estrutura e função quando comparada ao cultivo agroflorestais. A partir de 2013, o termo “agricultura sintrópica” foi definitivo, tendo como princípios a conservação da cobertura vegetal natural associado a plantação de culturas comerciais, visando o balanço energético positivo e processos de sucessão natural sem insumos externos (MICCOLIS et al., 2016; PASINI, 2017).

A Agricultura Sintrópica tem se difundido amplamente pelas diversas regiões do Brasil, trazendo consigo soluções inovadoras e sustentáveis para os desafios enfrentados pela agricultura contemporânea. Esta abordagem, que combina técnicas agroecológicas com princípios de conservação ambiental, tem despertado interesse e ganhado adeptos em todo o país (SILVA, 2023). Conforme destacado por Miccolis et al. (2016), a adoção de técnicas como o plantio em consórcio e a integração de árvores nas áreas de produção tem contribuído para aumentar a produtividade e reduzir a dependência de insumos externos.

Para melhor compreensão do desenvolvimento e qualidade do solo sob diferentes métodos de cultivo, a análise dos agregados estáveis é uma das propriedades físicas chave que indicam sua qualidade. Isso se deve ao fato de que práticas de manejo inadequadas podem causar danos substanciais à estrutura do solo. Entre esses danos, a perda de agregação pode resultar em maior compactação, redução na formação de raízes e atividade microbiana, diminuição da porosidade, restrição na absorção de água e aumento da suscetibilidade à erosão (REINERT & REICHERT, 2006).

Os agregados são estruturas compostas pela união de partículas primárias (areia, silte e argila), que se unem através da ação de agentes agregantes, como a matéria orgânica. Quando incorporada, essa matéria orgânica funciona como um agente cimentante, unindo as partículas minerais do solo. Além disso, os agregados também podem ser formados pela ação de raízes e microorganismos (CARDOSO et al., 2013). Quanto à sua classificação morfológica, os agregados podem ser de origem fisiogênica ou biogênica.

A formação fisiogênica corresponde à união das partículas pela adição de matéria orgânica em conjunto aos ciclos de umedecimento e secagem. Por outro lado, a estrutura biogênica depende dos agentes cimentantes das raízes e organismos para consolidar as partículas (BARBOSA, 2011). Em função disso, os agregados biogênicos servem como armazenamento de carbono orgânico e habitat para organismos que fornecem húmus e formam poros e galerias no solo (BRUSSAARD et al., 2007).

Portanto, o objetivo deste trabalho foi caracterizar e quantificar os agregados de acordo com a influência do tempo e tipo de manejo realizado em cada sistema na formação biogênica e fisiogênica presente nos sistemas SAFs de diferentes idades e como referência utilizou-se a mata secundária.

METODOLOGIA

Área de estudo e delineamento experimental

O estudo foi conduzido na propriedade rural Ecovila landê, localizada em Santa Bárbara do Pará, com as seguintes coordenadas: 1°09'43,89"S 48°15'31,99"O (**Figura 1**). Os tipos de solo predominantes na região são Latossolos Amarelos em áreas de relevo plano e Gleissolos nas baixadas, além de áreas de Espodossolos também presentes

na região (IBGE, 1999). Quanto ao clima, de acordo com a classificação de Köppen, é considerado equatorial úmido. As áreas de estudo estão inseridas em uma biossequência que compartilha a mesma classe de solo, porém com diferentes práticas de manejo.

Essas áreas incluem parcelas de 5, 4 e 2 anos sob sistemas agroflorestais sintrópicos (SAFs5, SAFs4 e SAFs2, respectivamente), além de uma parcela de mata secundária que servirá como referência (MS).

O delineamento experimental utilizado foi o inteiramente casualizado com cinco repetições aleatórias em cada sistema.

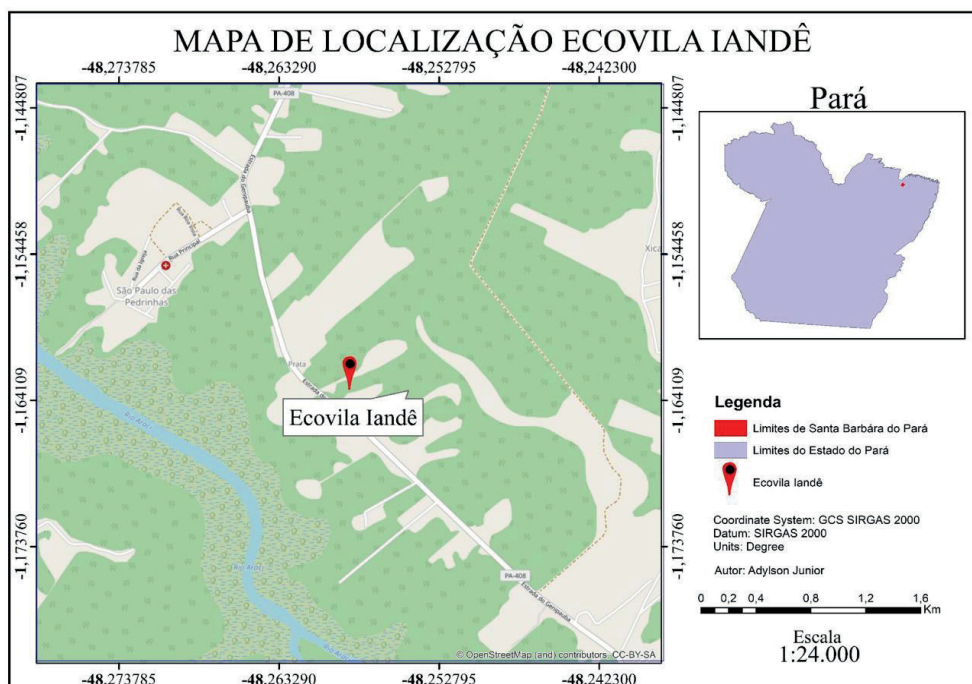


Figura 1. Mapa de localização da área de estudo

Fonte: Autores (2024)

Coleta das amostras e análise

Para a coleta das amostras, em cada área (sistema) foram abertas cinco mini trincheiras com dimensões aproximadas de 40x40x40 cm. Coletou-se amostras do tipo indeformadas nas profundidades 0-5, 5-10 e 10- 20 e em seguida foram armazenadas em potes plásticos com aproximadamente 500 g e levadas ao Laboratório de Gênese e Morfologia do Solo (UFRA, Belém) para posterior análise.

O procedimento de análise dos agregados consistiu no peneiramento das amostras, através do conjunto de peneiras granulométricas com aberturas 9,5; 8,0; 4,0 e 2,0 mm. (**Figura 2**). Os agregados retidos em cada peneira foram separados e analisados de acordo

com suas características e padrões morfológicos para formação biogênica e fisiogênica, conforme proposto por Velasquez et al. (2007) e Rossi et al. (2016). A identificação dos agregados fisiogênicos foi realizada pela observação de formas angulares, enquanto os biogênicos apresentam formas arredondadas, resultantes da passagem pelo trato intestinal animal, além da presença de raízes e coloração mais escura. (**Figura 3**). Após a separação, foi realizada a determinação da umidade residual e a quantificação dos agregados em termos de seu percentual de massa em relação ao total.



Figura 2. Peneiras granulométricas para separação dos agregados

Fonte: Autores (2024)



Figura 3. Agregados oriundo de formação biogênica

Fonte: Autores (2024)

Analise de dados

Após a obtenção dos dados, foi realizado a avaliação de normalidade (Shapiro-Wilk) e homocedasticidade (Bartlett) dos resíduos. Para os conjuntos de dados que não atingiram tais parâmetros realizou-se a transformação Box-Cox a -1 e a 2.35, após isso, foi feita a análise de variância através das comparações de médias entre si pelo teste t-student a 5% no software Rstudio 4.0.1.

RESULTADOS E DISCUSSÕES

ÁREA	%MAB		%TS	
0-5				
MS	52,35	a	47,21	ab
SAFs 5	43,53	a	55,55	ab
SAFs 4	37,47	a	62,52	a
SAFs 2	60,32	a	39,67	b
5-10				
MS	62,53	b	37,443	b
SAFs 5	63,15	b	37,761	b
SAFs 4	39,50	c	62,093	a
SAFs 2	74,57	a	25,345	c
10-20				
MS	66,97	a	33,02	b
SAFs 5	63,54	a	36,45	b
SAFs 4	44,38	b	55,61	a
SAFs 2	68,13	a	31,86	b

MAB = média percentual de agregados biogênicos; TS= média percentual de terra solta (não agregados). Ms = Mata secundária; SAFs 5 anos = sistema de 5 anos; SAFs 4= sistema de 4 anos; SAFs 2 = sistema de 2 anos. Letras minúsculas comparam os sistemas em cada profundidade. Média seguida de mesmas letras não diferem entre si pelo teste t-student a 5% de probabilidade.

Tabela 1. Valores em porcentagem (%) de agregados biogênicos (MAB) e não agregados (TS) presente nos SAFs e Mata secundária

Fonte: Autores (2024)

Os resultados indicaram que os percentuais de massa de agregados biogênicos (MAB) na profundidade 0-5 dos SAFs (5, 4 e 2 anos) e MS (Mata secundária) não apresentaram diferença significativa. Isto pode ser atribuído à maior concentração de raízes na camada superficial do solo, onde há um constante acúmulo de material orgânico e microrganismos, criando um ambiente favorável para uma maior agregação na região, que se iguala em todos os sistemas. Essa observação está em linha com os achados de Loss et al. (2017) que não encontraram diferenças na proporção de agregados biogênicos e físicos entre os tratamentos, mesmo após a aplicação de diferentes tipos e quantidades de estrume suíno na camada de 0-5 cm.

Na camada de 5-10 cm, foram observadas diferenças significativas, com o sistema de 2 anos (SAFs 2) apresentando uma média percentual de agregados biogênicos (MAB) maior do que os demais sistemas, seguido pelo SAFs de 5 anos e pela MS, que apresentaram médias semelhantes, e pelo SAFs de 4 anos, que teve a menor média. Esse padrão também foi observado na camada de 10-20 cm, onde o SAFs de 2 anos manteve a maior média de agregados, enquanto o SAFs de 4 anos teve a menor média novamente.

O sistema de 2 anos demonstrou melhor desenvolvimento estrutural, apresentando também menor média de massa de não agregados (TS) nas profundidades 0-5, 5-10 e 10-20. Nesse sentido duas hipóteses foram formuladas, a primeira estar relacionada a influência do sistema radicular de gramíneas, visto que alguns autores (WENDLING et al, 2005 e NICHOLS et al, 2013) deletaram que as raízes das gramíneas influenciaram na quantidade e estabilidade estrutural de agregados. Outra hipótese diz respeito ao manejo realizado com podas frequentes de material verde que são mantidos em superfície, contribuindo para conservação e aumento da umidade e diminuição das perdas de solo por erosão e aumenta o teor de matéria orgânica e formação de agregados (PERIN et al, 2003)

Em contra partida, o sistema de 4 anos apresentou uma menor média de porcentagem de agregados em todas as profundidades (0-5, 5-10, 10-20). Isso se deve ao fato de que o solo onde o SAFs foi implantado possuía uma maior presença de areia em comparação às outras áreas. No entanto, o manejo desse sistema foi realizado com cobertura de matéria orgânica à base de caroços de açaí e esterco, o que contribuiu para a formação de agregados, embora não tenha sido suficiente em comparação com os outros. Por outro lado, para a formação de agregados são necessárias duas condições essenciais: primeiro, a presença de forças mecânicas (como raízes, macro e microfauna, ciclo de umedecimento e secagem) para unir as partículas primárias do solo; segundo a presença de agentes cimentantes, como a fração argila, óxidos e matéria orgânica (CARTER, 2004). Portanto, um solo com maior teor de areia tende a ter uma agregação menos eficiente em curto prazo, uma vez que, de acordo com Chan et al. (2001), os agregados também dependem da junção de partículas de argila em conjunto com a matéria orgânica para sua formação.

CONCLUSÃO

O SAFs mais novo (SAFs 2) obteve maior influência na quantidade de agregados em relação aos SAFs mais antigos (5 e 4 anos) e Mata secundária. Tendo como hipóteses, que tal influencia na agregação pode estar relacionada ao manejo com podas frequentes de material verde e ao sistema radicular de gramíneas que podem ter estimulado o aumento da atividade biológica do sistema.

REFERÊNCIAS

- BATISTA, I. **Atributos edáficos e fauna do solo em áreas de integração lavoura-pecuária no bioma Cerrado**, Mato Grosso do Sul. Dissertação (Mestrado) – Universidade Federal Rural do Rio de Janeiro, Seropédica. p. 86, 2011
- BULLOCK, P., FEDEROFF, N., JONGERIUS, A., STOOPS, G., TURSINA, T. et al. **Handbook for Soil Thin Section Description**. Albrighton, England: Waine Research Publications, 1985.
- BRUSSAARD, L.; PULLEMAN, M. M.; OUE DRAOGO, E.; MANDO, A.; SIX, J. **Soil fauna and soil function in the fabric of the food** web. *Pedobiologia*, Jena, v. 50, n. 6, p. 447-462, 2007.
- CARTER, M. R. **Researching structural complexity in agricultural soils**. *Soil & Tillage Research*, p. 1-6, 2004
- CARDOSO, E.; VASCONCELLOS, R.; BINI, D.; MIYAUCHI, M. **Soil health: Looking for suitable indicators. What should be considered to assess the effects of use and management on soil health?** *Scientia Agricola*. V. 70, n. 4, pag. 219-303, 2013.
- CHAN, K. Y.; BOWMAN, A.; OATES, A. **Oxidizable organic carbon fractions and soil quality changes in an oxic paleustalf under different pasture ley**. *Soil Science*, New Brunswick, v. 166, n. 1, p. 61-67, 2001.
- EMBRAPA. **Manual de Métodos de análise de solo**. 2a ed. Rio de Janeiro, 1997. 212p.
- IBGE. Banco de Dados de Recursos Naturais da Amazônia Brasileira. Rio de Janeiro. IBGE/CISCEA/SIVAM. SIG, Produto Digital, 1999.
- LOSS, A. et al. **Soil fertility, physical and chemical organic matter fractions, natural 13C and 15N abundance in biogenic and physico-genic aggregates in areas under different land use systems**. *Soil Research*, v. 52, p. 685-697, 2014.
- Loss, A, et al. **Carbon, nitrogen and natural abundance of 13C and 15N in biogenic and physico-genic aggregates in a soil with 10 years of pig manure application**. *Soil Till Res* 166: 52-58. (Argentina) 38 (1): 12-20
- MICCOLIS, A. et al. **Restauração Ecológica com Sistemas Agroflorestais: Como conciliar conservação com produção. Opções para Cerrado e Caatinga**. Brasília: ISPN/ICRAF, 2016. 266p.
- NICHOLS, K. A. et al. **Roles of biology, chemistry, and physics in soil macroaggregate formation and stabilization**. *The Open Agriculture Journal*, Beijing, v.7, p.107-117, 2013.
- PASINI, F.S. **A Agricultura Sintrópica de Ernst Götsch: história, fundamentos e seu nicho no universo da Agricultura Sustentável**. 104f. Dissertação (Mestrado em Ciências Ambientais e Conservação) - Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2017.
- PERIN, A; GUERRA, J. G. M. & TEIXEIRA, M. G. **Cobertura do solo e acumulação de nutrientes pelo amendoim forrageiro em plantio direto**. *Pesquisa Agropecuária Brasileira*, v.38, p.791-796, 2003.
- PULLEMAN, M. **Interactions between soil organic matter dynamics and soil structure as affected by farm management**. PhD thesis. Wageningen University dissertation, pag. 146, 2002.

VELASQUEZ, E. et al. This ped is my ped: **Visual separation and near infrared spectra allow determination of the origins of soil macroaggregates**. *Pedobiologia*, v. 51, p. 75-87, 2007.

Wendling, Beno et al. **Carbono orgânico e estabilidade de agregados de um Latossolo Vermelho sob diferentes manejos**. *Pesquisa Agropecuária Brasileira* [online]. 2005, v. 40, n. 5 [Acessado 4 Outubro 2022], pp. 487-494.

YOUNGBERG, G.; DEMUTH, S. P. **Organic agriculture in the United States: A 30-year retrospective**. *Renewable Agriculture and Food Systems*, 2013.

REINERT, Dalvan José; REICHERT, José Miguel.; VEIGA, Milton.; SUZUKI, Luís Eduardo Akiyoshi Sanches. **Qualidade física dos solos**. In: *Reunião Brasileira de Manejo e Conservação do Solo e da Água*, 16, 2006.

ROSSI, C. Q. et al. **Vias de formação, estabilidade e características químicas de agregados em solos sob sistemas de manejo agroecológico**. *Pesquisa Agropecuária Brasileira*, v. 51, n. 9, p. 1677-1685, set. 2016

EFEITOS DA NUTRIÇÃO DE MATRIZES GESTANTES NO IMPRINTING FETAL EM SUÍNOS: REVISÃO

Acceptance date: 02/05/2024

Jessica Mansur Siqueira Crusóé

Universidade de Federal Viçosa
Florestal – Minas Gerais (Brasil)
<https://orcid.org/0009-0007-4210-8430>

Leonardo França da Silva

Universidade de Federal Viçosa
Viçosa – Minas Gerais (Brasil)
<https://orcid.org/0000-0002-9710-8100>

Genelício Crusóé Rocha

Universidade de Federal Viçosa
Viçosa – Minas Gerais (Brasil)

Leonardo Fonseca Faria

Universidade de Federal Viçosa
Viçosa – Minas Gerais (Brasil)

Victor Crespo de Oliveira

Universidade Estadual Paulista
Botucatu – São Paulo (Brasil)
<https://orcid.org/0000-0003-2719-9972-5380>

Matheus Faria Souza

Universidade de Federal Viçosa
Viçosa – Minas Gerais (Brasil)

Diego de Ávila Martins Braga

Universidade de Federal Viçosa
Viçosa – Minas Gerais (Brasil)

Denis Medina Guedes

Universidade de Federal Viçosa
Florestal – Minas Gerais (Brasil)
<https://orcid.org/0009-0004-9847-8471>

Michael Bergsten Eliphelete Rodrigues Barbosa

Universidade de Federal Viçosa
Florestal – Minas Gerais (Brasil)

Cauã Godinho Gomes Fraga

Universidade de Federal Viçosa
Florestal – Minas Gerais (Brasil)

Pedro Arthur Rezende Aguiar Moura

Universidade de Federal Viçosa
Florestal – Minas Gerais (Brasil)

Juarez Lopes Donzele

Universidade de Federal Viçosa
Viçosa – Minas Gerais (Brasil)

RESUMO: Com os avanços do melhoramento genético, os nutricionistas observaram a necessidade de adequar os programas nutricionais ao potencial genético e a capacidade de produção das matrizes atualmente disponíveis no mercado, afim de garantir melhor desempenho e produtividade dos leitões nascidos. No manejo nutricional da fêmea

gestante, deve ser considerado o desenvolvimento adequado da placenta e cordão umbilical para garantir o aporte de nutrientes e oxigênio para o desenvolvimento fetal. O aumento do número de fetos no útero leva a um aumento na competição de oxigênio e nutrientes pelos fetos acarretando em nascimento de leitões com menor peso e mais fracos que são sinais característicos do chamado crescimento intra-uterino retardado (CIUR). Quando não há adequado desenvolvimento, os leitões leves apresentam desempenho limitado ao longo do ciclo de produção, necessitando de estratégias que diminuam a frequência de nascidos na categoria de baixa viabilidade. Resultados positivos têm sido alcançados com a suplementação da ração de gestação, aumentando a sobrevivência embrionária, a distribuição de nutrientes, o peso do feto e conseqüentemente melhorando o desempenho pós-natal da leitegada. Diante do exposto, são destacados na presente revisão os efeitos da nutrição de matrizes gestantes no desenvolvimento fetal.

PALAVRAS-CHAVE: nutrientes, porcas, feto, placenta

ABSTRACT: With advances in genetic improvement in swines, it is important to adapt nutritional programs to the genetic potential and production capacity of sows, in order to guarantee better performance and productivity of piglets born. In the nutritional management of the pregnant female, adequate development of the placenta and umbilical cord should be considered to ensure nutrient and oxygen supply for fetal development. The increase in the number of fetuses in the uterus leads to an increase in competition of oxygen and nutrients by the fetuses leading to the birth of smaller and weaker piglets that are characteristic signs of retarded intrauterine growth. When there is no adequate development, piglets with low weight birth present limited performance throughout the production cycle, necessitating strategies that reduce the frequency of low birth weight piglets. Positive results have been achieved with supplementation of the feed gestation, increasing embryo survival, nutrient distribution, fetal weight and consequently improving the postnatal performance of the litter. The effects of the nutrition of pregnant sows on fetal development are highlighted in the present review.

KEYWORDS: nutrients, sows, fetus, placenta.

INTRODUÇÃO

A nutrição de fêmeas suínas tem evoluído consideravelmente nos últimos anos. Com os avanços do melhoramento genético, os nutricionistas observaram a necessidade de adequar os programas nutricionais ao potencial genético e a capacidade de produção das matrizes atualmente disponíveis no mercado.

As taxas de ovulação dessas matrizes estão cada vez maiores, originando as chamadas fêmeas hiperprolíficas ou de alta prolificidade. A hiperprolificidade caracterizada pela produção de leitegadas numerosas, associada às mudanças no manejo, possibilitou em alguns sistemas a produção superior de 30 leitões desmamados/fêmea/ano (AGRINESS, 2015). A hiperprolificidade é uma característica desejável, visto a produção de um maior número de leitões para o sistema de produção, no entanto, leitões de baixa viabilidade resultantes da maior lotação intrauterina requerem maior atenção e mão de obra, sendo parte dos problemas advindos das matrizes atuais.

Quando ocorre aumento do número de fetos, o fluxo sanguíneo uterino também aumenta, porém, não em proporção suficiente, o que reduz o fluxo sanguíneo para os fetos, diminuindo o suprimento de nutrientes e oxigênio para cada (FOXCROFT et al., 2006). Assim, é possível verificar uma correlação negativa entre o peso ao nascer e o número de nascidos, reduzindo de 25 a 35 gramas no peso médio para cada leitão adicional (DEVILLERS et al., 2007).

No manejo nutricional da fêmea gestante, deve ser considerado o desenvolvimento adequado da placenta e cordão umbilical para garantir o aporte de nutrientes e oxigênio para o desenvolvimento fetal (LIU et al., 2012). O aumento do número de fetos no útero leva a um aumento na competição de oxigênio e nutrientes pelos fetos acarretando em nascimento de leitões com menor peso e mais fracos que são sinais característicos do chamado crescimento intrauterino retardado (CIUR) (FOXCROFT et al., 2006). Esse aumento na competição por nutrientes pode causar deficiência nutricional nestes animais ainda no útero, que para aumentar as chances de sobrevivência se adaptam através de alterações fisiológicas e metabólicas. No entanto, essas modificações ocorrem em nível de genoma, podendo permanecer ao longo da vida do animal, o que é chamado de programação pré-natal (WU et al., 2004). Quando não há adequado desenvolvimento, os leitões leves apresentam desempenho limitado ao longo do ciclo de produção (alta mortalidade, pior conversão alimentar, menor ganho de peso e piora na qualidade da carne), necessitando de estratégias que diminuam a frequência de nascidos na categoria de baixa viabilidade (GONDRET et al., 2006).

Leitões de baixo peso ao nascimento apresentam menores chances de sobrevivência, devido ao menor nível de reservas corporais, alta sensibilidade ao frio e dificuldades de realização da primeira mamada. Além disso, estes animais de baixo peso tem um crescimento limitado pela hipertrofia de suas fibras musculares, pois o crescimento do número de fibras musculares é estabelecido durante o desenvolvimento fetal (WIGMORE & STICKLAND, 1983).

Em trabalho avaliando o peso ao nascer e desempenho subsequente, REHFELDT & KUHN (2006) verificaram que existe um efeito do peso ao nascer sobre o desempenho dos leitões na fase de creche e terminação. Estes autores concluíram que os leitões com peso ao nascimento entre 0,8 e 1,2 kg apresentam um menor peso vivo e menor peso de carcaça aos 182 dias de idade quando comparados aos leitões médios ou pesados ao nascimento.

O crescimento e desenvolvimento fetal é um processo biológico complexo que apesar de ser guiada pelo genoma, essa regulação genética pode ser influenciada pelo ambiente intrauterino e pela nutrição materna. Esses fatores podem interferir no tamanho e capacidade funcional da placenta, transferência de nutrientes e oxigênio da matriz para o feto, viabilidade de nutrientes para o feto e em vias metabólicas, podendo causar problemas irreversíveis nos animais. Assim, fica evidenciado que o crescimento pré-natal em mamíferos placentários é sensível aos efeitos diretos e indiretos da nutrição materna

em todos os estágios desde a maturação do óócito até o nascimento (REHFELDT et al., 2006; FERGUSON, 2005).

Além dos estudos mostrando o comprometimento no desenvolvimento de leitões mais leves ao nascimento, também é evidenciado perda na qualidade da carne (GONDRET et al., 2006; REHFELDT & KUHN, 2006). Assim, o peso ao nas cimento está diretamente relacionado à qualidade do leitão, que, por sua vez, está relacionado à sua capacidade de sobrevivência e a seu desempenho pós-natal. Dessa forma, o peso ao nascer é uma importante característica econômica para a suinocultura.

O entendimento dos processos relacionados ao desenvolvimento fetal em animais de produção tem sido alvo de pesquisas recentes. A partir desse conhecimento, torna-se possível a adoção de estratégias alimentares durante os diferentes estágios da gestação que possam resultar em incrementos na deposição de tecido muscular, o que resultaria em curto prazo em maior uniformidade de leitegada e em médio/longo prazo, em melhorias no desempenho da progênie bem como na qualidade da carne destes animais.

Crescimento intrauterino retardado

O crescimento intrauterino retardado (CIUR) pode ser definido como a redução no crescimento e desenvolvimento de embriões e fetos de mamíferos ou de seus órgãos durante a gestação. Em granjas, devido à facilidade de mensuração do peso fetal relativo à idade gestacional ou ao nascimento, estes parâmetros podem ser usados como critério prático para se detectar o CIUR (WU et al., 2006).

Perdas significativas de embriões e fetos podem ocorrer durante todo o período de gestação (GEISERT & SCHMITT, 2002; JONKER, 2004). Além da contribuição genética dos pais, o crescimento e desenvolvimento fetal são afetados pelo ambiente e por uma variedade de fatores. Entre estes, inclui a nutrição materna (baixa ou alta ingestão de alimentos ou desequilíbrio de nutrientes), má absorção intestinal materna, ingestão de substâncias tóxicas, temperatura ambiental e estresse, distúrbios no metabolismo materno ou fetal entre outros (MELLOR, 1983; MCEVOY et al., 2001; REDMER et al., 2004; WU et al., 2004). Assim, vários fatores podem prejudicar o crescimento fetal e causar o CIUR, sendo os dois principais fatores a capacidade uterina insuficiente e a nutrição materna.

Entre os animais domésticos, a ocorrência de CIUR em suínos é maior. Antes dos 35 dias de gestação, os embriões suínos são distribuídos de forma uniforme nos cornos uterinos (ANDERSON & PARKER, 1976). Depois deste período, a capacidade uterina se torna um fator limitante para o crescimento fetal, embora a distribuição destes esteja uniforme (KNIGHT et al., 1977). A diferença de crescimento entre os fetos no útero é mais evidente no final da gestação, onde o número de fetos excede cinco por corno uterino (PERRY & ROWELL, 1969).

Diferente de equinos e ruminantes, os suínos geralmente têm maior habilidade para mobilização de reservas corporais para direcionar para a nutrição dos fetos. Portanto, uma moderada restrição alimentar não seria suficiente para causar CIUR. Em trabalho realizado por ATINMO et al. (1974), foi observado que a restrição de 50% de ração para porcas gestantes não influenciou o peso dos leitões ao nascimento. Em contrapartida, BUITRAGO et al. (1974) observaram que uma restrição alimentar mais severa durante todo o período de gestação, de 8.0 para 2.0 Mcal de energia digestível diária, resulta em redução do peso e redução das fibras musculares de leitões ao nascimento.

Quando a matriz não recebe uma nutrição adequada durante o período de gestação, o feto sofrerá adaptações que o permitam sobreviver após o nascimento. Ocorrerão mudanças na taxa metabólica do feto com alteração da produção hormonal e redirecionamento do fluxo sanguíneo para proteger os órgãos-chave, tais como cérebro. Portanto, um animal acometido por CIUR possui órgãos menores, com exceção do cérebro. Este fenômeno é conhecido como “Brain sparing effect” (efeito de poupar o cérebro). Dessa forma, uma boa medida para se determinar a existência de CIUR seria a relação entre o peso do cérebro e do fígado. Em animais normais, esta relação é menor que um. A ocorrência de placentas pouco desenvolvidas também pode estar associada ao CIUR, visto que o peso das placentas e o fluxo sanguíneo placentário estão correlacionados ao peso dos fetos (TOWN et al., 2004).

Além de comprometer a sobrevivência do animal, o CIUR deixa sequelas permanentes que acometem determinados parâmetros zootécnicos tais como composição corporal, conversão alimentar, qualidade da carne, desempenho reprodutivo e saúde ao longo da vida do animal (WU et al., 2006). Estes mesmos autores também observaram maior mortalidade de leitões com CIUR antes do desmame e aqueles que sobrevivem sofrem atraso de crescimento permanente.

Geralmente, leitões com peso ao nascimento menor que 1,1 kg têm crescimento mais lento, são menos eficientes e possuem carcaças com maior proporção de gordura e menos músculos, quando comparados com leitões mais pesados com o mesmo peso ao abate (GONDRET et al., 2006).

O crescimento fetal restrito está associado com estruturas, metabolismo e funções anormais do intestino (WU et al., 2006). A redução do crescimento fetal causado por uma nutrição materna inadequada ou por superlotação uterina tem efeitos deletérios no intestino ocorrendo uma diminuição de sua massa e conseqüentemente má absorção de nutrientes. WANG et al. (2008) utilizando análises proteômica, verificaram diferenças na expressão de 11 proteínas no intestino delgado de leitões com CIUR, sendo essas proteínas relacionadas com o metabolismo energético, resposta imune, estruturas celulares e funções antioxidantes. Estes autores também observaram alterações no proteoma do fígado de suínos recém-nascidos com CIUR relacionados com o metabolismo do ferro. As transferrinas são proteínas responsáveis pelo transporte de ferro e a redução dessas

proteínas transportadoras no fígado de neonatos com CIUR pode resultar em deficiência de ferro causando anemia nesses animais. Também foi constatada a alteração em 12 proteínas relacionadas com a síntese e degradação proteica, que está relacionado com a diminuição do número de fibras musculares em leitões acometidos com CIUR.

Dessa forma, pode-se concluir que o CIUR tem efeitos no proteoma do intestino delgado, fígado e músculo esquelético de leitões recém-nascidos, causando alteração na sinalização celular, resposta imune, aumento da proteólise, redução da síntese de polipeptídeos, comprometendo a saúde e o desempenho futuro desses animais.

Um fator importante na regulação fetal e crescimento da placenta é o fator de crescimento semelhante à insulina I (IGF-1) é um polipeptídeo produzido pelo fígado em resposta ao hormônio de crescimento (GH).

Este fator promove a proliferação, diferenciação, migração, agregação e inibem a apoptose das células de mamíferos (CLEMMONS, 1997). CHEN et al. (2011) avaliaram a expressão de mRNA de IGF-1 no músculo longissimus dorsi, fígado e rins de leitões normais e com CIUR e verificaram que a expressão de IGF-1 tem uma correlação positiva com o peso corporal, dos rins e fígado. A expressão de IGF-1 foi maior em leitões com peso normal quando comparado a aqueles com CIUR.

Além disso, as concentrações dos aminoácidos arginina, ornitina, prolina, glutamina e poliaminas são menores no músculo esquelético de fetos suínos com CIUR. Dessa mesma forma, as concentrações de prolina são bem menores também no alantoide e fluido amniótico destes animais (WU et al., 2008).

Fases da gestação da matriz suína

A duração da gestação em suínos é em média 114 dias, podendo variar em quatro dias, para mais ou menos, depende da genética, linhagem e ambiente (PANZARDI et al., 2007). A necessidade nutricional da matriz é determinada pela demanda para a manutenção, ganho materno e fetal durante a gestação, e essas proporções de ganho são influenciadas pela fase de gestação e prolificidade principalmente (ABREU et al., 2013).

As fases gestacionais são englobadas em três eventos: até 21 dias, onde ocorre a ligação do embrião e início da formação da placenta; dos 21 aos 75 dias, fase em que ocorre a formação das fibras musculares fetais; dos 75 dias até o parto, onde há um crescimento significativo do feto e das glândulas mamárias (KIM et al., 2009).

O primeiro terço da gestação é caracterizado pela ligação embrio-maternal, início da formação da placenta e anexos fetais, o que exige menor necessidade de ganho de peso e reserva energética da fêmea. Nesta fase, tanto a subnutrição como uma supernutrição pode ser prejudicial. Uma alimentação deficiente pode resultar em menor síntese de óxido nítrico e poliaminas resultando em menor vascularização placentária e transferência de nutrientes da mãe para o feto. Por outro lado, um maior consumo alimentar durante o início da gestação

também pode exercer influência negativa na sobrevivência embrionária. Isto é atribuído à redução da concentração de progesterona plasmática, devido ao aumento do fluxo sanguíneo e do catabolismo hepático deste hormônio, causados pelo alto consumo de alimento (DEN HARTOG et al., 1994). A progesterona influencia as atividades secretórias do útero e do oviduto necessárias para o embrião em desenvolvimento (FOXCROFT et al., 2000). O período crítico para sobrevivência embrionária compreende as primeiras 48 e 72 horas da gestação, onde então, recomenda-se limitar o consumo de ração (JINDAL et al., 1996).

Portanto, o manejo nutricional tem como objetivo melhorar a sobrevivência embrionária e suprir de forma adequada para que a placenta e seus anexos tenham uma formação adequada (ECHEVERRI, 2004). É de fundamental importância a estimulação da angiogênese nesta fase para ter melhor crescimento celular e troca de gases e nutrientes entre a mãe e o feto, garantindo assim, um melhor desenvolvimento fetal (ALMEIDA, 2009).

O terço médio da gestação é uma fase em que há a recuperação das reservas corporais das fêmeas, mobilizadas na lactação anterior. A nutrição após o período crítico inicial da prenhez e até o início do terço final da gestação influencia mais a composição corporal da fêmea do que o tamanho da leitegada ou o peso dos leitões (CLOSE & COLE, 2001), apesar de ser nessa fase em que há o estabelecimento do número de fibras musculares nos fetos. O número de fibras musculares dos fetos está relacionado com a eficiência do crescimento após o nascimento (DWYER et al., 1993). Quando o número de fibras é reduzido, ocorre a queda do desempenho e ganho de peso destes leitões (GONDRET et al., 2005). Sendo assim, a nutrição nesta fase, é importante visar a formação de fibras musculares primárias e secundárias dos fetos a fim de garantir melhor desempenho pós-natal.

No terço final da gestação é o período que ocorre o maior desenvolvimento da glândula mamária e crescimento fetal, dessa forma, é necessário um maior aporte proteico e energético quando comparado às fases inicial e intermediária, resultando em aumento das exigências nutricionais das fêmeas suínas (MCPHERSON et al., 2004).

Outro fato a ser considerado nesta fase final, é que após 90 a 95 dias, ainda há continuidade do processo de hipertrofia muscular dos fetos, logo, o fluxo de nutrientes e oxigênio somado à nutrição materna são importantes e podem influenciar o peso ao nascimento dos leitões (ALMEIDA, 2009). Deve ser considerado que o maior fornecimento de energia durante a gestação pode resultar em um menor consumo de ração durante a lactação.

Portanto, se faz necessária uma perfeita integração entre essas duas fases, para que seja alcançado um melhor desempenho reprodutivo das matrizes, e, conseqüentemente, uma maior longevidade das mesmas dentro do plantel reprodutivo.

Conhecendo as diferentes fases da gestação, torna-se possível adequar os programas nutricionais de cada uma das fases de modo a favorecer os principais eventos. Uma nutrição equilibrada terá como conseqüências uma melhor taxa de sobrevivência fetal e peso ao nascimento viável para o sistema de produção.

Placentação e crescimento fetal

No início da gestação, existe a necessidade do fornecimento de condições para a sobrevivência embrionária com consequente aumento da leitegada. Uma subnutrição ou supernutrição, neste período, pode ser prejudicial. A capacidade uterina pode afetar o tamanho da leitegada e o peso ao nascimento dos leitões, que é altamente dependente da quantidade de nutrientes fornecidos através da placenta. A formação placentária apresenta um desenvolvimento expressivo do 12º a 30º dia de gestação e após este período, a placenta encontrase completamente formada (ECHEVERRI, 2004).

A capacidade funcional da placenta é extremamente importante para o desenvolvimento dos leitões, uma vez que o desempenho pós-natal é determinado, em grande parte, pelo desenvolvimento intrauterino (FOXCROFT & TOWN, 2004). O desenvolvimento da placenta dos leitões é estimulado por fatores regulatórios do crescimento representados pelo fator de crescimento semelhante à insulina e fator de crescimento vascular endotelial (DANTZER & WINTHER, 2001). A placenta forma estruturas que se ligam ao endométrio, formando uma superfície de contato essencial para a troca de nutrientes, gases respiratórios e produtos do metabolismo entre as circulações materna e fetal. Essa estrutura que liga o embrião ao endométrio ocorre pela interdigitação do córion com o epitélio endometrial, que são locais de aberturas das glândulas uterinas as quais são recobertas pelo córion. As aréolas são estruturas de absorção especializadas que se desenvolvem a partir dos 15 dias de gestação, utilizadas para a transferência de macromoléculas. Assim, a superfície da placenta dos suínos é formada por complexos areolares e de subunidades interareolares responsáveis pela transferência de nutrientes e trocas sanguíneas (GEISERT & YELICH, 1997).

Para a nutrição e sobrevivência do feto, além do número de pontos de transferência de macromoléculas, é fundamental considerar o aporte sanguíneo através da vascularização. Na superfície do corioalantóide, a bicamada formada pelo epitélio do trofoblasto e endométrio desenvolve dobras microscópicas aos 35 dias de gestação, as quais são o ponto de contato entre os capilares fetais e maternos (VALLET et al., 2009).

Uma placenta pouco eficiente passa a ser a principal responsável pelo aumento da taxa de mortalidade pré-natal. As placentas mais eficientes na disponibilização de nutrientes para o feto são decorrentes de maior fluxo sanguíneo, como observado nas fêmeas da raça chinesa Meishan, as quais desenvolvem placentas menores ocupando reduzido espaço uterino, porém há intensa proliferação de vasos sanguíneos na membrana cório-alantoide, possibilitando maior sobrevivência embrionária, leitegadas numerosas e mais homogêneas (WILSON et al., 1998). Fatores que influenciam os aspectos de desenvolvimento e função da vascularização da placenta podem ter efeito marcante no crescimento fetal e, portanto, com consequências na sobrevivência e crescimento neonatal. Por exemplo, uma alimentação desequilibrada, no início da gestação, pode resultar em menor síntese de óxido nítrico e de

poliaminas, resultando menor vascularização placentária e transferência de nutrientes da mãe ao feto, levando a uma subnutrição fetal.

Entre os nutrientes, os aminoácidos são considerados os mais importantes para o crescimento fetal, devido à necessidade destes para ativar os mecanismos da síntese de proteína nas células (RHOADS & WU, 2009). Além disso, os aminoácidos regulam diversas rotas metabólicas a fim de garantir a sobrevivência, crescimento, desenvolvimento, reprodução e saúde de animais e humanos (WU et al., 2009). Segundo estes mesmos autores, os produtos do catabolismo da arginina, como o óxido nítrico e as poliaminas, desempenham papel importante no crescimento placentário.

Segundo MCPHERSON et al. (2004), a composição dos tecidos fetais sofre mudanças durante o período de gestação. Além disso, a deposição de proteína e a gordura ocorrem de forma mais acelerada depois de 69 dias de gestação. Esses resultados indicam que deve haver um suporte adequado de proteínas e lipídeos para porcas gestantes para assegurar um adequado crescimento do tecido fetal, principalmente depois de 69 dias de gestação.

O crescimento do fígado nos fetos ocorre rapidamente nos estágios iniciais da gestação e o seu crescimento vai diminuindo enquanto o crescimento fetal progride. Uma explicação por esse rápido desenvolvimento do fígado é o aumento da atividade eritropoiética neste órgão durante o início da gestação (DYCE et al., 1996).

Durante o crescimento pré-natal, a formação dos eritrócitos ocorre no fígado, baço e na medula óssea, ao passo que durante o crescimento pós-natal, a formação dos eritrócitos ocorre quase que exclusivamente na medula óssea (REECE, 1997).

Crescimento e desenvolvimento das fibras musculares nos fetos

A formação do tecido muscular esquelético ocorre durante a fase embrionária (COSSU & BORELLO, 1999). A regulação deste processo envolve a ativação, proliferação e diferenciação de várias linhagens de células miogênicas e dependem da expressão e atividade de fatores de transcrição, conhecidos como fatores de regulação miogênica (MRF). Durante o desenvolvimento do embrião, o comprometimento das células miogênicas do mesoderma com linhagem miogênica depende da sinalização oriunda de tecidos circundantes, tais como notocorda e o tubo neural (CHARGE & RUDINIKI, 2004). Estes sinais são responsáveis pela ativação de genes e/ou fatores de transcrição capazes de transformar células não musculares em células com fenótipo muscular (PAULINO & DUARTE, 2013).

A formação do tecido muscular é uma função de baixa prioridade na partição dos nutrientes quando comparado a órgãos como cérebro, coração e fígado (DU et al., 2010). Portanto, o desenvolvimento do músculo esquelético é particularmente vulnerável a disponibilidade de nutrientes (ZHU et al., 2006). Nas espécies múltiparas, como na suína,

há naturalmente uma variação no peso ao nascimento, sendo esta variação fortemente relacionada com o número de fibras musculares presentes. Leitões com menor peso ao nascer apresentam um menor número de fibras musculares, sendo decorrente de um menor número de fibras que se diferenciaram durante o período de miogênese pré-natal, por motivos genéticos (cruzamento, genótipo) ou maternos (nutrição).

O músculo esquelético constitui o principal componente da carcaça de animais destinados à produção de carne. O baixo peso dos leitões ao nascer parece estar associado à redução do número de fibras musculares estabelecidas ainda no útero (WIGMORE & STICKLAND, 1983). Leitões nascidos com menor número de fibras musculares normalmente tem menor potencial de crescimento que animais nascidos com maior quantidade de fibras, concluindo assim que um maior número de fibras musculares é necessário para um melhor crescimento e desempenho (DWYER et al., 1993).

Um dos principais fatores responsáveis pela redução da deposição de proteína no músculo esquelético e aumento da gordura em fetos com CIUR é a regulação metabólica anormal do turnover proteico, adipogênese e biogênese mitocondrial. Leitões recém-nascidos com CIUR têm maior abundância de proteossoma no músculo esquelético e fígado, o que aumenta a degradação de proteínas quando comparados com leitões nascidos com peso normal (WANG et al., 2008).

Estudos revelam a presença de quatro grupos de fatores reguladores da miogênese: myoD, myf5, myogenina e MRF4 (myf6), sendo que todos podem ativar a diferenciação da musculatura esquelética. Estes fatores reguladores da miogênese consistem num grupo de fatores de transcrição responsáveis pela regulação central do programa de desenvolvimento muscular esquelético (MALTIN et al., 2001). Experimentos demonstraram que o myoD possui uma função principal na formação e na sobrevivência dos mioblastos, desempenhando um importante papel na diferenciação do mioblasto, enquanto acredita-se que o miogenina está envolvido na diferenciação final em miotubos (RAWLS, et al., 1995).

Em um trabalho realizado por TOWN et al. (2004), foi verificado um efeito moderado do processo de superlotação uterina na expressão de fatores reguladores da miogênese, como miogenina e myoD. Os autores concluíram que a superlotação intrauterina aos 30 dias de gestação pode causar um impacto na diferenciação das fibras musculares em virtude da redução da expressão da miogenina.

A multiplicação do número de fibras musculares (hiperplasia) ocorre na fase fetal e o número de fibras está completo ao nascimento do animal. Dois tipos de fibras musculares são formados durante o desenvolvimento fetal, as primárias e secundárias. As fibras musculares primárias são formadas pela fusão rápida dos mioblastos formando os miotubos primários e as secundárias são formadas na superfície das fibras primárias. Nos suínos, as fibras primárias estão presentes aos 35 dias de gestação e seu número cresce gradualmente até os 60 dias. A formação das fibras secundárias ocorre rapidamente por volta dos 54 a 90 dias de gestação (WIGMORE & STICKLAND, 1983), completando o processo de hiperplasia por volta dos 90 dias de gestação.

As fibras primárias são mais resistentes aos fatores ambientais, enquanto as secundárias são mais susceptíveis a fatores relacionados a variações hormonais e nutricionais (HANDEL & STICKLAND, 1987). A principal razão da menor formação de fibras musculares no feto é a subnutrição uterina, que pode ser explicado pela distribuição dos fetos nos cornos uterinos, definindo uma diferenciação no aporte nutricional. Animais refugos normalmente são provenientes de placenta com menor peso e reduzido fluxo sanguíneo enquanto leitões de maior peso são provenientes de placenta com maior peso e fluxo sanguíneo indicando uma diferença no aporte nutricional (DWYER et al., 1993). A restrição de nutrientes durante o início e no meio do período de desenvolvimento reduz o número de fibras musculares, enquanto a restrição durante o final da gestação reduz o tamanho das fibras musculares e a formação de adipócitos intramusculares (DU et al., 2010).

Os mecanismos pelos quais os hormônios agem sobre o crescimento muscular fetal ainda não são bem conhecidos. É possível que exista uma relação entre os diferentes hormônios que estão ligados ao crescimento e as substâncias denominadas de repartidores de nutrientes. Estas substâncias são chamadas assim devido à sua capacidade de redirecionar a distribuição de nutrientes em função da alteração do metabolismo da célula. Dessa forma, os nutrientes utilizados para a produção de tecido adiposo seriam direcionados para aumentar a deposição de tecido muscular (RICKS et al., 1984).

Quando se tem um adequado aporte nutricional durante o segundo terço da gestação, onde ocorre a formação das fibras musculares nos fetos, observa-se uma melhora na capacidade oxidativa nos músculos (MARKHAM et al., 2009). Um maior número de fibras oxidativas podem produzir carne de melhor qualidade com cor mais intensa, pH adequado e redução da perda por gotejamento (CHANG et al., 2003).

Após o nascimento, o crescimento muscular ocorre por meio da hipertrofia das células musculares pré-existentes e esse processo é dependente das células satélites. As células satélites são mioblastos indiferenciados que permanecem entre a membrana plasmática da fibra muscular e a lâmina basal (CHARGER & RUDINIKI, 2004), que quando estimulada, é ativada, proliferando e fundindo com a fibra muscular pré-existente. Os núcleos derivados das células satélites começam a sintetizar proteínas, aumentando o volume muscular através da formação de novos sarcômeros. No entanto, se houver a formação insuficiente de fibras musculares durante a fase fetal, o crescimento do músculo pós-natal é severamente limitado.

Dessa forma, é de fundamental importância o conhecimento de como manipular o desenvolvimento intrauterino do animal com o objetivo de maximizar a formação de fibras musculares, proporcionando assim, maior potencial de crescimento do animal em sua vida pós-natal (PAULINO & DUARTE, 2013).

Suplementação de arginina

A arginina é um aminoácido utilizado para síntese proteica e é metabolizada em glutamina, glutamato, prolina, aspartato, asparagina, ornitina e citrulina, com grande importância biológica para o desenvolvimento placentário e fetal (WU et al., 2009).

A síntese da arginina ocorre principalmente no eixo intestino-renal, sendo que células do epitélio do intestino delgado produzem citrulina e células dos túbulos proximais, nos rins, extraem a citrulina da circulação sanguínea, convertendo-a em arginina, devolvendo-a para a circulação. A arginina, quimicamente denominada ácido 2-amino-5-guanidopentanóico, é considerada um aminoácido condicionalmente não essencial para suínos, haja vista que é primordialmente necessária dos 3 aos 21 dias de idade, momento em que o organismo animal é capaz de sintetizar cerca de 60% de suas exigências. Sendo assim, a suplementação de arginina em dietas para porcas gestantes e lactantes tem sido uma ferramenta para maximizar o desempenho de leitões lactantes.

A nutrição aminoacídica é primordial para o desenvolvimento dos fetos, influenciando de forma positiva a maturação dos principais sistemas (muscular, cardiovascular, digestório, respiratório, esquelético, entre outros). Nesse contexto, a arginina desempenha importante papel no desenvolvimento de fetos de mamíferos (WU et al., 2007).

A suplementação de aminoácidos na ração de porcas em gestação pode resultar em melhor desempenho reprodutivo com leitegadas mais pesadas e uniformes. A arginina é o aminoácido mais estudado na tentativa de minimizar e/ou reduzir os efeitos da superlotação uterina sobre o tamanho e qualidade da leitegada. Este aminoácido influencia na angiogênese e desenvolvimento vascular providenciando mais nutrientes e oxigênio da porca para os fetos (LIU et al., 2012).

A arginina é precursora do óxido nítrico e responsável pela síntese de poliaminas, sendo assim, os aminoácidos da família da arginina (arginina, prolina e glutamina) são substratos essenciais para um bom desenvolvimento da placenta e de fetos suínos (WU et al., 2004). O óxido nítrico e as poliaminas são essenciais ao crescimento placentário e para a angiogênese.

O óxido nítrico é um importante fator vaso-relaxante que regula o fluxo sanguíneo materno-fetal e, portanto, a transferência de oxigênio da mãe para o feto (BIRD et al., 2003). As poliaminas, que são sintetizadas na placenta suína a partir de substratos derivados da prolina, regulam o DNA e síntese proteica, estando diretamente relacionadas à proliferação e diferenciação celular (WU et al., 2005). Segundo estes mesmos autores, o fluido alantoide de suínos é rico em arginina aos 40 dias de gestação e essa abundância nos fluidos fetais está relacionada à elevada síntese de óxido nítrico e poliaminas pela placenta suína durante a primeira metade da gestação, quando o seu crescimento é mais rápido.

O número de leitões natimortos pode ser reduzido com a inclusão de arginina à dieta ao final da gestação, visto que o óxido nítrico melhora o fluxo sanguíneo e conseqüentemente a passagem de nutrientes da mãe para o feto, o que possibilita maior sobrevivência dos fetos. MATEO et al. (2007) observaram redução de 65% de leitões natimortos e aumento do peso da leitegada ao nascimento quando leitões receberam 1% de arginina dos 30 até os 110 dias de gestação. Resultado semelhante foi encontrado por REMAEKERS et al. (2006), que constatou que o fornecimento de 40 g/dia de Larginina dos 14 aos 28 dias de gestação aumentou o tamanho da leitegada de +0,8 leitão/leitegada. Em contrapartida, LIMA (2010) não verificou diferença no número total de leitões nascidos vivos em porcas suplementadas com arginina durante a gestação, pois o fornecimento das dietas foi iniciado na fase final da gestação (90 dias), de modo que o tamanho da leitegada já fora determinado no primeiro mês gestacional.

Arginina e glutamina podem estar envolvidas na melhora da miogênese de fetos, colaborando para um melhor desempenho pós-natal de leitões de matrizes suínas híperprolíficas. Em estudos realizados por BÉRARD & BEE (2010) foi observado que a suplementação de arginina para porcas em gestação afetou de forma positiva a formação das miofibras primárias nos fetos, que, como sabemos, pode ter efeitos positivos no crescimento do músculo após o nascimento, composição e qualidade da carcaça desses animais. Entretanto, segundo HAZELEGER et al. (2007), a melhoria na formação das miofibras primárias não se deve diretamente a suplementação de arginina na dieta de gestação, e sim no aumento da angiogênese placentária.

A glutamina é abundante nas proteínas teciduais fetais e a sua deficiência é o principal fator que contribui para o aparecimento de CIUR. Portanto, a sua utilização na ração de porcas gestantes pode trazer benefícios como aumento do peso do leitão ao desmame e reduzir a incidência de CIUR. Já a suplementação simultânea de arginina e glutamina na ração de porcas em gestação melhora significativamente o desempenho reprodutivo das porcas primíparas (WU et al., 2011). Isso em virtude que os dois aminoácidos regulam a síntese proteica por ativarem a produção de poliaminas.

Desse modo, a suplementação com arginina para fêmeas gestantes parece não apresentar efeitos sobre o desempenho das fêmeas, mas sim da leitegada, influenciando positivamente alguns índices zootécnicos.

Suplementação de ractopamina

Outra forma de melhorar o desenvolvimento fetal é a utilização de agonistas β -adrenérgicos, onde o mais utilizado é a ractopamina, que é conhecida como um modulador agonista β -adrenérgico da classe das fenetanolaminas, um promotor do crescimento que age como modificador do metabolismo do animal proporcionando menor deposição de tecido adiposo e maior porcentagem de carne magra na carcaça, sendo análogo estrutural de hormônios denominados como catecolaminas (adrenalina e noradrenalina).

KIM et al. (1994) observou que leitões provenientes de porcas alimentadas com Salbutamol durante o primeiro terço da gestação apresentaram aumento no número de fibras musculares. HOSHI et al. (2005), suplementando porcas com ractopamina de 25 a 50 dias de gestação, constataram maior ganho de peso da progênie. Da mesma forma, GATFORD et al. (2009) constataram que a suplementação de 20 mg/kg de ractopamina melhorou o desempenho dos leitões nascidos com aumento de 9% no ganho de peso, e o desenvolvimento do tecido muscular esquelético. Em estudo mais recente, GARBOSSA et al. (2015) testaram a suplementação combinada de ractopamina e arginina para porcas gestantes e constataram que a combinação destes não resultou em efeitos aditivos, aumentando apenas o número de leitões nascidos vivos, porém, observaram melhora no desempenho e crescimento muscular das fibras do músculo semitendinoso de leitões recém-nascidos. Sendo assim, é necessário mais estudo sobre a combinação desses aditivos nas dietas de porcas gestantes para melhor entendimento de seus efeitos.

Embora sejam poucos trabalhos que estudam a relação dos agonistas β -adrenérgicos com o desenvolvimento fetal, estudos indicam que períodos de suplementação mais extensos não são necessariamente os responsáveis pelos melhores efeitos. Este comportamento se deve à diminuição da sensibilidade dos receptores beta aos agonistas após a exposição por períodos mais prolongados (MOODY et al., 2000). O processo de dessensibilização ocorre através da fosforilação da região C-terminal dos β -receptores. Essa fosforilação induz as proteínas G a se ligarem a outras proteínas, resultando na desativação dos receptores (PIPPIG et al., 1993).

O tecido placentário expressa receptores β adrenérgicos (MOORE & WHITSETT, 1982). Um experimento realizado *in vitro* por SIBLEY et al. (1986), pode-se concluir que a ractopamina é um β agonista que é capaz de regular a transferência de sódio na placenta de porcas. Esse efeito é importante devido à maioria dos aminoácidos serem transportados por transporte ativo dependente de sódio (BATTAGLIA & REGNAULT, 2001). Além disso, os agonistas β adrenérgicos podem aumentar o fluxo sanguíneo para os fetos através de receptores presentes na musculatura lisa dos vasos sanguíneos, elevando o AMPc causando vasodilatação, como demonstrado em artérias umbilicais humanas *in vitro* (KARADAS et al., 2007). Segundo Cantarelli et al. (2009), a ractopamina aumenta a retenção de nitrogênio, o que pode contribuir para maior síntese de proteína nos fetos, aumentando assim, a formação do tecido muscular.

A ação da hipertrofia sobre o músculo esquelético pode ser mediada pelo IGF-1 (Fator de crescimento semelhante à insulina I), que aumenta a síntese proteica e parece ser importante também na regulação do número de fibras musculares, por ser responsável pela proliferação e diferenciação dos mioblastos (ROSENTHAL & CHENG et al., 1995).

Outros aditivos e composição da ração de fêmeas suínas em gestação

O nível de energia da dieta também tem sido estudado como fator relacionado ao desenvolvimento fetal e da placenta. Segundo NOBLET et al. (1985), existe uma relação direta entre a nutrição materna e o peso fetal, no qual reduzindo a ingestão de energia após 80 dias de gestação, ocasionou uma redução do crescimento dos fetos em marrãs. Dessa mesma forma, LAWS et al. (2009) verificaram que a suplementação de 10% de óleo na dieta melhorou o peso ao nascimento e a uniformidade de leitões ao nascimento. MAHAN (1998) verificou melhoras no peso da leitegada ao nascimento quando avaliou diferentes níveis de fornecimento de ração para fêmeas suínas no período de gestação. Os animais que receberam 130 gramas de ração adicional por dia obtiveram maior número e peso de leitões ao nascimento quando comparados com a ração controle. Em contrapartida, LAWLOR et al. (2007), avaliando diferentes níveis de energia durante a gestação, não observaram influência no peso ao nascimento e uniformidade da leitegada. Diversos autores observaram efeitos negativos na mortalidade, número total de nascidos vivos, peso da leitegada e uniformidade quando forneceram quantidades elevadas de ração em diversas fases da gestação (MILLER et al., 2000; NISSEN et al., 2003; LAWLOR et al., 2007). O fornecimento elevado de ração durante este período pode alterar as concentrações de progesterona circulante, que, por sua vez, modifica o desenvolvimento e atividade secretora do endométrio, causando alteração na composição dos fluidos alantóicos que fornecem nutrientes para os fetos (EINARSSON & ROJKITIKHUN, 2003).

Efeitos negativos têm sido observados quando ocorre restrição proteica durante a gestação. Em estudos realizados por WU et al. (1998), foi observado concentrações reduzidas de aminoácidos como a arginina, ornitina e prolina na placenta e endométrio de marrãs recebendo dietas com baixos níveis de proteína. Com essa restrição severa de proteína, irá ocorrer redução nas atividades do óxido nítrico sintetase e a síntese de citrulina a partir da atividade da arginina e ornitina descarboxilase na placenta e endométrio. Esses resultados mostram a importância da nutrição materna, mais especificamente dos aminoácidos da família da arginina no crescimento fetal, uma vez que as funções afetadas estão relacionadas com a angiogênese e ao crescimento da placenta e fetal.

O alto ou baixo consumo de proteína (250 e 50%, respectivamente, das recomendações nutricionais) de porcas durante a gestação podem alterar o metabolismo fetal de aminoácidos podendo causar CIUR (REHFELDT et al., 2011). Diversos estudos revelam que o baixo consumo de proteína durante a gestação resulta em CIUR e desordens metabólicas na leitegada (DESAI et al., 1997; HOLEMANS et al., 2003). Os efeitos das dietas com alto teor proteico ainda não são tão conhecidos (DAENZER et al., 2002; KUCIA et al., 2011).

A utilização de aditivos pode ser uma alternativa para melhorar o desempenho da leitegada durante a gestação. O efeito da utilização de L-carnitina tem sido avaliado em vários estudos. Em estudo realizado POR MUSSEER et al. (1999), foi observado que fêmeas

alimentadas com L-carnitina durante a gestação (5° ao 112° dia) apresentaram maiores concentrações de insulina e IGF-I entre 60 e 90 dias, que é o período de desenvolvimento das fibras musculares secundárias do feto. A adição de L-carnitina na dieta melhorou a utilização dos nutrientes resultando em um aumento do peso das reservas de gordura e um maior peso ao nascimento. Os efeitos positivos da administração de L-carnitina estariam relacionados a possível elevação dos níveis de IGF-I, o que, segundo MAGRI et al. (1991) seria importante na proliferação e diferenciação das células miogênicas, melhorando o crescimento pós-natal dos leitões. De acordo com RAMANAU et al. (2004), leitões nascidos de fêmeas alimentadas com L-carnitina durante o período de gestação apresentaram maior taxa de crescimento durante a amamentação do que leitões do grupo controle.

Existem poucos estudos que relatam as exigências de vitamina D para marrãs e porcas durante a gestação ou lactação. A suplementação normalmente é na forma de vitamina D que é absorvido no intestino e transportado para o fígado onde ocorre hidroxilação, formando o 25- hidroxicolecalciferol (25OHD3).

Em trabalho realizado com suplementação de 25- Hidroxivitamina D3 (25OHD3) para porcas primíparas em lactação, ZHOU et al. (2016) observaram aumento no número de fibras musculares em leitões recém nascidos quando comparados com leitões nascidos de fêmeas que não foram suplementadas. Resultados semelhantes foram obtidos por HINES et al. (2013), que verificaram um aumento no número de fibras do músculo *Longissimus dorsi* em fetos aos 90 dias de gestação em porcas suplementadas com 25OHD3. Portanto, os efeitos desta vitamina no crescimento muscular dos leitões após o nascimento ainda não são conhecidos.

Algumas evidências têm mostrado que a vitamina D exerce uma variedade de efeitos sobre o desenvolvimento do músculo esquelético (GIRGIS et al., 2012; HAMILTON, 2009). A suplementação de 1,25 Hidroxicolecalciferol D3 resulta em alteração no RNA mensageiro e na expressão de proteínas moduladoras de determinados fatores de transcrição muscular, que aumentou significativamente o tamanho e diâmetro da fibra muscular (GARCIA et al., 2011).

CONSIDERAÇÕES FINAIS

O peso do leitão ao nascimento é um fator extremamente importante, inicialmente, para sua sobrevivência, e, posteriormente, para um bom crescimento e desenvolvimento até o momento de abate. Fêmeas hiperprolíficas produzem um maior número de leitões nascidos por leitegada, o que resulta em menor peso médio ao nascimento e, conseqüentemente, maior variabilidade de peso desses leitões. A capacidade uterina entra, neste aspecto, como um fator limitante. Portanto, características como eficiência placentária e tamanho de placenta devem ser incluídas em programas de melhoramento genético, uma vez que se tratam de aspectos vistos como essenciais para a produção de leitões mais homogêneos e de melhor viabilidade.

A nutrição e o manejo alimentar devem ser realizados sempre respeitando as diferentes fases reprodutivas em que as fêmeas se encontram, no intuito de proporcionar uma alta qualidade nutricional e, conseqüentemente, um bom peso do leitão ao nascimento. Associando estes dois fatores (genética e nutrição), certamente, auxiliarão no aumento da sobrevivência dos leitões durante a fase de lactação e com isso, contribuindo positivamente com a diminuição da taxa de mortalidade na maternidade e com o aumento do ganho econômico da produção.

REFERÊNCIAS

- ABREU, M.L.T et al. Atualizando a nutrição de porcas hiperprolíficas. In: SIMPÓSIO BRASIL SUL DE SUINOCULTURA, 6., 2013, Chapecó. Anais... Chapecó: [s. n.], 2013. p. 70-92. AGRINESS. Disponível em: <http://melhoresdasuinocultura.com.br/dados/edicoes>.
- ATINMO, T., W.; POND, G.; BARNES, R. H. Effect of maternal energy vs. protein restriction on growth and development of progeny in swine. *Journal of Animal Science*, v.39, p.703–711, 1974.
- BAZER, F. W.; FORD, J. J.; KENSINGER, R. S. Reproductive physiology. in *Biology of the Domestic Pig*. W. G. Pond and H.J. Mersmann, ed. Cornell Univ., Ithaca, NY, p.150-224, 2001.
- BÉRARD, J.; BEE, G. Effects os dietary L-arginine supplementation to gilts during early gestation on foetal survival, growth and myofiber formation. *Animal*, v.10, p.1680-1687, 2010.
- BUITRAGO, J. A.; WALKER, E. F.; SNYDER, W. I.; POND, W. G. Blood and tissue traits in pigs at birth and at 3 weeks from gilts fed low or high energy diets during gestation. *Journal of Animal Science*, v.38, p.766–771, 1974.
- CHANG, K. C.; COSTA, N.; BLACKLEY, R.; SOUTHWOOD, O.; EVANS, G.; PLASTOW, G.; WOOD, J. D.; RICHARDSON, R. I. Relationships of myosin heavy chain fibre types to meat quality traits in traditional and modern pigs. *Meat Science*, v.64, p.93–103, 2003.
- CHARGE, S. B. P.; RUDINIKI, M. A. Cellular and Molecular Regulation of Muscle Regeneration. *Physiol. Rev.*, v.84, n.1, p.209-238, 2004.
- CHEN, R.; YIN, Y.; PAN, J.; GAO, Y.; LI, T. Expression profiling of IGFs and IGF receptors in piglets with intrauterine growth restriction. *Livestock Science*, v.136, p.72-75, 2011.
- CLEMMONS, D.R., 1997. Insulin-like growth factor binding proteins and their role in controlling IGF actions. *Cytokine Growth Factor Rev.* v.8, p.45– 62, 1997.
- CLOSE W. H. & COLE D. J. A. *Nutrition of sows and boars*. 1.ed. Nottingham: Nottingham University Press, 377p, 2001.
- COSSU, G.; BORELLO, U. Wnt signaling and the activation of myogenesis in mammals. *The EMBO Journal*, V.18, n.24, p.6867-6872,1999.
- DAENZER, M.; ORTMANN, S.; KLAUS, S.; METGES, C. C. Prenatal high protein exposure decreases energy expenditure and increases adiposity in young rats. *Journal of Nutrition*, v.132, p.142-144, 2002.

- DANTZER & WINTHER. 2001. Histological and immunohistochemical events during placentation in pigs. *Reproduction*. v.58 (Suppl), p.209- 222, 2001.
- DEN HARTOG L. A.; VESSEUR P. C.; KEMP B. V. Nutrition-reproduction interactions in sows. In: Cole D.A., Wiseman J. & Valey M.A. (Eds). *Principles of pig science*. Nottingham: Nottingham University Press, p.215, 1994.
- DEVILLERS, N.; FARMER, C.; LE DIVIDICH, J. PRUNIER, A. Variability of colostrum yield and colostrum intake in pigs. *Animal*, Cambridge, v.1, n.7, p.1033–1041, 2007.
- DESAI, M.; BRYNE, C. D.; ZHANG, J.; PETRY, C. J.; LUCAS, A.; HALES, C. N. Programming of hepatic insulin-sensitive enzymes in offspring of rat dams fed a protein-restricted diet. *American Journal of Physiology*, v.272, G1083-G1090, 1997.
- DU, M.; TONG, J.; ZHAO, J.; UNDERWOOD, K. R.; ZHU, M.; FORD, S. P.; NATHANIELSZ, P. W. Fetal programming of skeletal muscle development in ruminant animals. *Journal of Animal Science*, v.88, n.13, suppl, p.E51-60, 2010.
- DWYER, C. M.; FLETCHER, J. M.; STICKLAND, N. C. Muscle cellularity and postnatal growth in the pig. *Journal of Animal Science*, v.71, p.3339- 3343, 1993.
- EINARSSON, S. T.; ROJKITTIKHUN. Effects of nutrition on pregnant and lactating sows. *Journal of Reproduction and Fertility*, v.48, p.229–239, 1993.
- ECHEVERRI H. M. Selection for placental efficiency in swine. 106f. Columbia, U.S.A. PhD Tesis (Faculty of the Graduate School) - University of Missouri, Columbia, 2004.
- FERGUSON, J. D. Nutrition and reproduction in dairy herds. *Veterinary Clinics: Food Animal Practice*, v.21, p.325-347, 2005.
- FOXCROFT G. R.; DIXON W. T.; TREACY B. K.; JIANG L.; NOVAK S.; MAO J.; ALMEIDA F. C. L. Insights into conceptus-reproductive tract interactions in the pig. *Journal of Animal Science*. v.77, p.1-15, 2000.
- FOXCROFT G.R.; TOWN S. Prenatal programming of postnatal performance – The unseen cause of variance. *Advanced Pork Production*. v.15, p.269–279, 2004. FOXCROFT, G. R.;
- DIXON, W. T.; NOVAK, S.; PUTMAN, C. T.; TOWN, S. C.; VINSKY, M. D. The biological basis for prenatal programming of postnatal development in pigs. *Journal of Animal Science*, v.84, p.105-112, 2006.
- GARBOSSA, C. A. P.; JUNIOR, F. M. C.; SILVEIRA, H.; FARIA, P. B.; SCHINCKEL, A. P.; ABREU, M. L. T.; CANTARELLI, V. S. Effects of ractopamine and arginine dietary
- GATFORD, K. L.; M. J. DE BLASIO, C. T.; ROBERTS, M. B.; NOTTLE, K. L.; KIND, W. H. E. J.; VAN WETTERE, R. J.; OWENS, J. A. Responses to maternal GH or ractopamine during early-
- GEISERT, R. D.; YELICH, J. V. Regulation of conceptus development and attachment in pigs. *Journal of Reproduction and Fertility*, v.52, p.133-149, 1997.

- GEISERT, R. D.; SCHMITT, R. A. M. Early embryonic survival in the pig: Can it be improved? *Journal of Animal Science*, v.80(E. Suppl. 1):E54–E65, 2002.
- GIRGIES, C. M.; CLIFTON-BLIGH, R. J.; HAMRICK, M. W.; HOLICK, M. F.; GUNTON, J. E. The roles of vitamin D in skeletal muscle: form, function, and metabolism. *Endocrine Reviews*, v.34, p.33-83, 2012.
- GONDRET, F.; LEFAUCHEUR, L.; JUIN, H.; LOUVEAU, I.; LEBRET, B. Low birth weight is associated with enlarged muscle fiber area and impaired meat tenderness of the longissimus muscle in pigs. *Journal of Animal Science*, v.84, p.93-103, 2006.
- HAMILTON, B. Vitamin D and human skeletal muscle. *Scandinavian Journal of Medicine & Science in Sports*, v.20, p.182-190, 2009.
- HANDEL, S. E.; STICKLAND, N. C. Muscle cellularity and birth weight. *Animal Production*, v.44, p.311-317, 1987.
- HAZELEGER, W.; RAMAEKERS, R.; SMITS, C.; KEMP, B. Effect of Progenos on placenta and fetal development in pigs. *Journal of Animal Science*, v.85, p.98, 2007.
- HINES, E.; COFFEY, J.; STARKEY, C.; CHUNG, T.; STARKEY, J. Improvement of maternal vitamin D status with 25-hydroxycholecalciferol positively impacts porcine fetal skeletal muscle development and myoblast activity. *Journal of Animal Science*, v.91, p.116-122, 2013.
- HOLEMANS, K.; AERTS, L.; VAN ASSCHE, F. A. Lifetime consequences of abnormal fetal pancreatic development. *Journal of Physiology*, v.547, p.11-20, 2003.
- HOSHI, E. H.; FONSECA, N. A. N.; PINHEIRO, J. W.; BRIDI, A. M.; SILVA, C. A. Muscle fiber number and growth performance of pigs from sows treated with ractopamine. *Asian-Australas. Journal of Animal Science*, v.18, p.1492–1497, 2005.
- JINDAL R.; COSGROVE J. R.; AHERNE F. X.; Foxcroft G. R. Effect of nutrition on embryo mortality in gilts: association with progesterone. *Journal of Animal Science*, v.74, p.620, 1996.
- JONKER, F. H. Fetal death: Comparative aspects in large domestic animals. *Animal Reproduction Science*, v. 82, p.415–430, 2004.
- KIM, Y. S.; SAINZ, R. D.; FERLAZZO, J.; TULLOH, N. M. Effect of maternal administration of salbutamol to sows on postnatal growth and carcass characteristics in the progeny. *Aust. J. Agric. Res.* v.45, p.271–278, 1994.
- KIM, S. W.; HURLEY, W. L.; WU, G.; JI, F. Ideal amino acid balance for sows during gestation and lactation. *Journal of Animal Science*, Champaign, v. 87, p. 123–132, 2009.
- KNIGHT, J. W.; BAZER, F. W.; THATCHER, W. W.; FRANK, D. E.; WALLACE, H. D. Conceptus development in intact and unilaterally hysterectomized-ovariectomized gilts: Interrelationships among hormonal status, placental development, fetal fluids, and fetal growth. *Journal of Animal Science*, v.44, p.620–637, 1977.
- KUCIA, M.; LANGHAMMER, M.; GORS, S.; ALBRECHT, E.; HAMMON, H. M.; NURNBERG, G.; METGES, C. C. High-protein diet during gestation and lactation affects mammary gland mRNA abundance, milk composition and pre weaning litter growth in mice. *Animal*, v.5, p.268- 277, 2011.

LAWLOR, P.; LYNCH, P. B.; O'CONNELL, M. K.; MCNAMARA, L.; REID, P.; STICKLAND, N.C. The influence of over feeding sows during gestation on reproductive performance and pig growth to slaughter. *Archiv. Tierz.*, v.50 (Special (Special Issue.)), p. 82-91, 2007.

LIMA, D. Dietas suplementadas com arginina para fêmeas suínas hiperprolíferas no período final da gestação e na lactação. Dissertação de Mestrado. 61p. Universidade Federal de Lavras, Lavras-MG, 2010.

MAGRI K. A.; EWTON D. Z.; FLORINI J. R. The role of the IGFs in myogenic differentiation. In: Raizada M.K. & Le Roith D. (Eds). *Molecular Biology and Physiology of Insulin-like Growth Factors*. New York: Plenum Press, p.57-76, 1991.

MAHAN, D.C. Relationship of gestation protein and feed intake level over a five-parity period using a high-producing sow genotype. *Journal of Animal Science*, v.76, p.533-541, 1998.

MARKHAM, T. C. W.; LATORRE, R. M.; LAWLOR, P. G.; ASHTON, C. J.; MCNAMARA, L. B.; NATTER, R.; ROWLERSON, A.; STICKLAND, N. C. Developmental programming of skeletal muscle phenotype/ metabolism. *Animal*, v.3, p.1001–1012, 2009.

MATEO, R. D.; WU, G.; BAZER, F. W.; PARK, J.C.; SHINZATO, I.; KIM, S. W. Dietary L-arginine supplementation enhances the reproductive performance of gilts. *Journal of Nutrition*, Philadelphia, v.137, p.652-656, 2007.

MALTIN C. A.; DELDAY M. I.; SINCLAIR K. D.; STEVEN J.; SNEDDON A. A. Impact of manipulations of myogenesis in utero on the performance of adult skeletal muscle. *Reproduction*. v.122, p.359-374, 2001.

MCEVOY, T. G.; ROBINSON, J. J.; ASHWORTH, C. J.; ROOKE, J. A.; SINCLAIR, K. D. Feed and forage toxicants affecting embryo survival and fetal development. *Theriogenology*, v.55, p.113– 129, 2001.

MCPHERSON, R. L.; JI, F.; WU, G.; BLANTON, J. R.; KIM, S. W. Growth and compositional changes of fetal tissues in pigs. *Journal of Animal Science*, v.82, p.2534-2540, 2004.

MELLOR, D. J. Nutritional and placental determinants of fetal growth rate in sheep and consequences for the newborn lamb. *British Veterinary Journal*, v.139, p.307–324, 1983.

MILLER, H. M.; FOXCROFT, G. R.; AHERNE, F. X. Increasing food intake in late gestation improved sow condition throughout lactation but did not affect piglet viability or growth rate. *Animal Science*, v.71, p.141-148, 2000.

MOODY, D. E.; HANCOCK, D. L.; ANDERSON, D .B. Phenethanolamine repartitioning agents. In: D'Mello, J. P F. (Ed) *Farm Animal Metabolism and Nutrition*, New York: CAB International, p.65- 95, 2000.

MUSSER R. E.; GOODBAND R. D.; TOKACH M. D.; OWEN K. Q.; NELSSSEN J. L.; BLUM S. A.; DRITZ S. S.; CIVIS C. A. Effects of Lcarnithine fed during gestation and lactation on sow and litter performance. *Journal of Animal Science*. v.77, p.3289-3295, 1999.

NISSEN, P. M.; DANIELSEN, V. O.; JORGENSEN, P. F.; OKSBJERG, N. Increased maternal nutrition of sows has no beneficial effects on muscle fiber number or postnatal growth and has no impact on the meat quality of the offspring. *Journal of Animal Science*, v.81, p.3018- 3027, 2003.

- NOBLET, J.; CLOSE, W. H.; HEAVENS, R. P.; BROWN, D. Studies on the energy metabolism of the pregnant sow. 1. Uterus and mammary tissue development. *British Journal of Nutrition*, v.53, p.251-265, 1985.
- PAULINO, P. V. R.; DUARTE, M. S. Programação fetal e seus impactos na produção e qualidade de carne bovina. IN: A tecnologia a serviço da bovinocultura de corte. VIII SIMPEC. Anais... Lavras-MG: Suprema, p.1-13, 2013.
- PERRY, J. S.; ROWELL, J. G. Variations in foetal weight and vascular supply along the uterine horn of the pig. *Journal of Reproduction and Fertility*, v.19, p.527-534, 1969.
- PIPPIG, S.; ANDEXINGER, S.; DANIEL, K. PUZICHA, M.; CARON, M. G.; LEFKOWITZ, R. J.; LOHSE, M. J. Overexpression of beta-arrestin and beta-adrenergic receptor kinase augment desensitization of beta 2 adrenergic receptors. *Journal of Biological Chemistry*, v.268, p.3201- 3208, 1993.
- RAMANAU A.; KLUGE H.; SPIKE J.; EDER K. Supplementation of sows with L-carnithine during pregnancy and lactation improves growth of the piglets during the suckling period through increased milk production. *Journal Nutritional*, v.134, p.86-92, 2004.
- RAWLS, A.; WILSON-RAWLS, J.; OLSON N. Genetic regulation of somite formation. *Current Topics in Developmental Biology*. v.47, p.131- 154, 1995.
- REDMER, D. A., J.; WALLACE, M.; REYNOLDS, L. P. Effect of nutrient intake during pregnancy on fetal and placental growth and vascular development. *Domestic Animal Endocrinology*, v.27, p.199-217, 2004.
- REHFELDT, C.; KUHN, G. Consequences of birth weight for postnatal growth performance and carcass quality in pigs related to myogenesis. *Journal of Animal Science*, v.84, (E Supplement), E113-E124, 2006.
- REHFELDT, C.; LANG, I. S.; GORS, S.; HENNIG, U.; KALBE, C.; STABENOW, B.; BRUSSOW, K. P.; PFUHL, R.; BELLMANN, O.; NURNBERG, G. Limited and excess dietary protein
- RAMAEKERS P.; KEMP, B.; VAN DER LENDE, T. Progens in sows increases number of piglets born. *Journal of Animal Science*, v.84, p.394, 2006.
- REECE, W. O. *Physiology of Domestic Animals*. Williams and Wilkins, Baltimore, MD, p.270-271, 1997.
- RICKS, C. A.; BAKER, P. K.; DALRYMPLE, R. H. Use of repartitioning agents to improve performance and body composition of meat animals. *Proc. Reciprocal Meat Conf.* v.37, p.5- 11, 1984.
- RHOADS, J. M.; WU, G. Glutamine, arginine, and leucine signaling in the intestine. *Amino Acids*, v.37, p.111-122, 2009.
- ROSENTHAL, S. M.; CHENG, Z. Q. Opposing early and late effects of insulin-like growth factor I on differentiation and the cell cycle regulatory retinoblastoma protein in skeletal myoblasts. *Proceedings of the National Academy of Sciences*, v.92, p.10307, 1995.
- TOWN, S. C.; PUTMAN, C.; TURCHINSKY, J.; DIXON, W. T.; FOXCROFT, G. R. Number of conceptuses in utero affects porcine foetal muscle development. *Reproduction*, v.128, p.443-454, 2004.

VALLET, J. L.; MILES, J. R.; FREKING, B. A. Development of pig placenta. *Control of Pig Reproduction VIII*, p.265-279, 2009.

WANG, J.; CHEN, L.; LI, D.; YIN, Y.; WANG, X.; LI, P.; DANGOTT, L. J.; HU, W.; WU, G. Intrauterine growth restriction affects the proteomes of the small intestine liver, and skeletal muscle in newborn pigs. *Journal of Nutrition*, v.138, p.60- 66, 2008.

WIGMORE, P. M . C.; STICKLAND, N. C. Muscle development in large and small pig fetuses. *Journal of Anatomy*, v.137, p.235-245, 1983.

WILSON, M. E.; BIENSEN, N. J.; YOUNGS, C. R.; FORD, S. P. Development of meishan and Yorkshire littermate conceptuses in either a meishan or Yorkshire uterine environment to day 90 of gestation and to term. *Biology of Reproduction*, Champaign, v.58, p.905-910, 1998.

WU, G.; MORRIS, S. M. J. Arginine metabolism: nitric oxide and beyond. *Biochemistry Journal*, v.336, p.1-17, 1998.

WU, G.; BAZER, F. W.; CUDD, T. A.; MEINIGER, C. J.; SPENCER, T. E. Maternal nutrition and fetal development. *Journal of Nutrition*, v.134, p.2169-2172, 2004.

WU, G.; BAZER, F. W.; HU, J.; JOHNSON, G. A.; SPENCER, T. E. Polyamine synthesis from proline in the developing porcine placenta. *Biology of Reproduction*, v.72, p.842-850, 2005.

WU, G.Y.; BAZER, F. W.; WALLACE, J. M.; SPENCER, T. E. Board-invited review: intrauterine growth retardation: implications for the animal sciences. *Journal of Animal Science*, v.84 p.2316–2337, 2006.

WU, G.; FULLER, W. B.; DAVIS, T. A.; JAEGER, L. A.; JOHNSON, G. A.; KIM, S. W.; KNABE, D. A.; MEININGER, C. J.; SPENCER, T. E.; YIN, Y. L. Important roles for the arginine family of amino acids in swine nutrition and production. *Livestock Science*, Atlanta, v.112, p.8- 22, 2007.

WU, G.; F. BAZER, F. W.; DAVIS, T. A.; KIM, S. W.; LI, P.; RHOADS, J. M.; SATTERFIELD, M. C.; SMITH, S. B.; SPENCER, T. E.; YIN, Y. L. Arginine metabolism and nutrition in growth, health and disease. *Amino Acids*, v.37, p.153–168, 2009. WU, G.; BAZER, F.W.; JOHNSON, G. A.; KNABE, D.A.; BURGHARDT, R.C.; SPENCER, T.E.; LI, X.L.; WANG, J.J. Triennial growth symposium: Important roles for Iglutamine in swine nutrition and production. *Journal of Animal Science*, v.89, p.2017- 2030, 2011. ZHU, M. J.; FORD, S. P.; MEANS, W. J.; HESS, B. W.; NATHANIELSZ, P. W.; DU, M. Maternal nutrient restriction affects properties of skeletal muscle in offspring. *The Journal of Physiology*, v.575, p.241-250, 2006.

PRODUCTION OF CACTUS PEAR IRRIGATED WITH BRACKISH WATER AND ORGANIC MATTER

Submission date: 01/04/2024

Acceptance date: 02/05/2024

Daniel Bezerra do Nascimento

Programa de Pós-Graduação em Zootecnia. Universidade Federal Rural de Pernambuco
Recife - Pernambuco, Brasil
<https://orcid.org/0000-0001-6704-2289>
<http://lattes.cnpq.br/3924251043370854>

André Luiz Rodrigues Magalhães

Programa de Pós-Graduação em Ciência Animal e Pastagens. Universidade Federal do Agreste de Pernambuco
Garanhuns - Pernambuco, Brasil
<https://orcid.org/0000-0003-2211-6795>
<http://lattes.cnpq.br/2621131411062422>

Glacyane Costa Gois

Programa de Pós-Graduação em Ciência Animal. Universidade Federal do Maranhão
Chapadinha – Maranhão, Brasil
<https://orcid.org/0000-0002-4624-1825>
<http://lattes.cnpq.br/4306037261876678>

Tuany Nathan Epaminondas de Amaral

Graduação em Zootecnia. Universidade Federal do Agreste de Pernambuco
Garanhuns - Pernambuco, Brasil
<https://orcid.org/0009-0002-7128-2394>
<http://lattes.cnpq.br/0413826335307009>

Túlio Vandeilton de Oliveira Albuquerque

Programa de Pós-Graduação em Ciência Animal e Pastagens. Universidade Federal do Agreste de Pernambuco
Garanhuns - Pernambuco, Brasil
<https://orcid.org/0000-0003-0146-2584>
<http://lattes.cnpq.br/8475370314741859>

Priscila Barreto da Silva

Programa de Pós-Graduação em Ciência Animal e Pastagens. Universidade Federal do Agreste de Pernambuco
Garanhuns - Pernambuco, Brasil
<https://orcid.org/0009-0000-2448-5354>
<http://lattes.cnpq.br/4152012653085297>

ABSTRACT: Due to the lack of food to offer the animals during the dry season, the irrigated cultivation of cactus pear is justified to accelerate the harvest. Given this, the supply of saline water as a water source to meet the needs of plants becomes an important alternative for the development of irrigated agriculture. However, irrigation with saline water when poorly managed, can compromise the production system. Animal manure can be used to minimize the harmful effects of irrigation water salinity on plants. For higher productivity of cactus pear crops,

the implementation of a production system with water supplementation using irrigation with brackish water from underground wells in the region and the adequate incorporation of organic fertilizer is an alternative for farmers. Therefore, this review aimed to gather information about the cultivation of cactus pear irrigated with saline water and fertilized with doses of organic matter.

KEYWORDS: Brackish water, Manure, *Opuntia stricta* Haw, Semiarid.

PRODUÇÃO DE PALMA FORRAGEIRA IRRIGADA COM ÁGUA SALOBRA E DOSES DE MATÉRIA ORGÂNICA

RESUMO: Devido à falta de alimentos para oferecer aos animais durante a estação seca, justifica-se o cultivo irrigado da palma forrageira para acelerar a colheita. Diante disso, o fornecimento de água salina como fonte hídrica para suprir as necessidades das plantas torna-se uma importante alternativa para o desenvolvimento da agricultura irrigada. No entanto, a irrigação com água salina, quando mal manejada, pode comprometer o sistema de produção. O esterco animal pode ser usado para minimizar os efeitos nocivos da salinidade da água de irrigação nas plantas. Para maior produtividade da cultura da palma forrageira, a implantação de um sistema de produção com suplementação hídrica utilizando irrigação com água salobra de poços subterrâneos da região e a incorporação adequada de adubo orgânico é uma alternativa para os agricultores. Portanto, esta revisão teve como objetivo reunir informações sobre o cultivo da palma forrageira irrigada com água salina e adubada com doses de matéria orgânica.

PALAVRAS-CHAVE: Água salobra, Esterco, *Opuntia stricta* Haw, Semiárido.

INTRODUCTION

In arid and semiarid regions, the uneven distribution of rainfall and the limited availability of freshwater can limit the development of competitive and economically viable livestock production (Silva, 2017). In this context, the use of water from underground wells, which mostly have high levels of salinity, appears as an alternative. When well managed, the use of this water in a rational and controlled way can represent an alternative for irrigation of some crops. According to Lima et al. (2018), irrigation in a production system depends on agro-economic performance, which is based on the productive response, net income, and economic viability.

Cactus pear responds positively to irrigation. According to Araújo Júnior et al. (2021), the Orelha de Elefante Mexicana (OEM) variety, when irrigated, provides an economic return 18 months after planting. However, caution is required in the irrigation of this plant with saline water, since when in excess in the soil, the salts can compromise the rational agricultural exploration because of the osmotic effects, ionic toxicity, and nutritional imbalance, causing a reduction in growth and development of the crops, consequently serious damage to agricultural activity (Andrade et al., 2019). In addition to promoting variations in the chemical and physical structures of the soil, culminating in the loss of fertility and susceptibility to erosion (Schossler et al., 2012).

Considering the damage that saline water can cause, salt toxicity has to be minimized and soil properties have to be improved by using economic approaches such as the application of organic fertilizer (Shaaban et al., 2013). The benefits of adding organic materials are due to their role in minimizing the deleterious effects of salts in the soil through improvement, and alteration of the chemical and physical properties of the soil, as well as its role as a fertilizer (Murtaza et al., 2020).

For greater productivity of cactus pear crops, the implementation of a production system with water supplementation using irrigation with brackish water from underground wells in the region and the adequate incorporation of organic fertilizer is an alternative for farmers in this region, mainly due to freshwater restriction. In this sense, this review aimed to gather information about the cultivation of cactus pear irrigated with saline water and fertilized with doses of organic matter.

PRODUCTIVE AND NUTRITIONAL POTENTIAL OF CACTUS PEAR

Cactus pear represents most of the food offered to animals during the dry season in the northeastern semiarid regions, which is justified by the following characteristics: rich in water, mucilage, and mineral residue, high coefficient of dry matter digestibility, and high productivity per unit area (Lopes et al., 2013). Which makes it a viable alternative to maintaining adequate levels of animal productivity in the semiarid region (Dubeux Júnior et al., 2010).

According to Abidi et al. (2009), cactus pear contains an average of 90% water in its composition. This high-water content tends to reduce water intake by animals (Magalhães et al., 2019), which favors animal production in periods of water deficit, common in the semiarid region. Some authors observed that water intake via a drinking fountain linearly decreased as the levels of cactus in the diet increased (Abidi et al., 2009; Soares, 2017). According to the NRC (2001), the requirement for water can be met through three different sources: voluntary intake of water; water intake from food; and water from the metabolism of nutrients in the body.

Among the cactus pear varieties, the Orelha de Elefante Mexicana (OEM) presents good agronomic responses, is less demanding in terms of nutrients, more tolerant to water stress conditions, and shows a higher production of dry matter per unit area compared to the Miúda cactus (Lopes et al., 2019). However, it presents variations in chemical composition (Table 1) according to plant age, harvest time, climate, fertilization management, and planting spacing.

References	DM ¹	OM ²	MM ²	CP ²	EE ²	NFC ²	NDF ²	ADF ²
	g.kg ⁻¹							
Araújo Júnior et al. (2021)	86,5	840,2	159,7	35,8	8,0	-	161,6	-
Conceição et al. (2018)	105,5	802,5	198	55,5	12,1	406,7	291,6	
Góes Neto et al. (2021)	103,4	811,4	-	74,1	16,2	342,8	421,2	281,0
Pessoa et al. (2020)	96,86	889,9	112,8	53,1	15,4	616,7	210	141,1
Monteiro et al. (2019)	123	914	86	55	-	550	259	-
Morais et al. (2019)	107,7	893,2	-	63,6		545,1	272,4	-
Silva et al. (2018)	94,0	881,2	-	60,0	12,7	547,3	262	-

DM= Dry matter, OM= Organic matter, MM= Mineral matter, CP= Crude protein, NFC= Non-fibrous carbohydrates, NDF= Neutral detergent fiber, ADF= Acid detergent fiber. ¹g.kg⁻¹ Natural matter. ²g.kg⁻¹ Dry matter.

Table 1. Chemical composition of cactus pear Orelha de elefante mexicana

The high productivity of cactus pear in the semiarid region is due to their adaptation to the climate of this region, which can be partially explained by the opening of the stomata essentially at night, when ambient temperatures are mild, which reduces water losses by evapotranspiration, thus showing a high water use efficiency (WUE) (Taiz & Zaiger, 2016).

Water use efficiency (WUE) for cactus pear is 50:1, that is, 50 kg water for each 1 kg dry matter produced, while C3 and C4 plants have efficiencies of 1000:1 and 500:1, respectively (Pereira et al., 2012). Water use efficiency can be different between cultivars, as demonstrated by Silva et al. (2014a, 2014b), who found that the Orelha de elefante mexicana cultivar presented a superior WUE than the other cultivars studied, considering the green matter production.

The cactus pear production system in the semiarid region is marked by the low adoption of technologies, which directly leads to productivity lower than the capacity of the crop given its multiple options for use that are not explored. Traditionally, under non-irrigated cultivation conditions, cactus pear is harvested every two years (Silva et al., 2015), however, the beginning and length of each vegetative stage may vary depending on the adopted management practices, postponing or anticipating the time of crop harvest (Araújo Júnior et al., 2021). Among these technologies, the use of irrigation, fertilization, denser planting with techniques that increase crop yield, and the combination of these can be highlighted.

The adoption of irrigation to increase the growth and survival of cactus pear plantations is an alternative that can be used by farmers. Lira et al. (2016) mention that when having an irrigation system, even if the amount of water is limited, that is, even in conditions where the water supply through irrigation does not meet the requirements of the crop, a small amount of water can result in positive responses in the plant, mainly in cactus pear.

Irrigation promotes and guarantees the survival of the cactus pear crop and generates a strategic green reserve of water. According to Cândido et al. (2013), the water potential of dense cultivation of the cactus pear cultivar Gigante with a production of 400 Mg of green

matter per hectare is high, with water content in the plantation around 90%, in which one hectare of the crop can supply around 360,000 liters water to cattle or goats in the semiarid region of Brazil.

USE OF BIOSALINE AGRICULTURE IN FORAGE PRODUCTION

Biosaline agriculture is a broad term used to characterize the cultivation of forage plants irrigated with saline well water (Masters et al., 2007). According to Silva et al. (2023), the use of saline water sources represents an alternative that can minimize the water supply crisis, especially in the arid and semiarid zones of the planet. These waters are used for irrigation and contain varying amounts of salts, which can interfere with crop development and production at certain concentrations.

The effects of salt stress on plants vary depending on different factors, such as the level of salt concentration, duration of exposure, phenological stage, interaction with environmental conditions, and resistance of the species or cultivar to salinity conditions (Toscano et al., 2019). The ability of the plant to survive and develop under saline stress is a function of its tolerance or escape mechanisms or a combination of both (Munns & Tester, 2008). According to the degree of tolerance to salinity, plants can be classified into halophytes: Plants that have a set of morphological, anatomical, and physiological adaptations that help them to germinate, grow, reproduce, and complete their life cycle in environments with high concentrations of salts (Nikalje et al., 2018); and glycophytes: Plants that are not adapted to tolerate salinity (Cheeseman et al., 2015).

Some halophytes have developed modified epidermal cells that accumulate excessive Na^+ in their vacuoles, which allows them to adapt to high salinity (Zhao et al., 2020), and can develop in environments with salt concentrations above 200 mM (millimolar) (Cheeseman et al., 2015).

In the Northeast, as in other regions of Brazil, the use of groundwater has increased in recent years. Soil drilling to reach water is a practice that farmers in semi-arid region use in search of a water source to meet the requirements of plants, thus becoming an important alternative for the development of irrigated agriculture (Santos et al., 2020).

Biosaline agriculture, when properly used, can increase crop production, however, when combined with inadequate management can cause great losses in a productive system, due to the excessive increase in the concentration of salts in the soil. The effects of salts on soil occur through the electrochemical interaction between salts and clay (Gheyi et al., 2016).

The increase in the exchangeable sodium concentration in the soil may make it denser, compact in dry conditions, dispersed, and sticky in wet conditions (Dias & Blanco, 2010). The concentration of sodium in the soil is capable of promoting the dispersion of clay particles, making the soil pulverized, causing clogging of micropores, reduced aeration, and water infiltration (Gasparetto et al., 2009).

Melloni et al. (2000) summarize the effects of soil salinity on plants into effect caused by the reduction of osmotic potential; nutritional imbalance due to the high ionic concentration and the inhibition of the absorption of other cations by the excess and toxic effect of sodium and chloride ions. The osmotic effect is due to the presence of salts in the soil that increase water retention forces, which reduces its availability for plants (Acosta-Motos et al., 2017) and, as a result, also reduces the availability of nutrients.

The increase in osmotic pressure caused by the excess of soluble salts in the soil solution may reach a level where plants will not have enough suction force to overcome the osmotic potential and, as a result, the plant will not absorb water, and consequently nutrients and this process is also called physiological drought (Dias & Blanco, 2010).

Another effect caused by salinization is the nutritional imbalance of the plant caused by disturbances in the absorption and/or distribution of nutrients. The reduction in Ca absorption, for example, can lead to loss of plasma membrane integrity, compromising the absorption capacity of some ions, mainly potassium (K) (Farias et al., 2009). In plants, the toxicity caused by saline water is mainly due to the presence of chlorine, sodium, and boron ions. These ions, when absorbed by plants, are accumulated in their tissues in concentrations high enough to cause damage to crops and reduce their yield (Silva et al., 2011).

One of the main determinants of livestock production in biosaline agriculture is the amount of biomass produced that can be consumed by the animals (Masters et al., 2007). The improvement in the use of brackish water has been the object of studies and, consequently, the effects of salt on the development and distribution of nutrients in plants have been better understood.

BRACKISH WATER FOR CACTUS PEAR IRRIGATION

The semiarid region has a network of very poor rivers, with low runoff volumes (Rocha & Soares, 2015). This can be explained by the temporal and spatial variability of rainfall and the dominant geological characteristics, where there is a predominance of shallow soils on crystalline rocks and, consequently, low water exchange between the river and the adjacent soil (Araújo, 2015).

In this scenario, the use of groundwater, specifically saline and brackish water, is important to increase agricultural and livestock production in the semiarid region. In addition, the availability of water for human consumption and agricultural practice has been gradually reduced both in quality and quantity, thus making the alternative use of water with higher salt contents necessary to meet the demand for agricultural irrigation in these regions (Silva et al., 2014a).

The quality of water used for irrigation is defined according to three criteria: its salinity, expressed as electrical conductivity, which assesses the risk of increasing the concentration

of soluble salts in the soil; its sodicity expressed as Sodium Adsorption Ratio, SAR, which assesses the risk of raising the percentage of exchangeable sodium, causing deterioration in the soil structure, and finally its toxicity which assesses the accumulation of certain ions in plant tissues (Almeida, 2010).

Irrigation with brackish water to optimize the growth and survival of crops is a strategic alternative that can be used by farmers located in regions characterized by the uneven temporal and spatial distribution of rainfall. Nevertheless, the use of saline water must be done in a rational way, since the lack of knowledge in the use of this resource can lead to the total loss of the crop, in addition to making the use of the soil unsuitable for other crops or even accelerating a process of desertification (Silva, 2017).

For Nobel (2001), soil salinity of 100 ppm (parts per million) inhibits atmospheric CO₂ uptake and *Opuntia* growth by 30%. These levels are usually exceeded in soils irrigated with saline water, as well as under natural conditions, when high temperatures cause high evaporation and, consequently, accumulation of salts on the soil surface. According to Araújo Júnior et al. (2021), cactus pears are not tolerant to saline stress, with general inhibition of root development and shoots of cactus pears in saline soils, because high levels of sodium in the soil inhibit CO₂ fixation (Dubeux Júnior et al., 2010).

Freire et al. (2018) evaluated irrigation frequency (7, 14, 21, and 28 days) and salinity levels (0.3; 0.5; 1.5, and 3.6 dS m⁻¹ NaCl) and noticed that the cactus pear does not tolerate the highest level of salts when irrigated with shorter intervals. In view of this, the tolerance of cactus pear and other plants to salinity for greater irrigation efficiency in each crop. When choosing to use brackish water in cactus pear irrigation, the soil must be well drained and irrigation should continue until the rainy season, since rain promotes the leaching of salts (Santos et al., 2020).

However, the use of irrigation systems with brackish water can increase the productivity of cactus pear plantations when well managed, as demonstrated by Fonseca et al. (2019), irrigating the cactus pear cultivar Gigante with 33% ETo with an interval of three days, which resulted in an increase in cactus height and the number of total cladodes in the plant.

ORGANIC MATTER FOR CACTUS PEAR CULTIVATION

The selection of the ideal planting system for cactus pear is influenced by several aspects such as climatic conditions, soil quality, property size, labor supply, technical assistance, the possibility of mechanization, costs of acquisition of inputs, availability of organic fertilizer, levels and sources of fertilizers, pests, and diseases, intercropped or monoculture cultivation, spacing used and among others (Padilha Júnior et al., 2016).

The mineral and organic maintenance fertilization is an important management measure for the cactus pear crop and should be carried out at each harvest, considering

the high extraction of nutrients with the removal of the paddles (Lemos, 2016). Mineral fertilizers have great efficiency in the availability of nutrients for plants, but due to their high cost, livestock farmers do not always have sufficient financial conditions to purchase in quantity and quality according to their needs (Macedo et al., 2018). Because of this, the use of organic fertilizer produced on the property is an option for farmers. The use of organic matter as a nutrient source on agricultural land improves the physical properties of the soil, in addition to being an environmentally friendly way of waste disposal (Nazli et al., 2016).

According to Finatto et al. (2013), organic fertilizer consists of residues of animal and plant origin, which, after decomposition, results in organic matter. In the past, organic sources were the only sources used in the soil, with the development of mineral sources, organic fertilization was being used less, and currently, due to the social appeal, from the conservationist and ecological point of view, this practice is gaining space in areas with agriculture or livestock (Macedo et al., 2018).

Among the various beneficial effects of organic matter in the soil in agricultural systems, the stimulation of the soil microbiota, soil physical conditioning (structure, porosity), biological and chemical buffer effect with the supply of negative charges, and increased Cation Exchange Capacity – CEC, nutrients (N, P, K, and S), thermal control and better water retention stand out (Ungera et al., 1991; Conceição et al., 2005; Boulal et al., 2011; Lemos, 2016).

Given the improvements in soil texture, organic fertilization can increase the carrying capacity capacity of the semiarid region. According to Peixoto et al. (2018), the production of green matter ($\text{Mg}\cdot\text{ha}^{-1}$) of cactus pear cultivar Gigante fertilized with bovine manure was 33.03% higher than the treatment without fertilization.

Regarding the amount of organic matter in cactus pear crops, Dubeux Júnior et al. (2010) recommend the use of 10 to 30 $\text{Mg}\cdot\text{ha}^{-1}$ cattle manure after each harvest, depending on the planting spacing used. In turn, Santos et al. (2002) suggest that in denser plantations 30 $\text{Mg}\cdot\text{ha}^{-1}$ can be used. Ramos et al. (2017) evaluated the growth of cactus pear cultivar Gigante as a function of fertilization with goat manure (0, 5, 10, 15, 20 $\text{Mg}\cdot\text{ha}^{-1}$), and observed a linear increase in the number of cladodes with the addition of organic fertilizer. Barros et al. (2016) also observed that the application of 90 $\text{Mg}\cdot\text{ha}^{-1}$ organic fertilizer to cactus pear cultivar Miúda promoted increments from 7.17 to 20.9 cladodes.

According to Macêdo et al. (2018), factors such as temperature, pH, and soil moisture are essential for the decomposition of organic matter to be more or less efficient. Souto et al. (2005) examined the decomposition rate of manure at different depths and reported that the decomposition was strongly influenced by the rainfall during the experimental period. According to Zhou et al. (2020), soil moisture acts on the dynamics of microorganisms that are essential for nutrient cycling and the fertility of agroecosystems, which stimulates plant growth and production. This is because water contributes to the solubilization of minerals present in the organic matter and uptake by the roots of cactus pear plants (Nunes, 2018).

FINAL CONSIDERATIONS

Irrigation with saline water can be a viable alternative to increase the productivity of cactus pear plantations, but proper management is required to minimize the effects of salts on the soil. Organic matter is a low-cost alternative that minimizes the deleterious effects of salts. Because of this, further research is necessary to evaluate the morphological, productive, and nutritional parameters of cactus pear irrigated with saline water and fertilized with doses of organic matter so that the production of cactus pear in the semiarid region can be improved.

REFERENCES

- Abidi, S., Salem, H. B., Vasta, V., & Priolo, A. (2009). Supplementation with barley or spineless cactus (*Opuntia ficus indica* f. *inermis*) cladodes on digestion, growth and intramuscular fatty acid composition in sheep and goats receiving oaten hay. *Small Ruminant Research*, 87; 9-16. DOI: 10.1016/j.smallrumres.2009.09.004
- Acosta-Motos, J. R., Ortuno, M. F., Bernal-Vicente, A., Diaz-Vivancos, P., Sanchez-Blanco, M. J., & Hernandez, J. A. (2017). Plant responses to salt stress: adaptive mechanisms. *Agronomy*, 7; 1-18. DOI: 10.3390/agronomy7010018
- Almeida, O. A. (2010). *Qualidade da água de irrigação*. Cruz das Almas: Embrapa Mandioca e Fruticultura, 228 p.
- Andrade, E. M. G., Lima, G. S., Lima, V. L. A., Silva, S. S., Gheyi, H. R., Araújo, A. C., Gomes, J. P., & Soares, L. A. A. (2019) Production and postharvest quality of yellow passion fruit cultivated with saline water and hydrogen peroxidem. *AIMS Agriculture and Food*, 4; 907-920. DOI: 10.3934/agrfood.2019.4.907
- Araújo, G. G. L. (2015) Os impactos das mudanças climáticas sobre os recursos hídricos e a produção animal em regiões semiáridas. *Revista Brasileira de Geografia Física*, 8; 598-609. DOI: 10.26848/rbgf.v8.0.p598-609
- Araújo Júnior, G. N., Silva, T. G. F., Souza, L. S. B., Souza, M. S., Araújo, G. G. L., Moura, M. S. B., Santos, J. P. A. S., Jardim, A. M. R. F., Alves, C. P., & Alves, H. K. M. N. (2021). Productivity, bromatological composition and economic benefits of using irrigation in the forage cactus under regulated deficit irrigation in a semiarid environment. *Bragantia*, 80; 1-12. DOI: 10.1590/1678-4499.20200390
- Barros, J. L., Donato, S. L. R., Gomes, V. M., Donato, P. E. R., Silva, J. A., & Padilha Júnior, M. C. (2016). Palma forrageira 'gigante' cultivada com adubação orgânica. *Revista Agrotecnologia*, 7; 53-65. DOI: 10.12971/5051
- Boulal, H., Gómez-Macpherson, H., Gómez, J. A., & Mateos, L. (2011). Effect of soil management and traffic on soil erosion in irrigated annual crops. *Soil & Tillage Research*, 115; 62–70. DOI: 10.1016/j.still.2011.07.003
- Cândido, M. J. D., Gomes, G. M. F., Lopes, M. N., & Ximenes, L. J. F. (2013). Cultivo da palma forrageira para mitigar a escassez de forragem em regiões semiáridas. *Informe Rural*, 7; 1-7.

- Cheeseman, J. M. (2015). The evolution of halophytes, glycophytes and crops, and its implications for food security under saline conditions. *New Phytologist*, 206; 557-570. DOI: 10.1111/nph.13217
- Conceição, M. G., Ferreira, M. A., Silva, J. L., Costa, C. T. F., Chagas, J. C. C., & Monteiro, C. C. F. (2018). Can cactus (*Opuntia stricta* [Haw.] Haw) cladodes plus urea replace wheat bran in steers' diet? *Asian-Australasian journal of animal sciences*, 31; e1627. DOI: 10.5713/ajas.17.0927
- Conceição, P. C., Amado, T. J. C., Mielniczuk, J., & Spagnollo, E. (2005). Qualidade do solo em sistemas de manejo avaliada pela dinâmica da matéria orgânica e atributos relacionados. *Revista Brasileira de Ciência do Solo*, 29; 777-788. DOI: 10.1590/S0100-06832005000500013
- Dias, N. S., & Blanco, F. F. (2010). Efeitos dos sais no solo e na planta. In: Gheyi, H. R., Dias, N. S., Lacerda, C. F. (Ed.). *Manejo da salinidade na agricultura: estudos básicos e aplicados*. Fortaleza: INCTSal. pp. 129-141.
- Dubeux Junior, J. C. B., Araújo Filho, J. T., Santos, M. V. F., Lira, M. A., Santos, D. C., Pessoa, R. A. S. (2010). Adubação mineral no crescimento e composição mineral da palma forrageira-Clone IPA-201. *Revista Brasileira de Ciências Agrárias*, 5; 129-135. DOI: 10.5039/agraria.v5i1a591
- Farias, S. G. F., Santos, D. R., Freire, A. L. O., & Silva, R. B. (2009) Estresse salino no crescimento inicial e nutrição mineral de gliricídia (*Gliricidia sepium* (jacq.) kunth ex steud) em solução nutritiva. *Revista Brasileira de Ciência do solo*, 33; 1499-1505. DOI: 10.1590/S0100-06832009000500040
- Finatto, J., Altmayer, T., Martini, M. C., Rodrigues, M., Basso, V., & Hoehne, L. (2013). A importância da utilização da adubação orgânica na agricultura. *Revista Destaques Acadêmicos*, 5; 85-93.
- Fonseca, V. A., Santos, M. R. D., Silva, J. A. D., Donato, S. L. R., Rodrigues, C. S., & Brito, C. F. B. (2019). Morpho-physiology, yield, and water-use efficiency of *Opuntia ficus-indica* irrigated with saline water. *Acta Scientiarum. Agronomy*, 41; 1-11. DOI: 10.4025/actasciagrion.v41i1.42631
- Freire, J. L., Santos, M. V. F., Dubeux Júnior, J. C. B. Bezerra Neto, E., Lira, M. A., Cunha, M. V., Santos, D. C., Amorim, S. A., & Mell, O. A. C. L. (2018). Growth of cactus pear cv. Miúda under different salinity levels and irrigation frequencies. *Anais da Academia Brasileira de Ciências*, 90; 3893-3900. DOI: 10.1590/0001-3765201820171033
- Gasparetto, E. C., Braidá, J. A., Carneiro, M., Scariot, J. J., & Tabolka, C. L. (2009). Grau de flocculação da argila de um latossolo vermelho utilizado com lavoura e mata nativa. *Synergismus Scientifica*, 4; 1-3.
- Gheyi, H. R., Dias, N. S., Lacerda, C. F., & Gomes Filho, E. (2016). *Manejo da salinidade na agricultura: Estudos básicos y aplicados*. 2nd ed. Fortaleza: INCTSal. 504 p.
- Góes Neto, P. E., Silva, J. G. M., Aguiar, E. M., Melo, A. A. S., Lima, G. F. C., Cardoso, D. B., & Silva, H. P. (2021). Native and introduced forage cacti in Saanen dairy goat diets. (2021). *Acta Scientiarum. Animal Sciences*, 43; e51029. DOI: 10.4025/actascianimsci.v43i1.51029
- Lemos, M. (2016). *Uso de esgoto doméstico tratado na produção de palma forrageira em assentamento rural do semiárido brasileiro*. 244f. Tese (Doutorado em Manejo do Solo e Água no Semiárido) – Universidade Federal de Rural do Semi-Árido – Mossoró-RN.

- Lima, L. R., Silva, T. G. F., Pereira, P. C., Morais, J. E. F., & Assis, M. C. S. (2018). Productive-economic benefit of forage cactus-sorghum intercropping systems irrigated with saline water. *Revista Caatinga*, 31; 191-201. DOI: 10.1590/1983-21252018v31n122rc
- Lira, M. A., Santos, M. V. F., Dias, F. M., Ferraz, A. P. F., Silva, M. C., Cunha, M. V., Mello, A. C. L., Dubeux Júnior, J. C. B., Santos, D. C., Freitas, E. V., & Arruda, D. M. P. (2016). Palma Forrageira: cultivo e usos. *Cadernos do Semiárido: riquezas & oportunidades*. 7; 10-22.
- Lopes, L. A., Cardoso, D. B., Camargo, K. S., Silva, T. G. P., Souza, J. S. R., Silva, J. R. C., Morais, J. S., Araújo, T. P. M. (2019). Palma forrageira na alimentação de ruminantes. *PUBVET*, 13; 1-10. DOI: 10.31533/pubvet.v13n3a277.1-10
- Lopes, E. B., Costa, L.B., Cordeiro Júnior, A. F., & Brito, C. H. (2013). Rendimento e aspectos fenológicos de espécies de palma forrageira em relação ao cultivo com dois tipos de cladódios. *Tecnologia & Ciência Agropecuária*, 7; 59-61.
- Macedo, A. J. S., Edvan, R. L., Santos, E. M., & Soares, M. N. (2018). Organic fertilization on tropical pastures: Review. *Revista Eletrônica de Veterinária*, 19; 1-19.
- Magalhães, A. L. R., Sousa, D. R., Nascimento Júnior, J. R. S., Gois, G. C., Campos, F. S., Santos, K. C., Nascimento, D. B., & Oliveira, L. P. (2019). Intake, digestibility and rumen parameters in sheep fed with common bean residue and cactus pear. *Biological Rhythm Research*, 50; 1-10. DOI: 10.1080/09291016.2019.1592351
- Masters, D. G., Benes, S. E., & Norman, H. C. (2007). Biosaline agriculture for forage and livestock production. *Agriculture, Ecosystems and Environment*, 119; 234-248. DOI: 10.1016/j.agee.2006.08.003
- Melloni, R., Silva, F. A. M., & Carvalho, J. G. (2000). Cálcio, magnésio e potássio como amenizadores dos efeitos da salinidade sobre a nutrição mineral e o crescimento de mudas de armoire (*Myracrodruon urundeuva*). *Cerne*, 6; 35-40.
- Monteiro, C. C. F., Ferreira, M. A., Vêras, A. S. C., Guido, S. I., Almeida, M. P., Silva, R. C., & Inácio, J. G. (2019). A new cactus variety for dairy cows in areas infested with *Dactylopius opuntiae*. *Animal Production Science*, 59; 479-485. DOI: 10.1071/AN17256
- Morais, G. S. O., Guim, A., Tabosa, J. N., Chagas, J. C. C., Almeida, M. P., & Ferreira, M. A. (2019). Cactus [*Opuntia stricta* (Haw.) Haw] cladodes and corn silage: How do we maximize the performance of lactating dairy cows reared in Semi-arid regions? *Livestock Science*, 221; 133-138. DOI: 10.1016/j.livsci.2019.01.026
- Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Biology*, 59; 651-681. DOI: 10.1146/annurev.arplant.59.032607.092911
- Murtaza, G., Sarwar, G., Sabah, N. S., Tahir, M. A., Mujeeb, F., Muhammad, S., Manzoor, M. Z., & Zafar, A. (2020). Judicious use of saline water for growing sorghum fodder through the application of organic matter. *Pakistan Journal of Agricultural Research*, 33; 1-7. DOI: 10.17582/journal.pjar/2020/33.1.106.112
- Nazli, R. I., Inal, I., Kusvuran, A., Demirbas, A., & Tansi, V. (2016). Effects of different organic materials on forage yield and nutrient uptake of silage maize (*Zea mays* L.). *Journal of Plant Nutrition*, 39; 912-921. DOI: 10.1080/01904167.2015.1109103

- Nikalje, G. C., Srivastava, A. K., Pandey, G. K., & Suprasanna, P. (2018). Halophytes in biosaline agriculture: Mechanism, utilization and value addition. *Land Degradation & Development*, 29; 1081-1095. DOI: 10.1002/ldr.2819
- Nobel, P. S. (2001). *Biologia Ambiental*. In: BARBERA, G.; INGLESE, P.; BARRIOS, E.P. *Agroecologia, cultivo e usos da palma forrageira*. João Pessoa: SEBRAE-PB/FAO. pp. 36-48.
- NRC. (2001). National Research Council. *Nutrient requirements of dairy cattle*. 7th ed. Washington, DC: The National Academies Press. 363p.
- Nunes, T. C. M. D. (2018). *Sistemas bioassalinos de produção de palma forrageira recebendo crescentes lâminas de água e doses de matéria orgânica*. 46f. Dissertação (Mestrado em Ciência Animal) – Universidade Federal do Vale do São Francisco, Petrolina – PE.
- Pereira, L. S., Cordery, I., & Iacovos, I. (2012). Improved indicators of water use performance and productivity for sustainable water conservation and saving. *Agricultural Water Management*, 108; 39-51. DOI: 10.1016/j.agwat.2011.08.022
- Padilha Júnior, M. C., Donato, S. L. R., Silva, J. A., Donato, P. E. R., & Souza, E. S. (2016). Características morfométricas e rendimento da palma forrageira ‘Gigante’ sob diferentes adubações e configurações de plantio. *Revista Verde*, 11; 67-72. DOI: 10.18378/rvads.v11i1.3710
- Peixoto, M. J. A., Carneiro, M. D. S., Amorim, D. S., Edvan, R. L., Pereira, E. S., & Costa, M. R. G. F. (2018). Características agrônômicas e composição química da palma forrageira em função de diferentes sistemas de plantio. *Archivos de Zootecnia*, 7; 35-39. DOI: 10.21071/az.v67i257.3489
- Pessoa, D. V., Andrade, A. P., Magalhães, A. L. R., Teodoro, A. L., Santos, D. C., Araújo, G. G. L., Medeiros, A. N., Nascimento, D. B., Valença, R. L., & Cardoso, D. B. (2020). Forage cactus of the genus *Opuntia* in different with the phenological phase: Nutritional value. *Journal of Arid Environments*, 181; 104243. DOI: 10.1016/j.jaridenv.2020.104243
- Ramos, J. P. F., Souza, J. T. A., Santos, E. M., Pimenta Filho, E. C., & Ribeiro, O. L. (2017). Crescimento e produtividade de *Nopalea Cochenillifera* em função de diferentes densidades de plantio em cultivo com e sem capina. *Revista Eletrônica de Veterinária*, 18; 1-12.
- Rocha, R., Soares, R. R. (2015). Water scarcity and birth outcomes in the Brazilian semiarid. *Journal of Development Economics*, 112; 72-91. DOI: 10.1016/j.jdeveco.2014.10.003
- Santos, D. C., Farias, I., Lira, M. A., Santos, M. V. F., Arruda, G. P., Coelho, R. S. B., Dias, F. M., Warumby, J. F., & Melo, J. N. (2002). Manejo e utilização da palma forrageira (*Opuntia* e *Nopalea*) em Pernambuco: cultivo e utilização. Recife: IPA. 45 p.
- Santos, M. R., & Donato, S. L. R. (2020). Irrigação da palma forrageira, *Revista Agrotecnologia*, 11; 75-86.
- Shaaban, M., Abid, M., & Abou-Shanab, R. A. I. (2013). Amelioration of salt affected soils in rice paddy system by application of organic and inorganic amendments. *Plant Soil Environment*, 59; 227-233. DOI: 10.17221/881/2012-PSE
- Schossler, T. R., Machado, D. M., Zuffo, A. M., Andrade, F. R., & Piauilino, A. C. (2012). Salinidade: Efeitos na fisiologia e nutrição mineral de plantas. *Enciclopédia Biosfera, Centro Científico Conhecer*, 8; e1563.

- Silva, R. R., Medeiros, J. F., Queiroz, G. C. M., Sousa, L. V., Souza, M. V. P., Nascimento, M. A. B., Morais, F. M. S., Nóbrega, R. F., Silva, L. M., Ferreira, F. N., Clemente, M. I. B., Cordeiro, C. J. X., Granjeiro, J. C. C., Constante, D. C., & Sá, F. V. S. (2023). Ionic response and sorghum production under water and saline stress in a Semi-Arid environment. *Agriculture*, 13; e1127. DOI: 10.3390/agriculture13061127
- Silva, R. C., Ferreira, M. A., Oliveira, J. C. V., Santos, D. C., Gama, M. A. S., Chagas, J. C. C., Inácio, J. G., Silva, E. T. S., & Pereira, L. G. R. (2018). Orelha de Elefante Mexicana (*Opuntia stricta* [Haw.] Haw.) spineless cactus as an option in crossbred dairy cattle diet. *South African Journal of Animal Science*, 48; 516-525. DOI: 10.4314/sajas.v48i3.12
- Silva, R. H. D. (2017). Crescimento de palma forrageira irrigada com água salina. 56f. Dissertação (Mestrado em Zootecnia) – Universidade Federal de Viçosa – Viçosa-MG.
- Silva, T. G. F., Araújo Primo, J. T., Morais, J. E. F., Diniz, W. J. S., Souza, C. A. A., & Silva, M. C. (2015). Crescimento e produtividade de clones de palma forrageira no semiárido e relações com variáveis meteorológicas. *Revista Caatinga*, 28; 10-18.
- Silva, J. L. A., Medeiros, J. F., Alves, S. S. V., Oliveira, F. A., Silva Junior, M. J., & Nascimento, I. B. (2014a). Uso de águas salinas como alternativa na irrigação e produção de forragem no Semiárido Nordestino. *Revista Brasileira de Engenharia Agrícola e Ambiental*, 18; 66-72. DOI: 10.1590/1807-1929/agriambi.v18nsuppS66-S72
- Silva, T. G. F., Araújo Primo, J. T., Silva, S. M. S., Moura, M. S. B., Santos, D. C., Silva, M. C., & Araújo, J. E. M. (2014b). Indicadores de eficiência do uso da água e de nutrientes de clones de palma forrageira em condições de sequeiro no Semiárido brasileiro. *Bragantia*, 73; 184-191. DOI: 10.1590/brag.2014.017
- Silva, I. N., Fontes, L. O., Tavella, L. B., Oliveira, J. B., & Oliveira, A. C. (2011). Qualidade de água na irrigação. *Agropecuária Científica no Semi-Árido*, 7; 1-15. DOI: 10.30969/acsa.v7i3.134.
- Soares, M. S. (2017). Palma forrageira: Aspecto do cultivo e desempenho animal. *Nutritime Revista Eletrônica*, 1; 6041-6055.
- Souto, P. C., Souto, J. S., Santos, R. V., Araújo, G. T., & Souto, L. S. (2005). Decomposição de esterco dispostos em diferentes profundidades em área degradada no Semiárido da Paraíba. *Revista Brasileira de Ciência do Solo*, 29; 25-130. DOI: 10.1590/S0100-06832005000100014
- Taiz, L., & Zieger, E. (2016). *Fisiologia vegetal*. 6th ed. Porto Alegre: Artmed. 719p.
- Toscano, S., Trivellini, A., Cocetta, G., Bulgari, R., Francini, A., Romano, D., & Ferrante, A. (2019). Effect of preharvest abiotic stresses on the accumulation of bioactive compounds in horticultural produce. *Frontiers in Plant Science*, 10; e1212. DOI: 10.3389/fpls.2019.01212
- Ungera, P. W., Stewarta, B. A., Parrb, J. F., & Singhc, R. P. (1991). Crop residue management and tillage methods for conserving soil and water in semi-arid regions. *Soil & Tillage Research*, 20; 219-240. DOI: 10.1016/0167-1987(91)90041-U
- Zhao, C., Zhang, H., Song, C., Zhu, J. K., & Shabala, S. (2020). Mechanisms of plant responses and adaptation to soil salinity. *The innovation*, 1; e100017. DOI: 10.1016/j.xinn.2020.100017
- Zhou, Y., Bastida, F., Zhou, B., Sun, Y., Gu, T., Li, S., & Li, Y. (2020). Soil fertility and crop production are fostered by micro-nano bubble irrigation with associated changes in soil bacterial community. *Soil Biology and Biochemistry*, 141; e107663. DOI: 10.1016/j.soilbio.2019.107663

EFEITO DA ADUBAÇÃO DE NITROGÊNIO NA CULTURA DO MILHO (ZEA MAYS) COMPARADO ENTRE OS DOIS HÍBRIDOS

Acceptance date: 02/05/2024

Kentaro Tomita

Ph.D.

Faculdade de Ciências Agropecuárias y Desenvolvimento Rural, Universidade Nacional de Pilar – Paraguai (Ex-professor visitante)

Faculdade de Ciências da Vida, Escola Superior Politécnica do Litoral (ESPOL): Campus Gustavo Galindo, Guayaquil, Equador [Voluntário Sênior de Agência de Cooperação Internacional do Japão: JICA em Paraguai y em Equador

RESUMO: O autor realizou várias pesquisas para o cultivo de milho no Panamá, Brasil, Paraguai e Equador como cultura básica. Neste artigo, explicou-se os resultados obtidos no Paraguai com os companheiros de América Latina. Na cidade de Pilar, Paraguai, realizou-se uma investigação para determinar a variação no rendimento da cultura do milho comparado entre os dois híbridos TNH0032100 e TNH0033100, a partir de diferentes níveis de adubação nitrogenada num Entisol. Avaliaram-se quatro doses de N (0, 50, 100 e 200 kgN/ha), combinando dois híbridos ao acaso com três repetições na cultura do milho onde utilizou-se o delineamento

experimental em parcelas subdivididas. Dos resultados da análise de variância, observou-se a diferença significativa a 1% para os híbridos, quanto ao percentual de proteína bruta no grão, também obteve-se altamente diferença significativa a 1% para os níveis do grão N aplicado na cultura. O rendimento e o % de proteína bruta para o híbrido TNH0032100 foram superiores aos para o híbrido TNH0033100. Por isso recomendou-se o cultivo do híbrido TNH0032100 no campo. Dos resultados obtidos, sobre a recuperação eficiente aparente de N, no tratamento 50kgN/ha foi de 38,3% no híbrido TNH0032100, com lixiviação e/ou volatilização deste elemento na cultura. Por outro lado, considera-se liberação nitrogenada a partir da matéria orgânica do solo (1,56%) pela mineralização por atividade microbiana na cultura do milho. No experimento, a adubação de N realizou-se na época da semeadura, aos 30 e 60 dias após a semeadura. Os resultados obtidos, determinou-se 50kgN/ha como a adubação econômica para o híbrido TNH 0032100.

PALAVRAS-CHAVE: adubação nitrogenada, híbridos de milho, produtividade, recuperação eficiente aparente do N absorvido

EFFECT DE NITROGEN APPLICATION ON THE CORN (*ZEA MAYS*) CULTURE COMPARED WITH TWO HYBRIDS

ABSTRACT: The author carried out various research for corn culture on Panama, Brazil, Paraguay and Ecuador as a basic crop. On the article, it is explained the obtained results in Paraguay with the Latin American companions. At Pilar city in Paraguay, it carried out an investigation to determine the variation on corn crop yield, the hybrids TNH0032100 and TNH0033100, from different levels of nitrogen fertilization on an Entisol. It was evaluated four levels of N (0, 50, 100 and 200kgN/ha) combined with two hybrids at random with three replications on corn where it was used the experiment design in split plots. From the results of analysis of variance, it was showed significant difference at 1% for these hybrids, and it was also obtained highly significant difference at 1% levels N applied to the crop on the percentage of crude protein in the grain. Yield and % of crude protein for hybrid TNH0032100 were superior to those for hybrid TNH0033100. Therefore, it is recommended that the hybrid TNH0032100 culture in the field. From the results obtained on the efficient recovery of apparent N in the treatment of 50kgN/ha was 38,3% in the hybrid TNH0032100, with leaching and/or volatilization of this element inside the crop. On the other hand, it is considered the nitrogen release from the soil organic matter (1,56%) by the mineralization by microbial activity within the corn crop culture. On the experiment, it carried out the N application at sowing, 30 and 60 days after sowing. It was determined 50kgN/ha as an economic application for the hybrid TNH 0032100.

KEYWORDS: apparent efficient recuperation of nitrogen absorption, corn, hybrids, nitrogen fertilization, yield

INTRODUÇÃO

A região latino-americana, na maior parte das definições, compreende todos os países do continente americano, exceto os Estados Unidos e o Canadá. A agricultura na América Latina é uma das suas principais atividades econômicas e destaca os países integrantes no cenário mundial. Na produção agrícola na América Latina, pode-se distinguir dois grandes grupos tais como minifúndios e latifúndios. A agricultura representa uma atividade econômica importante nos países latinos, pois grande parte das suas terras é utilizada para o cultivo. Uma característica interessante da região é que cerca de 70% da produção agrícola vêm de pequenas e médias propriedades, produtoras de feijão, batata, inhame, milho e mandioca. Apesar de terem uma grande participação no mercado, essas propriedades não adotam tecnologias avançadas, o que justifica sua baixa produtividade. A falta desse tipo de recurso é explicada, em grande parte, pela falta de incentivos financeiros e pela pouca divulgação de informações que as permita modernizar seus meios de produção.

Atualmente, para os grandes latifúndios, realiza produção de monocultura para exportação, com lavouras de cana-de-açúcar, café, cacau, trigo, frutas regionais e soja. Para Milho, também, mais uma vez, o Brasil e a Argentina são os grandes responsáveis pelo destaque da América Latina na produção de grãos no mundo, dessa vez o milho. Segundo dados das Nações Unidas, em 2016 a produção do grão foi de 171 milhões de toneladas.

No Paraguai, também, é muito importante para produção de Milho como dieta humana e animal. Para Milho, pela página de Web, produziu-se 64.143.414 toneladas no Brasil, enquanto que, para a Argentina, 39.792.854, para o Paraguai, 5.152.320 toneladas, respectivamente. Mundialmente, 1.060.247.727 toneladas de milho são produzidas por ano. Os Estados Unidos da América são o maior produtor de milho do mundo, com 384.777.890 toneladas de volume de produção por ano, respectivamente. A Tabela N°1 mostra a produção de milho nos 4 países onde o autor trabalhou sobre manejo da fertilidade do solo.





Bandera	País	Producción (t)	Área cultivada (ha)	Rendimento (kg/ha)
	Panamá	130000	68903	1886,7
	Brasil	64143414	14958862	4288,0
	Paraguay	5152320	960000	5367,0
	Ecuador	1199075	378335	3169,3

Tabela No1. Produção de milho no Panamá, Brasil, Paraguai e Equador.

Fonte: Página de web modificado pelo autor

Especialmente, observou-se o menor rendimento no Panamá, levando em consideração alta doação de solo muito ácido tal como Ultissolo.

Atualmente, existem os maiores desafios da agricultura latino-americana. Segundo a publicação Perspectivas da Agricultura e do Desenvolvimento Rural nas Américas, o maior desafio para a agricultura na América Latina é alcançar uma produtividade sustentável. A ideia é que vantagens econômicas sejam distribuídas com igualdade entre os trabalhadores rurais. Apesar do grande avanço nas práticas agrícolas percebido nos últimos anos, o que se reflete no crescimento nas safras, o esforço ainda tem sido insuficiente para trazer uma melhoria real na realidade social dos cidadãos do campo. Um desenvolvimento realmente sustentável deve se basear em três eixos:

- Proteção ao meio ambiente;
- Inclusão social;
- Expansão econômica,

Como a opinião do autor, é necessário educacional para os jovens que querem realizar agricultura. Acompanhando essas dificuldades, vêm os desastres naturais, como inundações, secas e o fenômeno El Niño. Dessa forma, os desafios se acumulam, somados à crescente demanda populacional de alimentos.

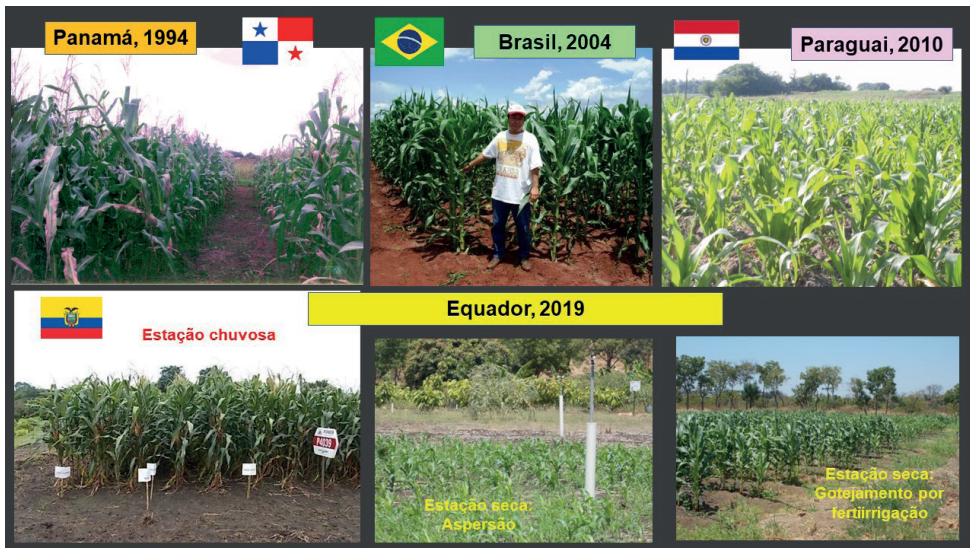


Foto N°1. Cultura de milho baixa condição de manejo da fertilidade do solo na Finca experimental em Panamá (1994), Brasil (2004), Paraguai (2010) e Equador (2019).

Para o milho, é um produto múltiplo, como dieta humana e animal e bioenergia... etc. De qualquer forma, é muito importante que se estabeleça um manejo integral da fertilidade do solo para a produção sustentável deste cultivo com baixos insumos e seleção do híbrido que pode-se adaptar em cada região. Levando em consideração as mudanças climáticas e a guerra (falta de materiais adubados)...etc, é necessário buscar o híbrido ou variedade adequado para o milho em cada solo com diferentes níveis de fertilizantes para estabelecer o sistema com baixos insumos.

O autor realizou várias pesquisas para o cultivo de milho no Panamá, Brasil, Paraguai e Equador como cultura básica (ver a Foto N°1) com os conpaheiros dos países. Neste artigo, explicou-se os resultados obtidos no Paraguai.



Figura Nº1. Situação do experimento na Cidade de Pilar no Departamento de Ñeembucú, Paraguai

MATERIAIS E MÉTODOS

Realizou-se o experimento num Entisol na Escola Agrícola San Isidro Labrador na Cidade de Pilar no Departamento de Ñeembucú, Paraguai (ver a **Figura Nº1**). Para isso, implementou-se um desenho experimental de parcelas divididas do campo da mesma Escola, onde plantou-se milho, híbrido TNH 0032100 e TNH 0033100 com diferentes níveis de adubação química nitrogenada no solo, a fim de assim comparar seu comportamento em resposta à adubação aplicada, consultando o método de Reyes (2003).

Adota-se uma metodologia quantitativa de acordo com os objetivos propostos, onde pretendeu-se avaliar a produtividade em função do híbrido da cultura do milho sob diferentes níveis de adubação de N.

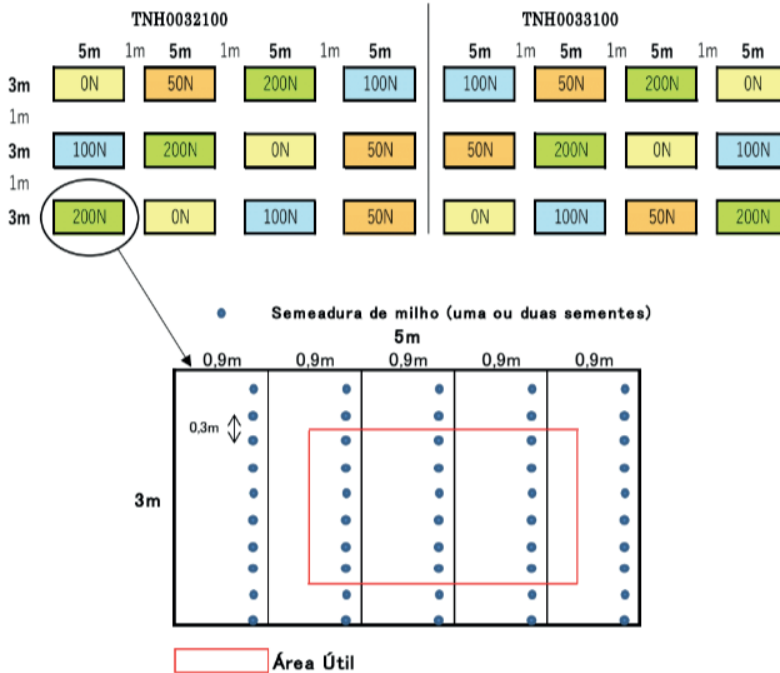


Figura N°2. Desenho experimental e área útil

Nesse arranjo, utilizou-se dois tamanhos de parcelas: a Parcela grande atribuída aos fatores (Híbrido) e o Sub-parcelas, atribuídas aos níveis do outro fator (Nitrogênio). A unidade de análise foi composta pelo Sub-parcelas, com uma população total de plantas de 2.400. A densidade de plantas foi a seguinte: 90cm entre fileiras e 30cm entre plantas, deixando 2 plantas por cova; obtendo assim 1200 plantas para cada Parcela grande e 100 plantas para cada Sub-parcela. O terreno principal tinha 264 m² (11m x 24m) para a variedade, e o Sub-Parcela de 15m² (5m x 3m) para níveis de adubação nitrogenada. A área útil era de 4,86 m² (2,7m x 1,8m), em que tiveram 36 (2 x 3 x 6) chumbadas em cada Sub-Parcela (Ver a **Figura N°2** e **Foto N°2**).



Foto Nº2. Cultura de Milho no campo experimental

Foto: um estudante, 2011

Como híbrido de milho, utilizaram-se TNH0032100 e TNH0033100, preparou-se como a Parcela principal. Por outro lado, como Sub-parcela, se preparam-se os 4 níveis do N tal como Tratamento preparam-se 0, 50, 100 e 200 kgN/ha.

Para a adubação do Fósforo e Potássio, levando em consideração os resultados da análise do solo da parcela experimental onde observa-se teor meio para P e o baixo para K no solo, respectivamente. (Ver **Tabela Nº2**); precedeu-se à adubação de superfosfato triplo (na forma de P_2O_5) e cloreto de potássio (na forma de K_2O) na dose de 50kg/ha em cada tratamento, antes da semeadura, para suprir a deficiência desses elementos e favorecer a absorção de N para obter rendimento ótima na cultura.

Para a adubação nitrogenada, utilizou-se Ureia para a cultura de milho. A adubação da Ureia realizou-se em três etapas; na época da semeadura, 30 e 60 dias após a semeadura.

Para Análise do solo antes da adubação e semeadura, utilizou-se água destilada (água: solo = 1:1) para determinar o valor do pH, e a solução extratora de Mehlich Nº 1 ($0,05M HCl + 0,0125 M H_2SO_4$) para determinar P e K disponíveis; a solução salina 1M KCl para determinação de Ca, Mg e Al trocáveis e o método de Walkley-Black (análise química) para determinação da matéria orgânica do solo. Além disso, o (H+Al) trocável determinou-se após a realização do método de pH_{SMP} (Shoemaker, Mclean e Pratt), usando a Tabela da interação entre o valor de pH_{SMP} e o (H+Al) trocável. Por outro lado, utilizou-se o sistema Bouyoucos para determinar o teor de argila (%) na análise física do solo no Laboratório da Fundação Nikkei - CETAPAR (Centro Tecnológico Agropecuário do Paraguai).

Para Análise do tecido vegetal do grão, determinou-se o percentual de umidade no grão de milho antes de realizar a análise do tecido vegetal na Fundação Nikkei-CETAPAR, e procedeu-se a pesar o grão fresco colhido para determinar o percentual de umidade de ambas os híbridos de milho TNH0032100 e TNH0033100, posteriormente colocou-se na estufa a 65°C por 3 dias, obtendo assim o peso seco, para determinar a % de umidade do grão colhido, e assim calcular o rendimento com 14% de umidade. As amostras secas utilizaram-se para determinar a porcentagem de proteína bruta (kg/ha) no grão de milho.

Para a Análise do tecido vegetal, enviaram-se amostras secas dos diferentes tratamentos à Fundação Nikkei-CETAPAR, na que utilizou-se o método de Kjeldahl, utilizando ácido sulfúrico para determinar o teor de $\text{NH}_4^{+}\text{-N}$ no tecido vegetal. Para a determinação da proteína bruta utilizou-se o valor de $\text{NH}_4^{+}\text{-N}$ x 6,25 (coeficiente protéico).

RESULTADOS E DISCUSSÃO

Análise do solo antes da adubação e semeadura

A Tabela N°2 mostra os resultados da propriedade física e química do solo antes de adubação e semeadura.

Amostra	pH (H ₂ O)	pH SMP	Argila	P	K	Ca	Mg	Al	H+Al	M.O.
			%	mg/kg	cmol _c /kg	cmol _c /kg	cmol _c /kg	cmol _c /kg	cmol _c /kg	%
Valor	5,70	5,64	16,7	44,0	0,08	0,37	0,11	0,12	6,70	1,56
Amostra	SB	CTC	V	m	CTCe					
	cmol _c /kg	cmol _c /kg	%	%	cmol _c /kg					
Valor	0,56	7,26	7,77	1,58	0,68					

Nota: M.O. = Matéria orgânica

Além disso, realizaram-se cálculos de:

Soma por bases (SB) = $\text{Ca}^{2+} + \text{Mg}^{2+} + \text{K}^{+}$

Capacidade de troca catiônica (CTC) = $\text{SB} + \text{H}^{+} + \text{Al}^{3+}$

Saturação por bases (V) (%) = $\text{SB} / \text{CTC} \times 100$

Saturação por Al (m) (%) = $\text{Al}^{3+} / \text{CTC} \times 100$

Capacidade de Troca Catiônica efetiva (CTCe) = $\text{SB} + \text{Al}^{3+}$

Tabela N°2. Análise do solo antes da adubação e semeadura

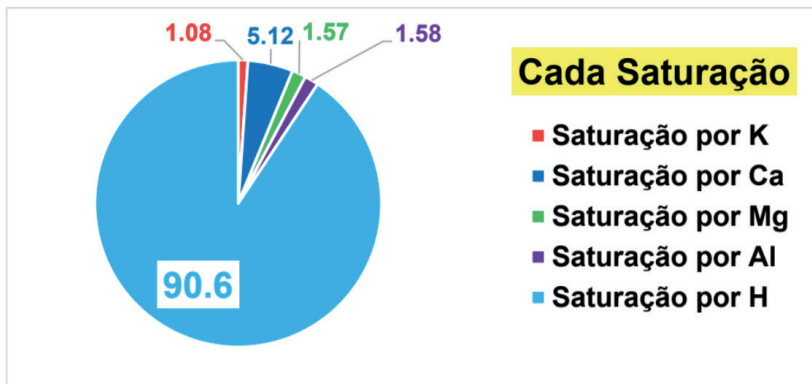
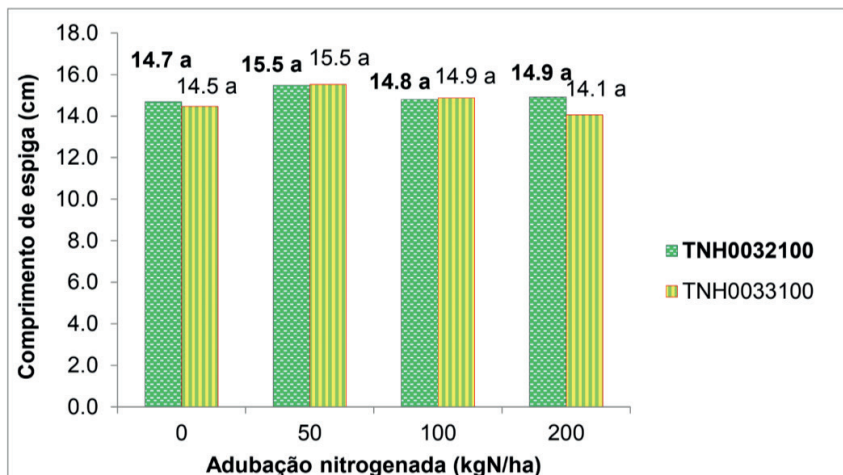


Figura N°3. Cada saturação por bases no solo

Pelo cálculo de cada saturação de acordo com a nota na Tabela N°1, são de 1,08 para a saturação para K, de 5,12 para a saturação por Ca, de 1,57 para a saturação por Mg, de 1,58 para a saturação por Al, de 90,6 para a saturação por H e de 92,2 (1,58 + 90,6) para a saturação por Al + H, respectivamente e A Figura N°3 mostra o gráfico redondo. Basicamente considera-se solo ácido com alto teor de H trocável e pode-se aplicar o trabalho para região de solos ácidos no Brasil.

Características agrônômicas da planta de milho - Comprimento da espiga (cm)

A Figura N°4 mostra a comparação do comprimento de espiga entre os dois híbridos em cada tratamento com diferentes níveis de N aplicado. Dos resultados da análise de variância, pode-se observar que não houve diferença significativa quanto ao comprimento das espigas para os diferentes tratamentos, porém, apresenta-se o maior valor meio no comprimento da espiga no duas variedades para o tratamento com 50kgN/ha, no entanto, tampouco observou-se estatisticamente significativa para sua diferença de comprimento sobre o teste de faixa múltipla de Duncan.

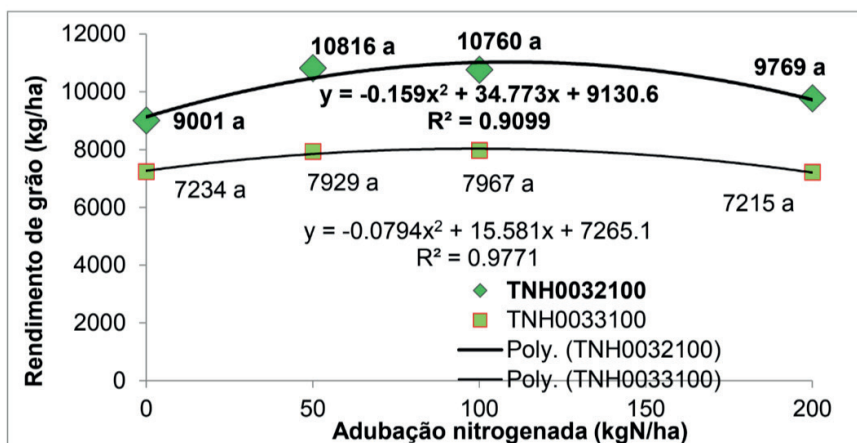


Nota: Médias com a mesma letra não são significativamente diferentes no teste de faixa múltipla de Duncan.

Figura N°4. Comparação do comprimento de milho entre os dois híbridos em cada tratamento com diferentes níveis de N aplicado

Rendimento do milho (kg/ha)

A Figura N°5 mostra a dinâmica do rendimento de grãos em cada híbrido de milho baixo condição de diferentes níveis de N aplicado. Dos resultados da análise de variância, observou-se a diferença significativa a 1% para o híbrido do milho e é superior para o híbrido TNH0032100.

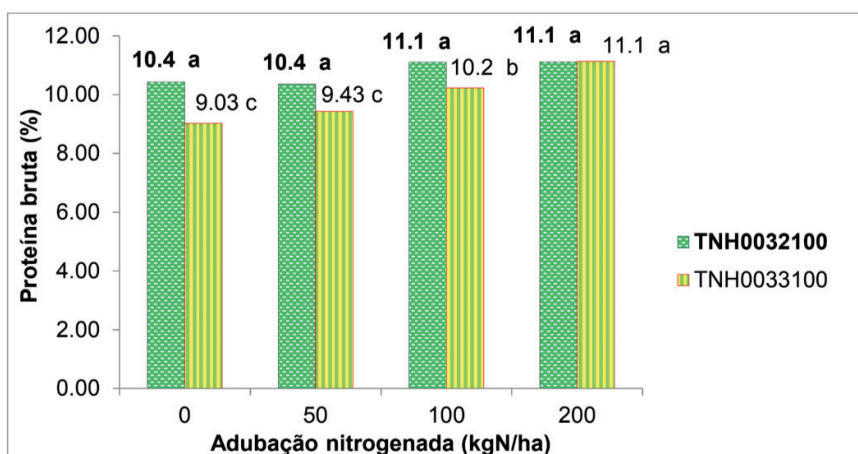


Nota: Médias com a mesma letra não são significativamente diferentes no teste de faixa múltipla de Duncan.

Figura N°5. Dinâmica do rendimento de grãos em cada híbrido de milho baixo condição de diferentes níveis de N aplicado

Análise do tecido vegetal no grão

A Figura N°6 mostra a comparação da proteína bruta (%) no grão entre os dois híbridos em cada tratamento com diferentes níveis de N aplicado. Dos resultados da análise de variância é possível apontar que houve diferença significativa a 1% não só para o híbrido sino também para os níveis de N aplicado em comparação com a testemunha. Pode-se observar uma alta porcentagem de proteína bruta nos tratamentos de 100 e 200kgN/ha para o híbrido TNH0032100, mas não observou-se a diferença significativa no Teste de faixa múltipla de Duncan. Nota-se também que o percentual de proteína bruta coincide nos dois híbridos (TNH0032100 e TNH0033100) com exceção do tratamento de 200kgN/ha.

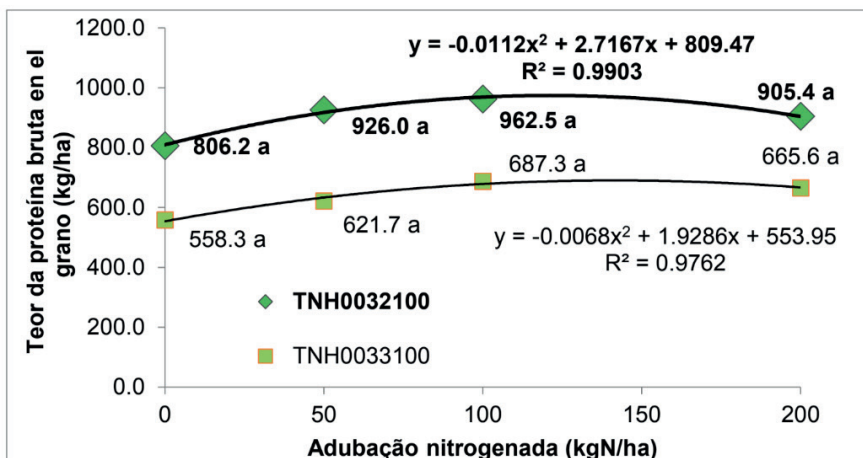


Nota: Médias com a mesma letra não são significativamente diferentes no teste de faixa múltipla de Duncan.

Figura N°6. Comparação da proteína bruta do milho entre os dois híbridos em cada tratamento com diferentes níveis de N aplicado

Teor de proteína bruta no grão (kg/ha)

A Figura N°7 mostra a dinâmica da proteína bruta no grão em cada híbrido de milho baixo condição de diferentes níveis de N aplicado. Dos resultados da análise de variância, observou-se a diferença significativa a 1% para o híbrido do milho e é superior para o híbrido TNH0032100 ao aplicar 50 a 100kgN/ha.



Nota: Médias com a mesma letra não são significativamente diferentes no teste de faixa múltipla de Duncan.

Figura N°7. Dinâmica do teor de proteína bruta no grão em cada híbrido de milho baixo condição de diferentes níveis de N aplicado

Recuperação eficiente aparente do N aplicado para cada híbrido de milho

Também, é muito importante avaliar a recuperação eficiente aparente do N.

Equação para determinar a recuperação eficiente aparente

$$\frac{\text{Absorção nitrogenada em cada tratamento (g)} - \text{absorção nitrogenada em testemunha(g)}}{\text{Aplicação nitrogenada (g)}} \times 100$$

$$= \text{Recuperação eficiente aparente do N (\%)}$$

Por exemplo, no tratamento 50kgN/ha para o híbrido TNH0032100, 926kg/ha / 6,25 (coeficiente protéico) = 148,2kg/ha como Nitrogênio amoniacal na Figura N°7.

Na Testemunha para mesmo híbrido, 806,2 / 6,25 (coeficiente protéico) = 129kg/ha como Nitrogênio amoniacal na Figura N°7.

De acordo com a equação, $(148,2 - 129) / 50 \times 100 = 38,4 \%$

Na Figura N°8 pode-se observar que a recuperação eficiente aparente de nitrogênio diminui à medida que aumentam as diferentes doses de N aplicado em ambos os híbridos de milho. A recuperação eficiência aparente do N para o híbrido TNH0032100 com 50kgN/ha foi de 38,3%, a qual obteve a maior porcentagem de recuperação entre todos os tratamentos. Atualmente, considerou-se alto teor de liberação de N inorgânico a partir da matéria orgânica quedada do solo dentro da cultura de milho e observou-se baixo valor para a recuperação nitrogenada aplicada de acordo com alta adubação nitrogenada.

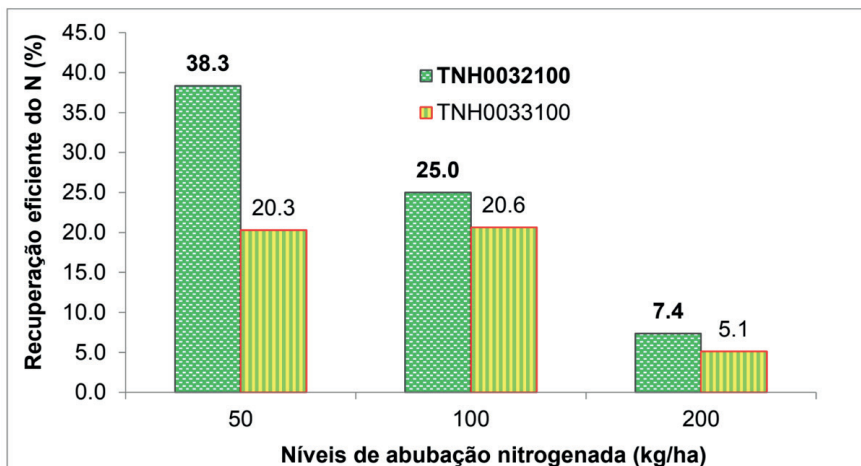


Figura N°8. Recuperação eficiente do N para cada híbrido

Os resultados obtidos (ver as Figuras N°5 e N°8), determinou-se a adubação econômica do N aplicado foi de 50kgN/ha para o híbrido TNH0032100.

CONCLUSÕES

De acordo com a análise dos dados obtidos durante a experimentação da cultura do milho, híbrido TNH0032100 e TNH0033100, com diferentes níveis de adubação nitrogenada (0kgN/ha, 50kgN/ha, 100kgN/ha e 200Nkg/ha), conclui-se que a diferença no rendimento entre os híbridos comparados resulta altamente significativa, onde destaca-se o híbrido TNH0032100, para adubação de 50kgN/ha; sendo também a combinação de híbrido e dose de N na qual apresenta maior rentabilidade (ao contrário com outras combinações); enquanto o híbrido TNH0033100, obteve maior rendimento com a adubação de 100kgN/ha, mas do ponto de vista econômica o tratamento que proporciona maior rentabilidade para o híbrido é 50kgN/ha, mesmo assim comparando os dois híbridos em estudo como aponta-se no híbrido TNH0032100 a que oferece maior retorno e determinou-se 50kgN/ha como a adubação econômica para o mesmo híbrido.

Atualmente, considerou-se alto teor de liberação de N inorgânico da matéria orgânica quedada a partir do solo dentro da cultura de milho e observou-se baixo valor para a recuperação nitrogenada aplicada de acordo com alta adubação nitrogenada.

AGRADECIMENTOS

Para este trabalho foi tese de María Lorena Galeano na Faculdade de Ciências Agropecuárias e Desenvolvimento Rural, Universidade Nacional de Pilar, Paraguai.

O autor dedicou-se à orientação com o Eng. MSc. Juan Alberto Bottino Fernández, e Eng. MSc. Gustavo Adolfo Retamozo Gimenez. Agradece aos paraguaios desenvolver a experiência educacional para o autor.

Atualmente, é muito importante realizar a produção sustentável de milho com baixos insumos e selecção de híbrido ou variedade que pode-se adaptar em cada região como um produto múltiplo como dieta humana, animal doméstico e bioenergia... etc. O autor traduziu do espanhol para o português para estender aos países de América Latina com o Brasil para este artigo.

Após a tradução do espanhol para o português pelo autor, o **Sr. Fernández Kondo** revisou a tradução para o português. Agradecê-lo.

REFERÊNCIAS

Arévalos, P., y Sunaga, S. 1999. Estudio de Suelo” Características Físico – Químicas de los Suelos del Área del Proyecto DERMASUR”. Proyecto de Desarrollo Rural y Mejoramiento Ambiental del Sur de Pilar (DERMASUR). 39 p.

Arias, Jiménez. C. A. 2007. Suelos Tropicales. Editorial Universidad Estatal a Distancia. San José, Costa Rica. 90-91.

Binder, D.L., D.H. Sander, and D.T. Walters. 2000. Maize response to time of nitrogen application as affected by level of nitrogen deficiency. *Agron. J.* 92: 1228-1236.

Bundy, L.G., and P.R. Carter. 1988. Corn hybrid response to nitrogen fertilization in the Northern Corn Belt. *J. Prod. Agric.* 1: 99-104.

Carlone, M.R., and W.A. Russell. 1987. Response to plant densities and nitrogen levels for four maize cultivars from different eras of breeding. *Crop Sci.* 27: 465-470.

Cox, W.J., S. Kalonge., D.J.R. Cherney, and W.S. Reid. 1993. Growth, yield and quality of forage maize under different nitrogen management practices. *Agron. J.* 85: 341-347.

Fiorenti, C. F. 2011. Influência da adubação nitrogenada no cultivo do feijoeiro em sucessão al milho e à Braquiária em sistema de semeadura direta. Universidade Estadual Paulista (UNESP) “Júlio de Mesquita Filho” Faculdade de Ciência Agrárias e Veterinárias Câmpus de Jaboticabal – SP-Brasil 86 p.

Galeano Lobos ML. 2012. Evaluación del rendimiento del cultivo de maíz (*Zea mays*) variedad híbrida TNH 0032100 y TNH0033100, con diferentes niveles de aplicación de Nitrógeno. 62 p. (Tesis de la Facultad de Ciencias Agropecuarias y Desarrollo Rural, Universidad Nacional de Pilar en el Departamento de Ñeembucú, Paraguay).

Jahn, E., P. Cofre, y P. Soto. 1995. Calidad del ensilaje de maíz con diferentes poblaciones y niveles de fertilización nitrogenadas. p. 23-24. XX Reunión Anual SOCHIPA A.G., Coquimbo, Chile. 19 y 20 de octubre. Sociedad Chilena de Producción Animal, Temuco, Chile.

<https://www.atlasbig.com/pt-pt/paises-por-producao-de-milho>

<https://blog.jacto.com.br/conheca-o-panorama-da-agricultura-na-america-latina/>

Hanway, J.J. 1963. Growth stages of corn (*Zea mays* L.). *Agron. J.* 55: 487-492.

Muchow, R.C., and T.R. Sinclair. 1994. Nitrogen response of leaf photosynthesis and canopy radiation use efficiency in field-grown maize and sorghum. *Crop Sci.* 34: 721-727.

Proaño, J. y K. Tomita. 2021. Evaluación de tres híbridos de maíz con niveles nitrogenados durante la estación lluviosa, Guayaquil, Ecuador. *ALFA, Revista de Investigación en Ciencias Agronómicas y Veterinarias enero-abril Vol. (5) (13):* 53-64.

Reyes, P. 2003. *Diseño de Experimentos Aplicados*, México: Trillas. 61-68, 219-244.

Scharf, P.C., W.J. Wiebold, and J.A. Lory. 2002. Corn yield response to nitrogen fertilizer timing and deficiency level. *Agron. J.* 94: 435-441.

Sinclair, T.R., and R.C. Muchow. 1995. Effect of nitrogen supply on maize yield. I. Modeling physiological responses. *Agron. J.* 87: 632-641.

Tomita, K., E. Márquez, C. Pardo y R. Sánchez. 2001. Efecto de cal y fósforo en el cultivo de maíz variedad Across-7728 y Guararé-8128 en un Ultisol, Panamá (Variedad adaptable para agricultores pequeños y aplicación económica de materiales). *Sociedad Colombiana de la Ciencia del Suelo. Bogotá, Colombia. Suelos Ecuatoriales. Vol. 31 (1):* 14-25.

Tomita, K. 2021. Efecto de la aplicación de yeso en el cultivo de girasol (*Helianthus annuus*) y maíz (*Zea mays*) en un suelo Oxisol (Rhodic Kandudox), Yguazú, Alto Paraná, Paraguay. *Agrárias: Pesquisa e Inovação nas Ciências que Alimentam o Mundo VII. Editora Artimes.* 156-168.

Tomita, K. y J. Proaño. 2022. Evaluación del rendimiento del grano con los tres híbridos asociados con tres niveles de la fertilización nitrogenada en el cultivo de maíz entre la aspersión y goteo por fertiriego durante la estación seca en un suelo Vertisol. *Agrárias: Pesquisa e Inovação nas Ciências que Alimentam o Mundo VIII. Editora Artimes.* 200-208.

Valarezo Torres PV. 2017. Evaluación de tres híbridos de maíz *Zea mays* L. con tres distancias de siembra (Bachelors thesis, Facultad de Ciencias Agrarias Universidad de Guayaquil). 44 p.

¿SE JUSTIFICA LA OVARIECTOMÍA EN VACAS? PRINCIPALES RIESGOS Y BENEFICIOS

Acceptance date: 02/05/2024

Gabriel Gerardo Aguirre Espíndola

Maestría en Ciencias
Departamento de Rumiantes
Facultad de Medicina Veterinaria y
Zootecnia. BUAP
<https://orcid.org/0009-0009-7101-7946>

Felicitas Vázquez Flores

Maestría en Ciencias
Departamento de Genética y Reproducción
<https://orcid.org/0000-0003-1267-1772>

Néstor Méndez Palacios

Doctorado en Ciencias
Departamento de Genética y
Reproducción
Facultad de Medicina Veterinaria y
Zootecnia. BUAP
<https://orcid.org/0000-0001-8985-7008>

Sandra Ortiz González

Doctorado en Ciencias
Departamento de Producción
Facultad de Medicina Veterinaria y
Zootecnia. BUAP
<https://orcid.org/0009-0007-4036-5310>

José Alfredo Galicia Domínguez

Doctorado en Eco-educación.
Facultad de Medicina Veterinaria y
Zootecnia. BUAP
<https://orcid.org/0000-0002-9117-7746>

RESUMEN: La ovariectomía bilateral, castración o esterilización, es un procedimiento quirúrgico que consiste en extirpar los ovarios, mediante diferentes técnicas siendo utilizado por productores de los sectores de vacas lecheras y vaquillas en corrales de engorde. La técnica previene la gestación y elimina la principal fuente de estrógeno a nivel ovárico. Las ventajas para las vaquillas esterilizadas incluyen: prevención de novillas preñadas en el corral de engorde, eliminación de aditivos alimenticios que suprimen el estro, no requiere de prueba de brucelosis y tuberculosis lo que facilita su movilización por transporte fuera del país, mejor ganancia diaria promedio y conversión alimenticia en comparación con novillas sin castrar, y las vacas adultas castradas obtienen mejor rendimiento de la canal y marmoleo de grasa en la masa muscular, generando una mejor relación costo beneficio al sacrificio. La Asociación Americana de Medicina Veterinaria, recomienda que se utilice la técnica aséptica adecuada y la sujeción en todos los procedimientos, y que la ovariectomía de flanco realizada sin anestesia es inhumana. El dolor y la incomodidad deben minimizarse tanto como sea posible antes, durante y después del

procedimiento quirúrgico. Realizar un diagnóstico de preñez antes de esterilizar las vaquillas podría reducir el riesgo de complicaciones.

ABSTRACT: Bilateral ovariectomy, castration or sterilization, is a surgical procedure that consists of removing the ovaries, using different techniques, being used by producers in the sectors of dairy cows and heifers in feedlots. The technique prevents pregnancy and eliminates the main source of estrogen at the ovarian level. The advantages for sterilized heifers include: prevention of pregnant heifers in the feedlot, elimination of feed additives that suppress estrus, does not require testing for brucellosis and tuberculosis, which facilitates their mobilization for transport outside the country, better average daily gain and feed conversion compared to non-castrated heifers, and castrated adult cows obtain better carcass yield and fat marbling in muscle mass, generating a better cost-benefit ratio at slaughter. The American Veterinary Medical Association recommends that proper aseptic technique and restraint be used in all procedures, and that flank oophorectomy performed without anesthesia is inhumane. Pain and discomfort should be minimized as much as possible before, during, and after the surgical procedure. Performing a pregnancy diagnosis before sterilizing heifers could reduce the risk of complications.

KEYWORDS: ovariectomy, risks and benefits.

INTRODUCTION

It is recognized that castration of cattle is important to inhibit heat behavior, improve the characteristics and quality of meat, and increase health security, by allocating cattle only for supply. Castration procedures cause pain and discomfort and studies indicate that preoperative use of non-steroidal anti-inflammatory agents as well as local anesthetics reduces the pain and distress associated with castration. The veterinarian should counsel clients and advocate for the use of procedures and practices that reduce or eliminate pain and distress (AVMA 2014). These include low-stress coping techniques and the use of clinically effective medications approved or permitted by the association. There are a number of acceptable castration techniques, as well as numerous pain management protocols considering relevant variables such as the animal's age, weight, temperament, skill level of the operator/technician, environmental conditions, available facilities and the human and animal safety (AVMA 2014).

MATERIALS AND METHODS

Ovariectomy in cows can be performed through a flank or vaginal approach. The flank approach begins with adequate asepsis, local anesthesia or block, and incising the left paralumbar fossa. (Coetzee, *et.al.* 2010), After opening the different surgical planes with an incision of approximately 15 cm, the hand is introduced to identify the ovaries and amputate with scissors or an ovariectomy instrument. The vaginal approach is performed by isolating the ovaries by rectal palpation. (Bronzuoli, Carlos. M. 2009). Disinfection of the vulva and

perineal region is performed, a low epidural block is applied, and a sterilization instrument is introduced into the vagina. A colpotomy incision is created by introducing the sterilization instrument through the vaginal fornix dorsolaterally to the cervix. There are variations in the world of vaginal methods (colpotomy) that act through the uterus to remove the ovaries or prevent blood supply to the fallopian tubes. Dutto ovariometer (Bronzuoli, Carlos M. 2009), Webb of the fallopian tube, Willis castrator (Petherick, *et.al.* 2014), Kimberly-Rupp cylindrical, Resinger and Richter epheminator with emasculating forceps, Riliu ovariometer for lassoing with clamp, linear strangulation with the Chassaignac fine chain bruiser, seal castration with nylon plastic clamp, linear extraction, (AVMA 2011). The surgical wound is inside the uterus, without sutures and not external to the skin (Kimberling and Rupp 1980). There is no damage to the skin, nor loss of meat in the carcass due to the scar (Moran, *et.al.* 2016). Animals recover in 4 days after surgery. They are faster procedures than flankotomy and reduce suffering and collateral damage. It is important to carry out the application of surgical protocols or procedures in relation to cleaning, disinfecting and shaving the incision site with a scalpel, manually locating the left ovary, introducing scissors for cutting and extraction, locating the right ovary, cutting and extraction. Both ovaries are checked. The superficial plane or skin is stapled and the muscles and peritoneum are not sutured and the wound is healed with methylene blue. The technique is widely accepted among surgeons

RESULTS

The different surgical procedures have been peer reviewed by the Animal Welfare Division of the American Veterinary Medical Association (AVMA), where you can mainly see a review of the scientific literature, which includes information obtained from proprietary data, legislative and regulatory review, market conditions and academic ethical evaluations. For the specific case of collection and transfer of cattle for completion in the United States, the dominant method authorized by the USDA is Flancotomy or extraction of the two ovaries with a 15-20 centimeter incision on the left flank, after asepsis of the paralumbar fossa and shaving of the area (Flores Delgado, Pamela. 2013). An aponeurosis is made by cutting the transversus abdominis muscle, it is manually explored in the peritoneum of the abdominal cavity until the ligament of the two ovaries is cut with curved serrated scissors or another oophorectomy instrument (Rizzo, *et.al.* 2016).

DISCUSIÓN

The AVMA recognizes that castration procedures cause pain and discomfort, and studies indicate that preoperative use of nonsteroidal anti-inflammatory agents and local anesthetics reduces the pain and distress associated with castration. The veterinarian should always counsel clients and advocate for the use of procedures and practices that

reduce or eliminate pain and distress. These include low-stress coping techniques and the use of approved or permitted clinically effective medications. Complications of transvaginal sterilization include hemorrhage and peritonitis; both can be fatal (Urrutia, *et.al.* 2012). Peritonitis can be caused by unintentional intestinal or rectal perforation (Piccinali, Ricardo. 2013). The mortality rate is much higher when the surgeon is inexperienced (Cattle Standards and Guidelines. 2013). Hemorrhage is more significant when the animal is pregnant, due to increased blood flow and the risk of cutting the uterine arteries (Cala. *et.al.* 2008). Castrating heifers is required, because in the USA the import of animals with reproductive capacity that are not genetically pure of a superior breed that allows genetic improvement is not allowed, even when their destination is the feedlot for finishing and slaughter (Gastelum. 2011). When considering the potential zoonotic risks, females and males should be castrated to the quarantine station on the border with the USA or many other international markets (Caceres. 2016). During pregnancy, Brucellosis increases the possibility of developing and spreading to other animals in the pen due to placentitis during parturition (Gastelum. 2011). The USA does not want to run the risk of an unexpected outbreak, which prevents them from exporting their meat to other parts of the world (SENASICA. 2015).

CONCLUSIONS

Little research and formal animal welfare assessments have been published regarding the pain and discomfort experienced by bovids during and after sterilization (Moran. 2016). Clinical signs that have been interpreted as indicators of postsurgical discomfort include stiff walking, prolonged prostration as well as raised head and tail (Ruiz. *et al.* 2015). In one study, the discomfort experienced by animals was considered comparable to that following normal rectal palpation (Prado. 2016). However, it should be noted that behavioral responses to painful stimuli may not be proportional to the degree of pain experienced. Pain is also evident when there is trauma to the vaginal wall due to childbirth, and the use of epidural anesthesia is common when procedures are performed to repair this tissue. Blood cortisol concentrations have been studied as indicators of physiological stress in animals, and it was found that, regardless of whether the oophorectomy was performed through the vaginal or flank approach, cortisol concentrations increased after the procedure (Rizzo *et al.* 2016). This study also evaluated serum haptoglobin concentrations, which in cattle may indicate a systemic inflammatory response. Serum haptoglobin concentrations were significantly higher in flank-spayed heifers compared to vaginally spayed heifers 8 to 96 hours after the procedure (Rizzo. *et.al.* 2016). Haptoglobin did not increase significantly in vaginally spayed heifers.

REFERENCES

- American Veterinary Medical Association. 2014. Literature review on the welfare implications of castration of cattle. 10 págs.
- Bronzuoli, Carlos M. 2009. Eficiencia de la castración método de Dutto.
- Caceres, Carlos V. 2016. Ventajas y desventajas en castración de bovinos.
- Cala, Ricaurte, Andrés Mauricio, Díaz Granados Ximena Gónima. 2008. Comparación entre el método tradicional de castración de campo VS castración quirúrgica convencional, evaluando el rendimiento en kilos y el bienestar animal. Universidad la Salle, Bogotá.
- Cattle Standards and Guidelines. 2013. Castration discussion paper. Australia. Febrero.
- Coetzee, Johann F., Nutsch Abbey L., Barbur Laura A., Bradburn Ryan M. 2010. A survey of castration methods and associated livestock management practices performed by bovine veterinarians in the United States. BMC Veterinary research 6:12.
- EFSA. 2012. Panel on animal health and welfare (AHAW); scientific opinion on the welfare of cattle kept for beef production and the welfare in intensive calf farming. EFSA Journal 10(5):2669. 166 páginas.
- Flores Delgado, Pamela. 2013. Exportación de bovino de carne de México. Tesis. Universidad Autónoma Antonio Narro.
- Garnero, Oscar J., Perusia Oscar R. 2016. Manual de anestésicos y cirugías de bovinos: Cirugías del aparato reproductor de la hembra. Universidad Nacional del Litoral, Santa Fe Argentina.
- Gastelum, Peralta José Ramón 2011. Experiencias en el control y erradicación de la brucelosis en el estado de Sonora. Castración de vaquillas: Alternativa para su comercialización en el Mercado de exportación
- Marroquín Pérez, Oscar Fernando. 2015. Evaluación de ganancia de peso en novillas de engorde con ovariectomía por vía transvaginal VS novillas tratadas con boldenona. Universidad de San Carlos de Guatemala. Octubre.
- Moran, María Celeste, Gual Fernando, Moscuzza Hernan, Garcia Espil Alberto. 2016. Técnicas quirúrgicas de ovariectomía en vacas de descarte y sus implicaciones productivas.
- Paiva Jorge, Cardozo Daniel S/F. Efecto de la castración en vaquillas suplementadas mantenidas a campo sobre la ganancia de peso hasta su terminación. FCA-Universidad Nacional de Asunción, Paraguay. Investigación Agraria. Vol. 4 Núm. 2 Pág. 15-18.
- Peiró Juliana R., Nogueira M., Nogueira Guilherme P., Perri Silvia H.V., Cardoso Daniel. 2009. Ovariectomy by flank approach in prepubertal Nelore (*Bos indicus*) heifers. The Canadian J. of Vet. Research 73:237-240.
- Piccinali, Ricardo. 2013. Descripción de una técnica quirúrgica para castrar vacas. Nota técnica. XLI Jornadas Uruguayas de Buiatría. EEA Concepción del Uruguay (INTA), entre Ríos. Pág. 137-139.
- Prado, Tulio M., Schumacher Jim, Dawson Lionel J. 2016. Surgical procedures of the genital organs of cows. Vet. Clin, Food. Anim 32: 727-752.

Quezada Parra, álvaro. 2013. Parámetros productivos de las becerras castradas para exportación. Universidad Autónoma Agraria Antonio Narro Unidad Laguna. Junio.

Rizzo A., Piccinno M., Lacitignola L., Sciorsci R.L. 2016. Application of an innovative technique for unilateral ovariectomy in dairy cows. Veterinary Record. Noviembre 5.

Ruiz Villa, Sandra Yolima 2015. Ovariectomía en bovinos. Universidad Tecnológica de Pereira, Colombia.

SAGARPA-SENASICA, 2013. Exportación de ganado bovino en pie a los EUA. Año2 Núm. 17. 21 de noviembre.

Urrutia C., Natalie 2012. Técnicas de castración de Ganado bovino. Pennsylvania State University. Junio.

GASTROINTESTINAL HELMINTHS OF SHEEP BREED IN SPREAD BELGRADE AREA

Acceptance date: 02/05/2024

Ivan Pavlovic

Academician, principal research fellow

Aleksandra Tasic

Research fellow, Scientific Institute of
Veterinary Medicine of Serbia

INTRODUCTION

Breeding of sheep represents a significant branch of livestock production. Despite the fact that the number of sheep in the social and individual sector of production varies from time to time, this branch of the economy and its improvement is given exceptional attention. The reason for this lies not only in tradition, but also in the knowledge that the breeding of small ruminants represents national wealth, both due to the production of wool and milk, as well as sheep and lamb meat, which is a highly sought after item on the world market.

The improvement of this production is related to solving a number of different problems, which aim primarily to increase

the economy while preserving the health and well-being of the animals. As part of these tasks, primary attention must be paid to health care.

There are many factors that contribute to the appearance, maintenance and spread of parasitosis. Among them are: joint keeping of animals of different age categories, joint grazing of animals of different age categories often of different owners, a large number of animals of older categories - carriers of a significant number of parasites, favorable climatic conditions for the development and survival of pararasitic stages and transitional hosts that are necessary for the development of certain a type of parasite in the external environment, and therefore the infection of animals.

The absence or inadequate implementation of control measures also contributes to the prevalence of parasitosis. The lack of enlightenment of the population, primarily livestock farmers, is one of the significant factors in the epizootiology of these diseases. The grazing diet allows

sheep to have constant contact with transitional hosts (oribatids, molluscs, etc.) and eggs and larval forms of parasites, so that there is no sheep that is not infected with at least one parasite species. Based on research in the world and in our country, diseases of parasitic etiology dominate in sheep both in terms of prevalence and incidence, and are accompanied by significant morbidity and moderate mortality.

The damage that occurs in sheep production is a consequence of the negative pathogenic effects of parasites on the host organism. In favorable conditions for the development and survival of the preparasitic stages in the external environment, conditions are created for infections of greater intensity, often with a greater number of parasite species, with different localization in the host organism. The consequence of this is the development of a clinically manifested disease, with the death of a large number of individuals, most often among the younger categories. The fact is, however, that in most cases, parasitic infections occur subclinically, that is, “imperceptibly” to the eye of the herdsman. Negative economic effects are also present in these situations and are manifested by a decrease in animal production, i.e. a decrease in the production of wool and milk, a poorer upbringing of the young, a decrease in general body resistance, i.e. an increased susceptibility to agents of other etiologies.

GASTROINTESTINAL HELMINTHS OF SHEEP

Distribution of gastrointestinal helminths in sheep

Numerous literature data support the fact that gastrointestinal helminths is the most common parasitic disease of small ruminants. All over the world, an extremely large number of older animals are infected with a large number of species of nematodes that participate in the etiology of this disease. It is of even greater importance that the infection of the young that occurs already in the first months of life. At the same time, adults are the main contaminant of pastures and are responsible for maintaining parasitic infections in herds. Parasitic gastroenteritis of ruminants is a disease caused by a large number of nematode species from the genera *Strongilidae*, *Trichostrongylidae* and *Ancylostomidae*. The disease is related to the grazing diet and the biological cycle of the parasite, which takes place without transitional hosts.

These studies were carried out in the north of Europe, where sheep and goat breeding were developed. In southern Greenland, sheep were dominated by *Nematodirus* spp. *Trichostrongylidae* spp. and *Ostertagia* spp. In Iceland, during the slaughter season, which lasts from September to October, they examined the kid and established an infection with *Teladorsagia* spp., *Trichostrongylus* spp., *Nematodirus* spp. *Oesophagostomum* spp. In Sweden, they investigated the distribution and eradication possibilities of *Haemonchus contortus*, and in Poland, they investigated the excretion of parasite eggs in sheep feces

and their role in pasture contamination, as well as the prevalence of certain helminthosis in sheep herds in that country.

In Western Europe, these studies were most present in countries with a long tradition of sheep production. In the British Isles, in Scotland, researches have shown the influence of climatic variations on the prevalence and distribution of certain helminthosis of sheep, especially *Haemonchus contortus*, *Nematodirus* spp. and *Trichostrongylus* spp., while studies of the natural dynamics of occurrence of gastrointestinal nematodes in sheep in England have established that it is more similar to the dynamics of occurrence in the continental part of Europe than in other parts of the British Isles. In France) found that in small ruminants the highest prevalence of nematodes from the genera *Ostertagia* spp., *Trichostrongylus* spp., *Nematodirus* spp., *Bunostomum* spp., *Capillaria* spp. and *Trichuris* spp. Differences in the distribution of individual types of helminths in the investigated areas were not significant due to the uniform microclimate in them.

In the Mediterranean area, where goat and sheep breeding is one of the main economic branches, extensive research was also conducted that monitored the prevalence of helminths in sheep during the grazing season, concluding that the seasonal variation is directly correlated with external conditions and that it reaches its peak at the beginning and end of the grazing season and that it depends on the type of helminth. In sheep and goats in Sicily, they established the presence of *Trichostrongylus* spp., *Nematodirus* spp., *Oesophagostomum* spp. and *Chabertia ovina*. In Spain, they investigated the etiology and epizootiology of gastrointestinal strongylids in the Toledo area and followed the occurrence of these parasites in goats in the dry areas of central Spain. The conclusion is that the seasonal distribution is certainly more significant in areas that are less susceptible to drying out and that are not conducive to the development of trichostrongylid larvae.

In the Western Balkan these studies were carried out in Macedonia, Bulgaria and Romania. The obtained results indicate that in lambs, infection with gastrointestinal nematodes is established very quickly after leaving for pasture, and that polyparasitism occurs as a regular occurrence in sheep and goats and is caused mostly by nematodes from the genus *Nematodirus* spp. *Trichostrongylidae* and *Strongyloides* spp.

In the Middle East, these studies are also in the center of attention due to the large number of herds of goats and sheep conditioned by climatic and religious factors. In Syria, the presence of *Haemonchus contortus*, *Ostertagia* spp. and *Trichostrongylu* spp. as the dominant parasite species of sheep and goats, and in sheep in Jordan, they came to the conclusion that representatives of the same genera dominate here as well. Research into the helminofauna of sheep and goats in Turkey has established that it does not fundamentally differ from neighboring countries in terms of composition and seasonal distribution.

African countries also have widespread farming of small ruminants. Climatic (deserts, semi-deserts, droughts, etc.), religious (large number of Muslim population) and economic conditions (most of the population is on the brink of existence and keeping goats or sheep

is the most profitable form of livestock production with the least investment) caused the presence of a large number of small herds along the entire African continent. In Morocco and Egypt, representatives of the genera *Haemonchus*, *Ostertagia* spp., and *Trichostrongyloidea* were identified as the most numerous species. In Ethiopia and Nigeria, he established the presence of *Haemonchus contortus*, *Trichostrongylus* spp., *Oesophagostomum* spp., *Cooperia* spp. and *Bunostomum trigonocephalum*. In his research, he states that mixed infections were the most common. In sheep in the southern forest regions of Ivory Coast, infections with *Haemonchus contortus*, *Trichostrongylus* spp., *Oesophagostomum* spp., *Cooperia* spp. and *Nematodirus* spp. as well as in sheep in Kenya and Cameroon. An identical faunistic composition of the parasite was also found in the very south of Africa, in sheep, goats and unborn lambs.

In Asia, sheep farming conditions are similar to helminth species. One part of the findings refers to research carried out in the former USSR, and then in Russia. However, the countries of the Indian subcontinent, Pakistan, India and Bangladesh, are certainly leading in this regard. In that area, the dominant species of nematodes are from the genera *Haemonchus*, *Trichostrongylus* spp., *Oesophagostomum* spp. and *Nematodirus* spp. In goats in Mongolia, the highest prevalence and intensity of infections was caused by nematodes from the genera *Ostertagia* spp., *Marshallagia* spp., and *Nematodirus* spp. In the People's Republic of China, research indicated that the highest prevalence and intensity of infections was with *Ostertagia* spp., *Trichostrongylus* spp., *Trichocephalus* spp. and *Nematodirus* spp.

The American continent is not spared from the presence of gastrointestinal strongylids. They are a constant health problem for sheep and goats. In the USA, a large number of researches were carried out as part of the US Food and Drug Administration experiment. The conclusion of the research is that the most common gastrointestinal strongylids from the genera *Nematodirus* spp. *Trichostrongylidae*, *Haemonchus contortus* and *Strongyloides papillosus*.

In South America, the situation is very similar. Small herds of sheep and goats are most often present along the high slopes of the Andes, and in smaller numbers on the pampas plains. As for the seasonal dynamics, it is identical to that occurring in Central European herds, and the parasitofauna is within the known strongylid species (*Ostertagia*, *Teladorsagia*, *Marshallagia*, *Haemonchus contortus*, *Nematodirus*, *Trichostrongylus*, *Bunostomum*, *Cooperia*, and *Oesophagostomum*), with several species within them, whose distribution is within the ecological valence of the environment.

In Australia, the dominant parasitic species are represented by certain species from the genera: *Ostertagia* spp., *Haemonchus*, *Trichostrongylus* spp., and *Chabertia*, while in certain areas of New Zealand representatives of the genera dominate: *Ostertagia* spp., *Cooperia* spp., *Oesophagostomum* spp., *Trichostrongylus* spp., and *Bunostomum* spp.

Biology characteristic of gastrointestinal helminths in sheep

Parasitic gastroenteritis of ruminants is a disease caused by a large number of nematode species from the genera *Strongilidae*, *Trichostrongylidae* and *Ancylostomidae*. The disease is related to the grazing diet and the biological cycle of the parasite, which takes place without transitional hosts.

Those genera include numerous subgenera, such as *Ostertagia*, *Teladorsagia*, *Haemonchus*, *Trichostrongylus*, *Cooperia*, *Nematodirus*, *Mecistocirrus*, *Bunostomum*, *Oesophagostomum*, and *Chabertia*. At the typical diagnostic stage, these trichostrongyle parasites (eggs on fecal flotation) are indistinguishable. Their life cycles are generally the same, differing only in slight nuances, such as the length of each stage, their location in the GI tract of adults, or their preferred environmental temperature ranges. Life cycles of all the trichostrongyles are direct and rely on animals to graze contaminated pastures to initiate infection. For these reasons, their diagnosis, treatment, control and prevention are very similar.

The developmental cycle of the parasite is straightforward. Females lay eggs that are eliminated in the external environment with feces. It is considered that the most fertile female is *H. contortus*, which lays 5,000-10,000 eggs per day. The eggs are of the strongylid type, 60-110 μm in size, with 8-16 (32) blastomeres. Eggs of *Nematodirus* spp. are 2 times larger, 130-260 μm , with 4-8 blastomeres. L3 of this species develop in eggs. In the external environment, embryogenesis, hatching of larvae, their molting and formation of infectious L3 occur. The infection is peroral through contaminated food or water, L3 enter the wall of the appropriate part of the digestive tract - the histotropic phase of development, and then return to the lumen and become adults. *Bunostomum* spp. percutaneous and p/o a L3 infection follow pulmonary-tracheal migration. The prepatent period is different for different species: *Oesophagostomum* usually 4-6 weeks, *Chabertia* 5-7 weeks, *Trichostrongylidae* 2-3 weeks, (if hypobiosis of the larvae in the mucosa has occurred, then adults emerge 4-6 months later). *Bunostomum* 40 -60 days. Parasites live in the host for several months to 1-2 years.

The development of eggs and larvae, as well as the activity of L3, depends on temperature, humidity, oxygen and sunlight. Temperature: *H. contortus* optimal temperature for egg embryonation and formation of L3 is 35°C; eggs of *Ostertagia*, *Trichostrongylus* and *Oesophagostomum* spp. embryonate at a temperature below 26°C, but for a longer time. They are more resistant to lower temperatures and desiccation - embryonation of eggs and hatching of larvae takes place even at 5°C. Eggs of *Ostertagia* spp. survive 20 days at -5°C. For embryonated eggs of *Nematodirus* spp. the optimal temperature is 21°C.

Embryonic development in eggs depends on soil moisture, atmospheric precipitation (dew). The position of the eggs in the breech, i.e. blocks of faeces, helps the survival of the eggs in many ways. Infectious L3 are negatively geotropic and positively phototropic to

moderate sunlight intensity. The humidity of the environment is necessary for their activity and the length of their survival in the external environment depends on it - during the winter and hot summers, the largest number of larvae die. The most infectious larvae are found on plants in April and September, less in May and October, and the least in July and August.

In natural conditions, every animal is infected - constant contamination of the pasture. This is contributed by the increased susceptibility of the already infected herd, the introduction of susceptible animals into the infected herd and the increase in the intensity of the infection in the already infected herd. Immunity is developed through continuous infections and then there is the elimination of the present parasites (self cure mechanism), complete or partial inhibition of the development of newly introduced larvae (spring rise) and complete or partial inhibition of the reproductive abilities of female parasites (spring rise). The seasonal dynamics of certain types of parasites, the degree of infection and the occurrence of diseases vary not only in different areas but also in the same area during the year. The parasite-host relationship is complex: physiological state and general condition, cultivation and feeding method, lambing time, soil configuration and macroclimate.

Recommendations for pasture rest times can be based on these factors; pastures can be rested for shorter periods of time when it is hot, but they need to be rested for longer periods of time in cool, dry weather. Similarly, humid, mild temperatures and rainfall events in temperate-arid climates increase the success of trichostrongyles and accelerate the rate of their development on pasture. Increased monitoring is required during these high-risk periods. In addition, trichostrongyles require vegetation to complete their life cycle, so animals on a dry lot will have a substantially decreased risk of detrimental infection.

Pathological importance of certain types of gastrointestinal helminths

Most GI parasite infections in ruminants are mixed, with several genera infecting a single animal. Notable exceptions are haemonchosis and type II ostertagiasis . In general, clinical signs are present in only a small proportion of a group of animals, even though all the animals have GI parasites. The clinical signs associated with GI parasitism are typically nonspecific and manifest as a chronic, slowly progressive illness unless a very high infectious dose is encountered or other concurrent risk factors are present.

Heavily infected animals experience gradual weight loss or decreased weight gain (in the case of young animals), ill thrift, gradually decreasing milk production, and poor hair coat. More severely affected animals show lethargy and weakness, and they may spend more time away from the group or lying down. Emaciation may occur in long-standing cases because of inappetence that progresses to anorexia, as well as poor nutrient absorption. Chronic GI parasitism may also predispose animals to secondary infections (bacterial, viral, fungal) that they would normally be able to resist.

Whether or not the animal has diarrhea, heavy GI parasitism causes a protein-losing enteropathy as a result of either increased loss from diarrhea or decreased absorption from direct damage to the mucosa by the parasites. Clinical signs of hypoproteinemia might include submandibular edema (bottle jaw) or ventral edema. In addition to inadequate protein absorption, heavily parasitized ruminants are likely to show poor absorption of critical vitamins and minerals from their diet.

Bloodsucking species of trichostrongyles (eg, *Haemonchus* spp) cause severe anemia; however, some anemia can also be found with most heavy trichostrongyle infections. Because trichostrongyles use cutting or slicing mouthparts to attach to abomasal or intestinal mucosa, blood can leak from these sites. Clinical anemia is commonly chronic, and many animals can compensate until their hematocrit is < 10%. Severe anemia presents as cardiovascular collapse: increased heart rate, increased respiratory rate and effort (open-mouth breathing, cheek puffing), poor perfusion, cold extremities, and sudden death. Acute decompensation may follow a quicker clinical course after heavy exposure (eg, after large rainfall events that accelerate larval development), or after an animal with chronic parasitism reaches critical levels of anemia, hypoproteinemia, and/or emaciation.

Oesophagostomosis

Oesophagostomum larvae develop in the mucosa and submucosa of the colon (cecum and colon) but also in the ileum, causing loss of blood and plasma proteins due to severe inflammation of the mucosa. Clinical symptoms include fever, diarrhea, inappetence and weight loss, death; in older categories - chronic course - with less pronounced symptoms, decline in productive abilities; solid immunity develops, but reinfections can lead to acute allergic enteritis (type III hypersensitivity) in the proximal part of the colon with eosinophilic infiltration, profuse secretion and hypertrophy. The larvae in the nodules cause acute ulcerative colitis, and the chronic course leads to death. Clinical signs are loss of appetite and anemia.

Chabertiosis

Infections with *Chabertia ovina* are usually of moderate intensity. Adults and larvae do not suck blood but may ingest some if a blood vessel is damaged. Nevertheless they harm the gut's lining where they attach, whereby they often change their attachment site multiplying the lesions. These causes small but numerous local ulcers, sometimes also bleeding, which can be significant in case of massive infections. In case of high intensity infection (over 800 parasites in small ruminants), colitis occurs and death is possible. Heavy infection with *Chabertia ovina* adults can severely damage the mucosa of the colon, with resulting congestion, ulceration, and small hemorrhages. Infected small ruminants are unthrifty, and their feces are soft, contain mucus, and may be streaked with blood. Immunity develops quickly, and outbreaks occur only in young animals or under conditions of severe stress.

Bunostomosis

It occurs frequently, and the parasites are highly pathogenic; animals at the age of 5-8 months are most often infected. An infection with 20-100 and the death of lambs is caused by 500-600 parasites leading to the manifestation of clinical symptoms. Clinical symptoms are anemia (they feed on blood and have a negative effect on hematopoietic), inappetence, weight loss, diarrhea with mucus and blood, hypoproteinemia, edema in the subjaw area, cachexia and will die.

Trichostrongylidosis

Heavy infections with *Trichuris* spp (whipworms) are not common; however, they may occur in young lambs or kids or during drought conditions when animals are fed supplemental feed on the ground. The eggs are very resistant to environmental conditions and can remain persistent on farms with contamination. Congestion and edema of the cecal mucosa develop, accompanied by diarrhea and unthriftiness. Both, larvae and adult parasites cause pathogenic effects. The course of the infection depends on the number of parasites and the species are hematophagous and pathogenic, the age and diet of the host; the disease manifests itself in animals younger than two years. The main pathogenic effects are hypoplastic gastritis and catarrhal enteritis.

Ostertagiosis

The parasitized glands of the gastric mucosa are destroyed; the cells that produce HCl are destroyed, protein digestion stops, pepsinogen cannot be converted into pepsin and therefore appears in the plasma (the permeability of the damaged mucosa is increased), which leads to the release of plasma proteins into the lumen of the abomasum and peripheral edema. The lesions are in the form of whitish raised nodules that merge, so the abomasal mucosa looks like cracked skin.

Hemonchosis

Acute course induce hemorrhagic anemia (normocytic hypochromic), dark-colored feces - melena, edema, general serious condition, decline in quality and quantity of wool and sudden death. Chronic course: inappetence, progressive weight loss, general serious condition, anemia, diarrhea rarely occurs, constipation is even more common.

Nematodirosis

Nematodirosis is characterized by sudden-onset unthriftiness, profuse diarrhea, and marked dehydration, with death as early as 2–3 days after an outbreak begins. Nematodirosis is commonly confined to lambs or weaner sheep, but in low-rainfall country where outbreaks are sporadic, older sheep may have heavy infections. Nematodirosis lesions usually consist

of dehydration and a mild catarrhal enteritis; however, acute inflammation of the entire small intestine may develop. Counts of $\geq 10,000$ worms, together with characteristic clinical signs and history, are indicative of clinical infections. Affected lambs may pass large numbers of eggs, which can be identified easily. If the onset of disease precedes maturation of the female worms, eggs will not be identified on fecal examination.

Trichostrongylosis

Anorexia, persistent diarrhea, and weight loss are the main clinical signs of *Trichostrongylus* infection. Villous atrophy (or stunting of villi) impairs digestion and malabsorption; protein loss occurs across the damaged mucosa. In these infections, dark hemorrhagic diarrhea with weight loss is typical. Infection of lambs with this parasite has particular pathogenic significance in some regions; a sudden increase in L3 on pasture during May and June can lead to high-intensity lamb infections and outbreaks of clinical symptoms. The parasite can be highly pathogenic because large numbers of larvae hatch over a short period at a time when young lambs are beginning to consume sizable quantities of grass. Disease may be associated with developing larval stages and may occur within 2 weeks of challenge (the prepatent period is 15 days).

Cooperiosis

Infection with *Cooperia* spp. has clinical signs are diarrhea (aqueous, green or black) with subsequent dehydration and weight loss as a consequence of poor food conversion. Other signs are the same as for other gastrointestinal roundworms: apathy, loss of appetite, reduced weight gains, etc. Massive infections are particularly harmful for young animals, which can become anemic. L4 larvae and adults burrow into the gut's wall, particularly in the first part (duodenum) and harm the tissues and blood vessels, but do not suck blood. As a general rule, *Cooperia* worms are less harmful than other gastrointestinal worms such as *Haemonchus* and *Ostertagia*. However, they worsen the damage caused by other worms.

Skrjabinemosis

Skrjabinema ovis are usually nonpathogenic pinworms. Adults are 8–10 mm long and live in the rectum and anus. Clinical signs include possible anal pruritus.

Localization of the most important gastrointestinal strongylids of sheep

genus	species	host	localisation
<i>Strongylidae</i>	<i>Oesophagostomum. venulosum</i>	small ruminant	large intestine
	<i>Oe. columbianum</i>	small ruminant	
	<i>Chabertia ovina</i>	small ruminant	
	<i>Haemonchus contortus</i>	small and large ruminant	
	<i>Ostertagia circumcincta</i>	mali preživari	abomasus
	<i>Ost. ostertagi</i>	small and large ruminant	
<i>Trichostrongylidae</i>	<i>Trichostrongylus axei</i>	small and large ruminant	small intestine
	<i>Trich.colubriformis</i>	small ruminant	
	<i>Cooperia curticei</i>	small ruminant	
	<i>Coop. punctata</i>	small ruminant	
	<i>Cooperia oncophora</i>	small and large ruminant	
	<i>Nematodirus filicolis</i>	small ruminant	
	<i>Nem. Spatiger</i>	small ruminant	
	<i>Nem. Battus</i>	small ruminant	
<i>Ancylostomatidae</i>	<i>Bunostomum trigonocephalum</i>	small ruminant	

GASTROINTESTINAL HELMINTHS OF SHEEP BREED IN SPREAD BELGRADE AREA

Breeding of sheep and goats were increased during last decade on Belgrade area. Today, small flocks of sheep and goats play an important role in providing animal protein for diet, especially for those people who live in village at mountains part of Belgrade area. Both, sheep and goats are milked and they produce the bulk milk supply.

Topography and climate condition of Belgrade

Belgrade is the capital and largest city of Serbia. It is located at the confluence of the Sava and Danube rivers and the crossroads of the Pannonian Plain and the Balkan Peninsula. The city has an urban area of 360 km² while together with its metropolitan area it covers 3,223 km². The spread area of Belgrade has extremely favorable conditions for modern agricultural production (climate, agricultural land, watercourses, developed processing industry). This economic branch is of strategic importance for supplying Belgrade with food products, along with the resources that abound in the wider environment (Vojvodina and Šumadija). The Belgrade region has a significant land potential of about 322,292 hectares of agricultural land, which makes up 70% of the total territory of the City of Belgrade.

Topography

Geographical and climate data about examined area was next: Belgrade is situated in South-Eastern Europe, on the Balkan Peninsula. It lies at the point where the river Sava merges into the Danube, on the slope between two alluvial planes. The river waters surround it from three sides, and that is why since ancient times it has been the guardian of river passages.

Belgrade lies 116.75 metres above sea level and is located at confluence of the Danube and Sava rivers. The city has an urban area of 360 square kilometres, while together with its metropolitan area it covers 3,223 km². On the right bank of the Sava (examined area), central Belgrade has a hilly terrain, while the highest point of Belgrade proper is Torlak hill at 303 m. The mountains of Avala (511 m) and Kosmaj (628 m) lie south of the city. Across the Sava and Danube, the land is mostly flat, consisting of alluvial plains and loessial plateaus.

After World War II, New Belgrade was built on the left bank of the Sava river, connecting Belgrade with Zemun. Smaller, chiefly residential communities across the Danube, like Krnjača, Kotež and Borča, also merged with the city, while Pančevo, a heavily industrialised satellite city, remains separate. The city has an urban area of 360 km² (140 sq mi), while together with its metropolitan area it covers 3,223 km².

One of the characteristics of the city terrain is mass wasting. On the territory covered by the General Urban Plan there are 1,155 recorded mass wasting points, out of which 602 are active and 248 are labeled as 'high risk'. They cover almost 30% of the city territory and include several types of mass wasting. Downhill creeps are located on the slopes above the rivers, mostly on the clay or loam soils, inclined between 7 and 20%. The most critical ones are in Karaburma, Zvezdara, Višnjica, Vinča and Ritopek, in the Danube valley, and Umka, and especially its neighbourhood of Duboko, in the Sava valley. They have moving and dormant phases, and some of them have been recorded for centuries. Less active downhill creep areas include the entire Terazije slope above the Sava (Kalemegdan, Savamala), which can be seen by the inclination of the Pobednik monument and the tower of the Cathedral Church, and the Voždovac section, between Banjica and Autokomanda.

Landslides encompass smaller areas, develop on the steep cliffs, sometimes being inclined up to 90%. They are mostly located in the artificial loess hills of Zemun: Gardoš, Ćukovac and Kalvarija.

However, the majority of the land movement in Belgrade, some 90%, is triggered by the construction works and faulty water supply system (burst pipes, etc.). The neighbourhood of Mirijevo is considered to be the most successful project of fixing the problem. During the construction of the neighborhood from the 1970s, the terrain was systematically improved and the movement of the land is today completely halted

Climate condition

Belgrade's climate exhibits influences of oceanic, under the Köppen climate classification, has a humid subtropical climate (*Cfa*) bordering on a humid continental climate (*Dfa*) with four seasons and uniformly spread precipitation. Monthly averages range from 1.4 °C in January to 23.0 °C in July, with an annual mean of 12.5 °C. There are, on average, 44.6 days a year when the maximum temperature is at or above 30 °C 95 days when the temperature is above 25 °C. On the other hand Belgrade experiences 52.1 days per year in which the minimum temperature falls below 0 °C, with 13.8 days having a maximum temperature below freezing as well. Belgrade receives about 691 mm of precipitation a year, with late spring being wettest. The average annual number of sunny hours is 2,112. (<http://www.hidmet.gov.rs/>).

Agricultural production in the wider Belgrade area

The city is divided into 17 municipalities. Most of the municipalities are situated on the southern side of the Danube and Sava rivers, in the Šumadija region. Three municipalities (Zemun, Novi Beograd, and Surčin), are on the northern bank of the Sava in the Syrmia region and the municipality of Palilula, spanning the Danube, is in both the Šumadija and Banat regions. The wider area of Belgrade municipalities consists of many villages, and the land is suitable for agriculture, especially for the cultivation of agricultural crops and animal husbandry.

Breeding of sheep and goats were increased during last decade on Belgrade area. Today, small flocks of sheep and goats play an important role in providing animal protein for diet, especially for those people who live in village at mountains part of Belgrade area. Both, sheep and goats are milked and they produce the bulk milk supply, together with a large proportion of the meat that is consumed. Along with the increasing number of flocks began on examination of their health status. During those examination especially was paid to the parasitic infections

The first serious studies of parasitic fauna of sheep and goats in the area of Belgrade were done in the period 2009-2010. In the meantime, there has been an increase in the number of herds, changes in microlimatic conditions and environmental conditions. Due to the sudden urbanization, which is inevitably accompanied by pollution of land and water, an increase in the number of non-owner dogs, the approach of wild animals to human settlements (foxes, etc.)? This has affected the quality of the environment, the grazing areas where sheep are kept, as well as the global epidemiological and hygienic condition of the city. All this affects, together with parasitic infections, the production results (milk yield, growth, quantity and quality of wool) in sheep.

For these reasons, after ten years, we returned to examining the parasitic fauna of small ruminants in the area of Belgrade in order to see the current situation causing these changes.

Methods of examination

Faecal examination

Faeces examination was conducted by methods of direct smear and flotation. During our examination we always used fresh samples of faeces. For direct smear, a small quantity of faeces is placed on a slide, mixed with a drop of water, spread out and examined directly. At least three slides from different parts of faecal sample should be examined. This method is suitable for very rapid examination, but it will usually fail to detect low-grade infection. Flotation techniques use solutions which have higher specific gravity than the organisms to be floated so that the organisms rise to the top and the debris sinks to the bottom.

During our examination we used Zinc sulphate solution with specific gravity 1.36 (700 g ZnSO₄ in 1000 ml water). The intensity of infection was measured by McMaster method. The number of eggs per gram can be calculated as follows: count the number of eggs within the grid of each chamber ignoring those outside the squares and then multiply the total by 50 – this gives the number of eggs per gram of faeces (e.p.g.)

A centrifugation fecal flotation performed on individual animal feces and at composite samples from multiple animals within a herd enables a qualitative assessment of the types of GI nematodes shedding eggs in the feces and a subjective assessment of the magnitude of infection. Composite fecal sampling is a robust method for evaluating parasite burden within a herd that is a resource-efficient option for owners and producers.

Faecal egg counts can provide robust information about herd health, and with good record keeping they can create a strong clinical picture of infection patterns within herds over time.

Necropsy examination

During necropsy the abomasum was cut open and the contents were washed into a bucket, under a strong flow of water. More water was added to give a total volume of five litres. While agitating the bucket, four subsamples of 50 ml (altogether 1/25 of the total material) were taken. The rest was washed through a sieve with a grid aperture of 250 μm , and searched macroscopically for large nematodes. The subsamples were washed gently through a sieve with a grid aperture of 100 μm . Material remaining in the sieve was poured into a Petri dish and examined on a dark background under a binocular microscope. All adult, male nematodes, with well-developed spicules, were transferred to a microscope slide, identified by species and counted. In order to estimate the total adult nematode burden

of each species, the number of male nematodes of each species found in the subsamples was multiplied by 25 and then again by 2, accounting for the adult female nematodes.

The same method was used on the small intestine, except that the intestine was not cut open, but its content was pressed out and the remaining material washed out with running water. The large intestine was cut open and its contents washed through a sieve with a grid aperture of 250 μm . The contents of the sieve were put in small portions into a large tray, water was added, and the contents were macroscopically searched for large nematodes.

Found parasites either fixing in 10% formalin, were mounted in lactofenol for identification, and mounted in Canada balsam.

Determination of adult parasites and parasites eggs we performed by its morphological characteristic.

First study of endoparasites infection (2009-2010)

The first study about season distribution of gastrointestinal helminthes of small ruminant at spread Belgrade area was started in March 2009 and finished in January 2010. During study we examined a total of 91 flocks of goats and sheep from 6 Belgrade districts Mladenovac, Lazarevac, Obrenovac, Grocka, and Vozdovac (from the village Mladenovac, Vlaska, Mala Krsna, Velika Krsna, Medjuluzje, Senjak, Velika Ivanča, Orašac, Mala Vrbica, Rajkovac, Dubona, Šepšin, Resnik, Velike Granice, Granice, Koracica, Jagnjilo, Markovac, Lazarevac, Arapovac, Junkovac, Leskovac, Sokolovo Rabrovac, Vrbovno, Zvecka, Krtinska and Stepojevac).

During study we collected fecal samples at monthly intervals. A total of 910 fecal samples were analyzed using standard coprological techniques. A total of 89 sheep's we were analyzed by post-mortem examination. Total differential worm counts were performed on all the alimentary tract and lungs using the technique. The faecal samples were obtained from a different source all together as they were collected from flocks in the field, and the results support the other findings. These counts were also of value in providing some information on the egg rise. The number of guts and lungs examined in this survey thought small in number, but in combination with results of coprological examination, samples appeared to represent the population adequately.

During the period 2009-2010 the next helminth species were found: The prevalence of individual species was the next: *Ostertagia circumcincta* (95,23%), *O.trifurcata* (91,53%), *O.ostertagi* (23,33%), *Trichostrongylus axei* (100%), *T.colubriformis* (89,57%), *T.capricola* (62,85%), *Nematodirus spathiger* (100%), *N. filicolis* (43,31%), *Haemonchus contortus* (88,95%), *Marshallagia marshalli* (23,77%), *Skrjabinema ovis* (5,26%), *Bunostomum trigonocephalum* (13,28%), *Chabertia ovina* (64,14%), *Oesophagostomum venulosum* (28,39%), и *Cooperia curticei* (60.52%).

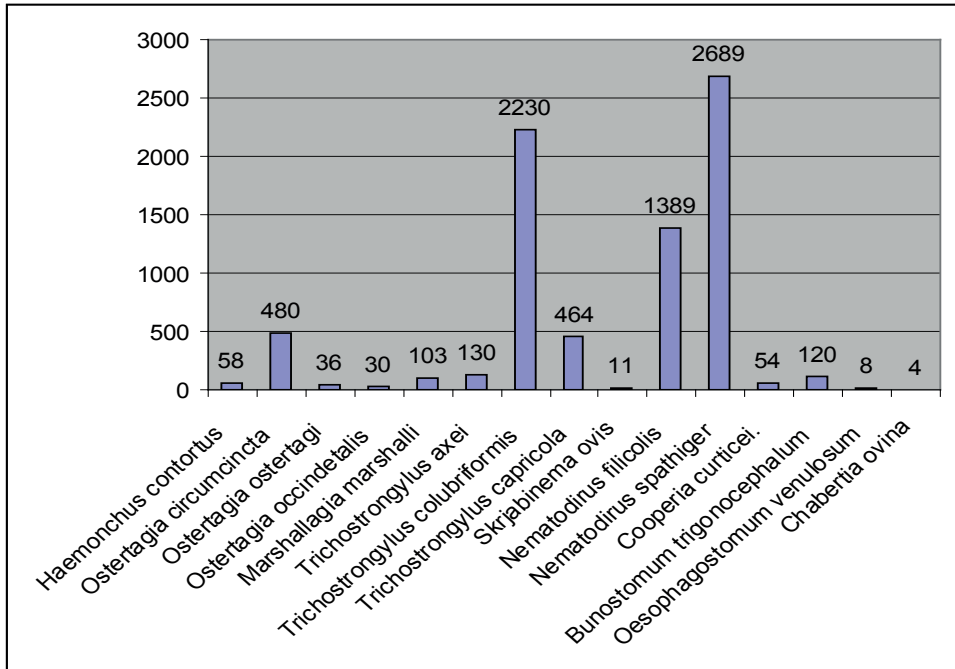
Species in the genus *Ostertagia*, *Trichostrongylus* and *Nematodirus* were present after the first appearance of those present during the entire study period.

Haemonchus contortus is ordered in animals during the warmer and *Marshallagia marshali* during the colder period of the year. Species in the genus *Cooperia*, and *Oesophagostomum*. *Bunostomum* were often present in lambs sacrificed during all the monitoring period. Species in the genus *Cooperia*, and *Oesophagostomum*. *Bunostomum* were often present in lambs sacrificed during the monitoring period

At the beginning of our research, conducted in March, the real extent of gastrointestinal infections strongilidae was 83.33%, after which he soon reached a level of 100% in the same way and moved to the end of follow-up period. Extensity of infection established genera gastrointestinal strongilidae was different. The distribution of parasites of the genera *Ostertagia*, *Trichostrongylus* and *Nematodirus* was reached during the monitoring period almost the maximum level.

The distribution of species within the established genera also varied. Within the genus *Ostertagia* most abundant were dominated by *Ostertagia circumcincta* and *O.trifurcata*. Prevalence of infection with *Ostertagia ostertagi* and *O.occidentalis* was higher during the colder periods of the year.

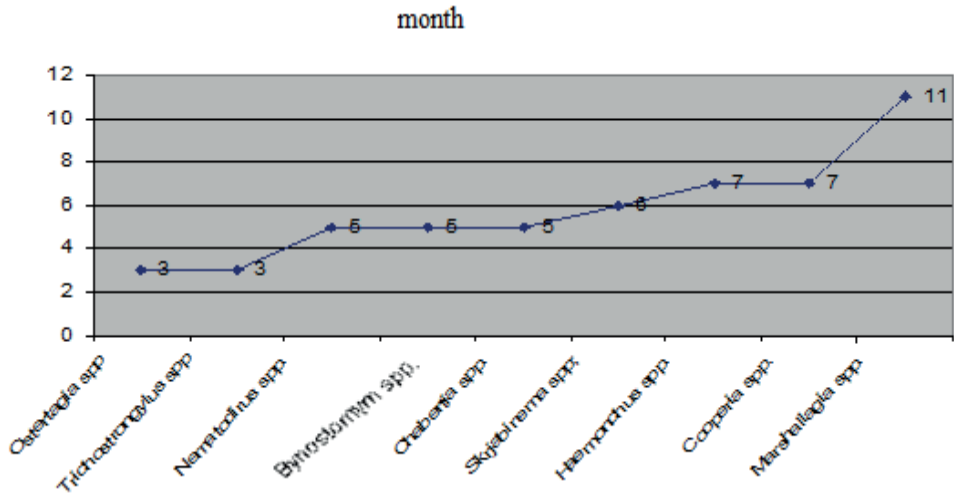
Among the species of the genus *Trichostrongylus* was the most prevalent *Trichostrongylus colubriformis*. Extensity of infection with *T.axei* and *T.vitrinus* varied, without any regularity. Extensity of infection with *Nematodirus filicollis* and *N.spathiiger* demonstrated a tendency to increase and leveled off at the highest level of the whole study period.



Intensity of infection of with gastrointestinal parasites

The dynamics of the first occurrence of established species of gastro-intestinal strongilida was as follows:

- In March: *Teladorsagia (Ostertagia) circumcincta*, *Ostertagia ostertagi*, *Trichostrongylus colubriformis*, *Nematodirus filicolis* and *N.spathiger*
- In May: *Ostertagia trifurcata*, *Trichostrongylus axei*, *Bunostomum trigonocephalum* and *Chabertia ovina*;
- In June: *Skirjabinema ovis*
- In July: *Haemonchus contortus*, *Cooperia curticei* and *Oesophagostomum venulosum*;
- In November: *Marshallagia marshalli*



The season dynamics of GI parasitesx in period 2009-2010

Second study of endoparasites infection (2018-2019)

The study of endoparasites infection performed during 2018-2019. we were carried out in 152 flocks of sheep originated from from 6 Belgrade districts Mladenovac, Lazarevac, Obrenovac, Grocka, and Vozdovac (from the village Mladenovac, Vlaska, Mala Krsna, Velika Krsna, Medjuluzje, Senjak, Velika Ivanca, Orašac, Mala Vrbica, Rajkovac, Dubona, Šepšin, Resnik, Ritopek, Vrčin, Vinča, Leštane, Pinosava, Grocka, Velike Granice, Granice, Koracica, Jagnjilo, Markovac, Lazarevac, Arapovac, Junkovac, Leskovac, Sokolovo, Rabrovac, Vrbovno, Zvecka, Krtinska and Stepojevac). During our examination we were examined total of 631 fecal samples. Examination we performed using standard coprological technique.

Total of 73 sheep and lambs we were examined by post-mortem examination. Total differential worm counts were done on the entire alimentary tract using the standard paristology necropsy technique. Determination of adult and eggs of parasites were done by morphological characteristic.

The faecal samples were obtained from a different source all together as they were collected from flocks in the field, and the results support the other findings. These counts were also of value in providing some information's on the peripartuirent egg rise. The number of guts and lungs examined in this survey thought small in number, but in combination with results of coprological examination, samples appeared to represent the population adequately.

In period 2018-2019 we found next helminth species: *Teladorsagia (Ostertagia) circumcincta* in 75,23%, *Ostertagia trifurcata* 71,53%, *O.ostertagi* 21.99%, *Trichostrongylus axei* 62,23%, *T.colubriformis* 69,57%, *T.vitrinus* 62,85%, *Nematodirus spathiger* 77,43%,

N.filicollis 33,31%, *Haemonchus contortus* 58,95%, *Marshallagia marshalli* 27,77%, *Skrjabinema ovis* 11,31%, *Bunostomum trigonocephalum* 13,28%, *Chabertia ovina* 63.85%, *Oesophagostomum venulosum* 27.91%, *Cooperia curticei* 60.52%, *C.oncophora* 28,39% and *C.punctata* 13,28%.

genus	species	intensity of infection		
		min	max	average
<i>Haemonchus</i>	<i>contortus</i>	12	104	58
<i>Ostertagia</i>	<i>circumcincta</i>	5	955	480
	<i>ostertagi</i>	8	64	36
	<i>occidentalis</i>	8	52	30
<i>Marshallagia</i>	<i>marshalli</i>	18	188	103
<i>Trichostrongylus</i>	<i>axei</i>	2	258	130
	<i>colubriformis</i>	3	4457	2230
	<i>capricola</i>	7	921	464
<i>Skrjabinema</i>	<i>ovis</i>	3	19	11
<i>Nematodirus</i>	<i>filicollis</i>	96	2700	1398
	<i>spathiger</i>	17	5361	2689
<i>Cooperia</i>	<i>curticei</i>	11	97	54
<i>Bunostomum</i>	<i>trigonocephalum</i>	8	232	120
<i>Oesophagostomum</i>	<i>venulosum</i>	2	14	8
<i>Chabertia</i>	<i>ovina</i>	2	6	4

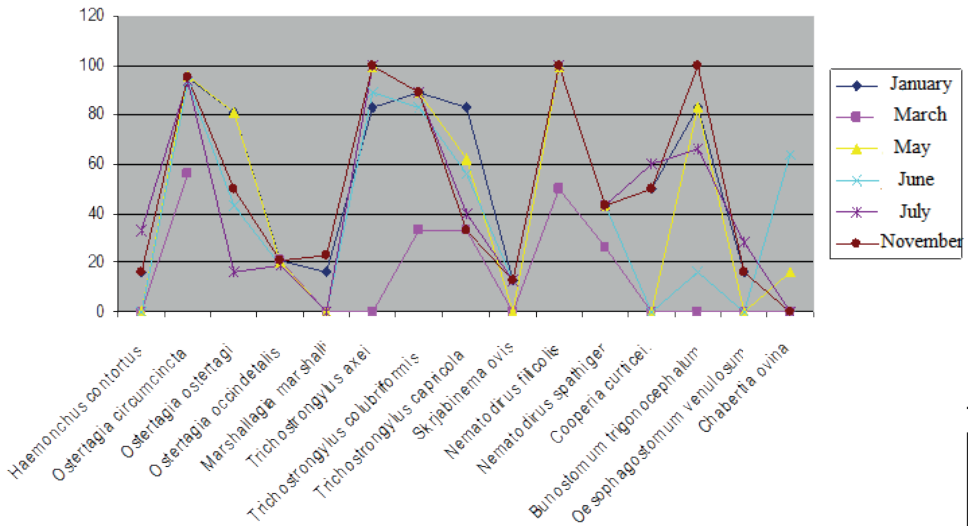
Intensity of infections (finding number of eggs/g feces)

Most prevalence species of nematode are *Trichostrongylus* and *Nematodirus* species. The distribution of species within the established genera also varied. Within the genus *Ostertagia* most abundant were dominated by *Ostertagia circumcincta* and *O.trifurcata*. Prevalence of infection with *Ostertagia ostertagi* and *Ostertagia occidentalis* was higher during the colder periods of the year. Among the species of the genus *Trichostrongylus* was the most prevalent *Trichostrongylus colubriformis*. Extensity of infection with *Trichostrongylus axei* and *T.vitrinus* varied, without any regularity. Extensity of infection with *Nematodirus filicollis* and *N.spathiger* demonstrated a tendency to increase and leveled off at the highest level of the whole study period.

The dynamics of the first occurrence of established species of gastro-intestinal strongilida was as follows:

- In March: *Teladorsagia (Ostertagia) circumcincta*, *Ostertagia ostertagi*, *Trichostrongylus colubriformis*, *T.vitrinus*, *Nematodirus filicollis* and *N.spathiger*
- In May: *Ostertagia trifurcata*, *Trichostrongylus axei*, *Bunostomum trigonocephalum* and *Chabertia ovina*;

- In June: *Skrjabinema ovis*
- In July: *Haemonchus contortus*, *Cooperia curticei*, *C. oncophora*, *C. punctata*, *Oesophagostomum venulosum*;
- In November: *Marshallagia marshalli*



The season dynamics of GI parasites in period 2018-2019

The seasonal dynamics itself varied within the limits that are usually found in the found parasite species. We will see that in the month of March - the period that coincides with the period of the first outing to pasture, the largest number of parasite species appeared ("spring-rise" phenomenon).

The next peak occurs in May, when we find four types of parasites, in July when there are three, while in June and November we have only one type of parasite each.

The ecological parameters that influence it are the micro and macro climate of the steam area, vegetation and the number of animals in the pasture. The population pressure on the pasture (number of animals per unit area) and the method of grazing - whether it is forced or stationary also affect the load on the pasture and the degree of its infection.

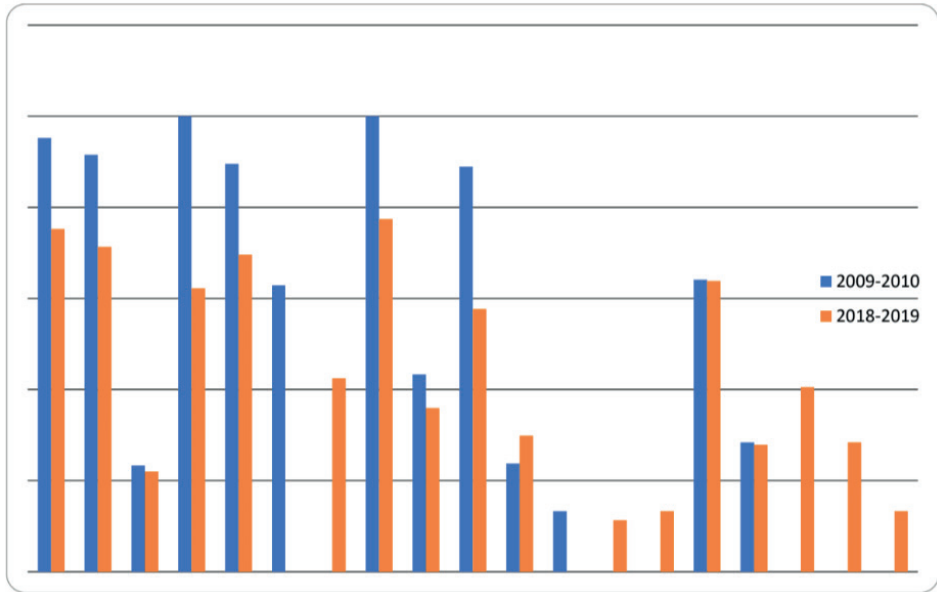
During our research done in the period 2018-2019, a difference in the biodiversity of GI helminths is noticed in comparison with the research done in the period 2009-2010. This research has for the first time identified parasites of the genus *Cooperia* that were not previously present in the Belgrade area. They are usually found in sheep flocks in southern and south-east Serbia.

Simultaneously with the increase in the number of GI helminth species, the prevalence of previously established species decreased. This is most noticeable in the three bridge prevalence genera of the GI nematode *Ostertagia*, *Trichostrongylus* and *Nematodirus* species.

If we make a comparison with the results we had during the research done in the period 2009-2010, it can be seen that the prevalence of certain types of parasites has decreased, but the number of parasite species has increased. There are many reasons for that, and the main one is that in the past period there has been a significant increase in the number of herds in the villages around the city. These were mainly animals that were procured from other parts of Serbia where these types of parasites are present.

YEARS			
2009-2010		2018-2019	
parasites species	%	parasites species	%
<i>Teladorsagia (Ostertagia) circumcincta</i>	95.23	<i>Teladorsagia (Ostertagia) circumcincta</i>	75.23
<i>Ostertagia trifurcata</i>	91.53	<i>Ostertagia trifurcata</i>	71.33
<i>Ostertagia ostertagi</i>	23.33	<i>Ostertagia ostertagi</i>	21.99
<i>Trichostrongylus axei</i>	100.00	<i>Trichostrongylus axei</i>	62.23
<i>Trichostrongylus colubriformis</i>	89.57	<i>Trichostrongylus colubriformis</i>	69.57
<i>Trichostrongylus capricola</i>	62.85	<i>Trichostrongylus vitrinus</i>	42.45
<i>Nematodirus spathiger</i>	100.00	<i>Nematodirus spathiger</i>	77.43
<i>Nematodirus filicolis</i>	43.31	<i>Nematodirus filicolis</i>	35.91
<i>Haemonchus contortus</i>	88.95	<i>Haemonchus contortus</i>	57.65
<i>Marshallagia marshalli</i>	23.77	<i>Marshallagia marshalli</i>	29.89
<i>Skrjabinema ovis</i>	5,26	<i>Skrjabinema ovis</i>	11.31
<i>Bunostomum trigonocephalum</i>	13,28	<i>Bunostomum trigonocephalum</i>	13.28
<i>Chabertia ovina</i>	64.14	<i>Chabertia ovina</i>	63.85
<i>Oesophagostomum venulosum</i>	28.39	<i>Oesophagostomum venulosum</i>	27.91
<i>Cooperia curticei</i>	60.52	<i>Cooperia curticei</i>	40.52
		<i>Cooperia oncophora</i>	28.39
		<i>Cooperia punctata</i>	13.28

Comparative prevalence of GI helminths of sheep in period 2009-210 and 2018-2019



Comparative prevalence of GI helminths of sheep in period 2009-2010 and 2018-2019

Although most of the gastro-intestinal species appear to follow this general pattern of seasonal distribution, some variations in intensity and duration of these characteristics with different worm species occurred. Thus with *Trichostrongylus* and *Nematodirus* species infection at mature sheep the spring peak was more pronounced than the autumn infection.

The season dynamics of the established parasites species during both period of examination (2009-2010 and 2018-2019) was as follows:

- In March have occurred *Ostertagia spp.* and *Trichostrongylus spp.*
- In May, the observed infection with *Nematodirus spp.*, *Bunostomum spp.* and *Chabertia spp. (ovina)*
- In June was the first record of *Skrjabinema spp.*;
- In July were established eggs of *Haemonchus spp. (contortus)* and *Cooperia spp.*
- In November showed the presence of *Marshallagia spp.*

Climate variations are a significant factor of seasonal distribution of certain species of sheep helminates. There are discrepancies in the seasonal distribution between certain regions in Serbia. Thus, certain species within the genera *Ostertagia*, *Trichostrongylus* and *Nematodirus* occur earlier in the plains (north Serbia Vojvodina) and the area of Belgrade than in hilly and mountainous areas.

Found parasites species were present at small ruminants in other parts of Serbia. This was confirmed by during examination performed in the hilly areas of Serbia (Šar

Planina, Stara Planina) (Vujić et al., 1911, 2015a), south, south-east and south-west part of Serbia, (Pavlović et al. 2013a, b, 2018a), at Timok District (Jovanović et al. 1991), Belgrade area (Pavlović et al. 2009, 2012) Vojvodina (Pavlović et al. 2017b) and Kosovo (Pavlović et al. 1995, Milanović et al. 2018). Same parasitic species were occurred at other Balkan countries like Macedonia or Bulgaria (Georgievski, 1989, Zurliski and Rusev, 1990).

Generally speaking the occurred parasites represent a global problem. Way of breeding usually at shepherds had prerequisite to a lot of infections including parasitoses. Pasture breeding make possible contact within sheep and eggs, larval stages and intermediate host of parasites. Those induce that there are no one sheep without parasites. The countries of Magreb, Middle East and Northern Africa are also in permanent problems with parasitic infections and losses ensued by them. Negative influence of parasitic infection reflected through lost of weight and decrement quantum of lactation (Bahgat et al. 1988, Dogana, et al. 1989, Ashraf and Nepote, 1989, Fakae, 1990, Smith, 1990, Quesada et al. 1990).

CONCLUSION

However, since the parasitic infections are in majority sub clinical this problem is not played due attention by a sheep breeder from the village in the Belgrade area. The prophylactic treatment is not conducted in the majority of flocks or it is only partially performed what can be seen by the records from the slaughter line and from production results. In aim of introducing parasites fauna of sheep and prepare measure to its control we must to continue our examination. This was the only way to obtain better product results, characteristics and quality of sheep and lambs meat.

REFERENCE

1. Akhmetov, A.A. Helminthes of sheep in Kokchetav oblast. in: Problems of Veterinary Parasitology in Kazakhstan. A Collection of Scientific Transactions. Kazakhstan Scientific Investigation Veterinary Institute, Almaty. 3-12, 1982
2. Angelovski T., Ilijev A. Study of gastrointestinal strongylidosis in sheep and the effect of thiabendazole on increasing milk production. Veterinarski glasnik 3: 177-182, 1972.
3. Ardeleanu D., Pivodă C., Neacșu M., Ida A. Bio-ecological phenomenon of poly-parasitism – actual major problem in breeding of sheep and goats. Lucrări științifice Zootehnie și Biotehnologii, vol. 40(2): 309-317, 2007
4. Ash R., Truong, P. The use of vetiver grass wetland for sewerage treatment in Australia. Proceeding of Third International Vetiver Conference, Guangzhou, China, 83-87, 2003.
5. Berezovski, V.S. (Seasonal and age dynamics of marshallagiosis of sheep in Kustanai oblast. In: Diseases of Sheep and Means of Their Control in Kazakhstan. Kazakhstan Scientific Research Veterinary Institute, Ministry of Agriculture of the Republic of Kazakhstan, Almaty. 121-122, 1975.

6. Bojkovski J., Relić R., Hristov S., Stanković B., Savić B.r, Pavlović I., Petrujkić T.: Influence of biological and chemical contaminants on health status of small ruminants. - Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 67 (2), 37-39, 2010.
7. Boag, B., Thomas R. J. The population dynamics of nematode parasites of sheep in northern England. *Research in Veterinary Science* 19: 293-295, 1975
8. Brown, T.H., Ford G.E., Miller D.W., Beveridge I. Effect of anthelmintic dosing and stocking rate on the productivity of weaner sheep in a Mediterranean climate environment. *Australian Journal of Agriculture Research*, 36, 845-855, 1985.
9. Charles, T. P. Seasonal prevalence of gastrointestinal nematodes of goats in Pernambuco state, Brazil. *Veterinary Parasitology* 30. 335-343, 1989.
10. Chartier C., Reche B. Gastrointestinal helminths and lungworms of French dairy goats: Prevalence and geographical distribution in Poitou-Charentes. *Veterinary. Research Communication*.16: 327-335, 1992.
11. Cherikaeva, N.S. and Martinov, V.V. The distribution of helminthes of sheep in Ural oblast. In: *Diseases of Sheep and Means of Their Control in Kazakhstan*. Kazakhstan Scientific Research Veterinary Institute, Ministry of Agriculture of the Republic of Kazakhstan, Almaty 321-324, 1975.
12. Diogo M.R., Goncalves A.C.B. Gastrointestinal strongylosis of sheep in mainland Portugal. *Repos. Trabalhos do LNIV* 21: 45-48, 1989
13. Euzeby J. *Diagnostic Experimental des Helminthoses Animales*, Tom 1, ITVS Paris, 1981.
14. Familton, A.S.; McAnulty, R.W. The epidemiology of gastrointestinal parasites of sheep - back to basics. *Proceedings of the 25th Seminar, Sheep & Beef Cattle Society of the NZ Veterinary Association*: 67-74, 1995.
15. Familton, A.S. McAnulty, R.W. Some challenges to current understanding of nematode epidemiology from Canterbury. *Proceedings of the 26th Seminar, Sheep & Beef Cattle Society of the NZ Veterinary Association*: 73-81, 1996.
16. Familton, A.S. McAnulty, R.W. Life cycles and development of nematode parasites of ruminants. In: *Sustainable control of internal parasites in ruminants*. Ed. G.K.Barrell. *Animal Industries Workshop*, Chapter 6: 67-80, 1997.
17. Farah, O. I., Ngatia T. A., Munyua K. W. Ethnoveterinary techniques practised by the Maasai pastoralist of Kajiado District, Kenya. *The African Pastoral Forum, Pastoral Information Network Program. Working Paper Series No. 8*, 1996.
18. Geerlings, E. C. L., Sheep husbandry and ethnoveterinary knowledge of Raika sheep pastoralists in Rajasthan, India. M.Sc. thesis, Wageningen University, Netherland, 65-83, 2001.
19. Georgievski B. Rasprostranjenost parazitskih infekcija ovaca na području opštine Prilep. *Specijalistički rad, Veterinarski fakultet Beograd*, 1991.
20. Giangaspero, M., F. Bahhady A., Orita G., Gruner L. Summer-arrested development of abomasal trichostrongylids in Awassi sheep in semi-arid areas of North-west Syria. *Parasitology Research* 78: 594-597, 1992.

21. Good, B., Hanrahan J. P., Crowley B. A., Mulcahy G. Texel sheep are more resistant to natural nematode challenge than Suffolk sheep based on faecal egg count and nematode burden. *Veterinary Parasitology* 136(3-4): 317-327, 2005.
22. Grace N.D. Prevention of trace element deficiencies in grazing ruminants: an evaluation of methods. *Proceedings of the New Zealand Grassland Association*, 54, 31-34, 1992.
23. Gronvold, J. Transmission of infective larvae of *Ostertagia ostertagi* and *Cooperia oncophora* (Trichostrongylidae: Nematoda). Dr Med. Vet. Thesis, Commissioned by DSR Booksellers, Copenhagen, 1989.
24. Hasslinger M. A., Schenkel F., Ogaylat S., Ulbricht G. Important endoparasites in sheep and goat flocks in Jordan. *Zentralbl. Veterinarmed. B.* 40(5): 329-336, 1993.
25. Ilić, G. Parasitic fauna and pathological changes caused by parasites in goats of Timočka Krajina. 1990. Doctoral dissertation, Faculty of Veterinary Medicine, Belgrade, 1991.
26. Ilić G., Jovanović D., Nešić D., Pavlović I. Goat parasitofauna and its pathomorphological significance in goats in the Timok region in the period 1986-1989. *Proceeding of I International Summer Conference for Advancement of Sheep and Goat Production*, Ohrid, 390-395, 1991.
27. Ilijev A. Fauna of endoparasites of sheep in the area of Pelagonija in SR Macedonia. Doctoral dissertation, Faculty of Veterinary Medicine, Belgrade, 1974.
28. Ivanović S., Pavlović I. Goat meat - safe food. NIVS Belgrade, 2015.
29. Kenyon F. Sargison N.D., Skuce P.J. Jackson F. Sheep helminth parasitic disease in south eastern Scotland arising as a possible consequence of climate change *Veterinary Parasitology* 163: 26 293–297, 2009.
30. Khalafalla R. E., Mahmoud A. Elseify M. A., Elbahi N.M. (2011). Seasonal prevalence of gastrointestinal nematode parasites of sheep in Northern region of Nile Delta. *Egypt Parasitology Research* 108, 2: 337-340, 2011.
31. Krdžalić P. Gastrointestinal nematodes of sheep of the Sjenica-Pešter Plateau with special reference to the dynamics and intensity of occurrence. Doctoral dissertation, Faculty of Veterinary Medicine, Belgrade. 1964.
32. Kristmundsson Á., Richter S.H. Lung- and gastrointestinal helminths of goats (*Capra hircus*) in Iceland. *Iceland Agriculture Science*, 13, 79–85, 2000.
33. Leathwick, D.M. Vlassoff, A.; Sutherland, I. Modelling drench resistance in parasites in sheep. *Parasite Notes* 11: A NZ Sheep Council & Merial NZ Ltd. Publication: 38-41, 1998.
34. Lepojević O., Cvetković L.J. Gastrointestinal strongylids of sheep in SR Serbia. *Archives of Biological Sciences*, 1-2:27-44. 1979.
35. Love S.C.J, Hutchinson G.W. Pathology and diagnosis of internal parasites in ruminants. In *Gross Pathology of Ruminants*, Proceedings 350, Post Graduate Foundation in Veterinary Science, University of Sydney, Sydney; Chapter 16:309-338, 2003.

36. Michel, J.F. Some thoughts on the control of parasitic gastro-enteritis. In: Symons, E.A.; Donald A.D.; Dineen, J.K. (Eds), *Biology and control of endoparasites*, Academic Press, Sydney. 113-131, 1982.
37. Milanovic V., Pavlovic I., Radovic B., Milošević B., Kragovc Đ., Ivanovic S., Bojkovski J.: Helminth fauna of small ruminants in north Kosovo Serbia. - The 17th International Symposium Prospects for 3rd Millennium Agriculture, Cluj-Napoca, Romania, Book of Abstracts No5/2018, 404, 2018
38. Nevenić V., Šibalić S., Cvetković L.J., Lepojević O., Kljajić D. A contribution to the knowledge of the study of gastrointestinal strongyloidosis in sheep from certain areas of the Republic of Serbia. *Veterinarski glasnik*, 14: 29-31, 1960.
39. Oka C.K., Zinsstag J., Pandey B.S., Fofana F., N-Depo A. (1999). Epidemiology of parasites of sheep in the southern forest zone of coast d'Ivoire. LANADA, Laboratoire central de pathologie animale de Bingerville, BP 206, Bingerville, Cote d'Ivoire. *Revue d'Elevage et de Medecine Veterinaire des pays Tropicaux* 52: 39-46, 1999.
40. Oladele S.B., Ibrahim N.D., Fatihu M.Y., Mohammed B., Sambo S.D., Aluko R.K., Twenty Six Years Retrospective Studies of Prevalence of Gastrointestinal Helminths Isolated From Necropsied Animals in Zaria, Nigeria. *Bull. Animal Health and Production in Africa* 234-240, 2006.
41. Omarov, B.B. The influence of the external surroundings on the development of eggs and larvae of *Nematodirus* in semidesert and hilly zones. in: *Invasive Diseases of Domestic Animals and Their Prophylaxis. A Collection of Scientific Transactions. Kazakhstan Scientific Investigation Veterinary Institute, Kazakhstan Academy of Agricultural Science, Almaty* 44-49, 1996.
42. Pavlović, I., Nešić D., Ilić G., Jovanović D., Vlatter D. Parasitofauna of sheep in the Timok region during 1990. *Proceeding of I International Summer Conference for Advancement of Sheep and Goat Production, Ohrid*, 387-389; 1991.
43. Pavlović I., Kulišić Z., Nešić D., Romanić S. : Endoparasitoses of sheep and goats in Prizren district. – 3rd International Conference of Sheep and Goat Production and 1st Symposium on the Reproduction of Domestic Animals, Ohrid, Macedonia, *Proceedings*, 106-110, 1995.
44. Pavlović I., Ivetić V., Savić ., Radanović O., Žutić M., Ivanović S.: Gastrointestinal strongyloidosis of goats and sheep in the area of Belgrade, *Zbornik naučnih radova Instituta PKB Agroekonomik*, 15 (3-4), 123-127, 2009.
45. Pavlovic, I., Savić B., Ivetić V., Radanović O., Žutić M., Jakić-Dimić D., Bojkovski J. The effect of parasitic infections to production results of sheep *Proceeding of IV Balkan Conference of Animal Science BALNIMALCON 2009, Challenges of the Balkan Animal industry and the Role of science and Cooperation, Stara Zagora, Bulgaria*, 389-391, 2009.
46. Pavlović I., Ivanović S., Žujović M., Tomić Z. Helminthoses of goats breeding at Belgrade area. *3rd International Congress, New Perspectives and Challenges of Sustainable Livestock Production, Beograd, Biotechnology in Animal Husbandry* 27: 1499-1504, 2011.
47. Pavlović I., Ivanovic S., Žujović M., Tomić Z.: Helminthoses of goats breeding at belgrade area. - *Biotechnology in Animal Husbandry* 27 (4), 1499-1504, 2011.
48. Pavlović I., Ivanović S., Žujović M., Tomić Z., Memiši N.: Endoparasites of goats in spread Belgrade area in period 2009-2010. VTH international conference: BALNIMALCON 2011 & Xth International Symposium of Animal Biology and Nutrition & 40th International Session of Scientific Communications of the Faculty of Animal Science, Bucharest, Romania; *Proceeding* 1-4, 2011.

49. Pavlović I., Ivanović S., Žujović M., Tomić Z. Influence of gastrointestinal helminths to goat health and production. Proceedings of 6th Central European Congress on Food, Novi Sad, Serbia, 1605-1607, 2012.
50. Pavlović I., Ivanović S., Žujović M., Tomić Z. Influence of gastrointestinal helminths to goat health and production. 6th Central European Congress on Food, Proceedings, Novi Sad, Serbia, 1605-1607, 2012.
51. Pavlović I., Ivanović S., Savić M., Ćirković D., Jovčevski Sr., Jovčevski St., Savić B., Bečkei Ž., Marčić D.: Gastrointestinal helminths of goats breeding at Stara plana area (Serbia). *Lucrări Științifice Medicină Veterinară Timisoara XLVIII (3)*, 159-166, 2015.
52. Pavlović I., Ivanović S., Stokić-Nikolić S., Bojkovski J., Šekler M., Savić B., Žutić M.: Season distribution of gastrointestinal helminths of goats in south-east Serbia. *Lucrări Științifice Medicină Veterinară* 2013, XLVI (3), Timișoara, 138-143, 2013.
53. Pavlović I., Stokić Nikolić S., Dobrosavljević I., Ilić Z.: Parasites fauna of goat at new established farm inhabit with gomas originated from warios area in Serbia. - 3rd International Epizootiology Days and XV Serbian Epizootiology Days, Niška Banja, Proceeding 241-242, 2013.
54. Pavlović I., Jovičić D., Žugić G., Jovčevski S., Ivanović S., Stanojević-Momčilović V., Bojkovski J.: Zoohygienic treatment of pastures in order to prevent parasitic infections of small ruminants.. XXV Savetovanje dezinfekcija, dezinsekcija i deratizacija - jedan svet jedno zdravlje-sa međunarodnim učešćem, Kovačica, Zbornik radova 59-63, 2014.
55. Pavlović I., Ivanović S.: The influence of environmental factors on the occurrence of gastrointestinal helminths of goats in Serbia. 4th International Congress New Perspectives and Challenges of Sustainable Livestock Production, Belgrade, Serbia, Proceeding, 549-557, 2015.
56. Pavlović I.: Helminth infections of small ruminants – gastrointestinal and pulmonary nematodes. One-day seminar UVVPS Leskovac mart 2017, Leskovac, Zbornik predavanja, 13-23, 2017.
57. Pavlović I., Becskei Z., Ivanović S., Petrović P.M., Savić M., Caro Petrović V., Bojkovski J.: Biodiversity of helminths of sheep breed in Vojvodina (Northern Serbia) – Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 74 (2), 162-166, 2017.
58. Pavlović I., Ivanović S., Ćirković D., Petrović P.M., Caro Petrović V., Maksimović N., Ivanović D.: Gastrointestinal helminths of sheep breeding at south west Serbia - *Bulgarian Journal of Veterinary Medicine* 20, Suppl. 1, 402-406, 2017.
59. Pavlović I., Hadžić I., Ivanović S., Petrović P.M., Caro Petrović V., Ružić-Muslić D., Bojkovski J.: The importance of proper grazing and eradication of pastures in the prevention of parasitic infections of small ruminants. Scientific expert meeting Sustainable primary agricultural production in Serbia - situation, possibilities, limitations and chances, Bačka Topola, Proceedings 115-121, 2018.
60. Pavlović I., Ivanović S., Hadžić I., Petrović P.M., Caro Petrović V., Bojkovski J., Pavlović M., Zvekić D.: Health protection of small ruminants in semi-intensive production. - National scientific meeting with international participation Sustainable agricultural production - the role of agriculture in environmental protection, Proceedings Bačka Topola, 165-172, 2019.
61. Pavlović I., Ivanović S.: Gastrointestinal helminths of goats breed in central part of Serbia. International symposium on animal science (ISAS) 2019, Herceg Novi, Montenegro, Proceedings, 143-148, 2019.

62. Pavlović I., Ivanović S., Petrović P. M., Caro Petrović V., Ružić-Muslić D., Maksimović N., Cekić B.: Season distribution of gastrointestinal helminths of goats kept under semi-intensive conditions in north west Serbia. 12th International Symposium Modern Trends in Livestock Production, Beograd, Serbia, Proceeding, 285-292, 2019.
63. Pavlović I., Caro Petrović V., Ružić Muslić D., Bojkovski J., Zdravković N., Relić R., Stefanović V.: Gastrointestinal helminths of sheep breed in Pomoravski and Rasina district. Proceedings of 13th International Symposium Modern Trends in Livestock Production, Belgrade, Serbia, 363-381, 2021.
64. Pavlovic I., Caro-Petrović V., Bojkovski J., Beckei Z., Zdravković N., Nešić K., Ružić-Muslić D. Gastrointestinal helminths of sheep breed in spread Belgrade area in period 2018-2019. Proceedings of III International Agricultural, Biological & Life Science Conference AGBIOL Edirne, Turkey, 585-592, 2021
65. Pavlovic I., Zdravković N., Bojkovski J. Biodiversity of gastrointestinal helminths of sheep breed in mountainous areas of Serbia. Abstract Book of 14th International Conference on Mathematics, Engineering, Natural & Medical Science, Pariz, France, 21, 2022.
66. Pavlovic I. Gastrointestinal helminths of sheep breed in central part of Serbia. Proceeding Book of Ankara International Congress on Scientific Research-VII, Ankara, Türkiye, 1057, 2022.
67. Pavlovic I., Bojkovski J., Caro-Petrovic V. Season distribution of ticks of sheep kept under semi-intensive conditions in Northeast Serbia. Abstract Books of International Euroasia Congress on Scientific Researches and Recent Trends, Antalya, Türkiye, 58, 2022.
68. Pavlovic I., Bojkovski J., Caro-Petrovic V., Mederle N., Zdravkovic N., Pavlović M., Tasic A. Biosecurity measures in the control of gastrointestinal parasitic infections of sheep in pasture Book of Abstract Multidisciplinary Conference on Sustainable Development, Section: Veterinary Medicine, Timișoara, Romania, 38, 2023.
69. Petrović P.M., Ilić Z., Caro Petrović V., Pavlović I. Successful and profitable sheep farming.. RANS, Beograd, 2021.
70. Quesada A., Cringoli G., Bochicchio V., Minnman P. Research on helminths of sheep and goats in Basilicata. note: aetio-epidemiological investigations. *Acta Medicina Veterinaria*, 36, 10, 41-59, 1990.
71. Roe, R., Southcott W.H., Turner N.H. Grazing management of native pastures in the New England region of New South Wales. I. Pastures and sheep production with special reference to systems of grazing and internal parasites. *Australian Journal of Agricultural Research*, 10, 530-534, 1969.
72. Rose C.H., Jacobs D.E., Jorgensen R.J., Nansen P. Studies on the helminth parasites of sheep in Southern Greenland. *Nordisk Vet.Medicin* 36: 77-87, 1984.
73. Skipp R.A., Hay F.S., Leathwick D.M., Popay I. Biological control of gastrointestinal nematodes of livestock in faeces and in pastures. Meat New Zeland Reporton Project 96PR36/1, 2000.
74. Smith M.C. Exclusion of infectious diseases from sheep and goat farms. *Veterinary Clinics of North America. Food Animal Practice* 6: 705-720, 1990.
75. Soulsby E.J.L. Helminths, Arthropods and Protozoa of Domesticated Animals. Baillier, Tindall and Cassell ed. London, 1977.

76. Šibalić S., Cvetković L.J., Nevenić V. (1961). Contribution to the knowledge of the harmful effect of gastrointestinal parasites on the growth of fattening lambs. *Veterinarski Glasnik* 5: 373-375, 1961.
77. Truong, P.N., Baker, D. Vetiver grass system for environmental protection. Technical Bulletin N0. 1998/1. Pacific Rim Vetiver Network. Royal Development Projects Board, Bangkok, Thailand, 1998.
78. Truong, P.N., Mason, F., Waters, D. and Moody, P. 2000. Application of vetiver Grass Technology in off-site pollution control. I. Trapping agrochemicals and nutrients in agricultural lands. *Proceeding of Second International Vetiver Conference, Bangkok, Thailand, 192, 2000.*
79. Vellinga T.V., Van den Pol-van Dasselaar A., Kuikman P.J. The impact of grassland ploughing on CO and N₂O emissions in the Netherlands. *Nutrition Cycle and Agroecosystem*, 70, 33–34, 2004.
80. Vlassoff A. Seasonal incidence of infective *Trichostrongyle* larvae on pasture: the contribution of the ewe and the role of the residual pasture infestation as sources of infection to the lamb. *New Zealand Journal of Experimental Agriculture*, 1.4, 281-284, 1976.
81. Vlassoff A. Biology and population dynamics of the free-living stages of gastrointestinal nematodes of sheep. In: A.D.Ross (edit.) *Internal parasites of sheep*. Animal Industries Workshop, Lincoln College, Perth, Australia, 11-20, 1982
82. Vujić, B., Anić, N. A contribution to the knowledge of some damages caused by subclinical parasitosis of sheep. *Veterinarski Glasnik* 12:604-608, 1958.
83. Vujić B., Bošković V., Savin Ž. The most important parasitic species of sheep and goats and their control.. I *International Conference of sheep and goat Production*. Ohrid, 375-381, 1991.
84. Whitehead D.C. *Nutrient elements in grassland. Soil plant animal relationships*. CABI, Wallingford, UK, 2000

TRENDS IN CELL-BASED SEAFOOD: THE USE OF BIOTECHNOLOGY FOR NUTRITION AND SUSTAINABILITY

Acceptance date: 02/05/2024

Narcilo Quadros Cardoso

Diretoria de Pesquisa e Produção (DPP),
Fundação Instituto de Pesca do Estado
do Rio de Janeiro (FIPERJ), Niterói, RJ,
Brazil

Departamento de Biotecnologia Marinha,
Instituto de Estudos do Mar Almirante
Paulo Moreira (IEAPM), Arraial do Cabo,
RJ, Brazil

Programa Associado de Pós-graduação
em Biotecnologia Marinha, Instituto
de Estudos do Mar Almirante Paulo
Moreira (IEAPM) / Universidade Federal
Fluminense (UFF), Arraial do Cabo, RJ,
Brazil

<https://orcid.org/0000-0003-2745-702X>

Carla Eliana Davico

Departamento de Biotecnologia Marinha,
Instituto de Estudos do Mar Almirante
Paulo Moreira (IEAPM), Arraial do Cabo,
RJ, Brazil

Empresa Brasileira de Pesquisa
Agropecuária (EMBRAPA) - Agroindústria
de Alimentos, Guaratiba, Rio de Janeiro,
Brazil

<https://orcid.org/0000-0002-9652-9747>

Michael dos Anjos dos Santos

Diretoria de Pesquisa e Produção (DPP),
Fundação Instituto de Pesca do Estado
do Rio de Janeiro (FIPERJ), Niterói, RJ,
Brazil

Departamento de Biotecnologia Marinha,
Instituto de Estudos do Mar Almirante
Paulo Moreira (IEAPM), Arraial do Cabo,
RJ, Brazil

Programa Associado de Pós-graduação
em Biotecnologia Marinha, Instituto
de Estudos do Mar Almirante Paulo
Moreira (IEAPM) / Universidade Federal
Fluminense (UFF), Arraial do Cabo, RJ,
Brazil

<https://orcid.org/0000-0002-2650-2362>

Rayanne Antunes Fernandes Sales

Diretoria de Pesquisa e Produção (DPP),
Fundação Instituto de Pesca do Estado
do Rio de Janeiro (FIPERJ), Niterói, RJ,
Brazil

Departamento de Biotecnologia Marinha,
Instituto de Estudos do Mar Almirante
Paulo Moreira (IEAPM), Arraial do Cabo,
RJ, Brazil

Programa Associado de Pós-graduação
em Biotecnologia Marinha, Instituto
de Estudos do Mar Almirante Paulo
Moreira (IEAPM) / Universidade Federal
Fluminense (UFF), Arraial do Cabo, RJ,
Brazil

<https://orcid.org/0000-0001-7507-9671>

Isabel Virgínia Gomes e Silva

Departamento de Biotecnologia Marinha, Instituto de Estudos do Mar Almirante Paulo
Moreira (IEAPM), Arraial do Cabo, RJ, Brazil
Programa Associado de Pós-graduação em Biotecnologia Marinha, Instituto de Estudos
do Mar Almirante Paulo Moreira (IEAPM) / Universidade Federal Fluminense (UFF),
Arraial do Cabo, RJ, Brazil
<https://orcid.org/0000-0002-4284-0038>

Letícia de Almeida Sant'Anna Trindade

Diretoria de Pesquisa e Produção (DPP), Fundação Instituto de Pesca do Estado do Rio
de Janeiro (FIPERJ), Niterói, RJ, Brazil
Departamento de Biotecnologia Marinha, Instituto de Estudos do Mar Almirante Paulo
Moreira (IEAPM), Arraial do Cabo, RJ, Brazil
<https://orcid.org/0000-0002-7802-9343>

Dayana Muniz Maia Montalvão

Departamento de Biotecnologia Marinha, Instituto de Estudos do Mar Almirante Paulo
Moreira (IEAPM), Arraial do Cabo, RJ, Brazil
Programa Associado de Pós-graduação em Biotecnologia Marinha, Instituto de Estudos
do Mar Almirante Paulo Moreira (IEAPM) / Universidade Federal Fluminense (UFF),
Arraial do Cabo, RJ, Brazil
<https://orcid.org/0000-0002-4619-3763>

Carolina Siqueira Safra Terra Melo

Departamento de Biotecnologia Marinha, Instituto de Estudos do Mar Almirante Paulo
Moreira (IEAPM), Arraial do Cabo, RJ, Brazil
Programa Associado de Pós-graduação em Biotecnologia Marinha, Instituto de Estudos
do Mar Almirante Paulo Moreira (IEAPM) / Universidade Federal Fluminense (UFF),
Arraial do Cabo, RJ, Brazil
<https://orcid.org/0000-0001-7838-2350>

Tailah Bernardo de Almeida

Departamento de Biotecnologia Marinha, Instituto de Estudos do Mar Almirante Paulo
Moreira (IEAPM), Arraial do Cabo, RJ, Brazil
<https://orcid.org/0000-0002-36954434>

Guilherme Búrgio Zanette

Diretoria de Pesquisa e Produção (DPP), Fundação Instituto de Pesca do Estado do Rio
de Janeiro (FIPERJ), Niterói, RJ, Brazil
<https://orcid.org/0000-0002-7084-0844>

Ricardo Coutinho

Departamento de Biotecnologia Marinha, Instituto de Estudos do Mar Almirante Paulo
Moreira (IEAPM), Arraial do Cabo, RJ, Brazil
<https://orcid.org/0000-0001-5430-2176>

Giselle Pinto de Faria Lopes

Departamento de Biotecnologia Marinha, Instituto de Estudos do Mar Almirante Paulo
Moreira (IEAPM), Arraial do Cabo, RJ, Brazil
Programa Associado de Pós-graduação em Biotecnologia Marinha, Instituto de Estudos
do Mar Almirante Paulo Moreira (IEAPM) / Universidade Federal Fluminense (UFF),
Arraial do Cabo, RJ, Brazil
<https://orcid.org/0000-0001-9502-2314>

ABSTRACT: In recent years, the animal protein industry has grown significantly due to population expansion. However, this has increased animal waste generated during meat production processes. To address this issue, cellular agriculture is becoming a promising biotechnological approach to sustainable production. The aim is to reduce animal dependency and suffering by developing cell-based protein sources, derived from animal tissues through cell cultures. This technology is rapidly advancing to meet the challenge of feeding the world's growing population a diet that is healthier, sustainable, animal-free, and environmentally friendly, and that generates minimal or no animal waste. This mini-review explores the advances and challenges in cell-based seafood production, highlighting the relevant methodologies for obtaining them from commercially important marine species and underscores the importance of developing alternative marine proteins with nutraceutical properties for the future.

KEYWORDS: alternative protein, cultivated meat, cellular agriculture, innovation.

TENDÊNCIAS EM FRUTOS DO MAR À BASE DE CÉLULAS: O USO DA BIOTECNOLOGIA PARA NUTRIÇÃO E SUSTENTABILIDADE

RESUMO: Nos últimos anos, o setor de proteína animal cresceu significativamente devido à expansão populacional. No entanto, isso resultou em um aumento dos resíduos animais gerados durante os processos de produção de carne. Para resolver esse problema, a agricultura celular está se tornando uma abordagem biotecnológica promissora para a produção sustentável. O objetivo é reduzir a dependência e o sofrimento dos animais por meio do desenvolvimento de fontes de proteína baseadas em células, derivadas de tecidos animais por meio de culturas de células. Essa tecnologia está avançando rapidamente para enfrentar o desafio de alimentar a crescente população mundial com uma dieta mais saudável, sustentável, livre de animais e ecologicamente correta, e que gere o mínimo ou nenhum resíduo animal. Esta mini-revisão explora os avanços e desafios na produção de frutos do mar com base em células, destacando as metodologias relevantes para a sua obtenção originada de espécies marinhas comercialmente importantes e ressalta a importância do desenvolvimento de proteínas marinhas alternativas com propriedades nutracêuticas no futuro.

PALAVRAS-CHAVE: proteína alternativa, carne cultivada, agricultura celular, inovação.

INTRODUCTION

The escalating global demand for animal-derived protein exacerbates the strain on ecosystems and biodiversity (FAO, 2018). Universal meat production significantly contributes to greenhouse gas emissions, with a substantial portion attributed to deforestation for grazing land (Steinfeld *et al.* 2006). Traditional agriculture is currently confronted with a formidable challenge. The consumption of animal protein and population growth exert pressure on biodiversity and deplete natural resources, thereby jeopardizing food security (Eibl *et al.* 2021; Mc Carthy *et al.* 2018).

Overfishing threatens marine biodiversity and food security, resulting in declines in seafood biomass and endangering animal populations with extinction (Palomares *et*

al. 2020). Over 15 years of exploitation, industrial fishing and fisheries are estimated to have reduced the ocean's biomass content by 80% (Myers; Worm, 2003). The demand for seafood is expected to rise due to its high content of quality protein, vitamins, trace elements, polyunsaturated fatty acids and minerals (Hassan *et al.* 2020).

Cultivated meat and seafood represent novel foods with the potential to offer an ethical, sustainable, and healthy protein source (Ong *et al.* 2021). Cell-cultured seafood production has the potential to alter several fundamental parameters considered constant in food production, including the generation of inedible excess tissue such as bones, blood, and skin, which are often discarded, leading to a negative environmental impact (Arvanitoyannis; Kassaveti, 2008). In addition to reducing environmental impact, cell-based food products can foster the development of local and autonomous markets by producing indirect environmental benefits. It can also shorten cycle times by streamlining supply chains and reducing production times from months to weeks to create functional foods (FAO; WHO, 2023).

As aquaculture transitions towards more intensive, controllable, and efficient systems, cell-cultured seafood production offers a new option to address the challenges associated with industrial aquaculture and marine fishing. This mini-review explores the opportunities and challenges in cell-based seafood production, addressing aspects such as marine cell culture, natural marine muscle tissue, and considerations regarding marine animals. We aim to summarize the trends and frontiers of cell-based food applied to seafood and to provide an overview of relevant methodologies for obtaining cells from commercially important marine invertebrate species.

SEAFOOD AS A SOURCE OF NUTRACEUTICALS

Nutraceuticals are substances, either in whole or in part, delivered as dietary supplements or ingredients clinically proven to offer benefits, including prevention and treatment of diseases. Marine nutraceuticals specifically pertain to compounds derived from sea (Ande *et al.* 2017). These compounds encompass oils (from fish, algae, seal blubber, and shark liver), which are rich in long-chain omega-3 polyunsaturated fatty acids, as well as shark cartilage, chitin, chitosan, and associated products, enzymes, peptides, protein hydrolysates, vitamins A, D and E, and other products (Alasalvar; Shahidi; Quantick, 2002). These marine nutraceuticals possess numerous unique features not found in nutraceuticals obtained from terrestrial resources, thus attracting increasing attention (Suleria *et al.* 2015).

Interest in seafood and other marine-derived compounds arises because most aquatic organisms have inherent mechanisms to survive hostile oceanic environments, including varying salinity, pressure, temperature, and illuminations. Most marine organisms produce several secondary metabolites that, while not directly involved in central physiological functions, contribute to their survival. They synthesize novel compounds with interesting

bioactivities, facilitating adaptation to these conditions (Venugopal, 2018). Nutraceuticals derived from seafood have already been recognized for their beneficial effects on human health, attributed to various physiological functions (McManus; Newton, 2011). Studies indicate that consuming seafood can reduce the risk of heart attack, stroke, obesity, and hypertension (Stanek *et al.* 2023; Giosuè *et al.* 2022; Riccardi, 2022; Anand *et al.* 2015). Seafood is low in saturated fat and higher in “heart healthy” polyunsaturated fat, including omega-3 fatty acids (Reames, 2012).

The Bivalvia class, which represents the shellfish, constitutes a significant food source and is part of the traditional diet of numerous cultures (Tabakaeva; Tabakaev, Piekoszewski, 2018). The nutritional value of these mollusks includes proteins and amino acids of high biological values, B-complex vitamins, saturated long-chain fatty acids, and minerals (Wright; Fan; Baker, 2018; Willer; Aldridge, 2020). The levels of saturated amino acids, omega-3 fatty acids, potassium, sodium, iron, and chlorine are higher in saltwater bivalves compared to freshwater bivalves (Moniruzzaman *et al.* 2021). Considering such nutritional composition, regular consumption of mollusks could improve immunity and reduce the risk of developing diseases (Chakraborty; Joy, 2020). The shells, soft tissues, and mucilage of mollusks are used in the pharmaceutical and food industry to develop medications for the treatment of various illnesses (Khan; Liu, 2019; Pissia, Matsakidou, Kiosseoglou, 2021; Lobine, Rengasamy; Mahomoodally, 2022).

Among the critical seafood, decapod crustaceans, which include lobsters, shrimps, and crabs, stand out for their significant role in the human food supply and the global economy (Mahmood Ghafor, 2020; FAO, 2022). Besides their ecological importance to the environment, decapod crustaceans are relevant for human health, providing abundant protein and micronutrients for nutrition (Behringer; Duermit-Moreau, 2021). The high commercial value of these animals attracts agribusiness, food industries and the marine ornamental business (Calado *et al.* 2003).

Aquaculture farming and fishing ensure a quality protein source for human consumption. Fish is a rich source of essential nutrients, including highly digestible proteins, vitamins A, and D3, trace minerals as iodine and selenium, and n-3 long-chain polyunsaturated fatty acids and its consumption is generally regarded as part of a healthy dietary pattern (Ramalho Ribeiro *et al.* 2019). In addition to their high nutritional value, fish proteins have functional properties such as water-holding capacity, gelling, emulsification, and textural properties. These characteristics play crucial roles in determining the textural attributes of these products, making them important quality parameters and strategic tools for cell-based sustainable biotechnology (Pal *et al.* 2018).

ADVANCES IN CELL-BASED SEAFOOD

Given livestock production's significant adverse impacts, establishing an *in vitro* meat production system is increasingly justified (Datar; Betti, 2010). Consumer demand for cellular meat production primarily arises from concerns about the environment and animal welfare. At the same time, secondary considerations include consumer and public health aspects of animal production and food security (Warner, 2019).

Producing meat through tissue engineering and cell culture is not new. Initially, early attempts were concentrated in universities and other research units. Before Mark Post showcased the world's first so-called lab-grown hamburger in late 2013 (Kupferschmidt, 2013), NASA had invested in producing edible fish muscle protein *in vitro*. The goal was to send high quantities of nutritional food to space with the least possible volume. To achieve this, researchers isolated muscle cells from the dorsal muscle of *Carassius* fish as an initial step (Benjaminson; Gilchrist; Lorenz, 2002).

Following the initial academic efforts, the concept of cell-based meat garnered attention in the food industry. Consequently, various startups related to the production of cell-based meat or the development of technologies enhancing its manufacture emerged, with many focusing on seafood (**Table 1**). While some focus has been on the biology and engineering required to optimize the manufacturing process, most of the debate has revolved around cultural, environmental, and regulatory considerations (Faustman *et al.*, 2020). As startups began to develop prototypes of structured foods, new regulatory concerns emerged. The first governmental approval for the commercialization of cell-based meat was granted by the Singapore Food Agency in late 2020, permitting *Eat Just*, a startup based in the United States, to sell cell-based chicken in Singapore (Southey, 2022).

Company's Name	Focus	Location	Year of Foundation
Fineless Foods	Fish	USA	2016
Wild Type	Fish	USA	2016
BlueNalu	Seafood	USA	2017
Avant Meats	Seafood	Hong Kong	2018
Shiok Meats	Seafood	Singapore	2018
ArtMeat	Beef and Seafood	Russia	2019
Bluu Biosciences	Seafood	Germany	2020
Cultured Decadence	Seafood	USA	2020
Magic Caviar	Caviar	Amsterdam	2020
Umami Bioworks	Seafood	Singapore	2020
Wanda Fish	Fish	Israel	2021
Mermade Seafood	Seafood	Israel	2021
Sustineri Piscis	Fish	Brazil	2023

Table 1: List of start-ups focusing on the production of cell-based seafood

Market entry of cell-based food products may require authorization at various levels, encompassing processes such as a food safety assessment of the cell-based food product, approval of planned and implemented quality controls, assurance protocols for the production process, and adherence to approved labeling requirements for the products. The essential elements for a practical regulatory framework for cell-based food are still considered in many countries (FAO, 2022). In the Brazilian regulatory context, the Good Food Institute Brazil (GFI) has launched a regulatory study to identify potential adjustments in the current regulatory frameworks. Scientifically grounded arguments support this endeavor and involves engaging the country's regulatory agencies (GFI, 2022). Recently, GFI Brazil published a Food Safety Plan for a cultivated meat burger, contributing to assessing safety aspects in cultivated meat production through applying the Hazard Analysis and Critical Control Points (HACCP) approach (Sant'Ana *et al.* 2023).

The recent evolution of cell culture techniques that facilitate the growth of edible animal tissue *in vitro* represents an example of potentially disruptive technology with many exciting aspects to consider (Van der Weele *et al.*, 2019; Stephens; Sexton; Driessen, 2019). With the current advancement in technology, lab-grown meat, also known as cultured meat, is expected to significantly impact the food market in the future (Ismail; Hwang; Joo, 2020).

CHALLENGES IN CELL-BASED SEAFOOD

The absence of fish and marine invertebrate cell lines means that cell culture research is conducted using primary cells isolated from these animals. A primary cell culture is initiated directly from the tissue (Jedrzejczak-Silicka, 2017). The isolation process involved in primary culture allows for precise control over hormonal, substrate, and physical conditions, which can influence cell and tissue function (Hightower; Renfro, 1988). Muscle and fat cells are crucial cell types aiming for the final product. As some of these cells can be hard to isolate, precursors of these cell types, such as satellite cells, fibro-adipogenic progenitors, pre-adipocytes, and mesenchymal stem cells, become strong candidates to be used as sources for the development of a cell-based seafood prototype (Bomkamp *et al.* 2023).

Isolation and Primary Culture

The techniques for obtaining primary cell cultures from seafood are similar to those used for mammals and other animals. Initially, the animal is sterilized with ethanol and anesthetized, after which the tissue of interest is removed under sterile conditions and subjected to a series of antibiotics to prevent microbial contamination. Subsequently, the desired tissue undergoes dissociation methods, such as enzymatic dissociation (Plotnikov, Karpenko; Odintsova, 2003; Oestbye; Ytteborg, 2019) or mechanical dissociation (Van der Merwe *et al.* 2010), to obtain a single-cell suspension for seeding in an appropriate culture medium.

Alternatively, the explant method can be employed instead of the single-cell suspension. In this case, no enzyme is used, and the original tissue is minced into smaller pieces, which are then placed in culture flasks. Cells begin to migrate out of the tissue pieces and adhere to the surface of the culture flask (Freshney, 2010; Potts *et al.*, 2020). Observations have been made of cell migration and growth from heart explants of the Indian Mud Crab *Scylla serrata* for over three weeks, demonstrating adherent cells with round, epithelioid-like, and fibroblastic morphologies (Sivakumar *et al.* 2019).

Previous studies have reported protocols for cell sorting to establish primary monogenic cultures. Techniques utilizing density gradient media to separate cells by density have yielded significant results (Gong *et al.* 2008; Odintsova; Dyachuk; Nezlin, 2010; Nogueira *et al.* 2013), particularly for shrimp hepatopancreatic cells (Toullec *et al.* 1992). Koiwai *et al.* (2019) recently isolated crustacean hemocytes using lectins and magnetic-activated cell sorting (MACS).

Over the past 30 years, researchers have attempted to create primary cultures of various crustacean species, with shrimps being the most commonly studied. Methods have been described for culturing shrimp ovaries, lymphoid tissues, cardiac, nerve, hematopoietic, hepatopancreatic, and epidermal cells (Nadala; Lu; Loh, 1993; Luedeman; Lightner, 1992; Tapay *et al.* 1995; Chen; Wang, 1999; Kasornchandra *et al.* 1999; Maeda *et al.* 2004; Anoop *et al.* 2021). Other crustaceans, such as crabs and lobsters, have also been the focus of primary cell culture research (Fadool; Michel; Ache, 1991; Stepanyan, 2004; Li; Shields, 2007; Sashikumar; Desai, 2008; Deepika, Makesh; Rajendran, 2014; Sivakumar *et al.* 2019). Among the primary tissues developed, cardiac tissue (Owens; Smith, 1999) and ovaries (Fraser; Hall, 1999) have shown more promising results, remaining viable in culture for more extended periods.

Various types of bivalve mollusk tissues have been experimented with to initiate primary cell cultures. Heart (Cecil, 1969; Wen; Kou; Chen, 1993), mantle (Perkins; Menzel, 1964), digestive glands (Le Pennec; Pennec, 2001), and gills (Gómez-Mendikute *et al.* 2005; Cornet, 2006) have shown substantial results in the growth of primary cell cultures of bivalve mollusks. Similarly, embryonic tissue (Boulo *et al.* 2000) and hemolymph (Ji *et al.* 2017) have also demonstrated significant results. Among these tissue sources, cultures from embryonic tissues show better potential for proliferation than cells from adult tissues (Odintsova; Khomenko, 1991).

Recent research has successfully cultivated adductor muscle cells of scallop *Patinopecten yessoensis*, obtaining fibroblast-like cells with multiple filopodia, similar to precursors of mature muscle cells in mammals. This was achieved using tissue explant methods and supplementing the medium with adductor muscle extract, fetal bovine serum, and supplements for insect cell culture (Suzuki *et al.* 2021).

***In vitro* culture conditions for marine animal cells**

Concerns regarding the culture conditions of mollusk cells have been addressed over the years. The growth medium commonly used for the cultivation of marine bivalve cells typically consists of a formulation of L-15 medium (Chen; Wang, 1999; Ladhar-Chaabouni *et al.* 2021) supplemented with soluble factors to enhance cell viability (Domart-Coulon *et al.* 1994). Adding taurine, an amino acid found in bivalve hemolymph, to the medium regulates osmolarity and improves cell viability (Lange, 1963). Medium osmolarity, pH, and incubation temperature are adjusted based on the specific animal species and body part. Generally, the growth medium's osmolarity must be like hemolymph's (Odintsova; Khomenko, 1991), typically ranging between 760 to 1100mOsmol for bivalves. The temperature of incubation is also a variable parameter. While low incubation temperatures reduce the risk of culture contamination, temperatures below 15° C do not contribute to cell migration.

The substrate to which cells adhere has significant effects on culture viability. Attached cells often exhibit increased metabolic activity (Ben-Ze'ev; Farmer; Penman, 1980). A desirable substrate promotes cell attachment and spreading *in vitro*, and considering that muscle cells are anchorage-dependent, selecting a compatible substrate is vital for bivalve muscle cells. It has been demonstrated that poly-D-lysine with a molecular weight exceeding 100 kDa promotes conditions for attachment of bivalve heart muscle cells *in vitro* (Buchananan *et al.* 1999).

Fish cell culture protocols are very similar to other established animal cell cultures, with some adaptations regarding incubation temperature and medium osmolality specific to different fish species (Fernandez *et al.* 1993). Commonly used growth media are Eagle's Minimum Essential Medium (EMEM), Leibovitz Medium L-15 or Medium 199 (Fryer; Lannan, 1994). According to Wolf and Ahne (1982), the more commonly used and elaborate media include vitamins and amino acids, often supplemented with fetal bovine serum (FBS). FBS is a joint supplement used in various protocols for animal cell cultures, containing a mixture of amino acids, proteins, vitamins, hormones, and other nutrients and factors that support the growth and survival of animal cells in culture (Barnes; Sato, 1980). The typical supplementation proportion of medium with FBS is 10% of the total medium volume, although some cell lines grow satisfactorily with only 5% serum, albeit at slightly reduced growth rates. Eagle's Minimal Essential Medium supplemented with fetal bovine serum is considered a versatile culture medium for mammals, birds, reptiles, amphibians, and fish cells (Lakra; Swaminathan; Joy, 2011) with appropriate adjustment in incubation temperatures. Unlike mammalian cell cultures, fish cells can thrive with infrequent subcultures (every 7-14 days or more) and rarely require changes in growth medium between subcultures (Fryer; Lannan, 1994).

The optimal growth temperature range reflects the donor fish species and its natural environment (Nicholson, 1989). The ease of growing fish cells at a lower temperature

compared to mammalian cells may provide cost benefits for cellular fish meat production. Moreover, blending tissue engineering with modern aquaculture techniques presents an attractive opportunity to utilize marine muscle cell culture for *in vitro* fish meat production (Goswami *et al.* 2022).

Cell immortalization and cell stemness

Cells obtained from primary cell culture undergo only a limited number of cell divisions before entering a state of senescence, wherein they experience stable growth arrest. The use of primary culture for cell-based seafood production becomes unsustainable in the long term due to the necessity of maintaining a donor animal as a cell source. To address this issue, the immortalization of target cells for alternative food production is a necessary goal to achieve large-scale production. Cell immortalization disrupts the mechanisms responsible for reaching senescence (Soice; Johnston, 2021).

Embryonic stem cells (ESC) represent a valuable repository of diverse cell morphotypes. Due to their plasticity, stem cells obtained from embryos, classified as totipotent cells, theoretically can differentiate into any cell type of the organism (Rippon; Bishop, 2004). Few researchers have achieved positive results in obtaining somatic cells from ESCs of marine animals under *in vitro* conditions (**Table 2**). These cells can undergo considerable differentiation, facilitated by supplementing the culture medium with specific factors (Holen; Kausland; Skjærven, 2010).

Species	Embryonic stage	Cellular differentiation	Reference
<i>Sparus aurata</i> (Fish)	Morula	neuron-like and epithelial-like	Vergès-Castillo <i>et al.</i> 2021
<i>Gadus morhua</i> (Fish)	Mid-blastula	fibroblast-like and neuronal-like	Holen, Kausland, e Skjærven, 2010
<i>Mytilus trossulus</i> (Mussel)	Trochophore larvae	ciliated cells, muscle cells and neuron-like	Odintsova, Dyachuk, e Nezhlin, 2010
<i>Loteolabrax japonicus</i> (Fish)	Blastula	neuron-like and muscle cells	Chen, Sha, e Ye, 2003
<i>Macrobrachium rosenbergii</i> (freshwater shrimp)	Fertilized egg	connective-tissue-like morphology	Sudarshan <i>et al.</i> 2024

Table 2: Cells obtained by differentiation of embryonic stem cells delivered from seafood animals

Cell lines are already available from seafood species. Establishing an immortalized lineage from bivalves has been a focal point for numerous researchers due to their social and ecological significance. Unsuccessfully, the only immortalized cell line originating from a mollusk species thus far is the embryonic delivery cell line provided by the freshwater snail *Biomphalaria glabrata* (Wang; Wang, 2019). Various transfection and cell hybridization techniques in crustaceans in shrimp cell culture are being explored as alternatives to

establish stable long-term cell lines, showing promising results (Ma; Zeng; Lu, 2017). Only three crustacean cell lines have been established, originating from the shrimp genus *Penaeus*. These cell lines are identified in the *Cellulosaurus* database as OKTr-1 (RRID: CVCL_9U40), OKTr-23 (RRID: CVCL_9U41), and PmLyO-Sf9 (RRID: CVCL_A8SX). Both cell lines OKTr-1 and OKTr-23 were described by Tapay *et al.* (1995) and categorized as transformed cells originating from the shrimp lymphoid tissues cell line (Oka). Conversely, the PmLyO-Sf9 cell line was derived from the hybridization of sf9 cells from the insect *Spodoptera frugiperda* with the lymphoid tissue cells of the shrimp *Penaeus monodon* (Anoop *et al.* 2021; Sathyabhama *et al.* 2021).

Most fish cell lines are derived from tissues such as skin, gill, heart, liver, kidney, spleen, swim bladder, brain, etc. Embryos and fins are the most frequently utilized tissue sources for primary culture. After the ovary, the fin is the second most common tissue used for cultivation due to its high regenerative ability (Fryer; Lannan, 1994). An increasing number of marine fish cell lines are available, likely in response to the growing interest in testing viral load, examining water toxicology, and developing vaccines for farmed fish. However, few of these cell lines have been utilized to produce edible fish, apart from a study on producing fish-based proteins for space voyagers on long journeys (Benjaminson; Gilchrist; Lorenz, 2002). Bairoch (2018) listed more than 139,500 cell lines in *Cellulosaurus* database, with about 856 being fish cell lines.

DISCUSSION

Climate change, food supply shocks caused by pandemics, and population growth threaten the traditional food system. Satisfying the demand for meat in the future will be a challenge if we intend to maximize the use of agricultural resources and reduce greenhouse gas production (FAO, 2017). Therefore, disruptive food technologies will be necessary for a more resilient, sustainable, and adequate food system. In this perspective, it is required to consider the factors influencing consumer perceptions of new food technologies, leading to greater acceptance of them (Siegrist; Hartmann, 2020). Informed decisions must be made to achieve scalability, reduce costs, and navigate regulatory challenges effectively. In addition to the core food safety assessments, regulatory considerations may be necessary for other issues such as labeling, consumer preference/acceptance, and ethical or religious aspects of cell-based food products (FAO, 2022).

To discuss the relevant technical issues of cell-based food production, it is important to use clear and consistent terminologies that all the stakeholders can accept. Terminologies and labels are also necessary and direct means of communicating information to consumers (FAO, 2021). Fernandes *et al.* (2019) suggest that, despite a clustering of recurring and highly relevant terms, cultured meat is a subject that spans various areas of knowledge. Nomenclature can significantly impact consumer perception, marketing efforts and relevant

regulatory actions such as labeling. While consumer acceptance is critical to the industry's success, the common or usual name chosen to label cell-based products must meet regulatory criteria, not just marketing needs (Hallman *et al.* 2023). A consistent nomenclature is crucial in bringing cultivated protein products to the commercialized market (Malerich; Bryant, 2022). A literature synthesis was conducted on various relevant terminologies by The Food and Agriculture Organization of the United Nations and the World Health Organization (FAO; WHO, 2023). The results showed that while some preferences differ among different sectors, "cell-based food" was less confusing, conveniently overarching, and generally well-accepted by consumers. However, it is essential to note that no term is 100 percent scientifically correct.

Although the terminology is still under discussion, cellular agriculture and cell-based meats have been considered the future of foods. There has been considerable buzz with the launch of next-generation meat alternatives. This field's growing excitement has prompted increasing research, value propositions, business investments, media coverage and discussion. Even so, crucial fundamental research to overcome key technical challenges must be carried out before cell-based meat production can be a reality. Alongside research progress, regulations and standardization of cell-based meat must keep up with the rapid progress in this field (Ong; Choudhury; Naing 2020).

Cultured meat is a promising but early-stage technology with critical technical challenges, including cell source, culture media, mimicking the *in vivo* myogenesis environment, animal-derived and synthetic materials, and bioprocessing for commercial-scale production (Stephens *et al.* 2018). Thus, one of the technical challenges of this technology is related to the use of animals and products derived from them. As Bhat *et al.* (2019) mentioned, cell-based meat does not involve slaughtering many animals. However, the initial source of cells and biopsies for starting cell cultures will certainly impact consumers' perceptions and decisions. Legislation regarding cell sourcing and isolation may exist concerning the acquisition of biopsies from live or deceased animals, which could raise animal welfare concerns. Cell bank regulations are also in place in several countries (EMA, 1998; FDA, 2010).

Besides, mass production of cell-based food utilizing traditional cell culture protocols will require hundreds of gallons of fetal bovine serum to produce a few pounds of meat. This implies the continuation of livestock production and an increase in animal exploitation. Due to ethical, environmental, and biological concerns, alternatives to fetal bovine serum or any animal-derived supplement for cell culture are needed. High-volume cell production in industrial bioreactors using serum-free medium is essential for commercial cultured meat manufacturing (Garrison *et al.* 2022). Numerous studies have focused on finding an optimal substitute for FBS. The use of fungi extracts (Benjaminson; Gilchrist; Lorenz, 2002), microalgae extracts (Ng *et al.* 2020), or cyanobacteria (Jeong *et al.* 2021) has shown success in eliminating or significantly reducing the use of FBS in culture. Cell-based food

production may also generate new biological or chemical by-products and waste, subject to specific regulations such as environmental legislation. Furthermore, these by-products may be utilized in feed applications if they meet feed safety requirements (FAO, 2022).

On the other hand, one potential future advantage of cell-based meats is the ability to design products with specific nutritional characteristics that are not typically achievable through conventional animal feeding approaches (Faustman *et al.* 2020). Cultivated meat technology can potentially disrupt the food industry; indeed, it is an inevitable reality. This new technology offers an alternative solution to address the environmental, health and ethical issues associated with the demand for meat products. The global market eagerly anticipates biotechnological advancements in the cultivated meat production chain (Santos *et al.* 2023).

A few years ago, cellular agriculture progressed with new research and publications to improve the selection of cell species and cell types to intensify cell-based meat production. Pressing issues such as global warming, environmental instability, and food security have propelled cell-cultured seafood into the spotlight (Rubio *et al.* 2019). The marine environment harbors many bioactive compounds with unique properties, offering significant potential for biotechnological applications (Boziaris, 2014). Seafood production from marine cell cultures represents a novel approach and an exciting opportunity for cellular agriculture. Cell-based seafood holds promising market penetration and sustainability potential, as it can produce meat from species that are challenging to cultivate in traditional aquaculture at competitive prices (Farzad, 2021). Consequently, the emergence of cell-based seafood industries has drawn the attention of aquaculture sectors. However, its market presence remains hypothetical due to consumer acceptance being contingent upon the approval of cell-based meat (Lindfors; Jakobsen, 2022).

Our mini-review of the relevant literature indicates that marine cell and tissue culture research has been largely overlooked. Recent advancements in cellular agriculture underscore the substantial environmental benefits that may result from substituting some industrially raised and processed meat with cultured meat alternatives. There are notable research gaps in marine cell culture that present valuable opportunities for further exploration (Munteanu *et al.* 2021; Rodríguez Escobar *et al.* 2021). Cell-based meat represents a promising strategy that could offer tools for nutritional enrichment and sustainable seafood generation without the environmental impact associated with traditional methods (Azhar *et al.* 2023).

The future generation of meat substitutes should focus on reducing saturated fat content and using fewer additives (Franca *et al.* 2023). Meanwhile, due to the advances in cultured meat technology worldwide and the slight emphasis that this area has received in no country, we may be missing significant opportunities in this market (GFI, 2022). According to Morais-da-Silva; Villar; Reis (2022), the potential of plant-based and cultivated meat production for creating new and high-skilled jobs has been highlighted. The impact of

novel food production systems on employment in conventional meat production may differ for each value chain stage. Technological advancements and investments in cultured meat research suggest that cultured meat will become a mainstream food commodity shortly (Post *et al.* 2020).

CONFLICT OF INTEREST

The authors declare that the research was conducted without any commercial or financial relationships that could potentially create a conflict of interest.

FUNDING

FAPERJ (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro), SEAPPA (Secretaria de Estado de Agricultura, Pecuária, Pesca e Abastecimento) and IEAPM (Instituto de Estudos do Mar Almirante Paulo Moreira) collaboration in the Scientific call by FAPERJ 03/2021, resulted in the scholarship awarded to Narcilo Quadros Cardoso (E-26/203.283/2021), Michael dos Anjos dos Santos (E-26/200.061/2022), and Rayanne Antunes Fernandes Sales (E-26/201.854/2022). GFI (The Good Food Institute), EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária), and IEAPM (Instituto de Estudos do Mar Almirante Paulo Moreira) collaboration in GFI Research Program Exploratory Research Grants, resulted in the fellowship awarded to Carla Eliana Davico.

REFERENCES

- Alasalvar, C.; Shahidi, F.; Quantick, P. 2002. Food and health applications of marine nutraceuticals: a review. In: **Seafoods - Quality, Technology and Nutraceutical Applications**. Berlin: Springer-Verlag Berlin Heidelberg. https://doi.org/10.1007/978-3-662-09836-3_15
- Anand, S.S. *et al.* 2015. Food consumption and its impact on cardiovascular disease: importance of solutions focused on the globalized food system: a report from the workshop convened by the World Heart Federation. **Journal of the American College of Cardiology** 66 (14): 1590–1614. <https://doi.org/10.1016/j.jacc.2015.07.050>
- Ande, M.P. *et al.* 2017. Marine nutraceuticals. **Aquaculture Times** 3 (2): 6-9.
- Anoop, B.S. *et al.* 2021. Immortalization of shrimp lymphoid cells by hybridizing with the continuous cell line Sf9 leading to the development of *PmLyO-Sf9*. **Fish & Shellfish Immunology** 113: 196–207. <https://doi.org/10.1016/j.fsi.2021.03.023>
- Arvanitoyannis, I.S.; Kassaveti, A. 2008. Fish industry waste: treatments, environmental impacts, current and potential uses. **International Journal of Food Science & Technology** 43 (4): 726–45. <https://doi.org/10.1111/j.1365-2621.2006.01513.x>
- Azhar, A. *et al.* 2023. Cell-based meat: The molecular aspect. **Frontiers in Food Science and Technology** 3: 1-23. <https://doi.org/10.3389/frfst.2023.1126455>

- Bairoch, A. 2018. The Cellosaurus, a cell-Line knowledge resource. **Journal of Biomolecular Techniques** 29 (2): 25–38. <https://doi.org/10.7171/jbt.18-2902-002>
- Barnes, D.; Sato, G. 1980. Serum-Free cell culture: a unifying approach. **Cell** 22 (3): 649–55. [https://doi.org/10.1016/0092-8674\(80\)90540-1](https://doi.org/10.1016/0092-8674(80)90540-1)
- Behringer, D.C.; Duermit-Moreau, E. 2021. Crustaceans, one health and the changing ocean. **Journal of Invertebrate Pathology** 186 (107500). <https://doi.org/10.1016/j.jip.2020.107500>
- Benjaminson, M.A.; Gilchrist, J.A.; Lorenz, M. 2002. *In vitro* edible Muscle Protein Production System (MPPS): Stage 1, Fish. **Acta Astronautica** 51 (12): 879–89. [https://doi.org/10.1016/S0094-5765\(02\)00033-4](https://doi.org/10.1016/S0094-5765(02)00033-4)
- Ben-Ze'ev, A.; Farmer, S.R.; Penman, S. 1980. Protein synthesis requires cell-surface contact while nuclear events respond to cell shape in anchorage-dependent fibroblasts. **National Library of Medicine. Cell** 21 (2): 365–372. [https://doi.org/10.1016/0092-8674\(80\)90473-0](https://doi.org/10.1016/0092-8674(80)90473-0)
- Bhat, Z.F. *et al.* 2019. Technological, regulatory, and ethical aspects of *in vitro* meat: a future slaughter-free harvest. **Comprehensive Reviews in Food Science and Food Safety** 18 (4): 1192-1208. <https://doi.org/10.1111/1541-4337.12473>
- Bomkamp, C. *et al.* 2023. Differentiation and maturation of muscle and fat cells in cultivated seafood: lessons from developmental biology. **Marine Biotechnology** 25(1): 1-29 <https://doi.org/10.1007/s10126-022-10174-4>
- Boulo, V. *et al.* 2000. Infection of culture embryo of the pacific oyster, *Crassostrea gigas*, by pantropic retroviral vectors. **In vitro Cellular & Developmental Biology - Animal** 36 (6): 395-399. [https://doi.org/10.1290/1071-2690\(2000\)036<0395:IOCECO>2.0.CO;2](https://doi.org/10.1290/1071-2690(2000)036<0395:IOCECO>2.0.CO;2)
- Bozaris, I.S. 2014. Food ingredients from the marine environment. Marine biotechnology meets food science and technology. **Frontiers in Marine Science** 1: 1-4. <https://doi.org/10.3389/fmars.2014.00066>
- Buchanan, J.T. *et al.* 1999. Improved attachment and spreading in primary cell cultures of the eastern oyster, *Crassostrea virginica*. **In vitro Cellular & Developmental Biology - Animal** 35 (10): 593–98. <https://doi.org/10.1007/s11626-999-0097-2>
- Calado, R. *et al.* 2003. Marine ornamental decapods—popular, pricey, and poorly studied. **Journal of Crustacean Biology** 23 (4): 963–973. <https://doi.org/10.1651/C-2409>
- Cecil, J.T. 1969. Mitoses in cell cultures from cardiac tissue of the surf clam *Spisula solidissima*. **Journal of Invertebrate Pathology** 14 (3): 407–10. [https://doi.org/10.1016/0022-2011\(69\)90170-0](https://doi.org/10.1016/0022-2011(69)90170-0)
- Chakraborty, K.; Joy, M. 2020. High-value compounds from the mollusks of marine and estuarine ecosystems as prospective functional food ingredients: An Overview. **Food Research International** 137: 1-36. <https://doi.org/10.1016/j.foodres.2020.109637>
- Chen, S.; Sha, Z.; Ye, H. 2003. Establishment of a pluripotent embryonic cell line from Sea Perch (*Lateolabrax japonicus*) embryos. **Aquaculture** 218 (1–4): 141–51. [https://doi.org/10.1016/S0044-8486\(02\)00570-7](https://doi.org/10.1016/S0044-8486(02)00570-7)

- Chen, S.N.; Wang, C.S. 1999. Establishment of cell lines derived from oyster, *Crassostrea gigas* thunberg and hard clam, *Meretrix lusoria* Röding. **Methods in Cell Science** 21 (4): 183–92. <https://doi.org/10.1023/A:1009829807954>
- Cornet, M. 2006. Primary mantle tissue culture from the bivalve mollusc *Mytilus galloprovincialis*: Investigations on the growth promoting activity of the serum used for medium supplementation. **Journal of Biotechnology** 123 (1): 78–84. <https://doi.org/10.1016/j.jbiotec.2005.10.016>
- Datar, I.; Betti, M. 2010. Possibilities for an *in vitro* meat production system. **Innovative Food Science and Emerging Technologies** 11 (1): 13–22. <https://doi.org/10.1016/j.ifset.2009.10.007>
- Deepika, A.; Marapan, M.; Rajendran K.V. 2014. Development of primary cell cultures from mud crab, *Scylla serrata* and their potential as an *in vitro* model for the replication of white spot syndrome virus. **In vitro Cellular & Developmental Biology - Animal** 50 (5): 406–16. <https://doi.org/10.1007/s11626-013-9718-x>
- Domart-Coulon, I. *et al.* 1994. Identification of media supplements that improve the viability of primarily cell cultures of *Crassostrea gigas* oysters. **Cytotechnology** 16 (2): 109–20. <https://doi.org/10.1007/BF00754613>
- Eibl, R. *et al.* 2021. Cellular agriculture: opportunities and challenges. **Annual Review of Food Science and Technology** 12: 51-73. <https://doi.org/10.1146/annurev-food-063020-123940>
- EMA. European Medicines Agency. 1998. **Quality of biotechnological products: derivation and characterization of cell substrates used for production of biotechnological/biological products**. Available online: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-5-d-derivation-characterisation-cell-substrates-used-production-biotechnological/biological-products-step-5_en.pdf (Accessed: march 2024)
- Fadool, D.A.; Michel, W.C.; Ache, B.W. 1991. Sustained primary culture of Lobster (*Panulirus Argus*) olfactory receptor neurons. **Tissue and Cell** 23 (5): 719–731. [https://doi.org/10.1016/0040-8166\(91\)90025-O](https://doi.org/10.1016/0040-8166(91)90025-O)
- FAO. Food and Agriculture Organization of the United Nations. 2017. **The future of food and agriculture - Trends and challenges**. Rome. Available online: <https://www.fao.org/3/i6583e/i6583e.pdf> (Accessed: march 2024)
- FAO. Food and Agriculture Organization of the United Nations. 2018. **World livestock: transforming the livestock sector through the sustainable development goals**. Rome. <https://doi.org/10.4060/ca1201en>
- FAO. Food and Agriculture Organization of the United Nations. 2021. **Food Labelling**. Rome. Available online: <https://www.fao.org/food-labelling/en/> (Accessed: march 2024)
- FAO. Food and Agriculture Organization of the United Nations. 2022. **Food safety aspects of cell-based food. Background document three – Regulatory frameworks**. Rome. <https://doi.org/10.4060/cc2353en>
- FAO; WHO. Food and Agriculture Organization of the United Nations; World Health Organization. 2023. **Food safety aspects of cell-based food**. Rome. <https://doi.org/10.4060/cc4855en>

Farzad, R. 2021. Cellular agriculture for production of cell-based seafood: FS432/FSHN21-2, 9/2021. **Food Science and Human Nutrition** 5: 1-4. <https://doi.org/10.32473/edis-fs432-2021>

Faustman, C. *et al.* 2020. Cell-based meat: the need to assess holistically. **Journal of Animal Science** 98 (8): 1–7. <https://doi.org/10.1093/jas/skaa177>

FDA. Food and Drug Administration. 2010. **Characterization and qualification of cell substrates and other biological materials used in the production of viral vaccines for infectious disease indications**. Available online: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/characterization-and-qualification-cell-substrates-and-other-biological-materials-used-production> (Accessed: march 2024)

Fernandes, A.M.; Fantinel, A.L.; Souza, A.R.L.; Révillion, J.P.P. 2019. Trends in cultured meat: a bibliometric and sociometric analysis of publication. **Brazilian Journal of Information Science: research trends** 13 (3): 56–67. <https://doi.org/10.36311/1981-1640.2019.v13n3.06.p56>

Fernandez, R.D. *et al.* 1993. Comparative growth response of fish cell lines in different media, temperatures, and sodium chloride concentrations. **Fish Pathology** 28 (1): 27–34. <https://doi.org/10.3147/jfsp.28.27>

Franca, P.A.P *et al.* 2022. Meat substitutes - past, present, and future of products available in Brazil: changes in the nutritional profile. **Future Foods** 5: 1-9. <https://doi.org/10.1016/j.fufo.2022.100133>

Fraser, C.A.; Hall, M.R. 1999. Studies on primary cell cultures derived from ovarian tissue of *Penaeus monodon*. **Methods in Cell Science** 21 (4): 213-218. <https://doi.org/10.1023/A:1009851809288>

Freshney, R.I. 2015. **Culture of animal cells: a manual of basic technique and specialized applications**. New Jersey, John Wiley & Sons.

Fryer, J.L.; Lannan, C.N. 1994. Three decades of fish cell culture: A current listing of cell lines derived from fishes. **Journal of Tissue Culture Methods** 16: 87-94. <https://doi.org/10.1007/BF01404816>

Garrison, G.L.; Biermacher, J.T.; Brorsen, B.W. 2022. How much will large-scale production of cell-cultured meat cost? **Journal of Agriculture and Food Research** 10: 1-8. <https://doi.org/10.1016/j.jafr.2022.100358>

GFI. The Good Food Institute Brazil. 2022. **Estudo Regulatório sobre Proteínas Alternativas no Brasil - Carne Cultivada**. Available online: <https://gfi.org.br/wp-content/uploads/2022/11/Estudo-Regulatorio-Carne-Cultivada-GFI-Brasil.pdf> (Accessed: march 2024)

Giosuè, A. *et al.* 2022. Relations between the consumption of fatty or lean fish and risk of cardiovascular disease and all-cause mortality: A systematic review and meta-analysis. **Advances in Nutrition** 13 (5): 1554-1565. <https://doi.org/10.1093/advances/nmac006>

Gómez-Mendikute, A. *et al.* 2005. Characterization of mussel gill cells *in vivo* and *in vitro*. **Cell and Tissue Research** 321 (1): 131–40. <https://doi.org/10.1007/s00441-005-1093-9>

Gong, N. *et al.* 2008. Culture of outer epithelial cells from mantle tissue to study shell matrix protein secretion for biomineralization. **Cell and Tissue Research** 333 (3): 493-501. <https://doi.org/10.1007/s00441-008-0609-5>

- Goswami, M. *et al.* 2022. Role and relevance of fish cell lines in advanced *in vitro* research. **Molecular Biology Reports** 49 (3): 2393–2411. <https://doi.org/10.1007/s11033-021-06997-4>
- Hallman, W.K *et al.* 2023. Cell-based, cell-cultured, cell-cultivated, cultured, or cultivated. What is the best name for meat, poultry, and seafood made directly from the cells of animals? **npj Science of Food** 7 (62): 1-16. <https://doi.org/10.1038/s41538-023-00234-x>
- Hassan, S. *et al.* 2020. Nutritional and health benefits of seafoods. In: Egbuna, C.; Tupas, G.D. (ed). **Functional Foods and Nutraceuticals**: 219–239. Cham: Springer International Publishing. https://doi.org/10.1007/978-3-030-42319-3_13
- Hightower, L. E.; Renfro, L.J. 1988. Recent applications of fish cell culture to biomedical research. **Journal of Experimental Zoology** 248 (3): 290–302. <https://doi.org/10.1002/jez.1402480307>
- Holen, E.; Kausland, A.; Skjærven, K. 2010. Embryonic stem cells isolated from Atlantic cod (*Gadus morhua*) and the developmental expression of a stage-specific transcription factor Ac-Pou2. **Fish Physiology and Biochemistry** 36 (4): 1029–39. <https://doi.org/10.1007/s10695-010-9381-z>
- Ismail, I.; Hwang, Y.H.; Joo, S.T. 2020. Meat analog as future food: a review. **Journal of Animal Science and Technology** 62 (2): 111-120. <https://doi.org/10.5187/jast.2020.62.2.111>
- Jedrzejczak-Silicka, M. 2017. History of cell culture. In: Gowder, S.J.T. (ed). **New Insights into Cell Culture Technology**. Croatia: InTech. <https://doi.org/10.5772/66905>
- Jeong, Y. *et al.* 2021. Marine cyanobacterium *Spirulina maxima* as an alternate to the animal cell culture medium supplement. **Scientific reports** 11(1). <https://doi.org/10.1038/s41598-021-84558-2>
- Ji, A. *et al.* 2017. Primary culture of Zhikong scallop *Chlamys farreri* hemocytes as an *in vitro* model for studying host-pathogen interactions. **Diseases of Aquatic Organisms** 125 (3): 217–26. <https://doi.org/10.3354/dao03145>
- Kasornchandra, J. *et al.* 1999. Progress in the development of shrimp cell cultures in Thailand. **Methods in Cell Science** 21 (4): 231–35. <https://doi.org/10.1023/A:1009828632486>
- Khan, B. M.; Liu, Y. 2019. Marine mollusks: food with benefits. **Comprehensive Reviews in Food Science and Food Safety** 18 (2): 548–64. <https://doi.org/10.1111/1541-4337.12429>
- Koiwai, K.; Kondo, H.; Hirono, I. 2019. Isolation and molecular characterization of hemocyte sub-populations in kuruma shrimp *Marsupenaeus japonicus*. **Fisheries science** 85: 521-532. <https://doi.org/10.1007/s12562-019-01311-5>
- Kupferschmidt, K. 2013. Lab burger adds sizzle to bid for research funds. **Science** 341 (6146): 602–3. <https://doi.org/10.1126/science.341.6146.602>
- Ladhar-Chaabouni, R. *et al.* 2021. Establishment of primary cell culture of *Ruditapes decussatus* haemocytes for metal toxicity assessment. **In vitro Cellular & Developmental Biology - Animal** 57 (4): 477–84. <https://doi.org/10.1007/s11626-021-00561-x>
- Lakra, W.S.; Swaminathan, T.R.; Joy, K.P. 2011. Development, characterization, conservation and storage of fish cell lines: a review. **Fish Physiology and Biochemistry** 37 (1): 1–20. <https://doi.org/10.1007/s10695-010-9411-x>

- Lange, R. 1963. The osmotic function of amino acids and taurine in the mussel, *Mytilus edulis*. **Comparative Biochemistry and Physiology** 10 (2): 173–79. [https://doi.org/10.1016/0010-406X\(63\)90239-1](https://doi.org/10.1016/0010-406X(63)90239-1)
- Le Pennec, G.; Pennec, M.L. 2001. Acinar primary cell culture from the digestive gland of pecten maximus (L.): an original model for ecotoxicological purposes. **Journal of Experimental Marine Biology and Ecology** 259 (2): 171–87. [https://doi.org/10.1016/S0022-0981\(01\)00232-5](https://doi.org/10.1016/S0022-0981(01)00232-5)
- Li, C.; Shields J. D. 2007. Primary culture of hemocytes from the Caribbean spiny lobster, *Panulirus argus*, and their susceptibility to *Panulirus argus* virus 1 (PaV1). **Journal of Invertebrate Pathology** 94 (1): 48–55. <https://doi.org/10.1016/j.jip.2006.08.011>
- Lindfors, E.T.; Jakobsen, S.E. 2022. Sustainable regional industry development through co-evolution - the case of salmon farming and cell-based seafood production. **Marine Policy** 135. <https://doi.org/10.1016/j.marpol.2021.104855>
- Lobine, D.; Rengasamy, K.R.R; Mahomoodally, M.F. 2022. Functional foods and bioactive ingredients harnessed from the ocean: Current status and future perspectives. **Critical Reviews in Food Science and Nutrition** 62 (21): 5794-5823. <https://doi.org/10.1080/10408398.2021.1893643>
- Luedeman, R.; Lightner, D.V. 1992. Development of an *in vitro* primary cell culture system from the penaeid shrimp, *Penaeus stylirostris* and *Penaeus vannamei*. **Aquaculture** 101 (3–4): 205–11. [https://doi.org/10.1016/0044-8486\(92\)90024-F](https://doi.org/10.1016/0044-8486(92)90024-F)
- Ma, J.; Zeng, L.; Lu, Y. 2017. Penaeid shrimp cell culture and its applications. **Reviews in Aquaculture** 9 (1): 88–98. <https://doi.org/10.1111/raq.12106>
- Maeda, M. *et al.* 2004. Replication of white spot syndrome virus in ovarian primary cultures from the kuruma shrimp, *Marsupenaeus japonicus*. **Journal of Virological Methods** 116 (1): 89–94. <https://doi.org/10.1016/j.jviromet.2003.10.013>
- Mahmood Ghafor, I. 2020. **Crustacean**. Diarte-Plata, G.; Escamilla-Montes. R. (ed). IntechOpen. <https://doi.org/10.5772/intechopen.89730>
- Malerich, M.; Bryant, C. 2022. Nomenclature of cell-cultivated meat & seafood products. **npj Science of Food** 6 (56): 1-13. <https://doi.org/10.1038/s41538-022-00172-0>
- Mc Carthy, U. *et al* 2018. Global food security – issues, challenges and technological solutions. **Trends in Food Science & Technology** 77: 11–20. <https://doi.org/10.1016/j.tifs.2018.05.002>
- McManus, A.; Newton, W. 2011. **Seafood, nutrition and human health: A synopsis of the nutritional benefits of consuming seafood**. Centre of Excellence Science, Seafood & Health, Curtin Health Innovation Research Institute, Curtin University of Technology. Available online: https://espace.curtin.edu.au/bitstream/handle/20.500.11937/32912/185277_53343_Seafood__nutrition_and_human_health.pdf?sequence=2&isAllowed=y (Accessed: march 2024)
- Moniruzzaman, M. *et al.* 2021. Nutritional evaluation of some economically important marine and freshwater mollusc species of bangladesh. **Heliyon** 7. <https://doi.org/10.1016/j.heliyon.2021.e07088>
- Morais-da-Silva, R.L.; Villar, E.G.; Reis, G.G. 2022. The expected impact of cultivated and plant-based meats on jobs: the views of experts from Brazil, the United States and Europe. **Humanities and Social Sciences Communications** 9 (297). <https://doi.org/10.1057/s41599-022-01316-z>

- Munteanu C. *et al.* 2021. Can cultured meat be an alternative to farm animal production for a sustainable and healthier lifestyle? **Frontiers in Nutrition** 4 (8). <https://doi.org/10.3389/fnut.2021.749298>
- Myers, R. A.; Worm, B. 2003. Rapid worldwide depletion of predatory fish communities. **Nature** 42: 280–83. <https://doi.org/10.1038/nature01610>
- Nadala, E.C.; Lu, Y.; Loh, P.1993. Primary culture of lymphoid, nerve, and ovary cells from *Penaeus stylirostris* and *Penaeus vannamei*. **In vitro Cellular & Developmental Biology** 29 (8): 620–22. <https://doi.org/10.1007/BF02634546>
- Ng, J.Y. *et al.* 2020. *Chlorella vulgaris* extract as a serum replacement that enhances mammalian cell growth and protein expression. **Frontiers in Bioengineering and Biotechnology** 8. <https://doi.org/10.3389/fbioe.2020.564667>
- Nicholson, B.L. 1989. Fish cell culture: an update. **Advances in Cell Culture**, 7: 1–18. <https://doi.org/10.1016/B978-0-12-007907-0.50007-4>
- Nogueira, L.S. *et al.* 2013. Isolation and fractionation of gill cells from freshwater (*Lasmigona costata*) and seawater (*Mesodesma mactroides*) bivalves for use in toxicological studies with copper. **Cytotechnology** 65: 773-783. <https://doi.org/10.1007/s10616-013-9647-2>
- Odintsova, N.A.; Dyachuk, V.A.; Nezlin, L.P. 2010. Muscle and neuronal differentiation in primary cell culture of larval *Mytilus trossulus* (Mollusca: Bivalvia). **Cell and Tissue Research** 339 (3): 625–37. <https://doi.org/10.1007/s00441-009-0918-3>
- Odintsova, N.A.; Khomenko, A.V. 1991. Primary cell culture from embryos of the japanese scallop *Mizuchopecten yessoensis* (Bivalvia). **Cytotechnology** 6 (1): 49–54. <https://doi.org/10.1007/BF00353702>
- Oestbye, T.K.; Ytteborg, E. 2019. Preparation and culturing of Atlantic salmon muscle cells for *in vitro* studies. **Methods in Molecular Biology** 1889: 319-330. https://doi.org/10.1007/978-1-4939-8897-6_19
- Ong, K.J. *et al.* 2021. Food safety considerations and research priorities for the cultured meat and seafood industry. **Comprehensive Reviews in Food Science and Food Safety** 20: 5421– 448. <https://doi.org/10.1111/1541-4337.12853>
- Ong, S.; Choudhury, D.; Naing, M.W. 2020. Cell-based meat: Current ambiguities with nomenclature. **Trends in Food Science & Technology** 102: 223-231. <https://doi.org/10.1016/j.tifs.2020.02.010>
- Owens, L.; Smith, J. 1999. Early attempts at production of prawn cell lines. **Methods in Cell Science** 21 (4): 207–12. <https://doi.org/10.1023/A:1009806606562>
- Pal, J. *et al.* 2018. A review on role of fish in human nutrition with special emphasis to essential fatty acid. **International journal of fisheries and aquatic studies** 6 (2): 427–30. <https://www.fisheriesjournal.com/archives/2018/vol6issue2/PartF/6-2-50-593.pdf>
- Palomares, M.L.D. *et al.* 2020. Fishery biomass trends of exploited fish populations in marine ecoregions, climatic zones and ocean basins. **Estuarine, Coastal and Shelf Science** 243. <https://doi.org/10.1016/j.ecss.2020.106896>

- Perkins, F.O.; Menzel, R.W. 1964. Maintenance of oyster cells *in vitro*. **Nature** 204 (4963): 1106–7. <https://doi.org/10.1038/2041106a0>
- Pissia, M.A.; Matsakidou, A.; Kiosseoglou, V. 2021. Raw materials from snails for food preparation. **Future Foods** 3. <https://doi.org/10.1016/j.fufo.2021.100034>
- Plotnikov, S.V.; Karpenko, A.A.; Nelly, A. O. 2003. Comparative characteristic of mytilus muscle cells developed *in vitro* and *in vivo*. **Journal of Experimental Zoology** 298 (2): 77–85. <https://doi.org/10.1002/jez.a.10274>
- Post, M.J. *et al.* 2020. Scientific, sustainability and regulatory challenges of cultured meat. **Nature Food** 1: 403–415. <https://doi.org/10.1038/s43016-020-0112-z>
- Potts, R.W.A *et al.* 2020. Developments in marine invertebrate primary culture reveal novel cell morphologies in the model bivalve *Crassostrea gigas*. **PeerJ** 8. <https://doi.org/10.7717/peerj.9180>
- Ramalho Ribeiro, A. *et al.* 2019. Farmed fish as a functional food: perception of fish fortification and the influence of origin – Insights from Portugal. **Aquaculture** 501: 22–31. <https://doi.org/10.1016/j.aquaculture.2018.11.002>
- Reames, E. 2012. Nutritional benefits of seafood. **Southern Regional Aquaculture Center** 7300. https://aquaculture.ca.uky.edu/sites/aquaculture.ca.uky.edu/files/srac_7300_nutritional_benefits_of_seafood.pdf (Accessed: march 2024)
- Regier, J. C. *et al.* 2010. Arthropod relationships revealed by phylogenomic analysis of nuclear protein-coding sequences. **Nature**, 463 (7284): 1079–1083. <https://doi.org/10.1038/nature08742>
- Riccardi, G. *et al.* 2022. Dietary recommendations for prevention of atherosclerosis. **Cardiovascular Research** 118 (5): 1188-1204. <https://doi.org/10.1093/cvr/cvab173>
- Rippon, H.J.; Bishop, A.E. 2004. Embryonic stem cells. **Cell Proliferation** 37 (1): 23–34. <https://doi.org/10.1111/j.1365-2184.2004.00298.x>
- Rodríguez Escobar, M.I.; Cadena, E.; Nhu, T.T.; Cooreman-Algoed, M.; De Smet, S.; Dewulf, J. 2021. Analysis of the cultured meat production system in function of its environmental footprint: current status, gaps and recommendations. **Foods** 10 (2941). <https://doi.org/10.3390/foods10122941>
- Rubio, N.R. *et al.* 2019. Cell-Based fish: A novel approach to seafood production and an opportunity for cellular agriculture. **Frontiers in Sustainable Food Systems Sec. Nutrition and Sustainable Diets** 3. <https://doi.org/10.3389/fsufs.2019.00043>
- Sant’Ana, A.S. *et al.* 2023. **Assuring the safety of cultivated meat: HACCP plan development and application to a cultivated meat target-product**. São Paulo: The Good Food Institute Brazil, 2023. Available online: https://gfi.org.br/wp-content/uploads/2023/10/Assuring-the-safety-of-cultivated-meat_HACCP-plan-development-and-application-to-a-cultivated-meat-target-product_GFI-Brazil.pdf (Accessed: march 2024)
- Santos A.C.A. *et al.* 2023. Tissue engineering challenges for cultivated meat to meet the real demand of a global market. **International Journal of Molecular Sciences** 24 (7): 1-22. <https://doi.org/10.3390/ijms24076033>

- Sashikumar, A.; Desai, P.V. 2008. Development of primary cell culture from *Scylla serrata*. **Cytotechnology** 56 (3): 161–69. <https://doi.org/10.1007/s10616-008-9152-1>
- Sathyabhama, A.B. *et al.* 2021. ‘PmLyO-Sf9-WSSV complex’ could be a platform for elucidating the mechanism of viral entry, cellular apoptosis and replication impediments. **Virology** 553: 102–110, <https://doi.org/10.1016/j.virol.2020.10.014>
- Siegrist, M.; Hartmann, C. 2020. Consumer acceptance of novel food technologies. **Nature Food** 1, 343–350. <https://doi.org/10.1038/s43016-020-0094-x>
- Sivakumar, S. *et al.* 2019. The development and characterization of a cell culture system from Indian mud crabs *Scylla serrata*. **Journal of Aquatic Animal Health** 31 (3): 244–58. <https://doi.org/10.1002/aah.10073>
- Soice, E.; Johnston, J. 2021. Immortalizing cells for human consumption. **International Journal of Molecular Sciences** 22 (21). <https://doi.org/10.3390/ijms222111660>
- Southey, F. 2022. Why Is EFSA yet to receive a novel food dossier on cell-based meat? **Food Navigator Europe**. Available online: <https://www.foodnavigator.com/Article/2022/10/31/Why-is-EFSA-yet-to-receive-a-novel-food-dossier-on-cell-based-meat> (Accessed: march 2024)
- Stanek, A. *et al.* 2023. The influence of dietary interventions on arterial stiffness in overweight and obese subjects. **Nutrients** 15 (6): 1–30. <https://doi.org/10.3390/nu15061440>
- Steinfeld, H. *et al.* 2006. **Livestock’s long shadow: Environmental issues and options**. Rome, Italy: FAO. Available online: <https://www.fao.org/3/a0701e/a0701e00.htm> (Accessed: march 2024)
- Stepanyan, R. 2004. Primary culture of lobster (*Homarus americanus*) olfactory sensory neurons. **Chemical Senses** 29 (3): 179–87. <https://doi.org/10.1093/chemse/bjh023>
- Stephens, N. *et al.* 2018. Bringing cultured meat to market: technical, socio-political, and regulatory challenges in cellular agriculture. **Trends in Food Science & Technology** 78, 155–166. <https://doi.org/10.1016/j.tifs.2018.04.010>
- Stephens, N.; Sexton A.E.; Driessen, C. 2019. Making sense of making meat: key moments in the first 20 years of tissue engineering muscle to make food. **Frontiers in Sustainable Food Systems** 3: 1–16. <https://doi.org/10.3389/fsufs.2019.00045>
- Sudarshan, G. *et al.* 2024. Development of long-term primary cell culture of *Macrobrachium rosenbergii*: morphology, metabolic activity, and cell-cycle analysis. **Frontiers in Marine Science**, 10: 1– 11. <https://doi.org/10.3389/fmars.2023.1322744>
- Suleria, H. *et al.* 2015. Marine-based nutraceuticals: an innovative trend in the food and supplement industries. **Marine Drugs** 13 (10): 6336–6351. <https://doi.org/10.3390/md13106336>
- Suzuki, M. *et al.* 2021. Cell culture and genetic transfection methods for the Japanese scallop, *Patinopecten yessoensis*. **FEBS Open Bio** 11 (8): 2282–91. <https://doi.org/10.1002/2211-5463.13237>
- Tabakaeva, O.V; Tabakaev, A.V; Piekoszewski, W. 2018. Nutritional composition and total collagen content of two commercially important edible bivalve molluscs from the Sea of Japan Coast. **Journal of Food Science and Technology** 55 (12): 4877–86. <https://doi.org/10.1007/s13197-018-3422-5>

- Tapay, L.M. *et al.* 1995. Transformation of primary cultures of shrimp (*Penaeus stylirostris*) Lymphoid (Oka) organ with Simian Virus-40 (T) antigen. **Experimental Biology and Medicine** 209 (1): 73–78. <https://doi.org/10.3181/00379727-209-43880>
- Tilami, K.S.; Sampels, S. 2018. Nutritional value of fish: lipids, proteins, vitamins, and minerals. **Reviews in Fisheries Science & Aquaculture** 26 (2): 243–53. <https://doi.org/10.1080/23308249.2017.1399104>
- Toullec, J.Y.; Chikhi, M.; Van Wormhoudt, A. 1992. *In vitro* protein synthesis and α amylase activity in F cells from hepatopancreas of *Palaemon serratus* (Crustacea; Decapoda). **Experientia** 48: 272–277. <https://doi.org/10.1007/BF01930474>
- Van der Merwe, M. *et al.* 2010. Investigating the establishment of primary cell culture from different abalone (*Haliotis midae*) tissues. **Cytotechnology** 62 (3): 265–77. <https://doi.org/10.1007/s10616-010-9293-x>
- Van der Weele, C.F.P. *et al.* 2019. Meat alternatives: an integrative comparison. **Trends in Food Science & Technology** 88, 505–512. <https://doi.org/10.1016/j.tifs.2019.04.018>
- Venugopal, V. 2018. Nutrients and nutraceuticals from seafood. In: Méryllon, JM., Ramawat, K. (eds) **Bioactive Molecules in Food**. Springer. https://doi.org/10.1007/978-3-319-54528-8_36-2
- Vergès-Castillo, A. *et al.* 2021. Establishment and characterization of single cell-derived embryonic stem cell lines from the gilthead seabream, *Sparus aurata*. **Comparative biochemistry and physiology** 256. <https://doi.org/10.1016/j.cbpb.2021.110626>
- Wang, X.; Wang, Y. 2019. Editorial: molecular physiology in molluscs. **Frontiers in Physiology** 10: 1–3. <https://doi.org/10.3389/fphys.2019.01131>
- Warner, R.D. 2019. Review: Analysis of the process and drivers for cellular meat production. **Animal** 13 (12): 3041–3058. <https://doi.org/10.1017/S1751731119001897>
- Wen, C.; Kou, G.; Chen, S. 1993. Establishment of cell lines from the Pacific Oyster. **In vitro Cellular & Developmental Biology - Animal** 29 (12): 901–3. <https://doi.org/10.1007/BF02634224>
- Willer, D.F.; Aldridge, D.C. 2020. Sustainable bivalve farming can deliver food security in the tropics. **Nature Food** 1 (7): 384–88. <https://doi.org/10.1038/s43016-020-0116-8>
- Wolf, K.; Ahne, W. 1982. Fish cell culture. **Advances in Cell Culture** 2: 305–28. <https://doi.org/10.1016/B978-0-12-007902-5.50014-2>
- Wright, A.C.; Fan, Y.; Baker, G.L. 2018. Nutritional value and food safety of bivalve molluscan shellfish. **Journal of Shellfish Research** 37 (4): 695–708. <https://doi.org/10.2983/035.037.0403>

COMPÓSITO REFORÇADO COM FIBRA DO PECÍOLO DA PALMEIRA DE DENDÊ: FABRICAÇÃO DE MATERIAL SUSTENTÁVEL

Acceptance date: 02/05/2024

Roberto Yuri Costa Dias

<http://lattes.cnpq.br/1066558959052005>

Rafael Vilhena Soares

Maria de Fátima Mendes Leal

Pedro Victor de Mendonça Maia

Roberto Tetsuo Fujiyama

PALAVRAS-CHAVE: Material Compósito, Fibra Natural, Sustentabilidade

COMPOSITE REINFORCED
WITH PALM PETIOLE FIBER:
PRODUCTION AIMING FOR THE
SEARCH FOR SUSTAINABLE
MATERIAL

RESUMO: Neste trabalho, são apresentados resultados de atividades desenvolvidas em uma disciplina da faculdade de engenharia mecânica da UFPA. Na atividade, pecíolos de folha de palmeira foram utilizados para fabricar material compósito utilizando matriz de poliéster. Após a separação das folhas, o pecíolo foi extraído. O pecíolo foi cortado em um comprimento de 10 mm. Após o corte, as placas de material compósito são fabricadas com as fibras orientadas aleatoriamente. O objetivo deste trabalho é apresentar um material com características sustentáveis. Ao final, foram produzidas placas de material compósito com fibras pecíolo e matriz de poliéster. O material produzido apresentou boas características em termos de configuração e distribuição da fibra.

ABSTRACT: In this work, results of activities developed in a discipline of the faculty of mechanical engineering at UFPA are presented. In the activity, palm leaf petioles were used to manufacture composite material using polyester matrix. After separating the leaves, the petiole was extracted. The petiole was cut to a length of 10 mm. After the cut, composite material plates are manufactured with the fibers oriented randomly. The objective of this work is to present a material with sustainable characteristics. At the end, composite material plates were produced with petiole fibers and polyester matrix. The material produced showed good characteristics in terms of fiber configuration and distribution.

KEYWORDS: Composite Material, Natural Fiber, sustainability

INTRODUÇÃO

O desenvolvimento de novas tecnologias, novos materiais e de formas de processos industriais, com o passar do tempo tem se tornado uma realidade natural da sociedade que avança de forma progressiva. Conforme a tecnologia avança, a busca por novos materiais é ampliada, a fim de suprir a necessidade de mercado na produção de bens que alimentam diversos setores produtivos das indústrias. Ainda neste contexto tem-se o envolvimento de materiais ecologicamente correto como forma de proteger o meio ambiente.

No Brasil a busca por novos materiais é intensificada, objetivando a utilização de componentes naturais, visto que o país é privilegiado por ter mais de 4 milhões de km² de floresta amazônica, segundo o IBGE (Instituto Brasileiro de Geografia e Estatística), facilitando a disponibilidade de material necessário para estudo. Dentro da gama de novos materiais que estão sendo explorados temos os materiais compósitos reforçados com fibras naturais.

O desenvolvimento de novos materiais têm sido objeto de pesquisa, desde a formulação à caracterização de compósitos constituídos com fibras naturais. Os materiais compósitos são obtidos através da combinação de dois ou mais materiais com diferentes propriedades químicas e físicas, sendo que um dos componentes é o matricial e os demais o reforço, no qual as propriedades são obtidas a partir da combinação das propriedades dos constituintes individuais (NAZARENO, 2018, 90).

Na região norte, temos como referência na produção de pesquisas relacionadas a materiais compósitos o laboratório do Grupo de Pesquisa em Materiais Compósitos da Universidade Federal do Pará, utilizando de reforço fibras naturais como, por exemplo, fibra de juta, malva, bambu e coco. As pesquisas no laboratório envolvem a aplicação de recursos ou matérias prima naturais, recicláveis e renováveis.

Segundo a Secretaria de Estado de Desenvolvimento Agropecuário e da Pesca, o Pará é o maior produtor nacional de óleo de palma de dendê com uma área plantada de 231.669 hectares e área colhida de 200.000 hectares, sendo 40 mil hectares em áreas de agricultores familiares.

No trabalho “Fabricação de materiais compósitos sanduíche reforçados com fibra de juta e resíduos de madeira” (DIAS, et. al) é realizada a produção de material compósito utilizando fibra de juta e utilizando um resíduo de madeira que seria descartado, mas buscou-se uma nova utilização, sendo a fibra de juta produzida a partir do caule da planta e o resíduo de madeira gerado de processamento de madeira para fins diversos.

As fibras vegetais são estruturas alongadas extraídas da natureza sem ter que passar por reações químicas de síntese ou de modificações estruturais. Algumas delas são partes integrantes de folhas, de caules, de sementes e de frutos. (GOMES, 2015)

O pecíolo, parte componente de uma folha, é a haste que possibilita movimentação, costumeiramente em formato cilíndrico, (ALMEIDA, M e ALMEIDA, C, 2018, p.57) que

no caso particular da palmeira do dendê são encontrados ao centro da folha conforme o esquema apresentado na figura YY.

O método mais simples de laminação consiste na impregnação de resinas de baixa viscosidade sobre as fibras, que geralmente encontram-se na forma de tecidos. Camadas deste tecido são empilhadas e revestidas com resina para que se obtenha a espessura desejada. Geralmente este processo ocorre sobre uma superfície plana ou molde aberto. A resina e o agente endurecedor são misturados imediatamente antes da aplicação e a cura é realizada a temperatura ambiente (CLYNE e HULL, 2019)

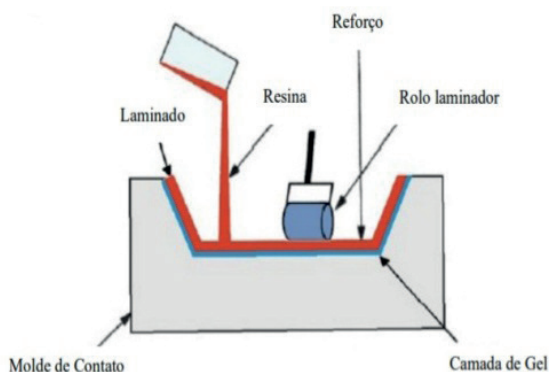


Figura 1- Ilustração do processo de laminação manual

Fonte: Adaptado de Chawala, 2019

Neste trabalho é proposto o uso do pecíolo das folhas da palmeira de Dendê na fabricação de placas de materiais compósitos, nas dimensões de 10x20mm, com o objetivo de identificar a viabilidade de utilização do mesmo como reforço e a realização de avaliação da qualidade superficial do compósito produzido buscando observar como o pecíolo da palmeira de dendê ficou disposto na matriz polimérica.

Pecíolo compreende a haste que possibilita a movimentação da folha, costumeiramente em formato cilíndrico

MATERIAIS E METODOLOGIA

Materiais

Para a produção dos compósitos, utilizou-se como matriz resina poliéster, comprada pelos entes do laboratório, e como reforço, fibras do pecíolo da folha da árvore de dendê, obtida a partir de uma palmeira dendezeiro, localizada na Universidade Federal do Pará, campus Belém.

Os materiais utilizados para confecção dos corpos de prova:

Pecíolo da Folha de Dendê; Resina Poliéster; Catalisador; Cera Desmoldante; Pincel; Molde de silicone. Na figura 1 tem-se a ilustração destes materiais citados, com exceção da folha do dendê.

- Utilizada com o intuito de reduzir a aderência entre a resina e o molde, facilitando a retirada do corpo de prova.
- Molde padronizado com dimensões especificadas pela norma ASTM D638.
- Tem por objetivo retirar o excesso de cera desmoldante que por ventura se aloje em certas regiões do molde de silicone.
- Resina Poliéster utilizada como matriz do material compósito.
- Tem como função acelerar o processo de cura ou solidificação do material.

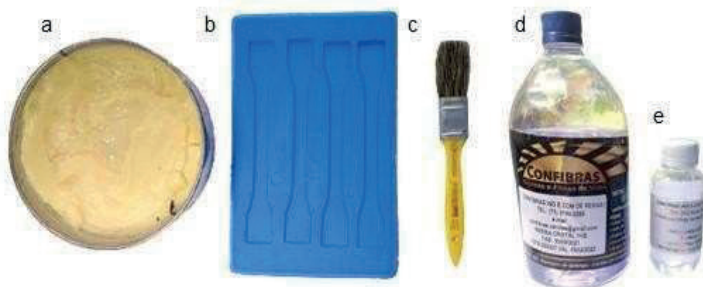


Figura 2: a) Cera Desmoldante b) Molde de Silicone c) Pincel d) Resina Poliéster e) Catalisador.

Fonte: Autoria Própria

Métodos

Primeiramente, realizou-se a extração do pecíolo da folha do dendê, para posterior corte do mesmo no tamanho de 10 mm. Após esta etapa, foram determinadas a fração mássica de fibra presente no compósito. Por fim, houve a mistura dos componentes, resina poliéster e pecíolo de dendê, que produziram os corpos de prova, os quais foram envazados no molde de silicone.

Extração e corte do pecíolo

A extração das folhas foi realizada de palmeiras de dendê encontradas no campus Universidade Federal do Pará, conforme a Figura 2.



Figura 3 – Dendezeiro.

Fonte: Autoria própria.

Em seguida, foi retirado o pecíolo da folha de dendê, que consiste na parte central da folha, dando início ao corte em tamanhos de 10 mm, utilizando gabarito. A Figura 3 é referente às duas partes separadas que compõem a folha da palmeira do dendê. Na Figura 4 tem-se o pecíolo cora no comprimento de 10 mm.



a b

Figura 4- a) Foliolo; b) Pecíolo.

Fonte: Autoria própria.



Figura 5- Fibras do pecíolo de dendê após o corte com 10 mm de comprimento.

Fonte: Aatoria própria.

Determinação das frações mássicas

A fração mássica é determinada pela divisão da quantidade de fibra pela quantidade de resina utilizada. A quantidade de reforço dentro da matriz polimérica pode alterar suas características mecânicas, por este motivo sua determinação é fundamental. Foi realizado o preenchimento do molde até o seu limite com as fibras dos pecíolos e posteriormente a pesagem dessa quantidade.



Figura 6 – Molde com fibras

Fonte: O autor

Preparação da composição antes do envase no molde

A matriz poliéster foi misturada com as fibras de pecíolo de dendê, já padronizadas com 10 cm de comprimento, em um recipiente plástico transparente, em seguida, a mistura foi vertida no molde de silicone de maneira a ocupar todo o interior destinado a formar o corpo de prova, a fim de configurar as dimensões preestabelecidas, vale ressaltar que todo este processo foi feito de forma manual. Para o preenchimento dos moldes foram utilizados 61,5 g de resina e 1% de catalisador.

RESULTADOS E DISCUSSÃO

Um dos parâmetros de influência nos compósitos reforçados com fibras está relacionado ao comprimento da fibra de reforço. Cabe ressaltar, que as características mecânicas de um compósito reforçado com fibras não dependem somente das propriedades da fibra, mas também do grau segundo o qual a carga aplicada é transmitida para as fibras pela fase matriz. Outro parâmetro a ser considerado é a orientação e concentração das fibras. O arranjo ou a orientação das fibras, a concentração das fibras e a sua distribuição apresentam influência significativa sobre a resistência e outras propriedades dos compósitos reforçados com fibras.

Fundamentado nisso, a Tabela 1 mostra que a eficiência de reforço por fibras distribuídas aleatoriamente e uniformemente é de apenas um quinto da eficiência na direção longitudinal de um compósito com fibras alinhadas; entretanto, as características mecânicas são isotrópicas.

<i>Orientações da Fibra</i>	<i>Direção da Tensão</i>	<i>Eficiência do Reforço</i>
Todas as fibras paralelas	Paralela às fibras	1
	Perpendicular às fibras	0
Fibras distribuídas aleatória e uniformemente em um plano específico	Qualquer direção no plano das fibras	$\frac{3}{8}$
Fibras distribuídas aleatória e uniformemente nas três dimensões no espaço	Qualquer direção	$\frac{1}{5}$

Tabela 1 – Eficiência de reforço de compósitos reforçados com fibras.

Fonte: H, Krenchel, 1964.

Mediante inspeção visual, notou-se que as placas apresentaram certas vacâncias de preenchimento do pecíolo do dendê, conforme mostra a Figura 5. Essas vacâncias podem influenciar na resistência do material pelo fato de não apresentar homogeneidade por toda região da placa de compósito.

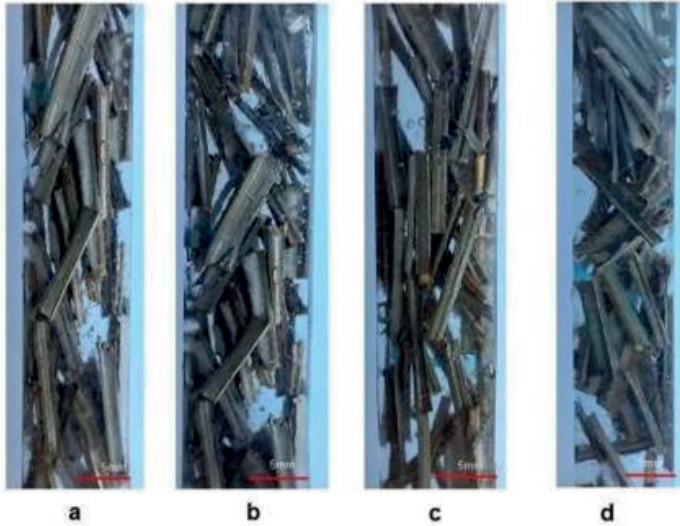


Figura 7 – Placas de compósito reforçado com pecíolo de dendê.

Fonte: Autoria própria.

Por fim, as placas de compósito apresentaram poucas porosidades, mas com formações de bolhas de ar. Indicativo para um maior cuidado no processo de envase no molde. Quanto à utilização dos pecíolos de dendê com a resina poliéster, foi observada uma boa trabalhabilidade oferecendo assim o tempo necessário para a realização do envase, proporcionando um bom tempo de execução nas produções das placas.

O pecíolo após o corte e secagem apresentou um diâmetro médio de 1,3 mm. Na figura 6 é possível observar a disposição do pecíolo dentro da matriz polimérica.



Figura 08 – Disposição do pecíolo de Dendê na matriz polimérica.

Fonte: Autoria própria.

CONSIDERAÇÕES FINAIS

Observando o cenário de desenvolvimento de materiais atual, é de grande importância pesquisas relacionadas à busca de materiais alternativos que possam suprir a demanda geracional. O presente trabalho buscou a avaliação da viabilidade de produção de compósitos através de pecíolos de dendê, e assim foi observado um promissor avanço na utilização do presente material. O resultado inicial é positivo, o material teve uma boa trabalhabilidade, se adequou bem ao molde utilizado. No entanto é necessária em pesquisas futuras a avaliação de suas propriedades, através de ensaios de caracterização mecânica, como o ensaio de tração, a fim de obter maior detalhamento do material produzido e posteriormente realizar a busca de utilização deste material como produto de utilidade em setores industriais e domésticos, trazendo para as indústrias uma alternativa de fabricação de material utilizando componentes naturais.

AGRADECIMENTOS

Agradecemos ao apoio recebido pela Faculdade de Engenharia Mecânica do ITEC-UFGA e aos alunos PIBIC bolsista da PROPESP-UFGA.

REFERÊNCIAS

- CUENCA, M. A. G. NAZARIO, C. C. **Importância e evolução da dendeicultura na região dos tabuleiros costeiros da Bahia de 1990 – 2002**. Aracaju: Embrapa Tabuleiros Costeiros, 2005. 23 p. (Documentos/Embrapa Tabuleiros Costeiros, 77)
- CAETANO, MÁRCIA COUTINHO; MOTA, DALVA MARIA DA; FERREIRA, Maria do Socorro Gonçalves. **Trabalho familiar na produção de dendê sob contrato na Amazônia brasileira: o caso de Santa Maria, Pará**. Século XXI (Santa Maria), 2018-11- 16, Vol.8 (1), p.417.
- DIAS, R. S. M. ; GOMES, I. S. ; VILHENA, E. S. ; PEREIRA, L. C. O. ; LOPES, C. E. P. ; VILHENA, E. S. ; OLIVEIRA, A. B. S. ; RIBEIRO, M. M. ; SILVA, S. C. ; ARAUJO, L. R. ; BITENCOURT JUNIOR, A. H. S. ; FUJIYAMA, R. T. . **Fabricação de materiais compósitos sanduiche reforçados com fibra de juta e resíduos de madeira**. Brazilian Journal of Development, v. 5, p. 6376-6384, 2019.
- LEVY NETO, F.; PARDINI, L. C., **Compósitos estruturais: ciência e tecnologia**. São Paulo: Edgar Blucher, 2006.
- LEÃO, M. A., **Fibras de licuri: um reforço alternativo de compósitos poliméricos**. 2008. 109 p. Dissertação (Mestrado em Engenharia Mecânica), Universidade Federal do Rio Grande do Norte, Natal, 2008.
- SATYANARAYANA, K. G.; GUIMARÃES, J. L.; WYPYCH, F.; **Studies on lignocellulosic fibers of Brazil. Part I: Source, production, morphology, properties and applications. Composites: Part A**, v. 38, p. 1694-1709, 2007.

Secretaria de Estado de Desenvolvimento Agropecuário e da Pesca, 2020. **DENDÊ**. Disponível em: <http://www.sedap.pa.gov.br/content/dend%C3%AA> Acessado em: 10/04/2022

TAKAHASHI, R., Desenvolvimento de material compósito de matriz polimérica reforçada a partir de pré-pregs de fibras naturais de curauá e de sisal. Monografia (grau de Engenheiro Mecânico), Universidade Federal do Pará, p. 17-19, 2011.

VEIGA, A.S.; FURLAN JUNIOR, J.; KALTNER, J.F Situação atual e perspectivas futuras da dendeicultura nas principais regiões produtoras: a experiência do Brasil. In: MÜLLER, A.A. FURLAN JUNIO, J (Ed.) **Agronegócio do dendê: Uma alternativa social, econômica e ambiental para o desenvolvimento sustentável da Amazônia**. Belém: Embrapa Amazônia Ocidental, 2001. p. 41-65.

GOMES, A.; COSTA, N.; MOHALLEM, N. **Os Tecidos e a Nanotecnologia**, 2015

ALMEIDA, M. ALMEIDA, C. V. **Morfologia da folha de plantas com semente**. Coleção Botanica (3). Piracicaba: ESALQ/USP, 2018. p. 57.

CLYNE, T. W.; HULL, D., **Na Introduction to Composite Materials**. 3 ed., Cambridge University Press, Cambridge, 2019.

RECILAR, I. G. **Efeito dos processos de laminação manual e laminação termomecânica nas propriedades mecânicas do material compósito carbono-epóxi**. Centro Universitario FEI, 2022.

COMPOSITE REINFORCED WITH PALM PETIOLE FIBER: PRODUCTION AIMING FOR THE SEARCH FOR SUSTAINABLE MATERIAL

SISTEMA CARDIOVASCULAR BOVINO: CONSERVAÇÃO E MONTAGEM

Acceptance date: 02/05/2024

Giovana Balbinot

Discente do Curso (Medicina Veterinária)
– Faculdade IDEAU – Passo Fundo/RS

Naiane Dierings

Discente do Curso (Medicina Veterinária)
– Faculdade IDEAU – Passo Fundo/RS

Géssica Gutierrez

Discente do Curso (Medicina Veterinária)
– Faculdade IDEAU – Passo Fundo/RS

Eliz Roberta Kuhn

Discente do Curso (Medicina Veterinária)
– Faculdade IDEAU – Passo Fundo/RS

Marciane Parizotto

Discente do Curso (Medicina Veterinária)
– Faculdade IDEAU – Passo Fundo/RS

Kleberson Leonardo Schafer

Discente do Curso (Medicina Veterinária)
– Faculdade IDEAU – Passo Fundo/RS

Eduardo Rebelato Sakis

Docente do Curso (Medicina Veterinária) –
Faculdade IDEAU – Passo Fundo/RS

Gabriela Tonello

Docente do Curso (Medicina Veterinária) –
Faculdade IDEAU – Passo Fundo/RS

Bárbara Thaisi Zago

Coordenadora do Curso de Medicina
Veterinária – Faculdade IDEAU – Passo
Fundo/RS

RESUMO: O sistema cardiovascular é composto pelo coração, sistema vascular sanguíneo e o sistema vascular linfático. Sendo o sistema vascular sanguíneo o que desempenha maior função, levando oxigênio e nutrientes para as diversas partes do corpo e que os resíduos metabólicos sejam transportados até seu local de eliminação. É formado pelo coração, artérias, vasos capilares e veias. Nesse sentido o objetivo desse trabalho é coletar e aplicar um método de conservação, buscando compreender as técnicas de preservação de estruturas anatômicas; realizar dissecação do órgão, conhecer as estruturas que compõem a anatomia topográfica, avaliação de tecidos e estrutura anatomofisiológica do sistema cardiovascular, suas patologias e aprofundar o conhecimento sobre a integração do sistema abordado que levam à homeostasia de cada organismo. Paralelamente a isso iniciou-se uma pesquisa de caráter qualitativo através de um estudo de caso

com pesquisa bibliográfica, com procedimentos de natureza aplicada. O material para o estudo foi uma doação de um coração da espécie bovina, onde seu abate ocorreu para consumo humano. O coração foi fixado no formol 10% para sua conservação e foi realizado a secção do tecido adiposo e do saco pericárdico que estava envolto a fim de deixar mais evidente a musculatura, veias e artérias do referido órgão. Esse trabalho buscou alinhar os conhecimentos teóricos e práticos, sendo possível identificar as particularidades do órgão de maneira macroscópica, sua função morfológica e funcional, e de que maneira se realiza a fixação e preservação de órgãos em formol.

PALAVRAS-CHAVE: Espécie bovina; Coração; Vascularização; Patologias.

ABSTRACT: The cardiovascular system is composed of the heart, the blood vascular system and the lymphatic vascular system. As the blood vascular system plays the greatest role, carrying oxygen and nutrients to different parts of the body and metabolic waste is transported to its elimination site. It is formed by the heart, arteries, capillaries and veins. In this sense, the objective of this work is to collect and apply a method of conservation, seeking to understand the techniques of preservation of anatomical structures; perform organ dissection, know the structures that make up the topographic anatomy, tissue evaluation and anatomical and physiological structure of the cardiovascular system, its pathologies and deepen the knowledge about the integration of the approached system that lead to the homeostasis of each organism. At the same time, a qualitative research was started through a case study with bibliographical research, with applied procedures. The material for the study was a donation of a bovine heart, where it was slaughtered for human consumption. The heart was fixed in 10% formalin for conservation, and the adipose tissue and the surrounding pericardial sac were sectioned in order to make the musculature, veins and arteries of that organ more evident. This work sought to align theoretical and practical knowledge, making it possible to identify the organ's particularities in a macroscopic way, its morphological and functional function, and how the fixation and preservation of organs in formaldehyde is carried out.

KEYWORDS: Bovine species; Heart; Vascularization; Pathologies.

INTRODUÇÃO

Os bovinos pertencem à família *Bovidae* e à subfamília *Bovinae*. Esses animais são herbívoros, e sua alimentação é composta basicamente por material vegetal. Além disso, eles são classificados como ruminantes, reconhecidos pela realização de movimentos de mastigação mesmo quando não estão se alimentando (ruminação). No estudo da fisiologia animal, o corpo é dividido em sistemas, e o presente trabalho abordará o sistema cardiovascular ou também conhecido como sistema circulatório.

O sistema cardiovascular é composto pelo coração, sistema vascular sanguíneo e o sistema vascular linfático. Sendo o sistema vascular sanguíneo, o que desempenha maior função, formado pelo coração, artérias, vasos capilares e veias. Este sistema caracteriza-se por ser um sistema fechado, circulando sangue ininterruptamente. Composto por duas circulações em série: a circulação pulmonar (pequena circulação), que é formada pelo átrio direito, ventrículo direito e pulmões; e a circulação sistêmica (grande circulação), integrado pelo átrio esquerdo, ventrículo esquerdo e órgãos sistêmicos.

Esse trabalho buscou aliar os conhecimentos teóricos e práticos, propiciando assim a realização de atividades relacionadas com os conteúdos estudados no semestre. Procurou-se vivenciar na prática a morfofisiologia dos sistemas orgânicos de animais domésticos a fim de gerar suporte para identificar e classificar os fatores etiológicos, compreender e elucidar a patogenia, interpretar sinais clínicos, exames laboratoriais, alterações morfofuncionais, além de avaliar e aprimorar o senso crítico.

Dessa forma o objetivo geral deste trabalho foi coletar e aplicar um método de conservação. E como objetivos específicos buscou-se compreender as técnicas de preservação de estruturas anatômicas; realizar dissecação das estruturas, avaliação de tecidos e estrutura anatomofisiológica do sistema cardiovascular; reconhecer as características estruturais, funcionais e morfológicas; identificar as características macroscópicas de tecidos que compõem o sistema cardiovascular; integração do sistema abordado e que levam à homeostasia de cada organismo; conhecer as estruturas que compõem a anatomia topográfica, fisiologia e morfologia histológica do sistema cardiovascular e relacionar a integração das atividades síncronas e assíncronas.

DESENVOLVIMENTO

No presente trabalho serão detalhados o referencial teórico, contendo abordagem de exposição ordenada e pormenorizada da anatomia do coração bovino, pericárdio, veias, artérias, vascularização e patologias que acometem o sistema cardiovascular dos bovinos. Em seguida a metodologia empregada para conservação do modelo anatômico em questão e os resultados encontrados.

Referencial Teórico

O sangue após circular nos tecidos retorna ao coração desoxigenado, sendo rico principalmente em dióxido de carbono, por meio da veia cava cranial (contendo sangue dos membros superiores do corpo) e da veia cava caudal (contendo sangue dos membros inferiores do corpo). O sangue venoso entra no átrio direito durante a diástole, período de relaxamento do átrio, o sangue é direcionado, através da valva tricúspide, ao ventrículo direito. No ventrículo ocorre a sístole, período em que se contrai e o sangue passa pelas válvulas semilunares pulmonares e segue para os pulmões, por meio das artérias pulmonares. Posteriormente a circulação do sangue pelos pulmões, ele retorna contendo sangue arterial, composto basicamente de oxigênio, ao coração por meio das veias pulmonares e direcionando-se ao átrio esquerdo, passa pela válvula mitral e chega ao ventrículo esquerdo, de onde é bombeado para todo o corpo por meio da artéria aorta (REECE, 2020).

Os vasos sanguíneos fazem um caminho permanente do sangue que deixa o coração e retorna a ele. Dos ventrículos para os átrios, os vasos sanguíneos compreendem a ordem: artérias, arteríolas, capilares, vênulas e veias (REECE,2020).

Em bovinos o volume sanguíneo corresponde a 6 a 8% do seu peso corporal. Uma célula sanguínea demora aproximadamente 30 segundos para deixar o coração, passar por todo o corpo e regressar (KÖNIG; LIEBICH, 2016).

Anatomia do coração bovino

O coração situa-se no mediastino, ou seja, na parte interior do saco pericárdico, onde fica a maioria da massa cardíaca no hemotórax esquerdo. A região ocupada pelo coração é oposta a parede torácica, grande no lado esquerdo do tórax e se estende a partir do bordo caudal da terceira costela e vai até o quinto espaço intercostal, e do lado direito se posiciona em meio ao terceiro e quartos espaços intercostais (CABRAL, 2008).

É composto pelo endocárdio (parte mais interna) o qual recebe as cavidades do coração, miocárdio (maior parte) e o epicárdio (parte mais externa). Possui também uma base que contém dois átrios (esquerdo e direito), um ápice, dois bordos, cefálico-direito e caudal- esquerdo e duas faces (atrial e auricular). A divisão interna do coração se dá em quatro câmaras, dois átrios de forma dorsal e dois ventrículos de forma ventral (LEMOS, 2017).

Por sua vez, os átrios (direito e esquerdo) são desligados de forma interna pelo septo interatrial, e os dois ventrículos pelo septo interventricular (Figuras 1 e 2). O átrio do lado direito faz comunicação com o ventrículo direito por meio do óstio atrioventricular direito, que contém uma válvula tricúspide. Já o átrio esquerdo se comunica com o ventrículo esquerdo por intermédio do óstio atrioventricular (CABRAL, 2008).

O miocárdio, ou músculo cardíaco, compõe a maior parte da parede cardíaca. Ele consiste em fibras de músculo estriado modificado, as quais se caracterizam por possuírem núcleos basais. Essas fibras formam anastomoses umas com as outras através de suas extremidades, o que resulta em um padrão de entrelaçamento com faixas mais leves que marcam a junção entre as células, os discos intercalares (KÖNIG; LIEBICH, 2016).

Os impulsos cardíacos são gerados a partir do auto despolarização do nó sinoatrial e a seguir, devido às comunicações entre as células cardíacas e a um sistema especializado de condução, formado pelos nós atrioventriculares, feixe (feixe de His) e fibras de Purkinje, os impulsos passam rapidamente para toda a musculatura cardíaca, de forma que o coração funciona como um sincício (como se fosse uma única célula), ocorrendo contração de suas células quase ao mesmo tempo. Essa contração de relaxamento sincronizado é importante para que desempenhe de forma adequada sua função de bomba (KLEIN, 2014).

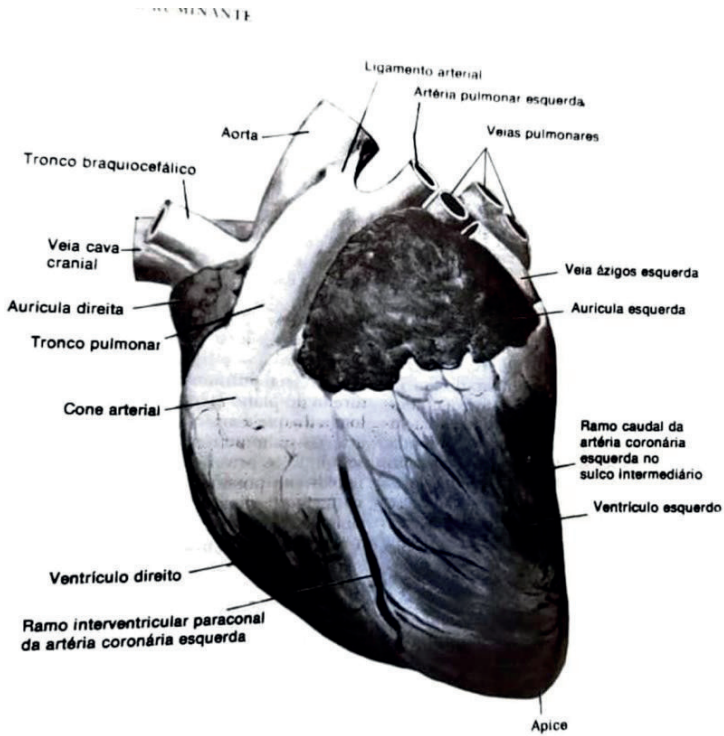


Figura 1: Coração Bovino, vista esquerda.

Fonte: Anatomia dos animais domésticos, Sisson/ Grossman 1986

Figura 33-2. Coração de bezerro, vista atrial (caudal direita).

1, Aorta descendente; 2, tronco braquiocefálico; 3, ligamento arterial; 4, artérias pulmonares; 5, veias pulmonares; 6, veia cava caudal; 7, veia cava cranial; 8, átrio direito; 9, veia ázigos subsinuata; 10, seio coronário; 11, veia cardíaca média; 12, ramo interventricular subsinuato; 13, artéria coronária direita; 14, ventrículo esquerdo; 15, ventrículo direito; 16, grande veia cardíaca.

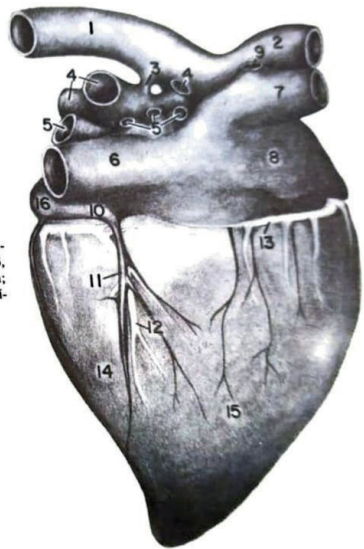


Figura 2 : Coração de bezerro, vista atrial (caudal direita)

Fonte: Anatomia dos animais domésticos, Sisson/ Grossman 1986

O peso do coração de um bovino adulto é de aproximadamente 2,5 kg, que coincide a 0,4% ou 0,5% do peso corporal. Contém um sulco intermédio ou caudal, que quando comparado a de um equino compreende um elevado comprimento de sua base ao ápice, é menor nos dois diâmetros, sendo assim, a parte ventricular possui forma cônica e mais pontiaguda (LEMOS, 2017).

Pericárdio

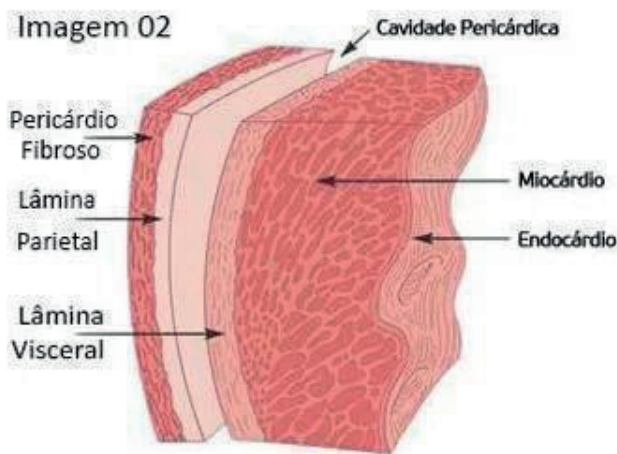


Figura 3: Cavidade pericárdica.

Fonte: STRAMA, 2019.

O coração é parcialmente cercado por uma membrana serosa chamada de pericárdio. O pericárdio é um saco fibro-seroso que envolve o coração e parte dos grandes vasos. Com a função de proteção e sustentação, corresponde a um saco completamente fechado de paredes resistentes. Divide-se em duas partes: o pericárdio fibroso e o pericárdio seroso (Figura 3). O pericárdio fibroso, fica mais externo, localiza-se unido ao esterno através dos ligamentos externos pericárdicos direito e esquerdo (DYCE, 2004).

O pericárdio seroso é constituído por duas lâminas, a parietal e a visceral. A lâmina parietal, que forra a face externa do pericárdio fibroso, é contínua à camada visceral na base do coração e reforçada por uma camada fibrosa superficial (o pericárdio fibroso), que por sua vez, é recoberta por uma camada de pleura mediastinal (também conhecida como pleura pericárdica). E a visceral, é a última camada do coração, mais interna que cobre a superfície do coração e topos justos cardíacos dos grandes vasos, são contínuas numa linha de reflexão sobre os grandes vasos e formam entre si a cavidade fechada (o espaço pericárdico) que contém um líquido seroso (líquido pericárdico), em quantidade suficiente para permitir uma fácil movimentação do coração contra o pericárdio, sem atrito entre as camadas na hora das contrações cardíacas (FAILS, 2019).

Vasos Sanguíneos

O coração tem a função de bombear o sangue por todo o corpo através de vasos sanguíneos que saem dele. Estes últimos são classificados como veias ou artérias conforme o sentido do fluxo do sangue e conforme ao tipo de sangue que elas carregam. As veias carregam sangue venoso, pobre em oxigênio, da periferia do corpo até o coração. Já as artérias fazem o caminho inverso, transportam sangue arterial, rico em oxigênio, do coração para o resto do corpo (DYCE, 2004).

As veias são classificadas em veias pequenas, médias e grandes calibres. Quanto mais próximo ao coração, são menos numerosas e de maior calibre e quanto mais longe, são mais numerosas e de menor calibre. São divididas em três partes: túnica íntima, que é uma camada subendotelial delgada feita de tecido conjuntivo frouxo, túnica média, que é uma camada mista de células musculares com fibras reticulares, e túnica externa ou túnica adventícia, que é bem grossa (JUNQUEIRA; CARNEIRO, 2017).

As vênulas são as menores veias encontradas e se estendem por todo corpo. Tem como principais funções, ser mediador da inflamação e alterar a permeabilidade vascular além de influenciar no fluxo de sangue nas arteríolas, recebem sangue dos capilares, tornam-se veias e retornam o sangue para o coração (JUNQUEIRA; CARNEIRO, 2017).

A veia cava é dividida em cranial e caudal, veia cava cranial recebe o sangue oriundo da cabeça, dos membros torácicos e da metade cranial do corpo, já a veia cava caudal recebe sangue que vem dos membros pélvicos e da parte caudal do corpo, as duas despejam seu conteúdo dentro do átrio direito. Merece destaque a veia porta e as veias pulmonares. A primeira tem grande importância no sistema pois ela recebe sangue do aparelho digestivo e o leva pelo fígado até a veia cava. Já a segunda, transporta o sangue rico em oxigênio de volta ao átrio esquerdo. Essa tem uma característica importante já que é única veia que transporta sangue rico em oxigênio enquanto as outras carregam sangue pobre em oxigênio chamado de sangue venoso (KÖNIG; LIEBICH, 2016).

As veias, principalmente as maiores, possuem dobras da túnica íntima que se projetam para o lúmen do vaso: as chamadas válvulas. Tais válvulas são compostas de tecido conjuntivo rico em fibras elásticas, revestidas por endotélio e consistem em estruturas que impedem o refluxo sanguíneo e direcionam o sangue venoso de volta para o coração (JUNQUEIRA; CARNEIRO, 2004)

O sistema cardiovascular é sempre o primeiro a se tornar fundamental nos mamíferos, os vasos sanguíneos incluem as artérias que transportam o sangue a partir do coração. Quando as artérias se ramificam e se dividem, elas acabam formando arteríolas, um diâmetro menor, que levam aos capilares, os quais apresentam o menor diâmetro dentre todos os vasos sanguíneos e permitem a passagem de células e nutrientes para os tecidos. A artéria coronária direita vem do seio direito do bulbo da aorta e faz passagem entre a aurícula do átrio direito e também do tronco pulmonar até o sulco coronário. Faz seu

seguimento ao redor da face cranial com base do coração e então se afunila em direção à origem do sulco interventricular subsinuoso. A artéria coronária direita se prolonga até o ápice e não irriga essa área os equinos e suínos (DYCE, 2004).

Temos uma grande variação no padrão das artérias coronárias em indivíduos, aquela que não é clinicamente importante na medicina veterinária, por outro lado tem diferença grande na medicina humana no que se refere à cirurgia e a infarto. As artérias coronárias são denominadas artérias finais porque não formam anastomoses, por isso a oclusão vascular de um ramo que não pode ser tolerada e acarreta infarto local do músculo cardíaco. A maioria das veias coronárias desemboca na veia cardíaca magna que ocorre paralelamente à artéria coronária esquerda (JUNQUEIRA; CARNEIRO, 2017).

Artérias de circulação pulmonar, o sangue é desoxigenado é transpassado do ventrículo direito para o pulmão pelas artérias da circulação pulmonar que faz que com o tronco pulmonar e as artérias pulmonares direita e esquerda, ventralmente à bifurcação da traquéia o tronco pulmonar se divide nas artérias pulmonares direita e esquerda. Cada artéria passa para o pulmão correspondente onde seus ramos seguem os brônquios até sua terminação. As artérias pulmonares são as únicas artérias no corpo que conduzem sangue desoxigenado. As artérias da circulação sistêmica compreendem as artérias que transportam o sangue oxigenado do ventrículo esquerdo do coração para os órgãos e tecidos corporais (KÖNIG; LIEBICH, 2016). Artéria subclávia é o tronco costocervical em qual se divide na artéria escapular dorsal, que ela se ramifica na base do pescoço e ao redor da cernelha, tem a artéria intercostal suprema (no cão é a artéria vertebral torácica), no qual se nutre as primeiras artérias intercostais. Artéria cervical profunda é o ramo terminal do tronco costocervical que se prolonga dorsalmente. A artéria vertebral passa cranialmente pelo canal transverso formado pelos forames transversos sucessivos das vértebras cervicais. Entra pelo canal vertebral do atlas. No bovino a artéria vertebral também vasculariza as partes caudal do encéfalo, esse fato ganha importância. A artéria torácica interna ocorre de forma caudal, acima do esterno e emite ramos intercostais, ela termina no diafragma e na artéria musculo frênica (KÖNIG; LIEBICH, 2016).

Os capilares ficam responsáveis por realizar as trocas gasosas entre as vênulas e as arteríolas, permitindo a troca de nutrientes entre o sangue e as células do corpo (EURELL; FRAPPIER, 2012), ainda segundo Dyce (2004), a quantidade de vasos capilares difere-se de acordo com o tipo de tecido que ele irriga.

Os capilares conectam as artérias e as veias e realizam a troca de gases e pequenas moléculas por difusão com os tecidos (REECE, 2017). A partir da árvore arterial, os vasos se abrem em volumosas redes de túbulos pequenos e de paredes uniformemente delgadas chamadas capilares (EURELL; FRAPPIER, 2012).

Patologias cardíacas em bovinos

As principais patologias clínicas que podem acometer o sistema cardíaco dos bovinos são as endocardites, pericardites, cardiomiopatias, miocardites, e os problemas cardíacos congênitos. Nestes animais, essas patologias são consideradas como sendo uma presunção a longo prazo, independentemente da sua causa, tornando mais difícil a decisão a tomar nesta situação (tratamento, refugio ou eutanásia) (JONES et al, 2000).

No entanto, o conhecimento dos principais sinais clínicos e anátomo-fisiológicos, podem ajudar a distinguir um diagnóstico quando encontrada em estágio inicial, e a conduta correta em relação a saúde do animal (BEXIGA et al, 2008).

Endocardite

A endocardite é a patologia valvular mais frequentemente encontrada em bovinos adultos. É adquirida e resulta em insuficiência da valva afetada. Apesar da válvula tricúspide ser a que se encontra comprometida com casos apresentados, também apresentam outras válvulas ou o endocárdio adjacente a estas podem, ocasionalmente, ser alvo de infecção (PEEK; MCGUIRK, 2008).

Esta é uma das poucas condições cardíacas passíveis de serem tratadas, de nem sempre ter uma taxa de sucesso elevada. Deste modo, é importante que as suspeitas, diagnóstico e tratamento ocorram o mais rápido possível para que o prognóstico seja mais favorável (PEEK; MCGUIRK, 2008). As endocardites em bovinos são maioritariamente de origem bacteriana, mas podem, ocasionalmente, ocorrer devido a parasitas (RADOSTITS et al, 2007).

Existem ainda dúvidas sobre se a infecção se inicia por adesão direta do microrganismo ao endotélio normal, ou se através de pequenas discontinuidades da superfície das válvulas ou por difusão hematogênica através dos capilares da base das válvulas (RADOSTITS et al, 2007).

Nos bovinos, a endocardite surge como consequência de uma doença crônica em uma região distante e com bactérias persistentes, sem lesões prévias no endotélio valvular (RADOSTITS et at, 2007).

Pericardites

A retículo-peritonite traumática é uma patologia comum em bovinos, pois o seu comportamento alimentar, pouco seletivo, faz com que estejam predispostos à ingestão acidental de corpos estranhos, principalmente metálicos. Devido à localização anatômica do coração nos bovinos, muito próximo da zona diafragmática do retículo, a presença de um corpo estranho perfurante no retículo pode levar ao desenvolvimento de uma pericardite, uma vez que o corpo estranho cria um trajeto fibroso levando bactérias que contaminam o fluido pericárdico, provocando uma pericardite fibrina-purulenta — pericardite traumática — uma das principais causas de pericardite nos bovinos (PEEK; MCGUIRK, 2008).

Uma vez que o diafragma, pericárdio e músculo cardíaco se encontram imediatamente cranialmente ao retículo e o fígado dorso medialmente a este, o avanço do corpo estranho que causou a retículo-peritonite pode levar ao desenvolvimento de uma pericardite séptica e/ou ao envolvimento dos outros órgãos no processo inflamatório (GUARD, 2002).

Na maioria dos casos desenvolve-se uma situação de toxemia, produzindo bactérias presentes no saco pericárdico. Caso existam bactérias produtoras de gás, este irá misturar-se com o fluido existente, sendo, por vezes, possível ouvir a auscultação (RADOSTITS et al., 2007).

Problemas cardíacos congênitos

Defeitos congênitos (DCs) são distúrbios na estrutura e/ou função de todo um sistema, ou parte dele, que ocorrem desde o nascimento. Em bovinos, os DCs têm prevalência variável entre 0,2-3% (LEIPOLD; DENNIS 1986). As perdas referentes aos DCs em um rebanho estão relacionadas ao aborto (ROUSSEAU, 1988), às malformações fenotípicas, às deficiências funcionais e ao subdesenvolvimento (PIMENTEL et al, 2007).

Os DCs podem ser hereditários ou terem causas infecciosas ou ambientais, ou ocorrerem por uma interação de ambos, agindo em um ou mais estágios do desenvolvimento fetal CÍTEK et al., 2009). A ocorrência de casos de DCs com aumento gradual no número de animais afetados, associado ao uso contínuo dos mesmos reprodutores em rebanho de uma única origem está relacionada à doença hereditária por genes recessivos. O nascimento de vários animais com DC em um único período reprodutivo é comum em casos induzidos por fatores ambientais ou doenças hereditárias por genes dominantes (SCHILD, 2007).

METODOLOGIA

O estudo desenvolvido caracteriza-se primeiramente de enfoque qualitativo através de um estudo de caso com pesquisa bibliográfica. Os procedimentos de natureza aplicada iniciaram-se com a doação do coração bovino.

O presente trabalho tem como estudo o sistema cardiovascular de um bovino da raça holandês com idade de dois anos e três meses, de aproximadamente seiscentos quilos, sendo que o animal era de pastagem, mas estava confinado há seis meses. O abate aconteceu na propriedade rural particular de um integrante do grupo, na cidade de Selbach-RS. O coração foi doado pelo colega que o levou até a instituição, onde iniciou-se o processo de conservação.

Para a coleta do órgão, foi utilizado: facas, serra, tesouras, avental de pvc, óculos e luvas. Após a coleta, o coração foi armazenado em uma caixa plástica a formol 10% como recomendado por Freitas et al, (2009). A solução foi preparada onde um litro de formol 37% foi diluído em nove litros de água. Ao final do processo toda extensão do órgão ficou

submersa na solução e após dez dias mudou sua coloração avermelhada para um tom mais esbranquiçado (Figuras 4 e 5). Permanecerá armazenado na instituição por tempo indeterminado. Estando o órgão conservado, reuniu-se o grupo no laboratório de anatomia da Faculdade IDEAU de Passo Fundo, onde foi realizada a secção do saco pericárdico, a fim de deixar mais evidente a musculatura do referido órgão. Para a realização desse procedimento foi utilizado equipamentos de segurança como jaleco, óculos, luvas e auxílio de um bisturi.

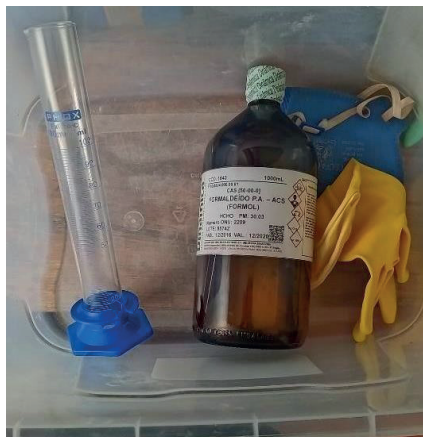


Figura 4 e 5: Materiais utilizados e coração imerso na solução de formol e água.

Fonte: Autores, 2021.

RESULTADOS E DISCUSSÃO

No processo de preparação do sistema cardiovascular, composto pelo coração e vasos sanguíneos, utilizou-se a técnica de conservação em formol a 10% e observou-se sendo eficaz, assim como descrito por Kimura e Carvalho (2010). Esta técnica compreende em preservar tecidos biológicos sem que aconteça a decomposição ou algum processo que irá prejudicar a peça anatômica. Durante a conservação da peça, ela teve uma mudança em sua coloração inicial, avermelhada, para um tom mais esbranquiçado após dez dias (Figura 6 e 7).



Figura 5 e 6: Resultado após imersão em solução de formol.

Fonte: Autores, 2021.

Assim como foi observado na peça trabalhada, parte da dificuldade na aprendizagem está relacionada com o fato de que o coração não é perfeitamente simétrico, podendo ser um tanto difícil discernir qual face (dorsal, ventral, direita ou esquerda) e quais vasos estão sendo visualizados.

Conforme relatado por Silva (2014), o coração é dividido, por um septo interventricular longitudinal, em lado esquerdo e lado direito. Cada um possui um septo transverso nos átrios, que recebem sangue e nos ventrículos que bombeiam sangue.

Segundo Konig e Liebich (2016), e visualizado na figura 7, o átrio direito recebe sangue da veia cava cranial, da veia cava caudal e do seio coronário dividindo-se em parte principal, seio da veia cava, e a aurícula do átrio direito.

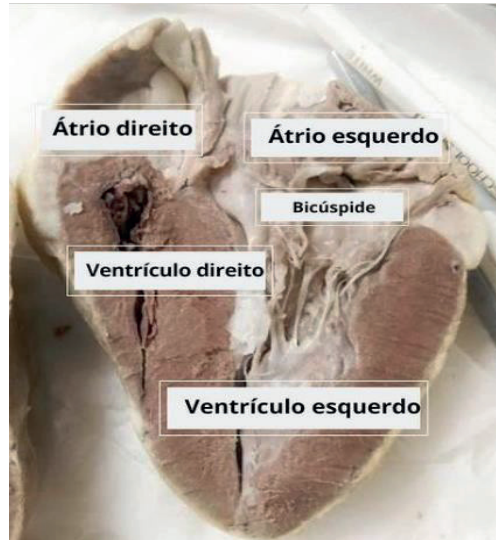


Figura 7: Átrios e Ventrículos.

Fonte: Biologycorner.com

O ventrículo direito designa a parte cranial direita da massa ventricular formando quase toda a borda cranial do coração, mas não vai até o ápice. Semelhante a algo triangular em seu contorno e tem a forma crescente, em corte transversal. É ornado pela valva atrioventricular direita (tricúspide) e pela valva pulmonar (CINELLI; SILVA, 2014).

Segundo o mesmo autor, o átrio esquerdo forma a parte caudal da base do coração. Localiza-se caudalmente ao tronco pulmonar e à aorta, e dorsalmente ao ventrículo esquerdo. As veias pulmonares abrem-se no átrio caudalmente ao mesmo e do lado direito. O óstio atrioventricular esquerdo está posicionado ventro cranialmente.

O ventrículo esquerdo é constituído pela parte caudal esquerda da massa ventricular. Ele possui sua forma mais regular e cônica do que o ventrículo direito e sua parede é muito mais espessa, exceto no ápice. Ele forma todo contorno caudal da parte ventricular e do ápice do coração. É ornado pela valva atrioventricular esquerda, também chamada de bicúspide ou mitral, e pela valva aórtica (CINELLI; SILVA, 2014).

Conforme cita Getty (2008), o músculo estriado cardíaco é o tipo de tecido muscular que forma as camadas musculares do coração, conhecida por miocárdio, também é chamado tecido muscular estriado cardíaco.

Conforme visto na peça anatômica trabalhada, coração é formado por três tipos principais de músculos: Ventricular, contrai de forma parecida com o músculo estriado, mas a duração de contração é maior; Atrial, contrai de forma parecida com o músculo estriado, mas a duração de contração é maior; Fibras musculares excitatórias e condutoras, só se contraem de modo mais fraco, pois contêm poucas fibrilas contráteis, ao contrário, apresentam ritmicidade e velocidade de condução variáveis, formando um sistema excitatório para o coração (GARTNER, 2007).

As mídias se dispõem lado a lado, juntando-se e separando-se entre si, através de “junções de abertura”. Uma grande vantagem neste tipo de disposição de fibras é que o impulso, uma vez atingindo uma célula, passa com grande facilidade às outras (BANKS, 1991).

Existem dois sincícios funcionais formando o coração: Sincício atrial e Sincício ventricular. Os dois são separados por uma membrana de tecido fibroso. Isso possibilita que a contração nas fibras que compõem o sincício atrial ocorra em tempo diferente da que ocorre no sincício ventricular (CUNNINGHAM, 2004).

Conforme relatado por Ashdwn (2011), isso ocorre para a perfeição do batimento cardíaco, ou seja, enquanto o átrio se contrai, denominado sístole o sangue é ejetado para o ventrículo, denominado diástole, e quando o átrio relaxa (diástole), o ventrículo se contrai (sístole) proporcionando assim o fechamento das válvulas e impulsionando o sangue para as artérias. Sendo assim, o “atraso” dos impulsos, ocasionado pela membrana de tecido fibroso entre átrios e ventrículos, causa diferença de contração entre eles. As contrações se caracterizam assim por ser rítmica, vigorosa e involuntária. As fibras musculares cardíacas têm discos (membranas que delimitam a célula) intercalados entre uma fibra e outra, possuindo contrações involuntárias, sendo controladas pelo sistema nervoso autônomo.

Observou-se que o órgão estava envolto de tecido adiposo, que foi parcialmente removido para melhor visualização dos vasos, além disso parte do saco pericárdio também foi seccionado com a mesma finalidade (Figura 8). O tecido adiposo é um tipo especial de tecido conjuntivo formado por adipócitos. Sendo a principal fonte reservatória de energia para o organismo, encontrado espalhado pelo corpo com função de sintetizar lipídios, proteger os órgãos internos em seus lugares e servir de isolante térmico (ALANIZ, 2006). O excesso de adipócitos pode acarretar obstrução de artérias e veias, além de afetar o metabolismo e qualidade de vida do animal.



Figura 8: Saco pericárdio seccionado e peça pronta.

Fonte: Autores, 2021.

O coração dos bovinos apresenta o osso cardíaco. Esse osso é uma calcificação de parte do esqueleto fibroso do coração e serve para estabilizar os grandes vasos durante a sístole, apresentando uma capa de gordura densa e possuem aspecto triangular (MELO, 2010).

Finalizando, ressalta-se a importância do estudo do sistema cardiovascular bovino, para conhecimento de suas estruturas e funcionamento, assim contextualizando com as demais estruturas para o melhor entendimento do organismo como um todo, favorecendo práticas de manejo e bem-estar desta espécie.

CONCLUSÃO

Conclui-se que, a partir desse trabalho se aliou a pesquisa e as aulas teóricas com a prática, favorecendo o aprendizado e desenvolvimento, o objetivo foi alcançado sendo possível identificar anatomicamente as particularidades do órgão de maneira macroscópica como as veias e artérias, sua função morfológica e funcional, a vascularização e percurso do sangue no corpo, patologias envolvidas e de que maneira se realiza a fixação e conservação de órgãos em formol 10%.

REFERÊNCIAS

ALANIZ, et al. **O tecido adiposo como centro regulador do metabolismo**. Departamento de Fisiologia e Biofísica, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, 2006.

ANDRADE, E. N., ROÇA, R. O., SILVA, R. A. M. S., PINHEIRO, R. S. B. **Abate de bovinos no pantanal Sul Mato-grossense e lesões em carcaças**. 2008. Disponível em: <http://www.sovergs.com.br/conbravet2008/anais/cd/resumos/R0845-1.pdf>. Acesso em: 08 de outubro de 2021.

BANKS, W. J. **Histologia Veterinária Aplicada**. Ed. Manole LTDA. 2ª Edição. São Paulo. 1991.

BROCK, J. C. **A Natural History of Domesticated Mammals**, 2ª ed., Cambridge University Press, Cambridge, 1999, p. 248.

CABRAL, S da C. L. **Patologia cardíaca em bovinos**. Dissertação de mestrado integrado em medicina veterinária. Lisboa, 2008.

CECCATTO, L. **Biologycorner, heart Dissection Walk Through**. Disponível em: Biologycorner.com, 2014. Acesso em 11 de outubro de 2021.

CHAVES, A. R. D. et al. **Raças Bovinas e a qualidade da carne**. Anais X Mostra Científica- FAMEZ / UFMS, Campo Grande, p. 294-300, 2017.

CINELLI, F. R; SILVA, E. M. Acadêmicas do curso de Medicina Veterinária. **Sistema Cardiovascular**. Universidade Federal de Goiás - Campus Jataí 2014.

CUNNINGHAM, J. G. **Tratado De Fisiologia Veterinária**. Ed. Guanabara Koogan. 3ª edição. Rio de Janeiro. 2004.

DYCE, K. M.; SACK, K. O.; WENSING, C. J. G. **Tratado de Anatomia Veterinária**. Elsevier. Rio de Janeiro. 840p, 2010.

EURELL, J. A; FRAPPIER, B.L **Histologia veterinária de Dellmann**. Ed. 6a, Editora Manole. p 117, 2012.

FAILS, A. D. **Franson Anatomia e Fisiologia dos Animais de Produção**. 8 ed. Rio de Janeiro. Guanabara Koogan, 2019.

FERNANDES, T. A. et al. **Características comportamentais dos bovinos: Influências da domesticação e da interação homem**. Revista Electrónica de Veterinária. Espanha. Vol. 18, núm. 12, p. 8. 2017.

FREITAS I.B., Souza A.M. & Santos R.M.B. 2009. **Técnica anatômica aplicada na conservação de cortes segmentares em Canis familiaris e Decapterus Macarellus**. IX Jornada de Ensino, Pesquisa e Extensão, UFRPE, Recife, p.1-3. (Resumo) Disponível em <<http://www.eventosufrpe.com.br/jepepx2009/cd/resumos/R0721-2>>. Pd.

GARTNER. L. P; HIATT. J. L. **ATLAS COLORIDO DE HISTOLOGIA**. Ed. Guanabara Koogan. 4ª edição. Rio de Janeiro. 2007.

GETTY. R. SISSON; GROSSMAN. **Anatomia dos Animais Domésticos**. 5.ed, Rio de Janeiro- RJ, Guanabara-Koogan, v. 1, p.901. 1986

GHOSHAL, N. G. **Coração e Artérias do Carnívoro**, In: GETTY, R. Sisson/Gossman. Cora Sisson and Grossman: Anatomia dos animais domésticos. 5ª edição. p. 900-959. Rio de Janeiro: Guanabara Koogan S.A.1986.

JUNQUEIRA, L. C. U; CARNEIRO, J. **Histologia Básica Texto & Atlas**. 13ª ed. Rio de Janeiro- RJ. Guanabara Koogan. p.209, 2017.

KLEIN, B. G. Cunningham. **Tratado de fisiologia veterinária**. Elsevier Brasil, 2014.

KIMURA, A.K.E.; CARVALHO, W.L. **Estudo da relação custo x benefício no emprego da técnica de glicerinação em comparação com a utilização da conservação por formol**. Trabalho de Conclusão de Curso de Extensão em Higiene Ocupacional, Universidade Estadual Paulista, Araraquara, SP. 30p, 2010.

KÖNIG, Horst; LIEBICH, Hans-Georg. **Anatomia dos Animais Domésticos: texto e atlas colorido**. 6. ed. Porto Alegre, Artme, 2016.

LEMOS, V. W. **Anatomia comparada do coração de mamíferos aplicada ao ensino**. Dois vizinhos, 2017.

REECE, W. O; ROWE. E. W. **Dukes I Fisiologia dos Animais Domésticos**, 13a ed. Rio de Janeiro - RJ. Guanabara Koogan. p.277, 2017.

SAMUELSON, D A. **Tratado de histologia veterinária**. Rio de Janeiro: Elsevier, 2007.

SCHILD, A. L. **Defeitos congênitos**, p.25-55. Doenças de Ruminantes e Equídeos. 3ª ed. Pallotti, Santa Maria, 2007.

STRAMA, A. **Mediastino e pericárdio**. 2019. Disponível em: <<https://www.passeidireto.com/arquivo/62266282/3-mediastino-e-pericardio>>. Acesso em: 04 de setembro de 2021.

WATTIAUX, M. A; HOWARD, W. T. **Processo Digestivo na vaca de leite**. Instituto Babcock para Pesquisa e Desenvolvimento da Pecuária Leiteira Internacional. Essenciais em Gado de Leite. University of Wisconsin-Madison, 2014.

GYP SUM IN AGRICULTURE

Acceptance date: 02/05/2024

Alceu Linares Pádua Junior

Universidade do Vale do Jequitinhonha
and Mucuri, campus Unaí, Brazil

Hamilton César de Oliveira Charlo

Instituto Federal do Triângulo Mineiro,
campus Uberaba, Brazil

Jose Luiz Rodrigues Torres

Instituto Federal do Triângulo Mineiro,
campus Uberaba, Brazil

Ernane Miranda Lemes

Universidade Federal de Uberlândia,
campus Uberlândia, Brazil

ABSTRACT: Gypsum (calcium sulfate) for agricultural uses is a source of essential nutrients for plants (calcium and sulfur) and a soil and root environment conditioner in surface and subsurface soil layers. Applying gypsum to the soil reduces physical, chemical, and biological losses. Gypsum is primarily available as mined (sedimentary rocks) or as a by-product of industrial processes (e.g., acid manufacturing), food protein production (lacto-gypsum), or pollution control systems (e.g., flue gas desulfurization gypsum). Many plant nutrients in the soil can be managed by

applying gypsum alone or in combination with other components such as lime and organic amendments. Surface-applied gypsum improves nutrient distribution in the soil profile and reduces aluminum saturation in no-tillage cropping systems. There are multiple and simultaneous benefits of applying gypsum to the soil that may be responsible for increasing plant resistance to stresses (biotic and abiotic plant diseases) and increasing plant biomass, grain, fibers, and extract yields in traditional extensive agroenvironments or organic cultivation. However, the use of gypsum in agriculture depends on its availability and shipping costs compared to the expected soil and yield responses. When gypsum is economically viable, significant advances in yields and the overall efficiency of the production process can be achieved in various crops and soil conditions. The aim of this chapter is not to exhaustively cover all gypsum-related topics but to address concepts and studies that will (i) review the recent literature on the effects of gypsum application in the soil-plant-environment system; (ii) present the gypsum recommendation methodologies and considerations; (iii) discuss studies and cases of gypsum uses in different soils and environmental conditions; (iv) suggest

ways of managing gypsum to efficient and sustainable agriculture, and (v) present themes to be studied and explored to advance the knowledge of this agricultural tool. The information presented in this chapter is intended for farmers, researchers, natural sciences students, agricultural management consultants, environmental regulators, and agricultural gypsum producers and traders.

KEYWORDS: calcium sulfate, calcium, sulfur, soil parameters, plant nutrition, crop management, efficient food production, eco-friendly agriculture

The sustainability of a growing human population depends on the sustainability of agriculture. Food, feed, fibers, and fuel from crop plants must consider preserving and improving healthy environments and their ecological services. Therefore, agriculture must be conducted according to adequate technical principles and precise farming management. The correct amount and time of fertilizer applications for crop production are essential for enhanced results and reduced environmental impacts.

Modern techniques for field fertilization include using highly efficient fertilizers and soil conditioners (products that affect soil physical, chemical, and biological attributes) to supply crop plants during their cycle. Agricultural gypsum is a fertilizer and a soil conditioner, and despite its underuse for farming, it is among the most important nutritional amendments used for crop production worldwide.

GYPSUM ORIGINS

Concentrated gypsum (CaSO_4 , or calcium sulfate) contains about 23.3% calcium, 18.6% sulfur, and minor amounts of other elements; however, for agricultural purposes, hydrated gypsum ($\text{CaSO}_4 \times 2\text{H}_2\text{O}$, or gypsum) is more available to the farmers. The production of hydrated gypsum starts with gypsum natural rock (Deer et al., 1966), which is grounded and heated (190-200 °C) to remove more than two-thirds of its water content. This less hydrated mined gypsum is a neutral salt containing approximately 79% calcium sulfate and 21% water, and it is a moderately soluble (2.5 g L^{-1}) and a relatively common mineral (Curi et al., 1993; Chen and Dick, 2011; Wang and Yang, 2018). Anhydrite (anhydrous gypsum) is another natural rock available as a gypsum source; however, the hardness and low reactiveness make it less economically attractive.

The world's top five mined gypsum producers are the USA (22 million tons), China, Iran, Turkey, and Thailand (Crangle Jr., 2021). Brazil produces about 3 million tons of mined gypsum (Crangle Jr., 2021), and 80 mines have been exploring this resource recently (IBRAM, 2020), mainly concentrated in the north, northwest, and central-west sides of the country (van Raij, 2008). Gypsum for agricultural uses is also available as (i) a by-product of phosphoric, hydrofluoric, and citric acid production, (ii) a by-product of the pollution-control processes (e.g., neutralization of sulfuric acid and flue-gas desulfurization), and minor (iii) from dairy whey side streams (Alcordero and Rechcigl, 1993; Zoca and Penn, 2017; Bondi et al., 2021).

The gypsum by-product of the manufacture of phosphoric acid from the sulfuric acid attack on rock phosphate is named phosphogypsum (van Raij, 2008; Vitti et al., 2008). This sort of gypsum is widely available and may have impurities such as small proportions of phosphorus, potassium, magnesium, sodium, boron, fluorine, silicon, iron, aluminum, heavy metals, and radionuclides depending on the gypsum rock composition and geological origins (Alcordero and Rechcigl, 1993), but the calcium and sulfur relative concentrations still very similar among the types of mined gypsum available (van Raij, 2008).

BENEFITS AND EFFECTS OF GYPSUM USE

The positive results of gypsum application to the soil-plant-environment system are long known (Mayer, 1768; Crocker, 1922; Brasil et al., 2020). The first studies to highlight the positive effects of gypsum on agriculture in Brazil were reported by Malavolta et al. (1979) - which indicated crop improvements and higher root development in high-sodium soils - and by Ritchey et al. (1980) - which stated that single superphosphate and gypsum application resulted in (i) increased soil calcium contents, (ii) lower subsoil aluminum saturation, and (iii) improved maize (*Zea mays*) root development.

Ritchey et al. (1980) also highlighted that the leaching of soil bases such as potassium and magnesium could occur as the gypsum rate increases and that the improvements generated by the gypsum application are not exclusively due to the calcium sulfate as a source of calcium and sulfur. Syed-Omar and Sumner (1991) observed that exchangeable magnesium was reduced throughout the first 0.525 m soil layer, while no reduction in exchangeable potassium was observed below the 0.225 m soil layer. Their study indicates magnesium is more susceptible to leaching loss than potassium after surface gypsum application (2, 5, or 10 Mg ha⁻¹). It was also suggested that surface-applied gypsum be used as a soil ameliorant along with proper management of magnesium and potassium fertilizers.

Gypsum dissociates into calcium cation (Ca²⁺) and sulfate anion (SO₄²⁻) in soil solution. The calcium added to the soil complex displaces other cations, such as magnesium (Mg²⁺), potassium (K⁺), and aluminum (Al³⁺). These displaced cations react with the sulfate anion and originate less phytotoxic forms of aluminum and neutral ionic pairs (cation + SO₄²⁻), such as MgSO₄ and K₂SO₄, which are highly mobile ionic pairs in the soil profile (Carvalho and van Raij, 1997).

The magnesium leaching caused by gypsum application can be even more damaging to plant development if other soil nutrition strategies are not appropriately implemented. A plant magnesium deficiency in the Brazilian Cerrado was potentialized by gypsum application to a newly opened area (summer of 2023/2024) for grain cropping (Pádua Jr., A. L. – data not previously published). The affected area was treated with 3 Mg ha⁻¹ of calcitic lime (45-55% CaO, 1-4.99% MgO) 90 days before maize sowing and 1 Mg ha⁻¹ de gypsum 60 days before maize sowing. The soil magnesium content in the 0-0.2 m soil layer was

also low ($0.4 \text{ cmol}_c \text{ dm}^{-3}$). However, the recommendation for magnesium-poor soils where gypsum is needed is the application of dolomitic lime (25-35% CaO, $\geq 5\%$ MgO). Dolomitic lime (2 Mg ha^{-1}) was applied to only one section of the cropping area. The plants from where calcitic lime was applied presented leaf symptoms of magnesium deficiency (Figure 1).



Figure 1. Maize plants from where calcitic lime and gypsum were applied presented leaf yellowing due to magnesium deficiency (A). B. Initial maize leaf symptom of magnesium deficiency (interveinal chlorosis).

Source: Pádua Jr, A. L.

Such a condition of maize plants responding to low magnesium availability was caused by (i) the excess of calcium cations from lime and gypsum application, (ii) the increased cation leaching potential generated by gypsum application, and (iii) the low soil magnesium content. Thus, in this case, gypsum potentialized the abiotic stress caused by the soil magnesium shortage. Magnesium sulfate (9% magnesium) was applied via foliar to reduce damage to the crop yield. Still, a nutritional soil repositioning of magnesium must be done before the next crop season. The effects and recommendations on soil cation management and the joint application of gypsum and lime will be further discussed.

Moreover, gypsum application does not provide only calcium and sulfur; other nutrients are added with gypsum application (Alcordero and Rechcigl, 1993). According to van Raij (2008), some gypsums can add up to about 1 kg ha^{-1} of boron for each 2 Mg ha^{-1} of gypsum applied to the cropping system. Boron is essential for root system growth, cell wall formation, and root expansion in the soil volume.

The regular gypsum positive effects include calcium and sulfur source in superficial and deeper soil profiles but also have positive effects on soil physical, chemical, and biological conditioning; reclaimed soils; reduced damage caused by subsoil acidity

(exchangeable aluminum) and pollutants on plant growth; increased the abundance of soil microorganisms; improved nutrient redistribution and its plant-use-efficiency in soil profile; enhanced seedling, shoot, and root development; lower nutrient losses in agricultural biological waste composting; increased rainfall absorption; lower soil particle dispersion; improve soil stability; lower surface crust formation, runoff, and soil erosion (Ritchey et al., 1980; Ritchey et al., 1995; Toma et al., 1999; Martins et al., 2002; Soratto and Crusciol, 2008; Chen and Dick, 2011; Cañadas et al., 2014; Batte and Forster, 2015; Qayyum et al., 2017; Zoca and Penn, 2017; Bossolani et al., 2018; Cuervo-Alzate and Osorio, 2020; Qu et al., 2020; Charlo et al., 2022; Goiba et al., 2023; Garbowski et al., 2023; Jin et al., 2023; Li et al., 2023; Niaz et al., 2023; Outbakat et al. 2023; Robinson et al., 2023; AbouRizk et al., 2024). Gypsum can also have other purposes and be carbonated with carbon dioxide (CO₂) to sequester it from the atmosphere; one megagram or one ton of hydrated gypsum can react with about 0.26 Mg of CO₂ and form lime (Wang et al., 2021).

About three decades ago, Wallace (1994) presented many reasons why gypsum is essential for agriculture maintenance on many soils. The main advantages of gypsum application to the soil include accumulating more soil organic matter and aggregate stability, improved water drainage into the soil, prevention, and correction of soil sodicity, and faster seed emergence. The author also highlighted that gypsum is an industrial waste product available at a relatively low cost in many locations.

Some agricultural areas may still be far from a gypsum source. The beneficial soil-plant-environment returns of gypsum must be considered before gypsum acquisition since shipping costs can be restrictive. The expenses of gypsum benefits naturally include purchasing the material, transporting it from industry to the crop area, and spreading it on the soil (Chen and Dick, 2011). Despite its many benefits, the commercial-scale use of gypsum still depends on its logistics and investment return compared to the yield responses achieved with its application, the cost/benefit ratio (Shainberg et al., 1989).

The review of Rashmi et al. (2018) resumed many of the impacts of gypsum application to crops, especially oilseed crops such as soybean (*Glycine max*) and mustard (*Brassica juncea*). The authors presented gypsum's fertilizer and soil conditioner roles and gypsum's impacts on plant biometrics, chemical composition, crop yield, and soil parameters. Although, as pointed out by Chen and Dick (2011), most farmers are unfamiliar with the field application of gypsum and consequently have not seen the gypsum benefits. Thus, there is a considerable lack of knowledge about the best management practices for using gypsum as an agricultural amendment.

Moreover, the soil chemical balancing for plants requires regular applications of minerals containing calcium, such as limestone (lime calcium carbonate, CaCO₃) and gypsum, to achieve cation balance on the soil exchange sites (Brock et al., 2020). The base cation saturation ratio of 13:2:1 (calcium, magnesium, and potassium contents, respectively) is usually indicated as a reference to support optimum crop development (Chaganti and

Culman, 2017). However, optimum crop development has been observed in commercial crop areas in Brazil for base cation saturation ratios of 8-10:3:1 (Pádua Jr., A. L. - personal information). Potassium luxury uptake by plants and reduced phosphorus deficiency are also observed in soils with chemically balanced cation saturation ratios (Kopittke and Menzies, 2007).

Gypsum can even be used as a phosphorus sorbing and retaining material in soils (Penn and Bryant, 2006; Bryant et al., 2012; McGrath et al., 2013; Endale et al., 2014; Penn and McGrath, 2014; Watts et al., 2021; Mao et al., 2022; Ekholm et al., 2024), reducing its losses up to about 66% (Murphy and Stevens, 2010; Kumaragamage et al., 2022). King et al. (2016) reported that the surface application of flue gas desulfurization gypsum (FGDG) (further discussed) considerably reduced dissolved reactive phosphorus and total phosphorus concentrations and loadings in drainage waters (runoff and tile solutions). Favaretto et al. (2012) also reported that gypsum application reduces water pollution by phosphorus but increases soil ammonium (NH_4^+) mobility. It is worth noting that the effects of gypsum application on soils and crops could take several years before demonstrable benefits (Farina and Channon, 1988; McKibben, 2012), but these effects can last for years (Toma et al., 1999).

The combination of gypsum with other materials frequently outcomes improved results. Tubail et al. (2008) reported that combining FGDG with organic waste (nitrogen-rich streams) results in a product with decreased nitrogen potential loss and reduced odors associated with ammonia volatilization during the composting process. Bossolani et al. (2020), after a long-term study, reported that the combined application of lime (13.04 Mg ha^{-1}) and gypsum (10 Mg ha^{-1}) increased soil fertility and biological nitrogen fixation to an extended level. These authors also reported (i) reduced soil nitrification and denitrification in maize rhizosphere intercropped with Ruzi grass (*Urochloa ruziziensis*), (ii) altered nitrogen cycle genes in the soil biota, (iii) reduced aluminum saturation, (iv) balanced micronutrient availability (especially manganese), (v) improved calcium and magnesium availability in soil, (vi) increased nitrogen acquisition and (vii) increased maize grain yield.

However, the effectiveness of the benefits from gypsum application depends significantly on the physical and textural qualities of the gypsum reaching the field. Factors such as particle size, humidity, purity, and impurities play crucial roles in influencing the overall impact of gypsum effects on soil properties, plant growth, and yield responses. The moisture content of the gypsum to be applied must be low, and the gypsum material must be dry enough to be handled without clumping together due to high water content. Gypsum moisture content between 10 and 22% presents no significant physical limitations for field application [Paolinelli et al. (1986) in van Raij (2008) p. 34]. Occasionally, gypsum can arrive in the cropping area excessively wet (Figure 2), especially during high gypsum demands by crop management agendas.



Figure 2. Excessively wet phosphogypsum. Saturated load was dripping calcium sulfate solution (A) from a mass in the back of a truck (B) that was unloaded as a compacted material (C and D) physically inappropriate for field application.

Source: Guareschi, G.

Such a situation of excessive water content in gypsum constitutes two significant problems besides losing gypsum's overall agronomic efficiency: (i) wet gypsum cannot be homogeneously applied over the field using regular mechanical spreaders, and (ii) after wet gypsum dries, it becomes a hard solid material that needs to be grounded and sieved again before its application.

LESS EXPRESSIVE GYPSUM EFFECTS

Numerous reports of improvements in the soil system and crop yield increments are not always detected or consistent due to variations in soil type, crop species, and prevailing climate conditions. These observations challenge the identification of the precise improvements responsible for yield increases since many simultaneous physical and chemical interactions occur in soil (Zoca and Penn, 2017). Thus, it is more reasonable to understand crop yield improvements (when they occur) due to the synergic effects of the gypsum application to the soil-plant-environment system (Shainberg et al., 1989).

The improvements generated by the gypsum application are not always consistent and present mixed effects on soil, plant, and environmental parameters (Churka Blum et al., 2013; Tirado-Corbala et al., 2013; Buckley and Wolkowski, 2014; Bortolanza and Klein, 2016; Chaganti et al., 2019; Sun et al., 2019; Brignoli et al., 2021; Popp et al. 2021). For example, Adams et al. (2022) found that the surface runoff in grassland systems was reduced - regardless of management (grazing or hay) - by pasture aeration (spike aerator) following broiler litter application (5.6 Mg ha^{-1}), especially when compared to surface-broadcast traditional practices; however, the authors observed that gypsum application did not affect soil infiltration rates.

Kost et al. (2018) studied the effects of FGDG and mined gypsum application on soil, plant, and water parameters across ten sites distributed in the USA (Alabama, Arkansas, Indiana, New Mexico, North Dakota, Ohio, and Wisconsin states) via a data meta-analysis. The authors found relatively few significant effects of gypsum applications on the response variables. Some crop yield responses to gypsum were detected in some sites, but the overall results indicated no significant differences between the gypsum sources and the untreated control. Additionally, Charlo et al. (2020) reported that maize yield and plant biometric attributes were not influenced by gypsum or K_2O doses; they also reported that gypsum caused cation (potassium and magnesium) displacement to deeper soil layers (0.2-0.4 m), which was not enough to improve maize responses.

Gypsum application usually reduces the adverse effects of polluted soils on plants. However, Dubrovina et al. (2021) found no effect on alleviating metal phytotoxicity in the contaminated soil they studied [O horizon (forest litter) and A horizon (mineral soil)]. Instead, gypsum increased the concentrations of soluble metals in the soil solution and enhanced the metal plant uptake. The calcium cations from solubilized gypsum possibly displaced

the metals in the exchangeable soil complex, making them more available to the plants and increasing the environmental hazard; thus, gypsum was ineffective and considered inappropriate as a soil remediation method to ameliorate soils polluted by metals. Argüello et al. (2022) also found limited effects of gypsum in cadmium-contaminated soils cultivated with cacao and, in some cases, even increased cadmium dissolution and plant-available by forming cadmium sulfate (CdSO_4) complexes.

GYPSUM AS BY-PRODUCT

Using industrial residues for soil amelioration and crop production can be a sustainable practice, and it has become of great interest in recent years. By-products from treated slags, such as yellow gypsum (Ali and Shahram, 2007; Ashrit et al., 2016; Ashrit et al., 2020; Prakash et al., 2020; Laxmanarayanan et al., 2022), and from pollution-control processes, such as the FGDG (Baligar et al., 2011; Watts and Dick, 2014; Wang and Yang, 2018) have demonstrated significant and positive results for the soil-plant-environment system. The yellow gypsum is produced from the Linz-Donawitz slag treated with concentrated sulfuric acid and neutralized with lime. The product (yellow gypsum) has about 87.98% of gypsum, plus other important nutrients such as iron (3.53%), silicon (1.79%), magnesium (0.78%), phosphorus (0.37%), titanium (0.15%), and other trace elements (Ashrit et al., 2015). The FGDG is primarily produced during the wet sulfur removal from fuel combustion gases in thermal power facilities, coal-fired power generation industries, smelters, and large-scale boilers (Koralegedara et al., 2019; Liu et al., 2021). Therefore, yellow gypsum and FGDG are mainly used in countries where their production is abundant, like China, the USA, India, and Germany.

The FGDG delivers similar benefits to the soil-plant-environment system as the gypsum from other origins (e.g., mined, by-product of acids production, dairy whey side streams). Reports of positive FGDG effects, even in long-term studies, are regular. Such effects include plant macro and micronutrients fertilization, reclaiming soil physicochemical attributes and polluted soils, stimulation of soil microbiota activity and ecological services, control of soil and nutrient erosions, improved plant development, and increased crop yield (Dick et al., 2006; Baligar et al., 2011; Watts and Dick, 2014; Marchis et al., 2016; Panday et al., 2018; Wang and Yang, 2018; Wang et al., 2021; USEPA, 2023). Zhao et al. (2019) studied the physical-chemical attributes and heavy metal contaminations after 17 years of FGDG application on sandy loam soil. After that period, the authors observed (i) an increased occurrence of soil macroaggregates ($> 250 \mu\text{m}$), (ii) no significant differences in the soil heavy metal contents (arsenic, cadmium, chromium, copper, mercury, nickel, lead, and zinc), and (iii) the soil reclamation effect caused by FGDG on sodic soils persisted and extended to deep soil layers.

FGDG can also be applied as an encapsulated or non-encapsulated soil amendment (Codling, 2017; Koralegedara et al., 2019). Additionally, FGDG typically presents a small and uniform size, with more than 95% of the particle sizing less than 150 microns, but it can be processed to form large-sized granules (Chen and Dick, 2011). However, gypsum produced from pollution-control processes may contain calcium carbonate, calcium sulfite, quartz (SiO_2), heavy metals, and other impurities that must be analyzed for safe environmental use before agricultural application (Chen and Dick, 2011; Wang and Yang, 2018; Koralegedara et al., 2019; Kong et al., 2023); therefore, for the environmentally responsible use of FGDG, it is necessary to accurately determine the contents of elements (plant nutrients or pollutants) in its composition (Chen and Dick, 2011; USEPA, 2023).

Chen et al. (2014) studied FGDG and mined gypsum application across many soils in the USA (Ohio, Indiana, Alabama, and Wisconsin states) to determine gypsum's ability to affect the concentration of trace elements (arsenic, barium, cobalt, chromium, copper, molybdenum, nickel, niobium, lead, antimony, selenium, strontium, vanadium, and zinc) in soils and earthworms. The authors found that (i) only mercury was slightly increased in some soils and earthworms when FGDG was applied; (ii) in some soils that received FGDG, selenium in earthworms was higher than in the untreated control but not higher than in mined gypsum treatment, and (iii) the bioaccumulation factor (ratio of element concentration in earthworm and element concentration in soil) where FGDG was applied were similar, or lower, to the untreated control and mined gypsum.

Additionally, Lee et al. (2007) indicated that the autumn surface application of FGDG in no-tillage systems managed before spring cropping would allow enough time for oxidation and dissolution reactions without causing significant negative effects on the soil biota. Thus, when properly managed and applied at the correct periods, FGDG represents a considerable input into agricultural systems.

GYPSUM ON SOIL ATTRIBUTES

The soil's physical characteristics are also essential for sustainable plant development. The soil infiltration rate (solution flux through a surface area per time) and water storage are usually improved by gypsum application (McIntyre et al., 1982; Truman et al., 2010; Muller et al., 2012; Zoca and Penn, 2017; Crusciol et al., 2019). The effects of gypsum on soil infiltration and water storage capacity originated from the flocculation and aggregation of subsoil components and improved root development. A developed root system also increases subsoil aggregation and reduces soil compaction; thus, gypsum, more precisely, calcium in gypsum, can minimize soil dispersion (Summer et al., 1990; Norton, 2008). As previously mentioned, gypsum can prevent soil surface crusting and reclaim calcareous non-sodic soils (Amezketta et al., 2005).

According to the Soil Science Society of America (1997), gypsum should be used as a calcium source in sodic soils, while the Brazilian legislation (Brasil, 2006) considers gypsum a soil sodicity corrector (sodium saturation reducer) and soil conditioner (soil attribute improver). These effects reduce soil erosion and improve water bodies' quality, particularly when applying gypsum with other management techniques. Soil aggregation induced by gypsum and polysaccharide (glucose) amendments can be enhanced (Walia et al., 2018). Such improved soil aggregation is usually attributed to the glucose gluing activity and the gypsum (calcium) binding-stabilizing activity.

Additionally, reports of positive effects of gypsum application on soil physics - soil structure (Tirado-Corbalá et al., 2019), water infiltration and drainage (Jayawardane and Blackwell, 1986; Tirado-Corbalá et al., 2013; Watts and Dick, 2014), bulk density (Buckley and Wolkowski, 2014), penetrometer resistance (Ellington, 1986) - are regular and important to the cropping system and raise the interest in the regular use of gypsum (Zoca and Penn, 2017; Rashmi et al., 2018). Gypsum is also used to reclaim sodic soils and to improve soil water infiltration decreased by low electrolyte concentration (Oster, 1982). However, the magnitude of soil and crop responses to gypsum application is affected by multiple variables such as soil characteristics, the history of the cropping area (previous agricultural practices), and crop variety (Shainberg et al., 1989). Despite many favorable reports in the literature, gypsum value is still poorly disseminated among farmers.

Gypsum is occasionally reported to change soil pH, especially soil water pH, either increasing or decreasing it (Farina and Channon, 1988; Shamshuddin and Ismail, 1995; Caires et al., 2006; Chen and Dick, 2011; Zoca and Penn, 2017; Tavakkoli et al., 2021). However, the range of soil pH changes caused by gypsum is modest and usually of a low extent (0.2-0.3 pH units). The soil pH response to the gypsum application is the product of the reactions between the gypsum and soil components. The replacement of hydrogen and aluminum in the cation exchange capacity (CEC) with calcium, the replacement of hydroxyl (OH⁻) with sulfate (SO₄²⁻), the precipitation of the solid soil phase, the ion-pair formation, and the self-liming effect are some of the gypsum reactions able to affect the soil pH significantly (Sumner, 1993). These reactions depend on the soil mineralogy and CEC ion composition, and the magnitude of each response dictates the influence of gypsum on soil pH (Zoca and Penn, 2017). However, when soil pH is high, presenting a soil pH of low acidity to alkaline conditions, the lime application is usually avoided. Thus, gypsum becomes an option for calcium and sulfur sources without significantly changing the soil pH in the soil profile.

Many areas worldwide present degraded soils or soil with severe chemical and physical limitations, raising concerns about food security (Kopittke et al., 2019; Agim et al., 2021; Kraamwinkel et al., 2021). Most of those degraded soils are highly weathered tropical soils, which need corrections to become highly crop-productive and sustainable. The soil acidity (low soil pH) is corrected with lime (CaCO₃ and MgCO₃). Still, the lime's beneficial effects are regularly limited to the soil layer of incorporation (usually up to 0.2 m deep) or the

first centimeters if applied to the soil surface (usually up to 0.1 m deep). However, Fontoura et al. (2019) reported that the lime application to the soil surface of a moderately acidic Oxisol under no-tillage rapidly lowered the subsoil acidity up to 0.6 m deep in the soil profile. Other studies also reported the effects of lime and gypsum surface application in deep soil layers (Crusciol et al., 2019; Besen et al., 2021a).

The time needed for gypsum reaction in soil and significant effects arising from its application depends on regular factors but are not limited to soil type, gypsum granulometry, soil temperature, and gypsum way of application (on the soil surface, soil incorporated, and dissolved in irrigation). Table 1 presents a study done with gypsum application in an Oxisol (60% clay) cultivated with soil tillage and cropped with maize in 2013 (Pádua Jr., A. L. – data not previously published). Basic soil analysis was performed every 0.2 m up to 1 m depth before and after four months of gypsum (6 Mg ha⁻¹) application. The gypsum rate was applied on the soil surface 40 days before maize sowing.

Soil layer (m)	pH (CaCl ₂)		SOM (dag dm ⁻³)		Phosphorus (mg dm ⁻³)		Calcium (cmol _c dm ⁻³)		Sulfur (mg dm ⁻³)		CEC (cmol _c dm ⁻³)		V (%)		m (%)	
	t ₀	4 m	t ₀	4 m	t ₀	4 m	t ₀	4 m	t ₀	4 m	t ₀	4 m	t ₀	4 m	t ₀	4 m
	0-0.2	4.4	5.4	3.2	3.3	2.2	1.8	2.2	4.5	2.5	6.4	8.1	8.7	49	75	5
0.2-0.4	4.0	4.8	2.3	2.1	0.7	1.2	1.1	2.9	1.2	34.4	7.9	8.5	30	52	32	4
0.4-0.6	3.9	4.7	1.5	1.7	0.5	0.7	0.6	2.3	0.7	18.1	6.9	7.5	22	45	50	11
0.6-0.8	4.0	4.5	1.1	1.4	1.0	0.7	0.6	1.4	1.0	10.7	6.3	6.6	20	32	50	19
0.8-1	4.0	4.4	1.1	1.0	0.2	0.6	0.5	1.4	1.4	5.3	6.2	6.7	18	31	57	32

SOM: soil organic matter; CEC: cation exchange capacity; V: soil base saturation; m: soil aluminum saturation. Source: Pádua Jr., A. L.

Table 1. Basic soil chemical analysis at different soil layers before (t₀) and four months (4 m) after gypsum (6 Mg ha⁻¹) application in a Haplorthox (Oxisol).

The gypsum application increased the contents of calcium, sulfur, and bases in the soil, reducing aluminum saturation in all the soil layers evaluated (Table 1). The maize grain yield observed raised from 6,600 kg ha⁻¹ (control no gypsum) to 10,200 kg ha⁻¹ (6 Mg ha⁻¹), indicating that applying gypsum about 40 days before sown is enough for the presented situation to cause significant increments to maize grain productivity. Interestingly, during the soil sampling in this soil tillage area, it was possible to demonstrate that only four months was enough for gypsum to run through the soil profile from the soil surface to depths up to 1 meter (Figure 3).



Figure 3. Deposit of solubilized gypsum in the 0.8-1 m soil layer four months after its application to a soil tillage area. Soil sample extracted with the aid of a Dutch auger.

Source: Pádua Jr., A. L.

Even in no-tillage cropping systems, the benefits of recent gypsum applications could be observed in soybean crops, especially in tropical soils. The gypsum application (3 Mg ha^{-1}) 60 days before soybean sowing improved nutrient distribution in the soil profile, raised grain yield, and increased residual effect of lime and gypsum for the succeeding crop seasons in Oxisol (Pádua Jr., A. L. – data not previously published). The gypsum application increased soybean grain yield by about 240 to 720 kg ha^{-1} .

Another soybean study in Oxisol presented the positive effects of gypsum (2 Mg ha^{-1}) - applied 40 days after lime application (2 Mg ha^{-1}) and 20 days before soybean sowing - on some soil cations contents in deep soil layers (Pádua Jr., A. L. – data not previously published). Calcium and magnesium soil contents and aluminum saturation are compared between the year of gypsum application (2015) and two years after (Figure 4).

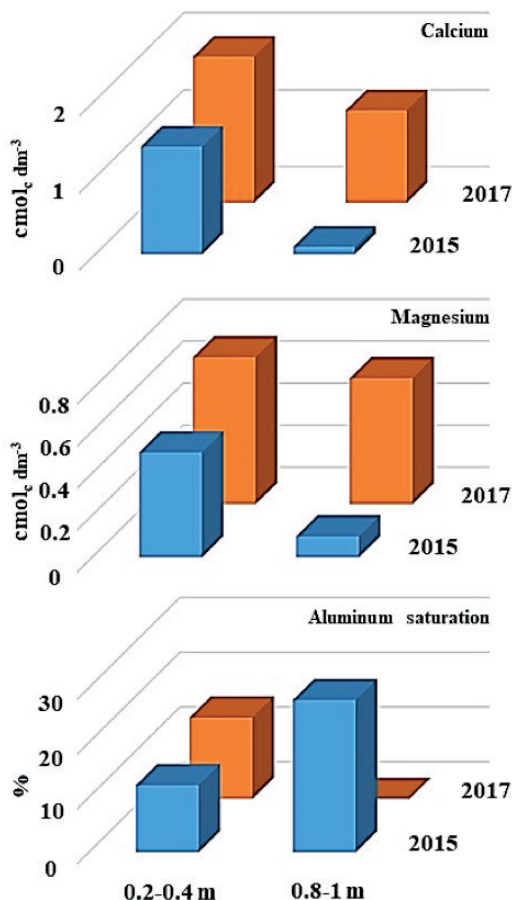


Figure 4. Soil calcium, magnesium, and aluminum contents in deep soil layers of an Oxisol in the year of gypsum application (2 Mg ha^{-1}) and two years after.

Source: Pádua Jr., A. L.

The increased soil aluminum saturation at the 0.2-0.4 m soil layer in 2017 is probably due to a boosted soybean root system cropped in the area. The gypsum application improved soil attributes in deeper soil layers and enhanced deeper root development conditions. This robust soybean root system would naturally absorb more soil cations and compensate for such extra absorption by exuding more hydrogen ions (H^+) in the soil solution. The increased H^+ availability reduced soil pH and increased aluminum availability and its saturation in soil. In the 0.8-1 m soil layer, the drop in the soil aluminum saturation was very expressive. It reflected the consequences of the arrival of solubilized soil cations from the gypsum applied above. In this soil depth (0.8-1 m), the influence of the soybean root system is lower than the effect of soil cation arrival from gypsum application, thus reducing the soil aluminum saturation.

Additionally, the greater volume of roots produced in the soil profile due to gypsum application increases soil phosphorus contents and cation exchange capacity over time after root decomposition.

GYPSUM AND LIME

The low lime solubility (about 172 times lower than gypsum) and the lack of soil disturbance in areas under no-tillage cropping systems reduce the effectiveness of the lime benefits to the soil profile when applied to the soil surface. In the Brazilian tropical soils, gypsum application frequently occurs from July to October, usually 40-60 days after lime and before routine fertilization and sowing. Still, no defined criteria for joint gypsum and lime application or the exact time for application is considered. This circumstance raises the need for studies including lime and gypsum (more soluble) in the soil surface to improve subsoil conditions for root systems development.

The interaction of gypsum and lime on soil attributes and crop yields is reported in the literature, especially but not exclusively for no-tillage cropping systems where the amendments must be applied to the soil surface (Caires et al., 2011a; Pauletti et al., 2014; Crusciol et al., 2016; Costa and Crusciol, 2016; Dalla Nora et al., 2017; Zoca and Penn, 2017; Fontoura et al., 2019; Anderson et al., 2021a). However, the results of the gypsum and lime interaction are occasionally contrasting.

In a no-tillage cropping system, the subsoil acidity is of significant concern since lime application to the soil surface could not solve this problem in deep soil layers. Due to the higher solubility and movement (deeper penetration) in the soil profile of the calcium-sulfate pair, the gypsum application can potentially reduce the subsoil acidity (aluminum saturation) (Ritchey et al., 1980; McBride, 1994; Zoca and Penn, 2017). The increment in exchangeable calcium (cation concentration effect) and sulfate [aluminum precipitation - $\text{Al}_2(\text{SO}_4)_3$] reduces the toxic aluminum effects in subsoil layers (Shainberg et al., 1989; Zambrosi et al., 2007).

According to Vitti and Mazza (2002), gypsum application to sugarcane (*Saccharum officinarum*) cropping areas must occur immediately after lime application, and the positive results are more expressive in Oxisols and quartz sand soils. However, according to Demattê (2005), the best efficiency of the gypsum reactions and effects in the soil is achieved three to six months after lime application. The author also exposed that such a procedure (gypsum three to six months after lime application) is counterproductive (timely) and suggested that good results are still observed when lime is applied before gypsum (two operations) and then incorporated.

Crusciol et al. (2019) evaluated the effects of lime (2.7 Mg ha^{-1}) and gypsum (2.1 Mg ha^{-1}) on (i) soil (sandy clay loam) attributes, (ii) plant nutrition, (iii) forage dry matter and crop yield, (iv) estimated cattle meat production, and (v) economic issues, and concluded

that the surface application of lime plus gypsum is essential for food production in acid soils under no-tillage in tropical agriculture. The authors reported that (i) lime increased soil pH, reduced the exchangeable acidity ($H^+ + Al^{3+}$) and the relative concentration of aluminum up to 0.6 m soil depth; (ii) gypsum increased calcium contents through the soil profile; (iii) lime (with or without gypsum) improved the nutrient acquisition by the crops cultivated in rotation; (iv) lime and gypsum raised the forage dry matter yield and crude protein concentration of palisade grass (*Urochloa brizantha*); (v) estimated meat production of the joint application (lime + gypsum) was 26% higher than lime alone and 225% higher than the untreated control, and (vi) increased the economic performance during four cropping seasons. The authors also emphasized that lime and gypsum applied one day apart can generate positive agronomic and economic results; however, the usual recommendation is to apply lime first to the soil, then gypsum application later or in the next crop season.

Field empirical experience has shown that crop responses to gypsum application in tropical soils occur more expressively when applied in alternated years with lime application –gypsum application in the first year, lime application the next year, and follows successively alternating fertilizers (Pádua Jr., A. L. - personal information). Moreover, Besen et al. (2024) reported that applying lime and gypsum to the soil surface is preferable in no-till cropping areas for improved soybean and wheat performance.

The combined application of lime and gypsum can also be an adequate alternative to supply calcium and magnesium in deep soil layers of stabilized no-tillage systems (> 10 years without significant soil tillage), especially in drylands. However, in irrigated areas, the conditions are different. In general, artificial watering over time improves the subsoil's chemical and physical properties and helps build the attributes along the soil profile. Thus, crops in areas artificially irrigated can be less responsive to gypsum applications.

Additionally, gypsum is more effective than lime for sodic soil reclamation as it increases the concentration of electrolytes in the soil solution and displaces sodium with calcium within the structure of the clay components (Oster, 1982; Raine and Loch, 2003). Many agricultural amendments [e.g., rice (*Oryza sativa*) straw, press mud (residue from sugarcane juice filtration), cow manure, combined organic residues, biochar, beneficial microorganisms, and phytohormones] have also been applied with gypsum to improve sodium removing, salt-leaching efficiency, soil biological properties, reclaim degraded lands, and crop yield (Kilpatrick, 2012; Schultz et al., 2017; Yamika et al., 2018; Ahmed et al., 2020; Basak et al., 2021; Bello et al., 2021; Rezapour et al., 2021; Yahya et al., 2022; Xu et al., 2023).

Zhao et al. (2019) studied buried layers of maize straw (6, 12, and 18 Mg ha⁻¹) and soil incorporated (0-0.2 m) FGDG (0.75 Mg ha⁻¹) on soil attributes and sunflower grain yield. Generally, the combined application (buried straw and incorporated gypsum) reduced soil pH and exchangeable absorption percentage. It increased the soil's electrical conductivity and grain yield (17.4% in the first year, 20.4% in the second year). The authors

also concluded combining organic residue and FGDC reduces soil salinity and sodicity. Moreover, gypsum application with *Atriplex halimus* (sea orache, Mediterranean saltbush, and a phytomedicine) can reclaim highly saline-sodic clay loam soils (Gharaibeh et al., 2011). The application of gypsum with compost (mix of plant and animal residues) and nanoparticles of manganese and selenium also improved some chemical and physical soil properties, water productivity, and yield of fava beans (*Vicia faba*) in salt-affected soil (Amer et al., 2023). The joint utilization of gypsum with lignin sludge as an organomineral fertilizer has also been a viable way to sustainably place gypsum, improve forest cultivation, and reclamation of disturbed lands, slopes of highways, and landfills of solid municipal waste (Matveeva et al., 2022).

CROP RESPONSES TO GYPSUM

Calcium is a low-mobility nutrient in plants and can not be mobilized from older tissues and redistributed in the plant via the phloem (Hanger, 1979; White and Broadley, 2003). Once calcium is associated with a compound within the plant structures, its relocation is slow, if at all, from one part of the plant to another. Fruits, for example, are at the end of the xylem transport system and are prone to receiving less calcium than other plant organs (Tonetto de Freitas and Mitcham, 2012; Song et al., 2018). Therefore, calcium must constantly be available to the roots (Chen and Dick, 2011); consequently, the crop responses, especially the yield responses, are prone to be sensitive to gypsum, a calcium source.

However, such yield responses are related to improved soil calcium and sulfur content increases and/or reductions in the subsoil layers' toxic aluminum (Al^{3+}) saturation. Additionally, the yield responses are frequently observed after enough time (months) of gypsum application to allow dissolved gypsum to be leached down into the subsoil layers (Sumner, 1993). However, Caires et al. (2011b) found a positive maize yield response to gypsum application in Oxisol right in the first year of application but no yield response to the soybean subsequently cropped.

In their chapter, Zoca and Penn (2017) concluded that all gypsum sources could generate positive crop yield responses and reduce the negative effects of stressful conditions. However, the authors highlighted that the impacts of gypsum application (positive or negative) must be evaluated for each specific objective, region (soil type and rainfall regime), crop species, and cultivation system. Rashmi et al. (2018) also concluded that gypsum could positively and negatively impact the soil-plant-environment system and that oilseed crops do not have a suitable recommendation for different soil types, climates, crop species, and cropping systems.

Despite some contrasting results, the positive effects also present a variety of ranges, going from substantial increases to a slight decrease in grain yield. In this sense, Pias et al. (2020) performed a meta-analysis comprising 129 harvests of six different crop species,

including barley, maize, rice, wheat (*Triticum sativum*), soybean, and white oat (*Avena sativa*), to identify the conditions under which grain yield responds to gypsum application in no-tillage areas (Figure 5).

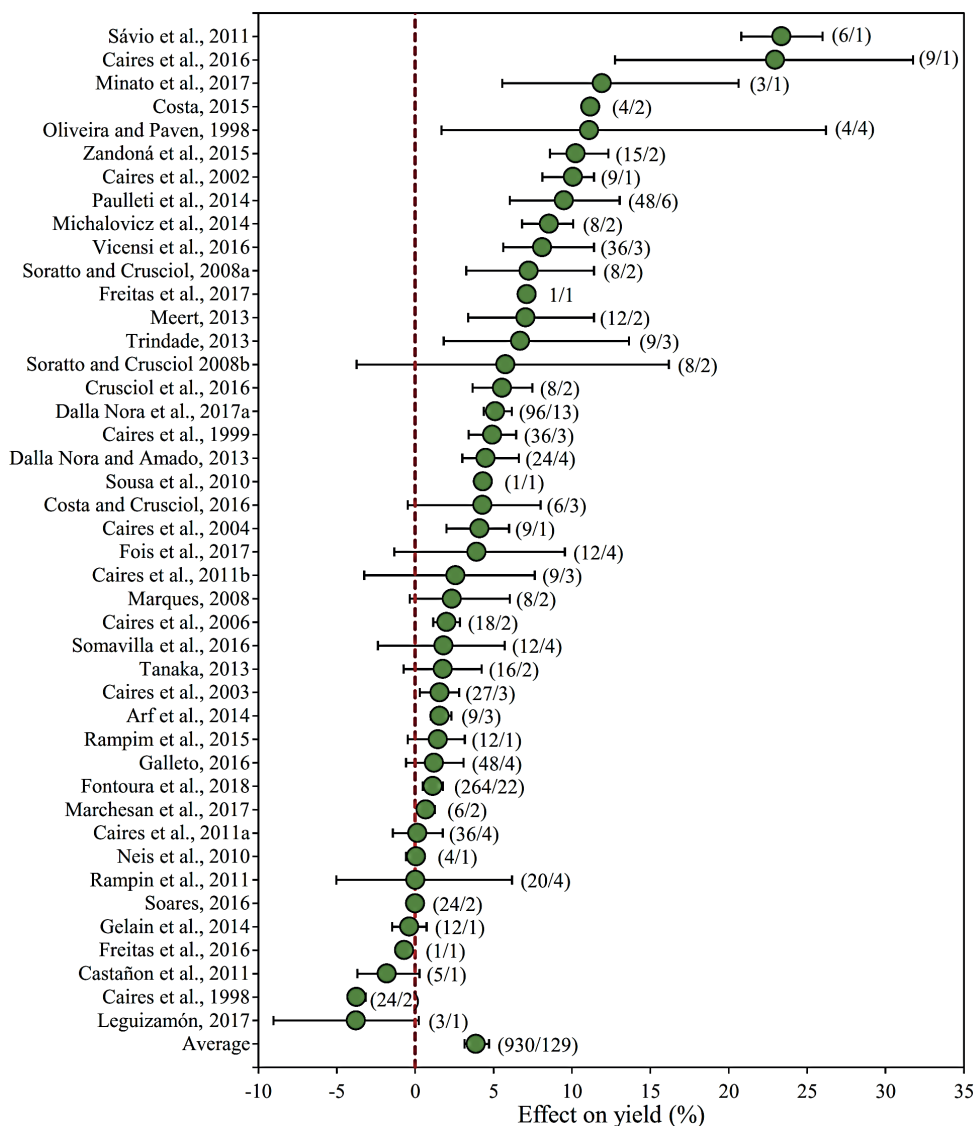


Figure 5. Mean effects (%) of gypsum application on crop grain yield in no-till soils in each primary study included in the meta-analysis. Error bars represent 95% confidence intervals of the means. The number of observation pairs and the total number of crop harvests (environment × year) included in each category are shown in parentheses.

Source: Pias et al. (2020)

Pias et al. (2020) found that (i) increased grain yield is very plausible (77-97%) when gypsum was applied to soils with aluminum saturation exceeding 5% in the 0.2-0.4 m soil layer - the decreased subsoil aluminum toxicity caused by the gypsum application allowed improved grain yields - (ii) the average increments in grain yield were 14 and 7% for cereal crops grown under water deficiency or not, respectively, and (iii) gypsum application should be avoided if aluminum saturation is below the critical thresholds since gypsum can cause excessive magnesium leaching across the soil profile. The authors also observed that soybeans positively responded to gypsum, even in areas with water deficit and aluminum saturation greater than 10%. The probability of a positive response was 88%, and the average yield increase was 12%. Such meta-analysis indicated that gypsum increased grain yield, decreased it, or presented no appreciable effect in 57, 5, and 38% of the evaluated studies, respectively.

Therefore, plant responses to gypsum application can be highly variable. For example, annual crops such as maize, wheat, and soybean, in general, present improved but extensively variable grain yield results (Dalla Nora et al., 2017; Soratto & Crusciol, 2008). Other studies do not reveal any positive gypsum effect on grain yield, maintaining similar results to the control (no-gypsum) (Marchesan et al., 2017; Fontoura et al., 2019), and there are studies even reporting minor drops in grain yield (Somavilla et al., 2016).

The variations in many reported results could be justified, in most cases, by the influence of other variables such as plant species and varieties, the time after gypsum application, gypsum application rates, soil chemical, physical, and biological properties, and climate (e.g., rainfall rates, average temperature) affecting the reports. Zoca and Penn (2017) also presented an excellent survey on agronomic gypsum studies varying from gypsum significantly improving soil attributes, crop performance, and yield results to no positive results.

The improvements in crop yield due to gypsum application are difficult to define, and delineating the exact positive effects besides a nutrient source due to many physical and chemical changes co-occurring in the soil is challenging. However, the impact of gypsum on soil characteristics and crop responses is primarily positive. It results from the synergic, accumulative, and additive effects of each potential change to the soil-plant-environment system caused by gypsum application.

GYPSUM TO GRAINS CROPS

In a long-term study (58 months), Caires et al. (2006) observed that (i) gypsum improved the subsoil chemical conditions, (ii) raised soil pH ($0.01 \text{ mol L}^{-1} \text{ CaCl}_2$), calcium, and sulfur contents, but also (iii) caused magnesium leaching in the soil profile. However, the authors could not identify a gypsum effect on soybean grain yield, but gypsum improved

soybean grain quality, presenting higher protein, sulfur, phosphorus, potassium, and calcium contents. The authors concluded that applying gypsum to no-tillage soybean crops is important to produce high-quality seeds.

The residual effects of lime and gypsum application (3.71 or 7.42 Mg ha⁻¹) on clayey soil and the grain yield of soybean and wheat in southern Brazil were studied by Besen et al. (2021a). The studied area has been cultivated under no-tillage since 1975. The authors found after 48 months of gypsum application that (i) aluminum contents decreased until 0.6 m soil depth if lime was incorporated (0.2 m) and until 0.3 m soil depth if lime was applied to the soil surface; (ii) lime incorporation reduced soil organic matter content in the surface soil layer; (iii) superficial lime application increased the magnesium contents in the 0.4-0.6 m soil layer; (iv) 7.42 Mg ha⁻¹ had a pronounced residual effect in subsoil layers but increased the vertical displacement of magnesium and potassium contents, and (v) soybean and wheat yields were not affected by the soil base saturation variations. Therefore, the authors concluded that the standard gypsum rate (3.71 Mg ha⁻¹) provided the best results and highlighted that the benefits of superficial gypsum application extend beyond surface soil layers in clayey soil from subtropical environments. Applying gypsum to the soil surface is also preferable to preserve the benefits of a continuous no-tillage cropping system.

Da Costa et al. (2016) reported different soil organic matter and soybean yield results. They found that (i) lime (2 Mg ha⁻¹) application, with or without gypsum (2.1 Mg ha⁻¹), can increase soil organic matter accumulation in the long term, and (ii) application of lime associated with gypsum to soil surface increased soybean and sorghum (*Sorghum bicolor*) calcium absorption and their respective grain yields. The authors also found that the subsoil's sulfur residual effects were observed after five years of gypsum application. In another study on sorghum, Charlo et al. (2022) reported that gypsum effectively increases soil calcium content by 60.5% in the 0-0.2 m soil layer and 34% in the 0.2-0.4 m soil layer. The authors also observed that (i) the highest gypsum dose studied (4,000 kg ha⁻¹) increased the soil availability of phosphorus and sulfur by 32.5 and 681%, respectively, and (ii) the nutrients increments would improve the crop's resistance to drought stress. Such improved nutrient availabilities mainly result from (i) the ions added to the soil with the gypsum application and (ii) the ion displacements in the soil complex caused by the added ions.

A noteworthy study was published by Fontoura et al. (2019), where they evaluated the surface gypsum and liming application on the chemical properties of four soil layers (0-0.1, 0.1-0.2, 0.2-0.4, and 0.4-0.6 m) in two periods 10 years apart (1 and 11 years after lime and gypsum application). Between the first and eleventh year of the experiment, 26 crops were cultivated [10 soybean crops, four white oats, three maize, three wheat, three barley (*Hordeum vulgare*), and three forage radish (*Raphanus sativus*)] under a no-tillage cropping system. The authors found (i) no synergic effect of gypsum and lime on soil chemical properties, crop yield, and most leaf-tissue macronutrients; (ii) yield increments were minor for cereals (4%) than for soybean (14%) and were limited to just 25% of cereal crop seasons,

and 40% for soybean; (iii) in the short term, gypsum raised more the exchangeable calcium content to 0.6 m soil depth than lime, but the latter presented more reductions in the soil acidity and extended residual effect (improved soil conditions for extended period), and (iv) the lime application to the soil surface under no-tillage lowered the subsoil acidity up to 0.6 m in the first year after its application, and improved soybean grain yield.

Flauridor et al. (2021) indicated no yield improvements for maize, alfalfa (*Medicago sativa*), or alfalfa-mixed grasses after two years of gypsum application. However, gypsum consistently increased sulfur concentrations in soil and crop tissues as soon as five months after each gypsum application. In the short term, the authors also observed that gypsum did not affect mineralizable soil carbon, penetrometer resistance, or unsaturated hydraulic conductivity. However, the second gypsum application reduced the soil protein and magnesium contents; the authors also reported magnesium leaching. Magnesium and potassium leaching to subsoil layers is regularly reported in the literature on gypsum and soil attributes. Therefore, planning magnesium and potassium fertilization before gypsum application must be considered as a way to improve soil fertility deeper in the soil profile.

In the long-term observations, Caires et al. (2011a) observed a residual effect on the subsoil sulfur content of an oxidic soil. After eight years of gypsum application, the sulfate concentrations in the subsoil were still high. After 7-10 years of surface-applied gypsum (no-tillage), maize grain yield improved, but soybean grain yield did not. The authors also reported that using gypsum in no-till systems is interesting when maize is frequently grown in crop rotations.

Additionally, it is essential to state that the residual effect of gypsum in the soil-plant-environment is unseparated from the soil attributes. Different soil characteristics (chemical, physical, and microbiological aspects) affect the extension of gypsum residual. Other significant factors that influence the gypsum residual include (i) the physical and chemical characteristics of the soil B horizon, (ii) the occurrence of impediments in lower soil layers, (iii) soil organic matter content, and (iv) the predominant soil structure.

De Moura et al. (2018) studied maize crop response in an Argisol (soil classified as Argisol in the Brazilian soil classification system) after five years of gypsum application and combinations with the residues of leguminous plant species [*Gliricidia sepium* (gliricidia) and *Acacia mangium* (acacia)]. They found that the mixed application of leguminous residues, urea, and gypsum (6 or 12 Mg ha⁻¹) reduced soil penetration strength and increased soil calcium content, soil organic matter, maize leaf area index, plant nitrogen amounts, and the maize grain yield. The authors concluded that managing gypsum and leguminous residues in humid tropic agrosystems is an appropriate strategy to improve maize productivity and crop sustainability.

Regarding the soil incorporation of such soil amendments, Besen et al. (2021b) evaluated lime incorporation approaches (incorporated or superficial) and the effect of reapplied lime and gypsum on soil chemical properties and grain yield of wheat and

maize in southern Brazil. Similar to other studies, the authors reported that (i) incorporating lime reduces soil acidity (increasing soil pH and reducing available aluminum), increases calcium and magnesium contents, and decreases the organic matter content in the revolved soil surface; (ii) gypsum increases sulfur and calcium availability in deeper soil layers; (iii) high gypsum rates ($> 7.42 \text{ Mg ha}^{-1}$) reduce the magnesium content in the surface layer; (iv) lime associated with gypsum and applied to the soil surface resulted in the highest wheat and maize yields indicating that the benefits of the continuous no-tillage system could be maintained. Besen et al. (2021b) also indicated the increasing sulfur content as the main factor for increased crop yields. Additionally, reductions in aluminum saturation in deeper soil layers were a common effect of the gypsum application to wheat, soybean, and maize (Rampim et al., 2011; Nora et al., 2014)

Also, on wheat, Rawat et al. (2020) studied the plant development (plant emergence, tillers per square meter, biometrics, yield) after applying nano-sized gypsum in silty clay loam soil and found positive results. The treatments that reduced the regular mineral fertilization (75%) plus nano-sized gypsum presented similar or superior plant development to the complete regular mineral fertilization (100%). Such treatments generated up to 25% economy compared to the recommended rates of mineral fertilizer with no grain yield penalty. Abbas et al. (2023) observed no noticeable impact of gypsum rate on the straw yield; however, the authors also observed that 3 Mg ha^{-1} of gypsum improved soil moisture conservation, nutrient uptake, and wheat grain yield with less input cost.

GYPSUM TO SUGARCANE CROP

Gypsum and lime ratios and application methods (incorporated, superficial, or applied in-furrow) in sugarcane were studied by Morelli et al. (1987) after 6 and 18 months of the application of the treatments. They concluded that (i) lime effectiveness was limited to the soil layer near the soil surface, regardless of incorporation method; (ii) gypsum improved base saturation and reduced aluminum saturation up to 0.75 m deep in the soil profile; (iii) calcium and magnesium saturation reduced in the second evaluation (18 months), and (iv) gypsum alone or with lime resulted in greater sugarcane yields than lime alone. A similar conclusion was pointed out by Lorenzetti et al. (1992).

Morelli et al. (1992) reported increased soil calcium contents and base saturation in deep soil depths after 18 months of gypsum application in another sugarcane study. The same was not observed when only lime was applied. The authors also reported that (i) the best calcium and magnesium distribution in the soil profile and the most prominent base saturation were observed when gypsum and lime were combined; (ii) the application of gypsum alone (without lime) caused magnesium leaching to deep soil layers; (iii) the combined application of gypsum and lime resulted in greater sugarcane yields than each one alone, and (iv) the highest sugarcane root biomass and yield - after four sugarcane

harvests - were observed when 4 Mg ha⁻¹ of lime were applied with 2 Mg ha⁻¹ of gypsum.

Crusciol et al. (2017) studied the surface application of gypsum with silicate, or lime, on sugarcane yield and the amendment of subsoil acidity in a 12-month study. The subsurface soil layer (0.2-0.4 m) presented reduced aluminum saturation and increased calcium, magnesium, potassium contents (base saturation), and sulfur contents with the surface gypsum application. The association of gypsum with silicate, or lime, increased sugarcane stalk, bagasse, trash yield, and energy yield; however, applying gypsum in association with silicate leads to the most superior profitability.

The dolomitic lime and gypsum surface application in green sugarcane ratoon was studied by Rossato et al. (2017) after 12 months of the treatment's application. They concluded that gypsum acted as a subsurface conditioner and contributed to the liming benefits of surface lime application to reach deeper soil layers. This deepening of the soil improvements caused by the gypsum and lime application allowed the development of the sugarcane root system to greater depths. It generated increments in sugarcane stalk, bagasse, trash yield, and sugar yield. However, the authors also indicated that gypsum might lead to the leaching of magnesium and potassium into deep soil layers.

Araújo et al. (2019) studied the influence of gypsum (5 Mg ha⁻¹) on soil carbon up to 2 m depth of an Oxisol. The carbon accumulation, its relationships with the soil's chemical properties, and the development of the sugarcane root system were evaluated next to the seventh sugarcane cut (87 months after gypsum application). The authors reported (i) increased calcium and sulfur contents and reduced aluminum saturation in the soil profile, which were responsible for the improved sugarcane root system development, and (ii) increased carbon sequestration in the deeper soil layers (1-2 m), where the leading supplier of soil acidity were the sugarcane roots.

GYPSUM TO OTHER CROPS

All living plants need calcium, sulfur (macronutrients found in gypsum), and other essential nutrients to complete their cycle. Crop plants of high productivity continuously need those nutrients and other beneficial nutrients in balanced proportions to develop and produce fully. Thus, several other crops present significant responses to the gypsum application. Most observed results are positive, highlighting the benefits of regular gypsum use and management for crop production and improved yield.

In coffee (*Coffea arabica*), the contents of soil calcium, magnesium, and potassium and their sulfate (SO₄²⁻) ionic pair along the soil profile (0-2.4 m deep) were evaluated by Ramos et al. (2013). The authors studied an Oxisol 16 months after gypsum application and reported that (i) 96% of ionic potassium (K⁺) in soil solution was at 0.35 to 0.45 m in its free form; (ii) the predominant leached chemical species occurred in the free forms (Ca²⁺, Mg²⁺, K⁺, SO₄²⁻), and (iii) the content of the chemical species of calcium and magnesium sulfate

was higher than the potassium sulfate chemical specie.

Anikwe et al. (2016) found that the combined application of lime (5 Mg ha⁻¹) and gypsum (2.5 Mg ha⁻¹) to an Ultisol improved soil physicochemical properties and cassava (*Manihot esculenta*) yield. The authors argued that the calcium applied via lime and gypsum flocculated the soil particles, enhancing the soil's physical attributes and pH, soil infiltration and aeration, soil phosphorus availability, and plant nutrient uptake for improved cassava growth. Corroborating the results observed by Anikwe et al. (2016), the effects of lime on soil physics are already known and usually affect soil flocculation, density, aggregates, and porous structure (Auler et al., 2019; Conradi et al., 2020).

Magnesium sources such as kieserite (standard magnesium source for agriculture - MgSO₄·H₂O), ground magnesium limestone [CaMg(CO₃)₂], and magnesium-rich synthetic gypsum (an industrial by-product that has > 70% of gypsum, 17.1% of magnesium hydroxide, 4.3% of calcium hydroxide, 2.3% of calcium carbonate, and pH 8.8) were evaluated in oil palm (*Elaeis guineensis*) by Ayanda et al. (2020). The authors concluded that the magnesium-rich synthetic gypsum is a viable soil conditioner for fertilizing and liming the soil. This source satisfied oil palm plants' calcium and magnesium requirements like other sources. Such a magnesium-improved gypsum would minimize the magnesium-induced deficiency caused by the magnesium-leaching potential when conventional gypsum sources are applied.

In eucalyptus (*Eucalyptus* sp.) seedlings, Gabriel et al. (2018) reported that lime decreased soil acidity and improved plant development; however, the authors also noted that the gypsum effect on the variables evaluated was insignificant and highlighted that gypsum could reduce seedling growth if excessive rates are applied. Ferreira et al. (2020) assessed the eucalyptus development for 36 months after applying up to 9.6 Mg ha⁻¹ of gypsum and reported no benefits to the dendrometric growth. The authors discussed the lack of plant response to gypsum benefits (source of calcium, sulfur, and a soil conditioner). They indicated a combination of factors responsible for the results' non-significance, such as soil type, eucalyptus tolerance to soil acidity, agronomic management, and climatic conditions.

Other perennial crops, such as turfgrasses, need corrections and supplementations in deep soil layers to achieve full development. Such improvements in deep soil layers can not be reached only with lime, especially when tilling is not an option (e.g., gardens and golf courses). Schlossberg et al. (2006) reported amelioration of the subsoil attributes up to 0.6 m deep after two years of soil surface gypsum application (10.6 or 20 Mg ha⁻¹). Thus, gypsum was a necessary soil amendment to manage macronutrients and potential soil acidity. The authors also reported that the turfgrass beneficial growth responses to the gypsum application vary among species.

Many horticultural crops, for example, presented improved yield, quality, shelf life, and profitability when gypsum is applied (Korcak, 1993; Brown, 2018; Lantzke, 2018; Santos et al., 2020; Charlo et al., 2021; Watts et al., 2021). Even crop production in soilless

growth media can benefit from gypsum. Media containing FGDG and organic composts have provided excellent plant production with no harmful environmental results (Chen and Dick, 2011). Bardhan et al. (2005) studied tomato (*Lycopersicon esculentum*) and wheat development in a low-cost, high-quality growth media for nursery, greenhouse, and landscape industries. The authors reported (i) improved plant growth (35 days after planting) for the tested media growth containing gypsum compared to the commercial media brand, and (ii) no toxic elements were detected in excess in the media solution leachates or the plant tissue evaluated.

Plants that exhibit rapid growth rates and are multipurpose, such as hemp (*Cannabis sativa*) and hops (*Humulus lupulus*) – both from the Cannabaceae botanical family – will positively respond to adequate fertilization and soil management (Brooks et al., 1961; Duke, 1983; Cannoy, 2015; Anderson et al., 2021b; Rehman et al., 2021). Industrial by-products applied as crop fertilizers and soil conditioners have also been tested for hemp production. Zielonka et al. (2017) studied the effects of sewage sludge (with and without gypsum) on hemp photosynthetic performance. The authors reported increased chlorophyll content in the leaves; however, this effect varied among hemp varieties. In hops, the gypsum benefits are long known (Mayer, 1768); however, no specific recommendation for calcium or sulfur rates has been reported yet (Gingrich et al., 2000; HÄPI, 2019). In these cases, where there is a lack of information regarding gypsum rate, the use of soil parameters - as indicated by other researchers for gypsum recommendations (Ernani et al., 1992; Sousa et al., 1992; van Raij et al., 1997; Sousa and Lobato, 2004; Sousa et al., 2007; Guimaraes et al., 2015; Pauletti and Motta, 2017; Tiecher et al., 2018) - is the most appropriated approach of determining a gypsum rate to be applied.

PLANT DISEASE RESPONSES TO GYPSUM

As highlighted here, gypsum is primarily a source of calcium and sulfur, especially in subsoil layers, which can provide adequate nutrition to improve the plant root system, absorption of nutrients, and natural disease control. Some reports of gypsum success management in controlling plant diseases have been published in the literature. The plant protection enhanced by gypsum application is probably related to improved calcium and sulfur plant nutrition, besides the soil improvements already mentioned.

Calcium is an essential factor for cell wall and membrane stability (structural functions) but also a secondary messenger in many physiological processes (signaling functions) that include the plant's responses to stresses via calcium-dependent proteins (Lecourieux et al.; 2006; Zheng et al., 2013; Thor, 2019). Sulfur plays an essential role in plant development by being a structural constituent of critical macro-biomolecules (amino acids, proteins, and oils) that regulate many processes regarding plant tolerance to environmental stresses (Zenda et al., 2021). Additionally, sulfur positively interacts with soil nitrogen, phosphorus,

and microorganisms, improving soil health.

Walker and Csinos (1980) evaluated the effects of gypsum application on peanut (*Arachis hypogaea*) yield, kernel grade, and pod rot (*Pythium myriotylum* and/or *Rhizoctonia solani*) damage in five peanut varieties in a sandy loam soil for three consecutive years. The authors detected reductions in pod rot severity, increments in kernel calcium content, and peanut yield for all cultivars as the gypsum rate increased. In watermelon (*Citrullus lanatus*), the rising rates of calcium applied as gypsum also reduced the incidence of blossom-end rot. This abiotic disease is caused by calcium deficiency, and the symptom is characterized by increasing dark rotten spots at the end of the watermelon fruit (Scott et al., 1993).

Narasimhan et al. (1994) studied the foliar applications of calcium sulfate, magnesium sulfate, ammonium molybdate, soil gypsum applications (500 kg ha⁻¹), and fungicide (carbendazim) application to manage sheath rot (*Sarocladium oryzae*) in rice. The sheath rot incidence was assessed 20 days after the treatment's application and was counted as the percentage of tillers affected by the disease. The gypsum application to the soil or the foliar fungicide application presented similar results regarding the management of sheath rot and grain yield increase. The authors also observed that all gypsum treatments reduced the incidence of sheath rot in rice, but this did not always translate into yield gains. However, Zahra and Sarwar (2015) found positive effects of gypsum and potassium silicate on rice yield parameters (plant height, productive tillers, straw, grain yield, and total biomass). The same authors also highlighted that the role of gypsum was more prominent than that of potassium silicate in improving the rice yield.

Messenger et al. (2000) reported that the application of gypsum reduced root rot (*Phytophthora cinnamomi*) in avocado (*Persea americana*) seedlings regardless of good or poor soil drainage. The authors highlighted that (i) the gypsum reduced the negative effect of root rot on the entire avocado seedling and root weight; (ii) seedlings grown in gypsum-amended soil were more resistant to root rot; (iii) reductions in root rot infection were not caused by avocado growth, improved root resistance, or reduced cell-membrane permeability in the roots, and (iv) the root rot infection was not profoundly affected by poor soil drainage when high gypsum rates were applied.

According to Fernando et al. (2021), gypsum is an important soil amendment to enhance plant growth, protection, and onion (*Allium cepa*) production. The authors reported that the best results for anthracnose (*Colletotrichum* spp.) control (non-occurrence of tip-burning or bulb rot) and onion bulb yield were observed when gypsum application was split in two (50 kg ha⁻¹ at planting and 50 kg ha⁻¹ two weeks after). This result indicates that a more constant nutrient availability by splitting the fertilizer rate is an adequate strategy to improve plant performance and sanity.

Applying gypsum (8 Mg ha⁻¹) on Mombaça grass (*Panicum maximum* cv. Mombaça) also generated significant improvements, increasing plant fresh-biomass, seed production, and root system volume in an Oxisol. Additionally, the sulfur in gypsum reduced leaf spot

occurrence and severity (*Bipolaris maydis*) (Figure 6).

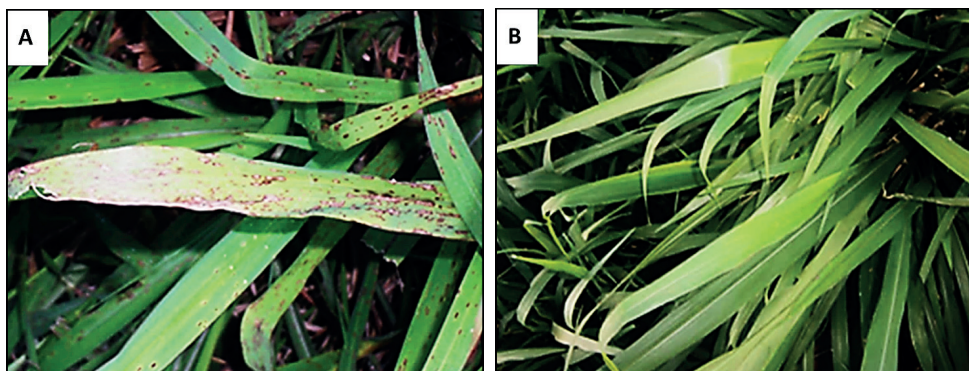


Figure 6. Incidence of leaf spot (*Bipolaris maydis*) on Mombaça grass (*Panicum maximum*). A: control treatment (no gypsum applied). B: gypsum treatment (8 Mg ha⁻¹).

Source: Pádua Jr., A. L.

GYPSUM AGRICULTURAL RATE RECOMMENDATIONS

Many of the gypsum interactions with the soil are known, and the results expected from its application are relatively well described, at least for the short-term experience with gypsum; however, the method of gypsum recommendation varies for different purposes and circumstances (van Raij, 2010). The positive effects of gypsum application in soil and plants are usually expected to occur when (i) root penetration is limited by available aluminum or low calcium contents in subsurface soil layers and when (ii) soil compaction can be alleviated by reducing the dispersion of clays in the soil. Ritchey et al. (1995) also indicated that (i) soils under periodic drought (water stress) or (ii) excessive rainfall (increased nitrogen leaching) and (iii) areas where the application of calcium can decrease the subsoil acidity are areas prone to present improved plant results from the gypsum application. Soils of dystrophic, dystrophic alic, aluminic, and acric characteristics are also prone to respond positively to gypsum application (Santos et al., 2018).

Gypsum application is standard for soil and crop management in many tropical soils, such as Brazil. Different methods of gypsum recommendations for crop production and soil conditioning are presented, and all of them are adequate for defined scenarios. However, a unique method for determining suitable gypsum application rates for all soils and cropping systems is unavailable. Many gypsum recommendations are based on empirical experiences and ranges of rate responses to gypsum. For example, Koske et al. (2005) reported for many Louisiana (USA) soils that if soil analysis indicates low calcium contents and soil pH of low acidity to alkaline, then 2,441 to 3,906 kg of gypsum should be added per hectare and incorporated by soil tillage before tomato planting in greenhouse areas.

Other imprecise recommendations include soil and medium ameliorations in greenhouses, nurseries, landscapes, and sports fields. These recommendations are usually

based on the same soil attributes considered for field crops; however, the rates applied to those specific places are generally higher to reduce the frequency of gypsum application (Chen and Dick, 2011). In most tropical soils, the regular gypsum rate recommendations for no-tillage (no soil disturbance) cropping systems are established on the subsoil contents of calcium, aluminum, sulfur, and phosphorus, the clay content, and the CEC. Those regular gypsum rate recommendations were naturally developed for conventional tillage cropping systems. However, Guimaraes et al. (2015) found a strong correlation between grain yield increase in no-tillage cropping areas and calcium saturation (78-84%) at 0-0.1 m soil depth. The authors used a machine learning technique and concluded that calcium saturation in the effective CEC, instead of aluminum saturation, is essential to estimate gypsum requirements in no-tillage cropping areas.

However, when the exchangeable calcium content is $0.5 \text{ cmol}_c \text{ dm}^{-3}$ or lower and/or subsoil aluminum saturation is 20% or greater, significant positive responses to the soil and crop plants are expected, according to Sousa and Lobato (2004), Sousa et al. (2007), and Pauletti and Motta (2017). Other recommendations are based on different variables. Demattê (1986) presented a gypsum recommendation for sugarcane crops based on the average soil CEC and soil base saturation (Table 2) in the 0-0.4 m layer of dystrophic sandy soil. The author also observed the (i) rise of soil base saturation in deep soil layers and (ii) 14 Mg ha^{-1} more sugarcane produced (three cuts average) when 2 Mg ha^{-1} of gypsum was applied to ratoon sugarcane.

CEC (mmol dm^{-3})	V (%)	Gypsum rate (Mg ha^{-1})
< 30	< 10	2
	10 - 20	1.5
	20 - 35	1
30 - 60	< 10	3
	10 - 20	2
	20 - 35	1.5
60 - 100	< 10	3.5
	10 - 20	3
	20 - 35	2.5

Table 2. Approximated gypsum rate according to the cation exchange capacity (CEC) and soil base saturation (V).

Source: Demattê (1986).

Oliveira et al. (2007) recommended gypsum application to sugarcane crops when the soil calcium contents are below $0.4 \text{ cmol}_c \text{ dm}^{-3}$ and the aluminum saturation is bigger than 20% in the 0.2-0.4 m soil layer. The authors recommended gypsum based on a fraction

of the recommended lime rate for sugarcane multiplied by a correction factor according to the soil layer: $G = (LR \times 0.3) \times (SL/0.2)$, where G is the rate of gypsum to be applied (Mg ha^{-1}), LR is the lime recommendation (Mg ha^{-1}) for the respective area, and SL is the soil layer depth interval that will be conditioned (m).

Vitti et al. (2005) also studied the application of gypsum to the sugarcane crop. The authors presented that if the calcium content is lower than $0.5 \text{ cmol}_c \text{ dm}^{-3}$, or the aluminum content is higher than $0.5 \text{ cmol}_c \text{ dm}^{-3}$, or the aluminum saturation is higher than 30%, or the base saturation is lower than 30% in the subsoil layer (0.2-0.4 m), then, significant effects of gypsum applications are expected to happen to the sugarcane crops. The gypsum recommendation proposed by Vitti et al. (2005) depends on the soil base saturation and CEC: $G = ((V_2 - V_1) \times \text{CEC})/50$, where G is the rate of gypsum to be applied (Mg ha^{-1}), V_2 and V_1 are the desired and the actual soil base saturation (%), respectively, and CEC is the cation exchange capacity ($\text{cmol}_c \text{ dm}^{-3}$).

The harmful subsoil conditions of low calcium content and acidity generate poor root system penetration, especially when exchangeable aluminum is highly available. These can be considered the most detrimental conditions for plant development and yield. Gypsum can help solve those limitations to improve plant development. According to Lorenzi et al. (1997), soils presenting less than $0.4 \text{ cmol}_c \text{ dm}^{-3}$ of calcium and/or aluminum saturation greater than 40% are significantly responsive to gypsum application. The gypsum recommendation in these soils was also based on the soil clay content: $G = 6 \times \text{clay}$, where G is the rate of gypsum to be applied (kg ha^{-1}), and clay is its amount (g kg^{-1}) in the subsoil layer (0.2-0.4 m).

Alvarez et al. (1999) indicated that if the calcium content is lower than $0.4 \text{ cmol}_c \text{ dm}^{-3}$, or the aluminum content is higher than $0.5 \text{ cmol}_c \text{ dm}^{-3}$, or the aluminum saturation is higher than 30% in the subsoil layer (0.2-0.4 or 0.3-0.6 m), then the positive effects of gypsum applications are expected to happen to the crops and soils. The gypsum recommendation is based on soil texture and varies from $0\text{-}400 \text{ kg ha}^{-1}$ (0-15% clay) to $1200\text{-}1600 \text{ kg ha}^{-1}$ (60-100% clay).

According to Sousa et al. (2001), the gypsum rate for soils cultivated with pasture can be recommended based on the soil clay content and soil texture when gypsum is intended as a soil conditioner and based on the sulfur content when gypsum is designed as a sulfur source. The gypsum rate estimated from the soil clay content is calculated as $G = 50 \times \text{clay}$, where G is the rate of gypsum to be applied (kg ha^{-1}), and clay is its amount (% or dag kg^{-1}) in the subsoil layer (0.4-0.6 m). According to soil texture, the gypsum recommendation varies from 700 kg ha^{-1} (sandy soils) to $3,200 \text{ kg ha}^{-1}$ (clayey soils). The residual effects of gypsum, recommended according to soil clay content or texture, can last at least five years.

The gypsum recommendation to supply sulfur to the plants is based on the average soil sulfur contents in the 0-0.4 m soil layer (Sousa et al., 2001). To soils with low sulfur contents ($\leq 4 \text{ mg dm}^{-3}$), the gypsum rate is $G = 10 \times \text{clay}$, where G is the rate of gypsum to be applied (kg ha^{-1}), and clay is its amount (% or dag kg^{-1}) in the soil. To soils with medium

sulfur contents ($5\text{--}9\text{ mg dm}^{-3}$), the gypsum rate is $G = 5 \times \text{clay}$. The residual effects of gypsum, recommended according to soil clay content or texture, can last at least two years. When the soil sulfur content is high ($\geq 10\text{ mg dm}^{-3}$), no gypsum application is needed unless the area is intended for pasture establishment or recovery and the soil sulfur content is lower than 4 mg dm^{-3} in the first 0.2 m of the soil profile.

Pias et al. (2019) also argued that (i) the no-tillage cropping system altered the soil sulfur dynamics, (ii) the emergence of high-yield crop varieties, (iii) the application of low-concentration sulfur fertilizers, and (iv) the reduction in atmospheric sulfur depositions makes the recommendation for sulfur fertilizers a priority since positive responses are expected, especially when the soil analysis ($0\text{--}0.2\text{ m}$ depth) is below 7.5 mg dm^{-3} .

Sousa and Lobato (2004) also indicated that if the calcium content is $0.5\text{ cmol}_c\text{ dm}^{-3}$ or lower and/or subsoil aluminum saturation is 20% or greater, and the gypsum rate is for an annual crop, then it can be estimated as $G = 50 \times \text{clay}$, where G is the rate of gypsum to be applied (kg ha^{-1}), and clay is its amount (% or dag kg^{-1}) in the subsoil layer ($0.2\text{--}0.4\text{ m}$); if the gypsum rate is for a perennial crop, then it can be estimated as $G = 75 \times \text{clay}$, where G is the rate of gypsum to be applied (kg ha^{-1}), and clay is its amount (% or dag kg^{-1}) in the subsoil layer ($0.2\text{--}0.4\text{ m}$)

According to Sousa et al. (1992), the study of subsoils in Cerrado (Savanna-like biome presenting weathered acid soils) indicated that significant responses to gypsum application might occur when subsoil exchangeable calcium contents below $0.1\text{ cmol}_c\text{ kg}^{-1}$, regardless of subsoil aluminum content. The authors also showed that when soil calcium contents exceed the plant's needs, the aluminum content dictates the occurrence of significant gypsum effects. In these conditions, significant soil and plant responses are expected to happen when aluminum saturation contents are above 65% and lower responses when aluminum saturation is below 35%. Sousa et al. (1992) presented a formula to determine gypsum recommendation also based on the soil clay content: $G = 17 + 6.508 \times \text{clay}$, where G is the rate of gypsum to be applied (kg ha^{-1}), and clay is its amount in the subsoil layer (g kg^{-1}). Moreover, acid soils with low CEC were reported to be similarly responsive to gypsum application (Demattê, 1992), including Oxisols, oxidic Ultisols, and low-CEC acid Inceptisols and Entisols. Sousa et al. (1992) also suggested using remaining phosphorus to indicate the gypsum rate; the extreme rate values varied from about 0.453 to 0 Mg ha^{-1} for 30 to 60 mg dm^{-3} of phosphorus and from 1.680 to 0.720 Mg ha^{-1} for 0 to 19 mg dm^{-3} of phosphorus.

Despite the many options, some inconsistencies regarding the gypsum recommendations and rates have been pointed out. Pivetta et al. (2019) evaluate cotton root development in an Oxisol related to aluminum and calcium activity and speciation in the soil solution as affected by gypsum rates based on soil clay content. The authors found that (i) the cotton root growth was more related to soil properties such as calcium content, aluminum saturation, base saturation, ratio calcium-effective cation exchange capacity, and ratio aluminum-calcium than to soil solution attributes, and (ii) that the current gypsum recommendations based on soil clay content are underestimating the gypsum rates needed

by the cotton crop.

Lately, Caires and Guimarães (2018) suggested a method for estimating gypsum rates based on the increase in calcium saturation to 60% in the effective CEC ($eCEC = \text{soil base sum} + \text{aluminum}$) at the subsoil layer (0.2-0.4 m). Over 10 years, the authors studied the effects of gypsum application on maize, soybean, wheat, and barley and, through an algorithm approach to regressions, proposed $G = (0.6 \times eCEC - Ca) \times 6.4$, where G is the rate of gypsum to be applied (Mg ha^{-1}), $eCEC$ is the effective cation exchange capacity ($\text{cmol}_c \text{ dm}^{-3}$), and Ca is the exchangeable soil calcium ($\text{cmol}_c \text{ dm}^{-3}$). The gypsum rates recommended by this method were closer to those associated with maximum economic yield than those indicated by other methods based on subsoil clay content, which is currently used in Brazil. Thus, the proposed method can be efficiently utilized when subsoil acidity is an important growth-limiting factor.

Therefore, among many gypsum rate recommendations for most agricultural situations, the selection of an appropriate rate usually depends on the contents of exchangeable ions (e.g., calcium, aluminum, sulfur, phosphorus), soil type, clay content, soil CEC and $eCEC$, base saturation, cropping system, rainfall regime, and the purpose of gypsum application (Sousa et al., 2001; Chen and Dick, 2011; Kost et al., 2014). The soil morphology is another variable that could be considered in the definition of adequate gypsum management, especially the rate of gypsum application and the moment of its reapplication. As reported by Cooper & Vidal-Torrado (2005), there are soil horizons, for example, characterized by extended development of structural pores (macropores), which favor water conduction processes (higher hydraulic conductivity), and less development of textural pores (micropores), reducing water retention in the horizon. Such conditions would affect the dynamic of gypsum results in soil and the time of its residual effect on plants.

In subtropical regions, gypsum application is still not a common practice. The knowledge of its effect on the soil-plant-environment system is less detailed, with no well-established parameter to decide for gypsum application. However, Tiecher et al. (2018) found significant and positive results with gypsum application when aluminum saturation was above 10% and calcium content was below $3 \text{ cmol}_c \text{ dm}^{-3}$ in subtropical subsoil layer (0.2-0.4 m) under no-tillage cropping system. Under these subsoil conditions (aluminum saturation > 10%, calcium < $3 \text{ cmol}_c \text{ dm}^{-3}$), maize and winter cereals grain yield increased by about 16 and 19%, whether the soil was water-deficient or not. Soybean grain yield only increased (27%) when gypsum was applied to soils of high subsurface acidity and water deficiency. The authors also highlighted that high gypsum rates ($6\text{-}15 \text{ Mg ha}^{-1}$) are applied to soils with low aluminum saturation and high calcium contents that may cause reductions in grain yield due to induced potassium and magnesium deficiency.

Additionally, Ernani et al. (1992) studied the application of gypsum to clayey soils with high CEC in temperate regions of regular rain distribution. The calcium contents in the studied soils were high, well above the level where calcium deficiencies would be expected.

Consequently, the authors observed low to no positive responses from the gypsum application, indicating the importance of identifying the subsoil characteristics that would positively respond to the gypsum application.

GYPSUM DOSAGE ERRORS

The under and overestimation of the gypsum rates can occur when soils present specific conditions with thick A or A-E horizons (over 0.4 m thick) – arenic and thick arenic soils – and soils with B horizons with high contents of available aluminum and clay. Therefore, the traditional methodologies for gypsum recommendation presented here might not reflect the adequate gypsum rate to deliver the expected soil-plant-environment benefits. In Oxisol and Nitosol soils (weathered soils with different B horizon attributes) of similar texture, the gypsum recommendation based on soil clay content might not meet the plant's requirements for its full development or the expected soil improvements.

In Nitosols, the water flow through soil layers is reduced by the occurrence of clay films and soil structure of subangular to prismatic blocks (B nitic) (Cooper and Vidal Torrado, 2005; Grego et al., 2011). The lower drainage of these soils increases the gypsum residual effects compared to the Oxisols (weak subangular blocky structure, with or without the granular structure, and no or faint clay film), thus affecting gypsum effects due to similar gypsum recommendations for soils of similar clay contents. Figure 7 exemplifies the differences observed between distinct soils cropped with soybeans.

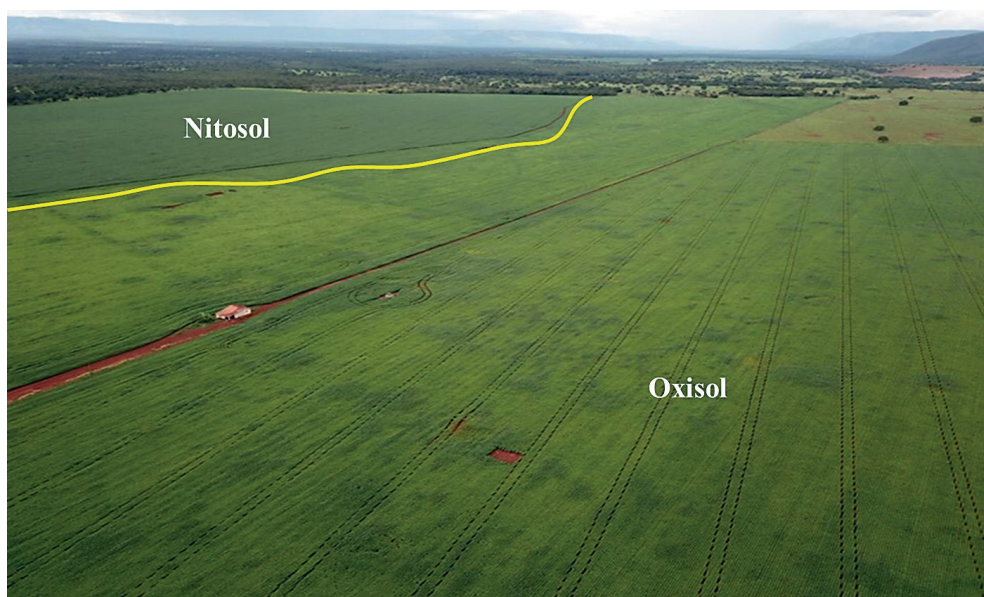


Figure 7. The residual effect of gypsum fertilization (4 Mg ha^{-1} applied 4 years before the picture register in the summer of 2022) in a Nitosol and Oxisol.

Source: Fachinetto, G. K.

The suggestion for situations where soil parameters affect the reaction of gypsum is to evaluate soil in more profound depths. Predicting adequate gypsum rates thus requires information about soil attributes that can reach 1 meter or deeper. Soil information from deep soil layers helps to understand the limitations and improvements needed for high gypsum efficiency and crop performance.

GYPSUM HANDLING AND APPLICATION

Gypsum (mined or FGDG) is not combustible or explosive and is not expected to produce any hazards under regular everyday use. No specific individual protection equipment is usually required for gypsum handling under ordinary conditions; still, always consult the current regulatory guidelines and policies before taking and storing any substance. However, a valid recommendation is to avoid, as much as possible, breathing gypsum dust, which may irritate in contact with the eyes, respiratory tract, throat, and skin. Gypsum is usually stored in the open at a strategic point that facilitates its future distribution in the cropping areas; however, when gypsum is stored in a closed-covered structure, enough ventilation must be provided to control airborne dust when generated.

Summarized gypsum rate recommendations, time of application, and method of application are presented by Chen and Dick (2011). The authors also recommended (i) annual gypsum application when gypsum is intended to be a nutritional source of calcium and sulfur and (ii) split gypsum application if high rates are required. In this latter situation, the initial gypsum rate should be higher to remediate the restrictive soil situation, and the subsequent rates should be for maintenance and annually applied. The gypsum application can be made any time of the year; however, its application during autumn and winter is usually the most common practice. The gypsum application in those seasons permits (i) the application after crops are harvested, (ii) soil drying, which implies lower damage caused by the drive of heavy spreading equipment across the field, and (iii) allows enough time for gypsum-soil reactions to provide the gypsum benefits to the next crop season.

As indicated here, there are many gypsum recommendation methods. At the same time, there is a lack of a reliable way of defining gypsum rates and frequency of application for many situations. This lack presents a potential difficulty for farmers, researchers, and consultants when determining adequate gypsum management. For those without a gypsum recommendation for a specific situation, we suggest consistent literature research (e.g., scientific papers, book chapters, bulletins, regional technical circulars, and advertisements) for particular soil conditions, climate, and crops intended to be cultivated. Then, identify the closest and most successful reports as references for a gypsum management strategy.

After the definition of gypsum management (rates and frequency of application), the method to use gypsum will depend on its granulometry (finer granulometry means faster

reaction in soil), gypsum source, and the urgency for its benefits. Crushed or powdered gypsum provides the same benefits as pelletized gypsum (granular form). However, pelletized gypsum offers important benefits such as (i) improved handling (pellets are easier to handle, store, transport, and apply; also, powdered gypsum has a great potential to clump together, clogging equipment and increasing maintenance requirements); (ii) better distribution (powdered gypsum is dusty and often becomes windblown reducing the precision of its application; also, pellets move quickly through standard spreading equipment), and, (iii) reduced product loss (powdered gypsum result in a significant amount of product losses due to its dusty origin which makes it easy to fly away from the targeted area). Conversely, pelletized gypsum might need extended time for its complete solubilization reaction in soil due to its low solubility. When deciding on powdered gypsum, avoiding its application during intense windy days is recommended to reduce material losses.

Gypsum is usually spread over the soil's surface in no-tillage cropping areas, or gypsum is incorporated downward into the subsoil. Gypsum incorporation is less frequent in tropical areas (hot, rainy areas). Gypsum incorporation is also an alternative to avoid and decrease gypsum erosion by wind and water; however, soil turning to incorporate gypsum, or any crop input is a pricy activity. Additionally, this soil management reduces the soil's water-holding capacity due to de-structuring during the soil incorporation process, which lowers the plant's resistance to occasional drought stress. Still, when soil turning is needed, many doubts arise regarding the frequency and cost-benefits of such activity together (or not) with gypsum management.

Nevertheless, dry-material spinners or drop spreaders regularly apply gypsum to the soil surface. Gypsum can also be dissolved in irrigation water if it is a fine powder (0.074 mm in size or smaller) for fast gypsum results in soil and uniform gypsum application (Chen and Dick, 2011). However, avoid foliar applications with gypsum and other agrochemicals, as they tend to form precipitates when the foliar spray solution dries on the plant's surfaces. This dried spray solution covers the plants with a thin film of gypsum, potentially blocking a fraction of sunlight and reducing the photosynthesis efficiency.

GYPSUM KNOWLEDGE GAPS AND FUTURES RESEARCH

Considering the points discussed here and the fact that the application of agricultural gypsum is still not a regular practice in many regions worldwide, we understand that to improve its usage, we must primarily stimulate and support public policies about the benefits of adequate gypsum management. These policies must reach the crop production system with technical criteria and initial professional support, especially to the smaller farmers who generally are the least benefited from technologies that seek sustainability in agriculture.

Even with all the research on the effects and benefits of gypsum application to the agricultural complex - including soil dynamics, plant physiology, environmental safety, and

economic thresholds - many critical issues are still not fully understood (Watts and Dick, 2014; Dalla Nora et al., 2017; Pias et al., 2020). Some aspects of the gypsum's long-term effects on (i) soil organic matter; (ii) nitrogen, magnesium, and phosphorus dynamics in soil; (iii) lime joint application and management; (iv) doses and frequency of regular fertilizer application; (v) the time needed to achieve significant results from the gypsum application; (vi) the economic value of gypsum crop management on yield (cost-benefits), and (vii) the environmental services are not well comprehended and need to be elucidated.

Additionally, other agricultural technologies are being developed and implemented to improve crop productivity (yield per area) and the sustainability of the agricultural activity, and indeed, they will affect the soil and plant responses and the agronomic recommendations for gypsum management. Such improved technologies include (i) smart fertilizers (e.g., slow and controlled release fertilizers, bioformulated fertilizers, nanofertilizers, beneficial nutrients) developed to enhance nutrient use efficiency and crop yield with low impacts on the natural environment (Raimondi et al., 2021; Karthik and Maheswari, 2021; Tayade et al., 2022; Verma et al., 2022; Abiola et al., 2023; Areche et al., 2023; Chakraborty et al., 2023); genetic engineering and genome editing techniques of crop plants to improve their resistance to stresses and use-efficiency of agricultural amendments (Jan and Shrivastava, 2017; Mackelprang and Lemaux, 2020; Clouse and Wagner, 2021; Lebedev et al., 2021; Raza et al., 2022); large-scale application of artificial lights (light supplementation) to field crops (Lemes et al., 2021), and digitalization-integration-robotization plus AI (artificial intelligence), DL (deep learning) and blockchain of agriculture (Krithika, 2022; Srivastava, et al., 2022; Adamides and Edan, 2023; Ali et al., 2023; Mahibha and Balasubramanian, 2023; Cheng et al., 2023; Mesías-Ruiz et al., 2023; Okolie et al., 2023; Wakchaure et al., 2023; Zeng et al., 2023) are emerging and represent some of the most recent advances for modern sustainable and productive agriculture.

Overall, we can assure you that gypsum is very important to maintain and improve soil health and its functionality for high crop nutrition and yield performance. Gypsum and other technologies can also lower production costs, reduce the negative impacts of agriculture on the natural and social environment, and increase world food safety.

ACKNOWLEDGMENTS

To *Universidade do Vale do Jequitinhonha and Mucuri*, to *Instituto Federal do Triângulo Mineiro*, and to *Universidade Federal de Uberlândia* for personnel and structural support.

To all previous researchers, cited or not here, who used their expertise and time to understand and improve gypsum agricultural management. Many thanks!

REFERENCES

- Abbas, F.; Siddique, T.; Fan, R.; Azeem, M. 2023. Role of gypsum in conserving soil moisture macronutrients uptake and improving wheat yield in the rainfed area. *Water*, 15: 1011. <https://doi.org/10.3390/w15061011>
- Abiola, W. A.; Diogo, R. V. C.; Tovihoudji, P. G.; Mien, A. K.; Schalla, A. 2023. Research trends on biochar-based smart fertilizers as an option for the sustainable agricultural land management: bibliometric analysis and review. *Frontiers in Soil Science*, 3: 1136327. <https://doi.org/10.3389/fsoil.2023.1136327>
- AbouRizk, J. S.; Dhar, A.; Naeth, M. 2024. Sandy soil with phosphogypsum improves hydraulic conductivity and leachate chemical properties for reclamation. *Environmental Sustainability*. <https://doi.org/10.1007/s42398-024-00302-2>
- Adamides, G.; Edan, Y. 2023. Human-robot collaboration systems in agricultural tasks: A review and roadmap. *Computers and Electronics in Agriculture*, 204, 107541. <https://doi.org/10.1016/j.compag.2022.107541>
- Adams, T. C.; Ashworth, A. J.; Owens, P. R.; Popp, M.; Moore, P.A.; Pennington, J. 2022. Pasture conservation management effects on soil surface infiltration in hay and grazed systems. *Journal of Soil and Water Conservation*, 77(1): 59-66. <https://doi.org/10.2489/jswc.2022.00182>
- Agim, L. C.; Ahukaemere, M. C.; Uzoh, I., Onwudike, S. U.; Osi, A. F.; Ihem, E. E.; Nkwopara, U. 2021. Soil degradation and the human condition, including the pandemic, interactions, causes, impacts, control measures and likely future prospects. In: Aide, M. T.; Braden, I. (Eds.), *Soil Science - Emerging Technologies, Global Perspectives and Applications*. IntechOpen. <https://doi.org/10.5772/intechopen.101153>
- Ahmed, K.; Qadir, G.; Nawaz, M. Q.; Riaz, M. A.; Nawaz, M.; Ullah, M. M. 2020. Combined effect of growth hormones and gypsum induces salinity tolerance in wheat under saline-sodic soil. *Pakistan Journal of Agricultural Sciences*, 58(6): 1749-1757. <https://doi.org/10.36899/japs.2021.1.0200>
- Alcordero, I. S.; Rechcigl, J. E. 1993. Phosphogypsum in agriculture: A review. In: Spark DL (Ed) *Advances in Agronomy*, 49: 55-118. [https://doi.org/10.1016/S0065-2113\(08\)60793-2](https://doi.org/10.1016/S0065-2113(08)60793-2)
- Ali, A.; Hussain, T.; Tantashutikun, N.; Hussain, N.; Cocetta, G. 2023. Application of smart techniques, internet of things and data mining for resource use efficient and sustainable crop production. *Agriculture*, 13, 397. <https://doi.org/10.3390/agriculture13020397>
- Ali, M. T.; Shahram, S. H. 2007. Converter slag as a liming agent in the amelioration of acidic soils. *International Journal of Agriculture and Biology*, 9(5): 715-720. Available at: https://www.fsublishers.org/Issue.php?no_download=published_papers/81882_..pdf&issue_id=1313
- Alvarez V. V. H.; Dias, L. E.; Ribeiro, A. C.; Souza, R. B. 1999. Uso de gesso agrícola. In: Ribeiro, A. C. Guimarães, P. T. G.; Alvarez V. V. H. (Ed.). *Recomendação para o uso de corretivos e fertilizantes em Minas Gerais: 5ª aproximação*. Viçosa, MG: Comissão de Fertilidade do Solo do Estado de Minas Gerais. p.67-78.
- Amer, M. M.; Aboelsoud, H. M.; Sakher, E. M.; Hashem, A. A. 2023. Effect of gypsum, compost, and foliar application of some nanoparticles in improving some chemical and physical properties of soil and the yield and water productivity of faba beans in salt-affected soils. *Agronomy*, 13(4): 1052. <https://doi.org/10.3390/agronomy13041052>

- Amezqueta, E.; Aragüés, R.; Gazol, R. 2005. Efficiency of sulfuric acid, mined gypsum, and two gypsum by-products in soil crusting prevention and sodic soil reclamation. *Agronomy Journal*, 97: 983-989. <https://doi.org/10.2134/agronj2004.0236>
- Anderson, G. C.; Pathan, S.; Hall, D. J. M.; Sharma, R.; Easton, J. 2021a. Short- and long-term effects of lime and gypsum applications on acid soils in a water-limited environment: 3. Soil solution chemistry. *Agronomy*, 11: 826. <https://doi.org/10.3390/agronomy11050826>
- Anderson, S. L. II; Pearson, B.; Kjelgren, R.; Brym, Z. 2021b. Response of essential oil hemp (*Cannabis sativa* L.) growth, biomass, and cannabinoid profiles to varying fertigation rates. *PLoS ONE* 16(7): e0252985. <https://doi.org/10.1371/journal.pone.0252985>
- Anikwe, M.; Eze, J.; Ibudialo, A. 2016. Influence of lime and gypsum application on soil properties and yield of cassava (*Manihot esculenta* Crantz.) in a degraded Ultisol in Agbani, Enugu Southeastern Nigeria. *Soil Tillage Research*, 158: 32-38. <http://dx.doi.org/10.1016/j.still.2015.10.011>
- Araújo, L. C.; Souza, D. M. G.; Figueiredo, C. C.; Rein, T. A.; Nunes, R. S.; Santos Jr, J. D. G.; Malaquias, J. V. 2019. How does gypsum increase the organic carbon stock of an Oxisol profile under sugarcane? *Geoderma*, 343: 196-204. <https://doi.org/10.1016/j.geoderma.2019.02.029>
- Areche, F. O.; Aguilar, S. V.; More López, J. M.; Castañeda Chirre, E. T.; Sumarriva-Bustinza, L. A.; Pacovilca-Alejo, O. V.; Camposano Córdova, Y. F.; Montesinos, C. C. Z.; Quincho Astete, J. A.; Quispe-Vidalon, D.; Brito Mallqui, C. H.; Camayo-Lapa, B. F.; Malpartida Yapias, R. J.; Corilla Flores, D. D.; Salas-Contreras, W. H. 2023. Recent and historical developments in chelated fertilizers as plant nutritional sources, their usage efficiency, and application methods. *Brazilian Journal of Biology*, 24(83): e271055. <https://doi.org/10.1590/1519-6984.271055>
- Argüello, D.; Dekeyrel, J.; Chavez, E.; Smolders, E. 2022. Gypsum application lowers cadmium uptake in cacao in soils with high cation exchange capacity only: A soil chemical analysis. *European Journal of Soil Science*, 73(2): e13230. <https://doi.org/10.1111/ejss.13230>
- Ashrit, S.; Banerjee, P. K.; Chatti, R. V.; Rayasam, V.; Nair, U. G. 2015. Synthesis and characterization of yellow gypsum from LD slag fines generated in a steel plant. *Current Science*, 109(4): 727-732. Available at: <https://www.currentscience.ac.in/Volumes/109/04/0727.pdf>
- Ashrit, S.; Chatti, R. V.; Sarkar, S.; Venugopal, R.; Udayabhanu, G. 2020. Potential application of yellow gypsum from LD slag as a soil conditioner. *Current Science*, 118(1). <https://doi.org/10.18520/cs/v118/i1/114-118>
- Ashrit, S.; Chatti, R.V.; Udpa, K. N.; Venugopal, R.; Nair, U. G. 2016. Process optimization of yellow gypsum synthesized from LD Slag fines-an opportunity for value addition of LD Slag. *Metallurgical Research and Technology*, 113(6): article 605. <https://doi.org/10.1051/metal/2016037>
- Auler, A. C.; Caires, E. F.; Pires, L. F.; Galetto, S. L.; Romaniw, J.; Charnobay, A. C. 2019. Lime effects in a no-tillage system on Inceptisols in Southern Brazil. *Geoderma Regional*, 15, e00206. <https://doi.org/10.1016/j.geodrs.2019.e00206>
- Ayanda, A. F.; Jusop, S.; Ishak, C. F. 2020. Othman, R. Utilization of magnesium-rich synthetic gypsum as magnesium fertilizer for oil palm grown on acidic soil. *PLoS ONE*, 15: e0234045. <https://doi.org/10.1371/journal.pone.0234045>

- Baligar, V. C.; Clark, R. B.; Korcak, R. F.; Wright, R. J. 2011. Flue gas desulfurization product use on agricultural land. *Advances in Agronomy*, 111: 51-86. <https://doi.org/10.1016/B978-0-12-387689-8.00005-9>
- Bardhan, S.; Chen, L.; Dick, W. A. 2005. Soilless media created from coal combustion products and organic composts: environmental and chemical properties. In: *Agronomy Abstracts*. American Society of Agronomy, Madison, Wis. Available at: <https://scisoc.confex.com/a-c-s/2005am/techprogram/P5391.HTM>
- Basak, N.; Sheoran, P.; Sharma, R.; Yadav, R. K.; Singh, R. K.; Kumar, S.; Krishnamurthy, T.; Sharma, P. C. 2021. Gypsum and pressmud amelioration improve soil organic carbon storage and stability in sodic agroecosystems. *Land Degradation & Development* 32(15): 4430-4444. <https://doi.org/10.1002/ldr.4047>
- Batte, M. T.; Forster, D. L. 2015. Old is new again: the economics of agricultural gypsum use. *Journal of the American Society of Farm Managers and Rural Appraisers*, 56-74. Available at: <https://www.usagypsum.com/wp-content/uploads/2016/04/economics-of-agricultural-gypsum-use.pdf>
- Bello, S. K.; Alayafi, A. H.; AL-Solaimani, S. G.; Abo-Elyousr, K. A. M. 2021. Mitigating soil salinity stress with gypsum and bio-organic amendments: a review. *Agronomy*, 11, 1735. <https://doi.org/10.3390/agronomy11091735>
- Besen, M. R.; Coneglian, C. F.; Cassim, B. M. A. R.; Kachinski, W. D.; Inoue, T. T.; Batista, M. A. 2021a. Forms of lime application and use of phosphogypsum in low acid soil in southern Brazil: soybean-wheat yield and soil chemical properties. *Revista Brasileira de Ciência do Solo*, 45: e0210001. <https://doi.org/10.36783/18069657rbc2021000x>
- Besen, M. R.; Ribeiro, R. H.; Esper Neto, M.; Minato, E. A.; Coneglian, C. F.; Kachinski, W. D.; Tormena, C. A.; Inoue, T. T.; Batista, M. A. 2021b. Lime and phosphogypsum application management: changes in soil acidity, sulfur availability and crop yield. *Revista Brasileira de Ciência do Solo*, 45: e0200135. <https://doi.org/10.36783/18069657rbc20200135>
- Besen, M. R.; Santos, G. L. A. A.; Cordioli, V. R.; Coneglian, C. F.; Inoue T. T.; Batista, M. A. 2024. Effects of applying lime and phosphogypsum in soybean and wheat nutrition. *Journal of Plant Nutrition*. <https://doi.org/10.1080/01904167.2024.2308195>
- Bondi, G.; Fenton, O.; Sawdekar, P.; Keane, H.; Wall, D. P. 2021. Potential of lacto-gypsum as an amendment to build soil quality. *Frontiers in Sustainability*, 1:625727. <https://doi.org/10.3389/frsus.2020.625727>
- Bortolanza, D. R.; Klein, V. A. 2016. Soil chemical and physical properties on an inceptisol after liming (surface and incorporated) associated with gypsum application. *Revista Brasileira de Ciência do Solo*, 40: e0150377. <https://doi.org/10.1590/18069657rbc20150377>
- Bossolani, J. W.; Crusciol, C. A. C.; Merloti, L. F.; Moretti, L. G.; Costa, N. R.; Tsai, S. M.; Kuramae, E. E. 2020. Long-term lime and gypsum amendment increase nitrogen fixation and decrease nitrification and denitrification gene abundances in the rhizosphere and soil in a tropical no-till intercropping system. *Geoderma*, 375: 114476. <https://doi.org/10.1016/j.geoderma.2020.114476>
- Bossolani, J. W.; Lazarini, E.; Santos, F. L.; Sanches, I. R.; Meneghette, H. H. A.; Parra, L. F.; Souza, L. M. 2018. Surface reapplication of lime and gypsum on maize cultivated sole and intercropped with *Urochloa*. *Communications in Soil Science and Plant Analysis*, 49(15): 1855-68. <https://doi.org/10.1080/00103624.2018.1475565>

Brasil, E. C.; Lima, E. V.; Cravo, M. S. 2020. Uso de gesso na agricultura. In: *Recomendações de calagem e adubação para o estado do Pará*. 2. ed. – Brasília, DF: Embrapa, p. 133-145. Available at: <https://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/1127247>

Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa SDA nº 35, 04-jul-2006. Normas sobre especificações e garantias, tolerâncias, registro, embalagem e rotulagem dos corretivos de acidez, de alcalinidade, de sodicidade e dos condicionadores de solo, destinados à agricultura. Diário Oficial da União, 12-jul-2006. Seção 1. Available at: <https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/insumos-agricolas/fertilizantes/legislacao/in-35-de-4-7-2006-correctivos.pdf>

Brignoli, F. M.; Gatiboni, L. C.; Mumbach, G. L.; Grando, D. L.; Souza Junior, A. A.; Iochims, D. A. 2021. Soybean agronomic performance does not change with gypsum application in a Cambisol submitted to water restriction in Southern Brazil. *Open Journal of Agricultural Research*, 1(1): 30-44. <https://doi.org/10.31586/ojar.2021.010106>

Brock, C.; Jackson-Smith, D.; Kumarappan, S.; Brown, C. 2020. Farmer and practitioner conceptions and experiences with soil balancing. *The Ohio State University*. 45 p.

Brooks, S. N.; Horner, C. E.; Likens, S. T.; United States - Agricultural Research Service. 1961. Hop production. *Agriculture Information Bulletin*, nº 240. Agricultural Research Service, U.S. Department of Agriculture. 46 p. Available at: <https://handle.nal.usda.gov/10113/CAT30947736>

Brown, B. W. 2018. Horticultural uses for flue gas desulfurization gypsum. PhD Dissertation. Horticulture, Auburn University. 98 p. Available at: <http://hdl.handle.net/10415/6221>

Bryant, R. B.; Buda, A. R.; Kleinman, P. J.; Church, C.; Saporito, L. S.; Folmar, G. J.; Bose, S.; Allen, A. L. 2012. Using flue gas desulfurization gypsum to remove dissolved phosphorus from agricultural drainage waters. *Journal of Environmental Quality*, 41(3): 664-671. <https://doi.org/10.2134/jeq2011.0294>

Buckley, M. E.; Wolkowski, R. P. 2014. In-season effect of flue gas desulfurization gypsum on soil physical properties. *Journal of Environment Quality*, 43(1): 322-327. <https://doi.org/10.2134/jeq2012.0354>

Caires, E. F.; Churka, S.; Garbuio, F. J.; Ferrari, R. A.; Morgano, M. A. 2006. Soybean yield and quality a function of lime and gypsum applications. *Scientia Agricola*, 63(4): 370-379. <https://doi.org/10.1590/S0103-90162006000400008>

Caires, E. F.; Guimarães, A. M. 2018. A novel phosphogypsum application recommendation method under continuous no-till management in Brazil. *Agronomy Journal*, 110(5): 1987-1995. <https://doi.org/10.2134/agronj2017.11.0642>

Caires, E. F.; Joris, H. A. W.; Churka, S. 2011a. Long-term effects of lime and gypsum additions on no-till corn and soybean yield and soil chemical properties in southern Brazil. *Soil Use Management*, 27: 45-53. <https://doi.org/10.1111/j.1475-2743.2010.00310.x>

Caires, E. F.; Maschietto, E. H.G.; Garbuio, F. J.; Churka, S.; Joris, H. A. W. 2011b. Surface application of gypsum in low acidic Oxisol under no-till cropping system. *Scientia Agricola*, 68(2): 209-216. <https://doi.org/10.1590/S0103-90162011000200011>

Cañadas, E. M.; Ballesteros, M.; Valle, F.; Lorite, J. 2014. Does gypsum influence seed germination? *Turkish Journal of Botany* 38: 141-147. <https://doi.org/10.3906/bot-1305-19>

Cannoy, D. C. 2015. Green gold - a *Cannabis sativa* L. Lucis suitability analysis for West Virginia. Theses. Marshall University. Available at: <https://mds.marshall.edu/etd/964/>

Carvalho, M. C. S.; van Raij, B. 1997. Calcium sulphate, phosphogypsum and calcium carbonate in the amelioration of acid subsoils for root growth. *Plant and Soil*, 192 (1): 37-48. <https://doi.org/10.1023/A:1004285113189>.

Chaganti, V. N.; Culman, S. W. 2017. Historical perspective of soil balancing theory and identifying knowledge gaps: A review. *Crop, Forage & Turfgrass Management*, 3(1): 4220-4230. <https://doi.org/10.2134/cftm2016.10.0072>

Chaganti, V. N.; Culman, S. W.; Dick, W. A.; Kost, D. 2019. Effects of gypsum application rate and frequency on corn response to nitrogen. *Agronomy Journal*, 111: 1109-1117. <https://doi.org/10.2134/ajgronj2018.10.0683>

Chakraborty, R.; Mukhopadhyay, A.; Paul, S.; Sarkar, S.; Mukhopadhyay, R. 2023. Nanocomposite-based smart fertilizers: a boon to agricultural and environmental sustainability. *Science of the Total Environment*, 10(863): 160859. <https://doi.org/10.1016/j.scitotenv.2022.160859>

Charlo, H. C. O.; Almeida, J. S. M.; Castoldi, R.; Moreira, E. F. A.; Torres, J. L. R.; Lemes, E. M. 2022. Soil attributes and productivity of sorghum as a function of gypsum and potassium doses. *Communications in Soil Science and Plant Analysis*, 53(13): 1630-1643. <https://doi.org/10.1080/00103624.2022.2063311>

Charlo, H. C. O.; Almeida, J. S. M.; Lana, R. M. Q.; Castoldi, R.; Moreira, É. F. A.; Franco Jr, M. R.; Santos, W. B. 2020. Changes in chemical soil and corn yield after application of gypsum and potassium doses. *Bioscience Journal*, 36(3): 810-826. <https://doi.org/10.14393/BJ-v36n3a2020-42443>

Charlo, H. C. O.; Almeida, J. S. M.; Moreira, E. F. A.; Castoldi, R.; Luz, J. M. Q.; Lemes, E. M. 2021. Gypsum and potassium doses on cauliflower nutritional status and production. *Revista Caatinga*, 34(2), 370. <https://doi.org/10.1590/1983-21252021v34n213rc>

Chen, L.; Dick, W. A. 2011. Gypsum as an agricultural amendment: general use guidelines. The Ohio State University Extension. Available at: <https://fabe.osu.edu/sites/fabe/files/imce/files/Soybean/Gypsum%20Bulletin.pdf>

Chen, L.; Kost, D.; Tian, Y.; Guo, X.; Watts, D.; Norton, D.; Wolkowski, R. P.; Dick, W. A. 2014. Effects of gypsum on trace metals in soils and earthworms. *Journal of Environmental Quality*, 43(1): 263-272. <https://doi.org/10.2134/jeq2012.0096>

Cheng, C.; Fu, J.; Su, H.; Ren, L. 2023. Recent advancements in agriculture robots: benefits and challenges. *Machines*, 11(1): 48. <https://doi.org/10.3390/machines11010048>

Churka Blum, S. C.; Caires, E. F.; Alleoni, L. R. F. 2013. Lime and phosphogypsum application and sulfate retention in subtropical soils under no-till system. *Journal of Soil Science and Plant Nutrition*, 13(2): 279-300. <https://dx.doi.org/10.4067/S0718-95162013005000024>

Clouse, K. M.; Wagner, M. R. 2021. Plant genetics as a tool for manipulating crop microbiomes: opportunities and challenges. *Front Bioengineering and Biotechnology*, 9: 567548. <https://doi.org/10.3389/fbioe.2021.567548>

- Codling, E. E. 2017. Effects of ammonium nitrate encapsulated with coal combustion byproducts on nutrient accumulation by corn and rye. *Journal of Plant Nutrition*, 40(12): 1702-1709. <https://doi.org/10.1080/01904167.2017.1310891>
- Conradi, E. Jr.; Gonçalves, A. C. Jr.; Seidel, E. P.; Ziemer, G. L.; Zimmermann, J.; Dias de Oliveira, V. H.; Schwantes, D.; Carlos, D. Z. 2020. Effects of liming on soil physical attributes: a review. *Journal of Agricultural Science*, 10: 278-286. <https://doi.org/10.5539/jas.v12n10p278>
- Cooper, M.; Vidal-Torrado, P. 2005. Caracterização morfológica, micromorfológica e físico-hídrica de solos com horizonte B nítico. *Revista Brasileira de Ciência do Solo*, 29: 581-595. <https://doi.org/10.1590/S0100-06832005000400011>
- Costa, C. H. M.; Crusciol, C. A. C. 2016. Long-term effects of lime and phosphogypsum application on tropical no-till soybean–oat–sorghum rotation and soil chemical properties. *European Journal of Agronomy*, 74: 119-132. <https://doi.org/10.1016/j.eja.2015.12.001>
- Crangle Jr, R. D. 2021. Gypsum. In: *Mineral Commodity Summaries*. U.S. Geological Survey. pp. 74-75. <https://doi.org/10.3133/mcs2021>
- Crocker, W. 1922. History of the use of agricultural gypsum. Gypsum Industries Association, Chicago, IL.
- Crusciol, C. A. C.; Artigiani, A. C. C. A.; Arf, O.; Carmeis Filho, A. C. A.; Soratto, R. P.; Nascente, A. S.; Alvarez, R. C. F. 2016. Soil fertility, plant nutrition, and grain yield of upland rice affected by surface application of lime, silicate, and phosphogypsum in a tropical no-till system. *Catena* 137: 87-99. <https://doi.org/10.1016/j.catena.2015.09.009>
- Crusciol, C. A. C.; Marques, R. R.; Filho, C. A. C. A.; Soratto, R. P.; Costa, C. H. M.; Neto, F. J.; Castro, G. S. A.; Pariz, C. M.; Castilhos, A. M.; Franzluebbers, A. J. 2019. Lime and gypsum combination improves crop and forage yields and estimated meat production and revenue in a variable charge tropical soil. *Nutrient Cycling in Agroecosystems*, 115, 347-372. <https://doi.org/10.1007/s10705-019-10017-0>
- Crusciol, C. A. C.; Rossato, O. B.; Foltran, R.; Martello, J. M.; Nascimento, C. A. C. 2017. Soil fertility, sugarcane yield affected by limestone, silicate, and gypsum application. *Communications in Soil Science and Plant Analysis*, 48(19): 2314-2323. <https://doi.org/10.1080/00103624.2017.1411507>
- Cuervo-Alzate, J. E.; Osorio, N. W. 2020. Gypsum incubation tests to evaluate its potential effects on acidic soils of Colombia. *Revista Facultad Nacional de Agronomía Medellín*, 73(3): 9349-9359. <https://doi.org/10.15446/rfnam.v73n3.85259>
- Curi, N.; Larach, J. O. I.; Kampf, N.; Moniz, A. C.; Fontes, L. E. F. 1993. *Vocabulário de ciência do solo*. Sociedade Brasileira de Ciências do Solo, Campinas (Brasil). 89 p.
- Da Costa, C. H. M.; Crusciol, C. A. C. 2016. Long-term effects of lime and phosphogypsum application on tropical no-till soybean-oat-sorghum rotation and soil chemical properties. *European Journal of Agronomy*, 74: 119-132. <http://dx.doi.org/10.1016/j.eja.2015.12.001>
- Dalla Nora, D.; Amado, T. J. C.; Nicoloso, R. S.; Gruhn, E. M. 2017. Modern high-yielding maize, wheat and soybean cultivars in response to gypsum and lime application on no-till Oxisol. *Revista Brasileira de Ciência Solo*, 41: 1-21. <https://doi.org/10.1590/18069657rbc20160504>

De Moura, E. G.; Portela, S. B.; Macedo, V. R. A.; Sena, V. G. L.; Sousa, C. C. M.; Aguiar, A. D. C. F. 2018. Gypsum and legume residue as a strategy to improve soil conditions in sustainability of agrosystems of the humid tropics. *Sustainability*, 10: 1006. <https://doi.org/10.3390/su10041006>

Deer, W. A. Howie, R. A.; Zussman, J. 1966. *An introduction to the rock-forming minerals*. New York: John Wiley, 582 p.

Demattê, J. L. I. 1986. Solos arenosos de baixa fertilidade: estratégia de manejo. In: 5° Seminário Agroindustrial e 29° Semana “Luiz De Queiroz”, Piracicaba. Anais... Universidade de São Paulo, Escola Superior de Agricultura Luiz de Queiroz.

Demattê, J. L. I. 1992. Aptidão agrícola de solos e o uso do gesso. II Seminário Sobre o Uso do Gesso na Agricultura. Anais... IBRAFOS, Uberaba, Brazil, pp. 307-324.

Demattê, J. L. I. 2005. Recuperação e manutenção da fertilidade dos solos. *Visão Agrícola*, 1: 48-59. Available at: <https://www.esalq.usp.br/visaoagricola/sites/default/files/cana-adubos-e-corretivos.pdf>

Dick, W. A., Kost, D.; Nakano, N. 2006. A review of agricultural and other land application uses of flue gas desulfurization products. Electric Power Research Institute, Palo Alto, CA. Report 101385. 97 p.

Dubrovina, T. A.; Losev, A. A.; Karpukhin, M. M.; Vorobeichik, E. L.; Dovletyarova, E. A.; Brykov, V. A.; Brykova, R. A.; Ginocchio, R.; Yáñez, C.; Neaman, A. 2021. Gypsum soil amendment in metal-polluted soils-an added environmental hazard. *Chemosphere*, Oct; 281: 130889. <https://doi.org/10.1016/j.chemosphere.2021.130889>

Duke, J. A. 1983. *Handbook of energy crops*. Purdue University. Available at www.hort.purdue.edu/newcrop/duke_energy/dukeindex.html

Ekholm, P.; Ollikainen, M.; Punttila, E.; Ala-Harja, V.; Riihimäki, J.; Kiirikki, M.; Taskinen, A.; Begum, K. 2024. Gypsum amendment of agricultural fields decreases phosphorus losses – evidence on a catchment scale. *SSRN*, 59 p. <http://dx.doi.org/10.2139/ssrn.4694131>

Ellington, A. 1986. Effects of deep ripping, direct drilling, gypsum and lime on soils, wheat growth and yield. *Soil and Tillage Research*, 8; 29-49. [https://doi.org/10.1016/0167-1987\(86\)90321-1](https://doi.org/10.1016/0167-1987(86)90321-1)

Endale, D. M.; Schomberg, H. H.; Fisher, D. S.; Franklin, D. H.; Jenkins, M. B. 2014. Flue gas desulfurization gypsum: implication for runoff and nutrient losses associated with broiler litter use on pastures on ultisols. *Journal of Environmental Quality*, 43(1): 281-289. <https://doi.org/10.2134/jeq2012.0259>

Ernani, P.R.; Cassol, P.C.; Peruzzo, G. 1992. Eficiência agrônômica do gesso agrícola no sul do Brasil. In: II Seminário sobre o Uso do Gesso na Agricultura, Uberaba, 1992. Anais. Uberaba, IBRAFOS. 1: .263-276.

Farina, M. P. W.; Channon, P. 1988. Acid-subsoil amelioration: II. Gypsum effects on growth and subsoil chemical properties. *Soil Science Society of America Journal*, 52(1): 175-180. <https://doi.org/10.2136/sssaj1988.03615995005200010031x>

Favaretto, N.; Norton, L. D.; Johnston, C. T.; Bigham, J.; Sperrin, M. 2012. Nitrogen and phosphorus leaching as affected by gypsum amendment and exchangeable calcium and magnesium. *Soil Science Society of America Journal*, 76: 575-585. <https://doi.org/10.2136/sssaj2011.0223>

- Fernando, M. S. W.; Silva, S. H. S. A.; Kanchana, S. 2021. Influence of gypsum application in disease management of onion (*Allium cepa* L.). International Journal of Innovative Science and Research Technology, 6(8): 900-905. Available at: <https://ijisrt.com/assets/upload/files/IJISRT21AUG592.pdf>
- Ferreira, C. F.; Bassaco, M. V. M.; Pereira, M.; Pauletti, V.; Prior, S. A.; Motta, A. C. V. 2020. Dendrometric analysis of early development of *Eucalyptus urophylla* x *Eucalyptus grandis* with gypsum use under subtropical conditions. FLORAM, 27(1): e20190095. <http://dx.doi.org/10.1590/2179-8087.009519>
- Fleuridor, L.; Herms, C.; Culman, S.; Dick, W. A.; Paul, P. A.; Doohan, D. 2021. Short-term responses of soils and crops to gypsum application on organic farms. Agronomy Journal, 113(5): 4220-4230. <https://doi.org/10.1002/agj2.20669>
- Fontoura, S. M. V.; Pias, O. H. C.; Tiecher, T.; Cherubin, M. R.; Moraes, R. P.; Bayer, C. 2019. Effect of gypsum rates and lime with different reactivity on soil acidity and crop grain yields in a subtropical Oxisol under no-tillage. Soil & Tillage Research, 193: 27-41. <https://doi.org/10.1016/j.still.2019.05.005>
- Gabriel, C. A.; Cassol, P. C.; Simonete, M. A.; Moro, L.; Pflieger, P.; Mumbach, G. L. 2018. Lime and gypsum applications on soil chemical attributes and initial growth of eucalyptus. Floresta, 48(4): 573-582. <https://doi.org/10.5380/ufv.v48i4.57455>
- Garbowski, T.; Bar-Michalczyk, D.; Charazińska, S.; Grabowska-Polanowska, B.; Kowalczyk, A.; Lochyński, P. 2023. An overview of natural soil amendments in agriculture. Soil and Tillage Research, 225: 105462. <https://doi.org/10.1016/j.still.2022.105462>
- Gharaibeh, M. A.; Eltaif, N. I.; Albalasmeh, A. A. 2011. Reclamation of highly calcareous saline sodic soil using *Atriplex halimus* and by-product gypsum. International Journal of Phytoremediation, 13(9): 873-83. <https://doi.org/10.1080/15226514.2011.573821>
- Gingrich, C.; Hart, J.; Christensen, N. 2000. Hops fertilizer guide. Oregon State University Extension. Available at: <https://catalog.extension.oregonstate.edu/fg79>
- Goiba, P. K.; Prakash, N. B.; Dhumgond, P.; Shruthi; Yogesh, G. S. 2023. Application of slag-based gypsum in rice crop and its effect on growth, yield and nutrient availability in acidic, neutral and alkaline soils. Communications in Soil Science and Plant Analysis, 54(11): 1510-1524. <https://doi.org/10.1080/00103624.2022.2161558>
- Grego, C. R.; Coelho, R. M.; Vieira, S. R. 2011. Critérios morfológicos e taxonômicos de Latossolo e Nitossolo validados por propriedades físicas mensuráveis analisadas em parte pela geoestatística. Revista Brasileira de Ciência do Solo, 35(2): 337-350. <https://doi.org/10.1590/S0100-06832011000200005>
- Guimarães, A. M.; Caires, E. F.; Silva, K. S.; Rocha, J. C. F. 2015. Estimating gypsum requirement under no-till based on machine learning technique. Revista Ciência Agronômica, 46: 250-257. <https://doi.org/10.5935/1806-6690.20150004>
- Hanger, B. C. 1979. The movement of calcium in plants. Communications in Soil Science and Plant Analysis, 10(1-2): 171-193. <https://doi.org/10.1080/00103627909366887>
- HĀPI - Hop Research Center (ed.). 2019. Fertility guide for hops. Hāpi Research Ltd. 29 p. Available at: <https://hapi.co.nz/wp-content/uploads/2019/10/Fertility-Guide-for-Hops-Oct-2019.pdf>

Instituto Brasileiro de Mineração (IBRAM). 2020. Informações sobre a economia mineral brasileira 2020 (ano base 2019). 1. ed. Brasília: IBRAM. 80 p. Available at: <https://portaldaminerao.com.br/wp-content/uploads/2021/03/Economia-Mineral-Brasileira-IBRAM-2020.pdf>

Jan, M.; Shrivastava, M. 2017. Genetically modified plants: a systematic review. *International Journal of Recent Scientific Research*, 8(4): 16471-16481. <http://dx.doi.org/10.24327/ijrsr.2017.0804.0155>

Jayawardane, N. S.; Blackwell, J. 1986. Effects of gypsum-slotting on infiltration rates and moisture storage in a swelling clay soil. *Soil Use and Management*, 2(3): 114-118. <https://doi.org/10.1111/j.1475-2743.1986.tb00693.x>

Jin, M.; Zhou, Y.; Wen, B.; Liu, L.; Liu, H. 2023. Progresses of gypsums for the improvement of saline-alkaline soil. *Journal of Nanjing Forestry University*, 47(2): 1-8. [10.12302/j.issn.1000-2006.202209047](https://doi.org/10.12302/j.issn.1000-2006.202209047)

Karthik, A.; Maheswari, M. U. 2021. Smart fertilizer strategy for better crop production. *Agricultural Reviews*. 42(1): 12-21. <https://doi.org/10.18805/ag.R-1877>

Kilpatrick, L. A. 2012. Sustainable growth of *Miscanthus* on marginal lands amended with flue gas desulfurization gypsum and sewage biosolids. *American Society of Agricultural and Biological Engineers*, 12-1337624. 37 p. <https://doi.org/10.13031/2013.41822>

King, K. W.; Williams, M. R.; Dick, W. A.; LaBarge, G. A. 2016. Decreasing phosphorus loss in tile-drained landscapes using flue gas desulfurization gypsum. *Journal of Environmental Quality* 45, 1722-1730. <https://doi.org/10.2134/jeq2016.04.0132>

Kong, F.; Ying, Y.; Lu, S. 2023. Heavy metal pollution risk of desulfurized steel slag as a soil amendment in cycling use of solid wastes. *Journal of Environmental Sciences*, 127: 349-360. <https://doi.org/10.1016/j.jes.2022.05.010>

Kopittke, P. M.; Menzies, N. W. 2007. A review of the use of the basic cation saturation ratio and the "ideal" soil. *Soil Science Society of America Journal*, 71(2): 259-265. <https://doi.org/10.2136/sssaj2006.0186>

Kopittke, P. M.; Menzies, N. W.; Wang, P.; McKenna, B. A.; Lombi, E. 2019. Soil and the intensification of agriculture for global food security. *Environment International*, 132: 105078. <https://doi.org/10.1016/j.envint.2019.105078>

Koralegedara, N. H.; Pinto, P. X.; Dionysiou, D. D., Al-Abed, S. R. J. J. 2019. Recent advances in flue gas desulfurization gypsum processes and applications - A review. *Journal of Environmental Management*, 251: 109572. <https://doi.org/10.1016/j.jenvman.2019.109572>

Korcak, R. F. 1993. High-gypsum byproducts as soil amendments for horticultural crops. *HortTechnology*, 3(2). 156-161. <https://doi.org/10.21273/HORTTECH.3.2.156b>

Koske, T. J.; Hall M.; Hinson, R.; Pollet, D.; Sanderlin, R. 2005. Commercial growing of greenhouse tomatoes. Available at: <http://www.lsuagcenter.com/NR/rdonlyres/7D6AEFA5-E5C2-494A-AF3B-DE10DDC1ADBE/10421/pub1808greenhousetomatoes1.pdf>

Kost, D.; Chen, L.; Guo, X.; Tian, Y.; Ladwig, K.; Dick, W.A. 2014. Effects of flue gas desulfurization and mined gypsums on soil properties and on hay and corn growth in eastern Ohio. *Journal of Environmental Quality*, 43: 312-321. <https://doi.org/10.2134/jeq2012.0157>

- Kost, D.; Ladwig, K. J.; Chen, L.; DeSutter, T. M.; Espinoza, L.; Norton, L. D.; Smeal, D.; Torbert, H. A.; Watts, D. B.; Wolkowski, R. P.; Dick, W. A. 2018. Meta-Analysis of gypsum effects on crop yields and chemistry of soils, plant tissues, and vadose water at various research sites in the USA. *Journal of Environmental Quality*, 47(5): 1284-1292. <https://doi.org/10.2134/jeq2018.04.0163>
- Kraamwinkel, C. T.; Beaulieu, A.; Dias, T.; Howison, R. A. 2021. Planetary limits to soil degradation. *Communications Earth & Environment*, 2: 249. <https://doi.org/10.1038/s43247-021-00323-3>
- Krithika, L. B. 2022. Survey on the applications of blockchain in agriculture. *Agriculture*, 12, 1333. <https://doi.org/10.3390/agriculture12091333>
- Kumaragamage, D.; Weerasekara, C. S.; Perry, M.; Akinremi, O. O.; Goltz, D. 2022. Alum and gypsum amendments decrease phosphorus losses from soil monoliths to overlying floodwater under simulated snowmelt flooding. *Water*, 14: 559. <https://doi.org/10.3390/w14040559>
- Lantzke, N. 2018. Gypsum recommendations for horticulture in Carnarvon. Carnarvon Growers Association. Farmnote, number 3. 4 p. Available at: <https://www.agric.wa.gov.au/sites/gateway/files/Farmnote%20-%20Gypsum%20recommendations.pdf>
- Laxmanarayanan, M.; Prabhudev, D.; Shruthi; Jahir Basha, C. R.; Supriya, S.; Prakash, N. B. 2022. Influence of yellow gypsum on nutrient uptake and yield of groundnut in different acid soils of Southern India. *Scientific Reports*, 12: 5604. <https://doi.org/10.1038/s41598-022-09591-1>
- Lebedev, V. G.; Popova, A. A.; Shestibratov, K. A. 2021. Genetic engineering and genome editing for improving nitrogen use efficiency in plants. *Cells*, 10(12): 3303. <https://doi.org/10.3390/cells10123303>
- Lecourieux, D.; Ranjeva, R.; Pugin, A. 2006. Calcium in plant defense-signaling pathways. *The New Phytologist*, 171(2): 249-269. <https://doi.org/10.1111/j.1469-8137.2006.01777.x>
- Lee, Y. B.; Bigham, J. M.; Dick, W. A.; Jones, E. S.; Ramsier, C. 2007. Influence of soil pH and application rate on the oxidation of calcium sulfite derived from flue gas desulfurization. *Journal of Environmental Quality*, 36: 298-304. <https://doi.org/10.2134/jeq2006.0050>
- Lemes, E. M.; Azevedo, B. N. R.; Domiciano, M. F. I.; Andrade, S. L. 2021. Improving soybean production using light supplementation at field-scale: a case study. *Journal of Agricultural Studies*, 9(3): 259-275. <https://doi.org/10.5296/jas.v9i3.18890>
- Li, C.; Dong, Y.; Yi, Y.; Tian, J.; Xuan, C.; Wang, Y.; Wen, Y.; Cao, J. 2023. Effects of phosphogypsum on enzyme activity and microbial community in acid soil. *Scientific Reports*, 13: 6189. <https://doi.org/10.1038/s41598-023-33191-2>
- Liu, S.; Liu, W.; Jiao, F.; Qin, W.; Yang, C. 2021. Production and resource utilization of flue gas desulfurized gypsum in China - A review. *Environmental Pollution*, 288: 117799. <https://doi.org/10.1016/j.envpol.2021.117799>
- Lorenzetti, J. M.; Rodrigues, J. C.; Morales, S. H.; Dematté, J. L. I. 1992. Uso de calcário e gesso em soqueira de cana-de-açúcar. *STAB*, 10: 14-18. <http://www.stab.org.br/revista.html>
- Lorenzi, J. O.; Monteiro, P. A.; Miranda Filho, H. S.; Van Raij, B. Raízes E Tubérculos. In: Van Raij, B.; Cantarella, H.; Quaggio, J. A.; Furlani, A. M. C. (Eds.) 1997. *Recomendações de adubação e calagem para o Estado de São Paulo*. Campinas: Instituto Agrônomo de Campinas, p. 221-229. (IAC. Boletim Técnico, 100). Available at: <https://www.iac.sp.gov.br/publicacoes/boletim100.php>

- Mackelprang R.; Lemaux, P. G. 2020. Genetic engineering and editing of plants: an analysis of new and persisting questions. *Annual Review of Plant Biology*, 71(1): 659-687. <https://doi.org/10.1146/annurev-arplant-081519-035916>
- Mahibha, G.; Balasubramanian, P. 2023. Impact of artificial intelligence in agriculture with special reference to agriculture information research. *Current Agriculture Research Journal*, 11(1). Available at: <https://bit.ly/3xx30KC>
- Malavolta, E.; Romero, J. P.; Liem, T. H.; Vitti, G. C. 1979. Gesso agrícola: seu uso na adubação e correção do solo. São Paulo: ULTRAFÉRTIL, 32 p.
- Mao, Y.; Li, X.; Dick, W. A.; Cao, L. 2022. Use of flue gas desulfurization gypsum to reduce dissolved phosphorus in runoff and leachate from two agricultural soils. *Soil Ecology Letters*. <https://doi.org/10.1007/s42832-022-0135-5>
- Marchesan, E.; Tonetto, F.; Teló, G. M.; Coelho, L. L.; Aramburu, B. B.; Trivisio, V. S. 2017. Soil management and application of agricultural gypsum in a Planosol for soybean cultivation. *Ciencia Rural*, 47, 1-7. <https://doi.org/10.1590/0103-8478cr20161102>
- Marchis, D.; Badulescu, C.; Nistor, M.-C. 2016. Benefits of using FGD gypsum from S.E. Turceni in agriculture. *Research Journal of Agricultural Science*, 48(4): 247-253. Available at: https://rjas.ro/download/paper_version.paper_file.8023d1dc03b3fb93.6d617263686973322e706466.pdf
- Martins, O. C.; Novais, R. F.; Alvarez, V. V. H.; Ribeiro, A. C.; Barros, N. F. 2002. Respostas à aplicação de diferentes misturas de calcário e gesso em solos. I. Alterações químicas no perfil do solo. *Revista Ceres*, 49: 123-35. Available at: <http://www.ceres.ufv.br/ojs/index.php/ceres/article/view/2798/0>
- Matveeva, V. A.; Smirnov, Y. D.; Suchkov, D. V. 2022. Industrial processing of phosphogypsum into organomineral fertilizer. *Environ Geochem Health*, 44: 1605-1618. <https://doi.org/10.1007/s10653-021-00988-x>
- Mayer, J. F. 1768. Die Lehre vom Gyps als einem vorzüglich guten Dung zu allen Erd-Gewächsen auf Aeckern und Wiesen, Hopfen- und Weinbergen [Instruction in gypsum as an ideal good manure for all things grown in soil on fields and pastures, hops yards and vineyards]. Anspach, (Germany): J.J. Palm. p. 61. <https://doi.org/10.3931/e-rara-45060>
- McBride, M. B. 1994. *Environmental chemistry of soils*. Oxford University Press, New York. 406 p.
- McGrath, J. M.; Penn, C. J.; Coale, F. J. 2013. A modeling approach to the design of in situ agricultural drainage filters. *Soil Use Management*, 29, 155-161. <https://doi.org/10.1111/j.1475-2743.2011.00381.x>
- McIntyre, D.S.; Loveday, J.; Watson, C.L. 1982. Field studies of water and salt movement in an irrigated swelling clay soil. I. Infiltration during ponding. II. Profile hydrology during ponding. III. Salt movement during ponding. *Australian Journal of Soil Research*, 20: 81-90, 91-99, 101-105. <https://doi.org/10.1071/SR9820081>, <https://doi.org/10.1071/SR9820091>, <https://doi.org/10.1071/SR9820101>
- McKibben, W. 2012. *The art of balancing soil nutrients: A practical guide to interpreting soil tests*. Acres, U.S.A. 304 p.
- Mesías-Ruiz, G. A.; Pérez-Ortiz, M.; Dorado, J.; Castro, A. I.; Peña, J. M. 2023. Boosting precision crop protection towards agriculture 5.0 via machine learning and emerging technologies: a contextual review. *Frontiers in Plant Science*, 14: 1143326. <https://doi.org/10.3389/fpls.2023.1143326>

- Messenger, B. J.; Menge, J. A.; Pond, E. 2000. Effects of gypsum soil amendments on avocado growth, soil drainage, and resistance to *Phytophthora cinnamomi*. *Plant Disease*, 84: 612-616. <https://doi.org/10.1094/PDIS.2000.84.6.612>
- Morelli, J. L.; Dalben, A. E.; Almeida, J. O. C.; Dematte, J. L. I. 1992. Calcário e gesso na produtividade da cana-de-açúcar e nas características químicas de um Latossolo de textura média álico. *Brasileira de Ciência do Solo*, 16: 187-194. <http://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=4316645>
- Morelli, J. L.; Nelli, E. J.; Dematte, J. L. I.; Dalben, A. E. 1987. Efeito do gesso e do calcário nas propriedades químicas de solos arenosos álicos e na produção de cana de açúcar. *STAB*, 6(1): 24-31. <http://www.stab.org.br/revista.html>
- Muller, M. M. L.; Tormena, C. A.; Genu, A. M.; Kramer, L. F. M.; Michalovicz, L.; Caires, E. F. 2012. Structural quality of a no-tillage red latosol 50 months after gypsum application. *Revista Brasileira de Ciência do Solo* 36, 1005-1013. <https://doi.org/10.1590/S0100-06832012000300030>
- Murphy, P. N. C.; Stevens, R. J. 2010. Lime and gypsum as source measures to decrease phosphorus loss from soils to water. *Water Air Soil Pollut Water, Air, & Soil Pollution*, 212: 101-111. <https://doi.org/10.1007/s11270-010-0325-0>
- Narasimhan, V.; Ramadoss, N.; Sridhar, V. V.; Kareem, A. A. 1994. Using gypsum to manage sheath rot in rice. *International Rice Research Newsletter*, 19(2): 27-28. Available at: <https://eurekamag.com/research/003/007/003007204.php>
- Niaz, S.; Wehr, J. B.; Dalal, R. C.; Kopitke, P. M.; Menzies, N. W. 2023. Wetting and drying cycles, organic amendments, and gypsum play a key role in structure formation and stability of sodic Vertisols. *SOIL*, 9: 141-154. <https://doi.org/10.5194/soil-9-141-2023>
- Nora, D. D.; Amado, T. J.; Bortolotto, R. F.; Ferreira, A. O.; Keller, C.; Kunz, J. 2014. Alterações químicas do solo e produtividade do milho com aplicação de gesso combinado com calcário. *Magistra*, 26(1): 1-10. Available at: <https://www3.ufrb.edu.br/magistra/index.php/magistra/article/view/432/114>
- Norton, L. D. 2008. Gypsum soil amendment as a management practice in conservation tillage to improve water quality. *Journal of Soil and Water Conservation*, 63: 46A-48A. <https://doi.org/10.2489/jswc.63.2.46A>
- Okolie, C. C.; Danso-Abbeam, G.; Groupson-Paul, O.; Ogundeji, A. A. 2023. Climate-smart agriculture amidst climate change to enhance agricultural production: a bibliometric analysis. *Land*, 12: 50. <https://doi.org/10.3390/land12010050>
- Oliveira, M. W.; Freire, F. M.; Macêdo, G. A. R.; Ferreira, J. J. 2007. Nutrição mineral e adubação da cana-de-açúcar. *Informe. Agropecuário*, 28: 30-43. Available at: http://www.nutricaoeplantas.agr.br/site/downloads/unesp_jaboticabal/oliveira_cana_informeagropec.pdf
- Oster, J. 1982. Gypsum usage in irrigated agriculture: a review. *Fertilizer Research*. 3: 73-89. <https://doi.org/10.1007/BF01063410>
- Outbakat, M.; Choukr-Allah, R.; Bouray, M.; EL Gharous, M.; EL Mejahed, K. 2023. Phosphogypsum: properties and potential use in agriculture. In: Choukr-Allah, R.; Ragab, R. (eds) *Biosaline Agriculture as a Climate Change Adaptation for Food Security*. Springer, Cham. https://doi.org/10.1007/978-3-031-24279-3_12

Panday, D.; Ferguson, R. B.; Maharjan, B. 2018. Flue gas desulfurization (FGD) gypsum as soil amendment. In: Rakshit, A.; Sarkar, B.; Abhilashis, P. C. (Eds.), *Soil Amendments for Sustainability: Challenges and Perspectives*. CRC Press, FL, pp 199-208. Available at: <https://digitalcommons.unl.edu/agronomyfacpub/1245>

Paolinelli, M. T.; Oliveira, P. M.; Santos, P. R. R. S.; Leandro, V. P.; Moraes, W. Y. 1986. Aplicação direta do fosfogesso. In: *Seminário Sobre o Uso de Gesso na Agricultura*, 1., Brasília. Anais... Brasília: DDT, p.197-207.

Pauletti, V.; Motta, A. C. V. 2017. Manual de adubação e calagem para o estado do Paraná. Sociedade Brasileira de Ciência do Solo - Núcleo Estadual Paraná, Curitiba. 289 p.

Pauletti, V.; Pierri, L.; Ranzan, T.; Barth, G.; Motta, A. C. V. 2014. Efeitos em longo prazo da aplicação de gesso e calcário no sistema de plantio direto. *Revista Brasileira de Ciência do Solo* 38: 495-505. <https://doi.org/10.1590/S0100-06832014000200014>

Penn, C. J.; Bryant, R. B. 2006. Application of phosphorus sorbing materials to streamside cattle loafing areas. *Journal of Soil and Water Conservation*, 61(5): 303-310. Available at: <https://www.jswnonline.org/content/61/5/303>

Penn, C. J.; McGrath, J. M. 2014. Chemistry and application of industrial by-products to animal manure for reducing phosphorus losses to surface waters. In: He, Z., Zhang, H. (Eds.), *Applied Manure and Nutrient Chemistry for Sustainable Agriculture and Environment*. Springer, The Netherlands, pp. 211-238. https://doi.org/10.1007/978-94-017-8807-6_11

Pias, O. H. D. C.; Tiecher, T.; Cherubin, M. R.; Mazurana, M.; Bayer, C. 2019. Crop yield responses to sulfur fertilization in Brazilian no-till soils: a systematic review. *Revista Brasileira de Ciência do Solo*, 43: 1-21. <https://doi.org/10.1590/18069657rbcS20180078>

Pias, O. H. D. C.; Tiecher, T.; Cherubin, M. R.; Silva, A. G. B.; Bayer, C. 2020. Does gypsum increase crop grain yield on no-tilled acid soils?: A meta-analysis. *Agronomy Journal*, 112: 675-692. <https://doi.org/10.1002/ajj2.20125>

Pivetta, L. A.; Castoldi, G.; Pivetta, L. G.; Maia, S. C. M.; Rosolem, C. A. 2019. Gypsum application, soil fertility and cotton root growth. *Bragantia*, 78(2): 264-273. <https://doi.org/10.1590/1678-4499.20180183>.

Popp, M.; Lindsay, K.; Ashworth, A.; Moore, P.; Owens, P.; Adams, T.; McCarver, M.; Roark, B.; Pote, D.; Pennington, J. 2021. Economic and GHG emissions changes of aeration and gypsum application. *Agriculture, Ecosystems & Environment*, 321: 107616. <https://doi.org/10.1016/j.agee.2021.107616>.

Prakash, N. B.; Dhumgond, P.; Shruthi, S. 2020. Performance of slag-based gypsum on maize yield and available soil nutrients over commercial gypsum under acidic and neutral soil. *Communications in Soil Science and Plant Analysis*, 51: 1780-1798. <https://doi.org/10.1080/00103624.2020.1791161>

Qayyum, M. F.; Rehman, M. Z. U.; Ali, S.; Rizwan, M.; Naeem, A.; Maqsood, M. A.; Khalid, H.; Rinklebe, J.; Ok, Y. S. 2017. Residual effects of monoammonium phosphate, gypsum and elemental sulfur on cadmium phytoavailability and translocation from soil to wheat in an effluent irrigated field. *Chemosphere*, 174: 515-523. <https://doi.org/10.1016/j.chemosphere.2017.02.006>.

Qu, J.; Zhang, L.; Zhang, X.; Gao, L.; Tian, Y. 2020. Biochar combined with gypsum reduces both nitrogen and carbon losses during agricultural waste composting and enhances overall compost quality by regulating microbial activities and functions. *Bioresour. Technol.* 314, 123781.

- Raimondi, G.; Maucieri, C.; Toffanin, A.; Renella, G.; Borin, M. 2021. Smart fertilizers: What should we mean and where should we go? *Italian Journal of Agronomy*, 16(2): 1794. <https://doi.org/10.4081/ija.2021.1794>
- Raine, S. R.; Loch, R. J. 2003. What is a sodic soil? Identification and management options for construction sites and disturbed lands. In: *Road, Structures and Soils in South East Queensland 29-30th* (Department of Main Roads, Queensland). 14 p.
- Ramos, B. Z.; Toledo, J. P. V. F.; Lima, J. M.; Serafim, M. E.; Bastos, A. R. R.; Guimarães, P. T. G.; Coscione, A. R. 2013. Gypsum applications to coffee: influence on calcium, magnesium and potassium contents and pH of the solution of a dystrophic red latosol *Revista Brasileira de Ciências do Solo*. 37(4): 1018-1026. <https://doi.org/10.1590/S0100-06832013000400019>
- Rampim, L.; Lana, M. C.; Frandoloso, J. F.; Fontaniva, S. 2011. Atributos químicos de solo e resposta do trigo e da soja ao gesso em sistema semeadura direta. *Revista Brasileira de Ciência do Solo*, 35: 1687-1698. <https://doi.org/10.1590/S0100-06832011000500023>
- Rashmi, I.; Mina, B. L.; Kumar, K.; Ali, S.; Kumar, A.; Kala, S.; Singh, R. K. 2018. Gypsum - an inexpensive, effective sulphur source with multitude impact on oilseed production and soil quality: A review. *Agricultural Reviews*, 39: 218-225. <https://doi.org/10.18805/AG.R-1792>
- Rawat, A.; Kumar, R.; Singh, V. P.; Bhatt, B. 2020 Effect of plant based nano-sized gypsum on growth parameters and yield of wheat (*Triticum aestivum* L.). *International Journal of Chemical Studies*, 8(4): 2991-2993. <https://doi.org/10.22271/chemi.2020.v8.i4aj.10105>
- Raza, A.; Mubarik, M. S.; Sharif, R.; Habib, M.; Jabeen, W.; Zhang, C.; Chen, H.; Chen, Z.-H.; Siddique, K. H. M.; Zhuang, W.; Varshney, R. K. 2023. Developing drought-smart, ready-to-grow future crops. *Plant Genome*, 16: e20279. <https://doi.org/10.1002/tpg2.20279>
- Rehman, M.; Fahad, S.; Du, G.; Cheng, X.; Yang, Y.; Tang, K.; Liu, L.; Liu, F. H.; & Deng, G. 2021. Evaluation of hemp (*Cannabis sativa* L.) as an industrial crop: a review. *Environmental Science and Pollution Research International*, 28(38): 52832-52843. <https://doi.org/10.1007/s11356-021-16264-5>
- Rezapour, S.; Asadzadeh, F.; Barin, M.; Nouri, A. 2021. Organic amendments improved the chemical-nutritional quality of saline-sodic soils. *International Journal of Environmental Science and Technology*, 19(6): 4659-4672. <https://doi.org/10.1007/s13762-021-03599-2>
- Ritchey, K. D.; Feldhake, C. M.; Clark, R. B.; Sousa, D. M. G. 1995. Improved water and nutrient uptake from subsurface layers of gypsum-amended soils. In: Karlen, D. L. et al. (Ed.), *Agricultural Utilization of Urban and Industrial By-Products*. ASA Spec. Publ., vol. 58. ASA, CSSA, and SSSA, Madison, WI, pp. 157-181. <https://doi.org/10.2134/asaspecpub58.c8>
- Ritchey, K. D.; Souza, D. M. G.; Lobato, E.; Correa, O. 1980. Calcium leaching to increase rooting depth in a Brazilian savannah oxisol. *Agronomy Journal*, 72: 40-44. <https://doi.org/10.2134/agronj1980.00021962007200010009x>
- Robinson, M. J. C.; Dhar, A.; Naeth, M. A.; Nichol, C. K. 2023. Phosphogypsum impacts on soil chemical properties and vegetation tissue following reclamation. *Environmental Monitoring and Assessment*, 195: 769. <https://doi.org/10.1007/s10661-023-11379-3>

- Rossato, O. B.; Foltran, R.; Crusciol, C. A. C.; Martello, J. M.; Rossetto, R.; Mccray, J. M. 2017. Soil fertility, ratoon sugarcane yield, and post-harvest residues as affected by surface application of lime and gypsum in southeastern Brazil. *Bioscience Journal*, 33(2): 276-287. <https://doi.org/10.14393/BJ-v33n2-32755>
- Santos, C. A.; Carmo, M. G. F.; Bhering, A. S.; Costa, E. S. P.; Amaral Sobrinho, N. M. B. 2020. Use of limestone and agricultural gypsum in cauliflower crop management and clubroot control in mountain farming. *Acta Scientiarum Agronomy*, 42: 2-11. <https://doi.org/10.4025/actasciagron.v42i1.42494>
- Santos, H. G.; Jacomine, P. K. T.; Anjos, L. H. C.; Oliveira, V. A.; Lumbreras, J. F.; Coelho, M. R.; Almeida, J. A.; Araújo Filho, J. C.; Oliveira, J. B.; Cunha, T. J. F. 2018. Sistema Brasileiro de Classificação de Solos. 5 ed. Brasília, DF: Embrapa. 356 p. Available at: <https://www.infoteca.cnptia.embrapa.br/handle/doc/1094003>
- Schlossberg, M. J.; Miller, W. P.; Kruse, J. 2006. Turfgrass growth and water use in gypsum-treated Ultisols. The ASA-CSSA-SSSA International Annual Meetings. Indianapolis, IN. November 2006. Available at: <https://acs.confex.com/crops/2006am/techprogram/P27169.HTM>
- Schultz, E.; Chatterjee, A.; DeSutter, T.; Franzen, D. 2017. Sodic soil reclamation potential of gypsum and biochar additions: influence on physicochemical properties and soil respiration. *Communications in Soil Science and Plant Analysis*, 48(15): 1792-1803. <https://doi.org/10.1080/00103624.2017.1395449>
- Scott, W. D.; McCraw, B. D.; Motes, J. E.; Smith, M. W. 1993. Application of calcium to soil and cultivar affect elemental concentration of watermelon leaf and rind tissue. *Journal of the American Society of Horticultural Science*, 118: 201-206. <https://doi.org/10.21273/JASHS.118.2.201>
- Shainberg, R.; Sumner, M.E., Miller, W.P., Farina, M.P.W., Pavan, M.A.; Fey, M.W. 1989. Use of gypsum on soils: a review. In: Stewart, B.A. (Ed), *Advances in Soil Science*, 9. Springer. p. 1-111 https://doi.org/10.1007/978-1-4612-3532-3_1
- Shamshuddin, J.; Ismail, H. 1995. Reactions of ground magnesium limestone and gypsum in soils with variable-charge minerals. *Soil Science Society of America Journal*, 59: 106-112. <http://dx.doi.org/10.2136/sssaj1995.03615995005900010017x>
- Soil Science Society of America. 1997. Glossary of soil science terms. SSSA, Madison (EUA). 134 p.
- Somavilla, L.; Pinto, M. A. B.; Basso, C. J.; Da Ros, C. O.; Silva, V. R.; Brun, T.; Santi, A. L. 2016. Response of soybean and corn to soil mechanical intervention and agricultural gypsum application to the soil surface. *Semina: Ciências Agrárias*, 37, 95-102. <https://doi.org/10.5433/1679-0359.2016v37n1p95>
- Song, W.; Yim, J.; Kurniadinata, O. F.; Wang, H.; Huang, X. 2018. Linking fruit Ca uptake capacity to fruit growth and pedicel anatomy, a cross-species study. *Frontiers in Plant Science*, 9: 575. <https://doi.org/10.3389/fpls.2018.00575>
- Soratto, R. P.; Crusciol, C. A. C. 2008. Dolomite and phosphogypsum surface application effects on annual crops nutrition and yield. *Agronomy Journal*, 100, 261-270. <https://doi.org/10.2134/agrojn12007.0120>
- Sousa, D. M. G.; Lobato, E. 2004. Cerrado: correção do solo e adubação. 2. ed. Planaltina: Embrapa Cerrados. 416 p. Available at: <https://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/555355>

- Sousa, D. M. G.; Lobato, E.; Ritchey, K. D.; Rein, T. A. 1992. Suggestions for diagnosis and recommendation of gypsum application for soil of the Cerrados. In: II Seminário Sobre o Uso do Gesso na Agricultura. Anais... IBRAFOS, Uberaba, Brazil, pp. 139-158.
- Sousa, D. M. G.; Miranda, L. N.; Oliveira, A. S. 2007. Acidez do solo e sua correção. In: Novais, R. F.; Alvarez, V. V. H.; Barros, N. F.; Fontes, R. L.; Cantarutti, R. B.; Neves, J. C. L. (eds.) Fertilidade do Solo. Viçosa, MG: Sociedade Brasileira de Ciência do Solo. p. 205-274.
- Sousa, D. M. G.; Vilela, L.; Lobato, E.; Soares, W. V. 2001. Uso de gesso, calcário e adubos para pastagens no cerrado. Circular Técnica 12. Planaltina, DF: Embrapa Cerrados. 22 p. Available at: <https://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/566086>
- Srivastava, S.; Pandey, V. K.; Singh, R.; Dar, A. H.; Dash, K. K.; Panesar, P. S. 2022. A critical review on artificial intelligence and robotic vision in food industry. International Journal of Science & Critics, 1(1): 8-17. Available at: <https://www.sciencecritics.com/wp-content/uploads/2023/01/Review-Article-IJSC22D002.pdf>
- Sumner, M. E. 1993. Gypsum and acid soils - the world scene. Advances in Agronomy, 51(51): 1-32. [https://doi.org/10.1016/S0065-2113\(08\)60589-1](https://doi.org/10.1016/S0065-2113(08)60589-1)
- Sumner, M. E.; Radcliffe, D. E.; McCray, M.; Carter, E.; Clark, R. L. 1990. Gypsum as an ameliorant for subsoil hardpans. Soil Technology, 3: 253-258. [https://doi.org/10.1016/0933-3630\(90\)90005-N](https://doi.org/10.1016/0933-3630(90)90005-N)
- Sun, L.; Ma, Y.; Liu, Y.; Li, J.; Deng, J.; Rao, X.; Zhang, Y. 2019. The combined effects of nitrogen fertilizer, humic acid, and gypsum on yield-scaled greenhouse gas emissions from a coastal saline rice field. Environmental Science and Pollution Research, 26: 19502-19511. <https://doi.org/10.1007/s11356-019-05363-z>
- Syed-Omar, S. R.; Sumner, M. E. E. 1991. Effect of gypsum on soil potassium and magnesium status and growth of alfalfa. Communication in Soil Science and Plant Analysis, 22: 2017-2028. <https://doi.org/10.1080/00103629109368554>
- Tavakkoli, E.; Uddin, S.; Rengasamy, P.; McDonald, G. K. 2021. Field applications of gypsum reduce pH and improve soil C in highly alkaline soils in southern Australia's dryland cropping region. Soil Use Management, 38: 466-477. <https://doi.org/10.1111/sum.12756>
- Tayade, R.; Ghimire, A.; Khan, W.; Lay, L.; Attipoe, J. Q.; Kim, Y. 2022. Silicon as a smart fertilizer for sustainability and crop improvement. Biomolecules, 12(8): 1027. <https://doi.org/10.3390/biom12081027>
- Thor, K. 2019. Calcium - nutrient and messenger. Frontiers in Plant Science, 10: 440. <https://doi.org/10.3389/fpls.2019.00440>
- Tiecher, T.; Pias, O. H. C.; Bayer, C.; Martins, A. P.; Denardin, L. G. O.; Anghinoni, I. 2018. Crop response to gypsum application to subtropical soils under no-till in Brazil: a systematic review. Revista Brasileira de Ciência do Solo. 42: e0170025. <https://doi.org/10.1590/18069657rbcS20170025>
- Tirado-Corbalá, R.; Slater, B. K.; Dick, W. A.; Bigham, J.; Muñoz-Muñoz, M. 2019. Gypsum amendment effects on micromorphology and aggregation in no-till Mollisols and Alfisols from western Ohio, USA. Geoderma Regional, 16, e00217. <https://doi.org/10.1016/j.geodrs.2019.e00217>

Tirado-Corbalá, R.; Slater, B. K.; Dick, W. A.; Bigham, J.; Mccoy, E. 2013. Hydrologic properties and leachate nutrient responses of soil columns collected from gypsum-treated fields. *Soil and Tillage Research*, 134, 232-240. <https://doi.org/10.1016/j.still.2013.08.007>

Toma, M.; Sumner, M. E.; Weeks, G.; Saigusa, M. 1999. Long-term effects of gypsum on crop yield and subsoil chemical properties. *Soil Science Society of America Journal*, 63: 891-895. <https://doi.org/10.2136/sssaj1999.634891x>

Tonetto de Freitas, S.; Mitcham, E. J. 2012. Factors involved in fruit calcium deficiency disorders. *Horticultural Reviews (chapter 3)*, 40: 107-146. <https://doi.org/10.1002/9781118351871.ch3>

Truman, C. C.; Nuti, R. C.; Truman, L. R.; Dean, J. D. 2010. Feasibility of using FGD gypsum to conserve water and reduce erosion from an agricultural soil in Georgia. *Catena*, 81: 234-239. <https://doi.org/10.1016/j.catena.2010.04.003>

Tubail, K.; Chen, L.; Michel Jr., F. C.; Keener, H. M.; Rigot, J. F.; Klingman, M.; Kost, D.; Dick, W. A. 2008. Gypsum additions reduce ammonia nitrogen losses during composting of dairy manure and biosolids. *Compost Science & Utilization*, 16(4): 285-293. <http://dx.doi.org/10.1080/1065657X.2008.10702390>

USEPA (United States Environmental Protection Agency). 2023. Beneficial use evaluation: flue gas desulfurization gypsum as an agricultural amendment. United States Department of Agriculture (EPA Contract No. EP-W-15-005). Available at: https://www.epa.gov/system/files/documents/2023-03/FGD_Ben_Use_Eval_with_Appendices_March_2023_508.pdf

Van Raij, B. 2008. Gesso na agricultura. Campinas: Instituto Agrônomo, Fundação IAC, n° 117. 233 p.

Van Raij, B. 2010. Melhorando o ambiente radicular em subsuperfície. In: Prochnow, L.I., Casarin, V., Stipp, S.R. ed. *Boas práticas para uso eficiente de fertilizantes: contexto mundial e técnicas de suporte*. v. 1. Piracicaba: INPI-Brasil, p. 349-382.

Van Raij, B.; Cantarella, H.; Quaggio, J. A.; Furlani, A. M. C. 1997. Recomendações de adubação e calagem para o Estado de São Paulo (Boletim Técnico, 100), 2 ed. Instituto Agrônomo/Fundação IAC, Campinas. 285 p. Available at: <https://www.iac.sp.gov.br/publicacoes/boletim100.php>

Verma, K. K.; Song, X. -P.; Joshi, A.; Tian, D. -D.; Rajput, V. D.; Singh, M.; Arora, J.; Minkina, T.; Li, Y. -R. 2022. Recent trends in nanofertilizers for sustainable agriculture under climate change for global food security. *Nanomaterials*, 12: 173. <https://doi.org/10.3390/nano12010173>

Vitti G. C.; Queiroz F. E. C.; Otto R.; Quintino T. A. 2005. Nutrição e adubação da cana-de-açúcar. Departamento de Solos e Nutrição de Plantas, ESALQ/USP, Piracicaba, SP. 78 p.

Vitti, G. C.; Mazza, J. A. 2002. Planejamento, estratégias de manejo e nutrição da cultura de cana-de-açúcar. POTAFOS - Informações Agronômicas, n° 97. 16 p. Available at: [http://www.ipni.net/publication/ia-brasil.nsf/0/504B40E488537AE083257AA2005EA7F6/\\$FILE/Encarte%2097.pdf](http://www.ipni.net/publication/ia-brasil.nsf/0/504B40E488537AE083257AA2005EA7F6/$FILE/Encarte%2097.pdf)

Vitti, G. S.; Luz, P. H. C.; Malavolta, E.; Dias, A. S.; Serrano, C. G. E. 2008. Uso de gesso em sistemas de produção. Piracicaba: GAPE, 104 p.

Wakchaure, M.; Patle, B. K.; Mahindrakar, A. K. 2023. Application of AI techniques and robotics in agriculture: a review. *Artificial Intelligence in the Life Sciences*, 3: 100057. <https://doi.org/10.1016/j.ailsci.2023.100057>

- Walia, M. K.; Dick, W. A. 2018. Selected soil physical properties and aggregate-associated carbon and nitrogen as influenced by gypsum, crop residue, and glucose. *Geoderma*, 320: 67-73. <https://doi.org/10.1016/j.geoderma.2018.01.022>
- Walker, M. E.; Csinos, A. S. 1980. Effect of gypsum on yield, grade and incidence of pod rot in five peanut cultivars. *Peanut Science*, 7 (2): 109-113. <https://doi.org/10.3146/i0095-3679-7-2-13>
- Wallace, A. 1994. Use of gypsum on soil where needed can make agriculture more sustainable. *Communications in Soil Science and Plant Analysis*, 25(1-2): 109-116, <https://doi.org/10.1080/00103629409369015>
- Wang, J. M.; Yang, P. L. 2018. Potential flue gas desulfurization gypsum utilization in agriculture: A comprehensive review. *Renewable and Sustainable Energy Reviews*, 82: 1969-1978. <https://doi.org/10.1016/j.rser.2017.07.029>
- Wang, Y.; Wang, Z.; Liang, F.; Jing, X.; Feng, W. 2021. Application of flue gas desulfurization gypsum improves multiple functions of saline-sodic soils across China. *Chemosphere*, 277: 130345. <https://doi.org/10.1016/j.chemosphere.2021.130345>
- Watts, D. B.; Dick, W. A. 2014. Sustainable uses of FGD gypsum in agricultural systems: Introduction. *Journal of Environment Quality*, 43(1): 246-252. <https://doi.org/10.2134/jeq2013.09.0357>
- Watts, D. B.; Runion, G. B.; Torbert, H. A. 2021. Influence of flue gas desulfurization gypsum on phosphorus loss from a horticultural growth medium. *Horticulturae*, 7: 199. <https://doi.org/10.3390/horticulturae7070199>
- White, P. J.; Broadley, R. 2003. Calcium in plants. *Annals of Botany*, 92: 487-511. <https://doi.org/10.1093/aob/mcg164>
- Xu, X.; Wang, J.; Tang, Y.; Cui, X.; Hou, D.; Jia, H.; Wang, S.; Guo, L.; Wang, J.; Lin, A. 2023. Mitigating soil salinity stress with titanium gypsum and biochar composite materials: Improvement effects and mechanism. *Chemosphere*, 321: 138127. <https://doi.org/10.1016/j.chemosphere.2023.138127>
- Yahya, K. E.; Jia, Z.; Luo, W.; YuanChun, H.; Ame, M. A. 2022. Enhancing salt leaching efficiency of saline-sodic coastal soil by rice straw and gypsum amendments in Jiangsu coastal area. *Ain Shams Engineering Journal*, 13(5): 101721. <https://doi.org/10.1016/j.asej.2022.101721>
- Yamika, W. S. D.; Aini, N.; Setiawan, A.; Purwaningrahyu, R. D. 2018. Effect of gypsum and cow manure on yield, proline content, and K/Na ratio of soybean genotypes under saline conditions. *Journal of Degraded and Mining Lands Management*, 5(2): 1047-1053. <https://doi.org/10.15243/jdmlm.2018.052.1047>
- Zahra, N.; Sarwar, G. 2015. Comparison of gypsum and potassium silicate for improving yield and yield components of rice. *International Journal of Agricultural and Applied Sciences*, 7(1): 1-6. Available at: https://www.academia.edu/download/49702340/comparison_of_gypsum_and_potassium_silic20161018-11950-1fp6o9v.pdf
- Zambrosi, F. C. B.; Alleoni, L. R. F.; Caires, E. F. 2007. Gypsum application and ionic speciation of the solution from an oxisol under no-till system. *Ciência Rural*, 37: 110-117. <https://doi.org/10.1590/S0103-84782007000100018>

Zenda, T.; Liu, S.; Dong, A.; Duan, H. 2021. Revisiting sulphur - the once neglected nutrient: It's roles in plant growth, metabolism, stress tolerance and crop production. *Agriculture*, 11: 626. <https://doi.org/10.3390/agriculture11070626>

Zeng, H.; Dhiman, G.; Sharma, A.; Sharma, A.; Tselykh, A. 2023. An IoT and Blockchain-based approach for the smart water management system in agriculture. *Expert System*, e12892. <https://doi.org/10.1111/exsy.12892>

Zhao, Y.; Wang, S.; Li, Y.; Zhuo, Y.; Liu, J. 2019. Sustainable effects of gypsum from desulphurization of flue gas on the reclamation of sodic soil after 17 years. *European Journal of Soil Science*, 70(5): 1082-1097. <https://doi.org/10.1111/ejss.12807>

Zheng, Z. Z.; Shen, J. Q.; Pan, W. H.; Pan, J. W. 2013. Calcium sensors and their stress signaling pathways in plants. *Hereditas*, 35(7): 875-884. <https://doi.org/10.3724/sp.j.1005.2013.00875>

Zielonka, D.; Nierebiński, M.; Kalaji, H. M.; Augustynowicz, J.; Prędecka, A.; Russel, S. 2018. Efficiency of the photosynthetic apparatus in *Cannabis sativa* L. fertilized with sludge from a wastewater treatment plant and with phosphogypsum. *Ecological Questions*, 28: 55-61. <https://doi.org/10.12775/EQ.2017.039>

Zoca, S. M.; Penn, C. 2017. An important tool with no instruction manual. *Advances in Agronomy*, 144: 1-44. <https://doi.org/10.1016/bs.agron.2017.03.001>

PHENOTYPIC CORRELATIONS BETWEEN LINEAR TRAITS AND GROWTH CURVES OF HOLSTEIN CALVES

Acceptance date: 02/05/2024

Leidiane Bora Cardoso

Veterinary Medicine Course - UniSanta Cruz, Curitiba - PR

Wellington Hartmann

Professor doctor - UniSanta Cruz, Curitiba - PR

ABSTRACT: The selection of productive traits have great importance for the good performance of dairy farms. Strategies are developed with the aim of promoting the genetic improvement of the desired traits in future generations. The general objective of this study was to establish the growth curve of the Holstein breed, considering factors that contribute to precocity, especially in relation to body weight and withers height. Monthly measurements of body weight and withers height, as well as rump measurements, were carried out, enabling the monitoring of the development of Holstein calves in a dairy farm in São José dos Pinhais, Paraná. The results showed high and positive correlations (r) between the different measurements, as follows: (a) withers height x rump length: $r = 0.91$; (b) rump ileus width x body weight: $r = 0.86$; (c) rump ischium width x body weight: $r =$

0.96 ; (d) rump ileus width x rump ischium width: $r = 0.84$; (e) rump length x rump ileus width: $r = 0.84$ and (f) rump length x rump ischium width: $r = 0.98$. Average daily weight gain was highest in the first six months of age, followed by a slowdown due to pre-puberty behavioral factors. The importance of growth curves for breeding programs is emphasized, demonstrating the need for efficient management to optimize future reproductive performance. The correlation between body development and the anticipation of heat externalization in calves is relevant for reproductive management.

KEYWORDS: Growth curve; Herd monitoring; Heifers.

INTRODUCTION

The body development of calves is the initial challenge in dairy cattle farming because it is the group of animals that will have the responsibility of replacing the cows in the herd. The goals established by the technical areas for the Holstein breed include: mortality of less than 10% and weaning at 60 days, when it is recommended that it presents 2.5 times

the birth weight, with consumption of 2.0 kg of concentrate/day, making it possible to reach sexual maturity early, at 13 months, with 60% of adult weight.

To achieve this goal, it is necessary to minimize the incidence of diseases in the first four months of life, following prophylaxis recommendations (AZEVEDO et al., 2016). Genetic characteristics related to the progenitors influence body development, and allied to these, nutritional factors. Right at birth, calves are monogastric and unable to use solid food in their diet, but with physiological and biochemical aptitude for milk use. However, with correct feeding management, in 45 days they can be weaned and considered ruminants (COELHO et al., 2009).

Attention to the category of calves begins during pregnancy. It is the period characterized by dramatic metabolic changes in the pregnant cow, which should have their effect minimized under the provision of an anionic diet. Cows have their lactation interrupted at 60 days before the expected date of calving, and this period is called the transition period. Meeting nutritional needs is directly related to the development of the fetus and the beginning of the cow's lactation curve in the postpartum period (WEICH et al., 2013).

With reference to the weaning phase, solid food should be provided to calves during the lactation period, promoting the development of rumen papillae. This is achieved with hay, and so weaning can take place at 60 days of age. At this stage, fermented foods, such as silage, are not yet recommended. After three months of age, the combination of silage and hay can be used, but only of optimal quality. It is important that water is constantly available to make up for the lack of saliva in the young calf and thus ensure a good fermentation in the rumen, thus stimulating the intake of concentrate (SANTOS et al., 2002). The concentrates fed to the calves must have a high grain size or coarse texture to cause the movement of the reticulum-rumen, rumination, salivation and the maintenance of adequate pH (COELHO et al., 2009).

It is of great importance to pay special attention to health care, with the administration of antiparasitic drugs from 60 days onwards, and the vaccination calendar established, taking into account: pneumoenteritis, brucellosis, clostridiosis (anthrax and botulism) and rabies. In the region studied, there is also a need for vigilance with reference to babesiosis and anaplasmosis. Vaccination against foot-and-mouth disease should be carried out in the states where it occurs, and Paraná is a free area without vaccination (OMSA, 2021).

The reproductive development of females depends on the age at which they give birth for the first time. Among the advantages of impregnating younger heifers are: shorter time to obtain return on investment, increased number of calves produced and lactations, translating into increased reproductive and productive life, and faster selection in the herd by reducing the interval between generations (COZLER et al., 2019). The high speed of growth in calves is desirable in dairy farms to result in the production of high quality heifers, which show early sexual maturity with minimal cost (BORO et al., 2016).

GOAL

The objective of the present study was to delineate the growth curve of the Holstein Breed in relation to body weight and withers height, considering the factors that impact positively sexual precocity, allowing the first birth to occur at two years of age. In addition, to estimate the phenotypic correlations between body weight, withers height, and rump length; and to obtain the mathematical models that explain the curves of body weight, withers height, and rump length in relation to age.

MATERIAL AND METHODS

In the period between March 2023 and March 2024, the development of forty-six Holstein heifers was monitored, from the suckling stage to one year of age, in a dairy farm located in São José dos Pinhais, Paraná. At the time of birth, each calf received four liters of colostrum and sanitary care was immediately performed, especially the treatment of the navel. In the first 60 days of life, milk was fed in the morning and late afternoon, corresponding to 10% of the body weight per day. After consumption, at the time of washing the buckets, good quality water was provided, remaining ad libitum. There was also the gradual introduction of 20% protein concentrate, in increasing amounts, from 0.5 to 2.0 kg per day.

At 50 days of age, they had access to hay. After this period, the weaning process was implemented, with the gradual remove of milk until 60 days, when the calves began to be fed exclusively with concentrate and hay. Silage was introduced after 100 days. The handling system adopted on this farm is semi-intensive, with the calves remaining for the first four months in a collective calf, where they share the space with animals of the same age group, and later transferred to a paddock formed by Tifton pasture (*Cynodon spp*), with a covered trough for concentrate and silage. In this phase, they consumed 0.9 to 1.8 kg/head/day of concentrate.

To evaluate development, monthly measurements were performed of the following dimensions: (1) withers height, (2) rump width at the ileum (RWI), (3) rump width at the ischium (RWIsc), (4) rump length (RL – measured from the sacral tuberosity of the ileum to the ischial tuberosity, following the line of the spine), (5) body weight (by chest circumference), and (6) withers height (from the withers to the ground). These measurements allowed us to estimate (7) croup surface area (ASR) and (8) average daily weight gain (ADWG). The database consisted of 4.416 pieces of information ($n = 4.416$).

Pearson's linear correlation coefficient (r) was calculated in order to investigate the relationships between data variability. For the study of multiple regressions, their accuracy and correlation studies, the statistical program Assistat® version 7.7 pt was used. The study of correlations is an important tool in genetic improvement and herd management, because it allows the use of positive bulls for one characteristic to obtain the concomitant improvement of others.

RESULTS

Rump development

The measurement of the rump is an important part of information in progeny tests, and should be a criterion for the selection of dairy heifers, because the greater the width, the easier it will be for the animal to give birth, in addition to providing better dorsal support of the udder (ARMELIN and HARTMANN, 2021). The study addressed the behavior of the rump surface in relation to calf development and its trend line. To calculate the rump surface area, the following equation was used: $RSA = ((RWI + RWIsc) / 2) \times RL$ (rump length).

There was a decrease in growth rate between 7 and 12 months of age, considered the prepuberty period. The trend line was obtained with the coefficient of determination: $R^2 = 0.9115$. The development of the rump surface in proportion to age can be seen in Figure 1.

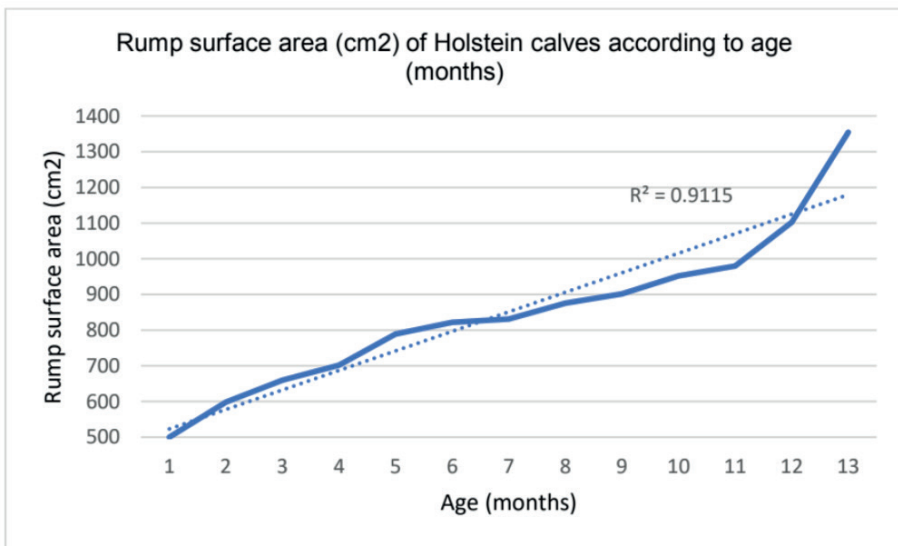


Figure 1 - Rump surface and age of Holstein calves in São José dos Pinhais, PR, from March 2023 to March 2024. N = 46.

Source: Cardoso e Hartmann (2024)

With reference to rump development, growth acceleration was observed after 12 months of age. There was uniform development between rump length and rump ileus width up to 9 months, as shown in Figure 2.

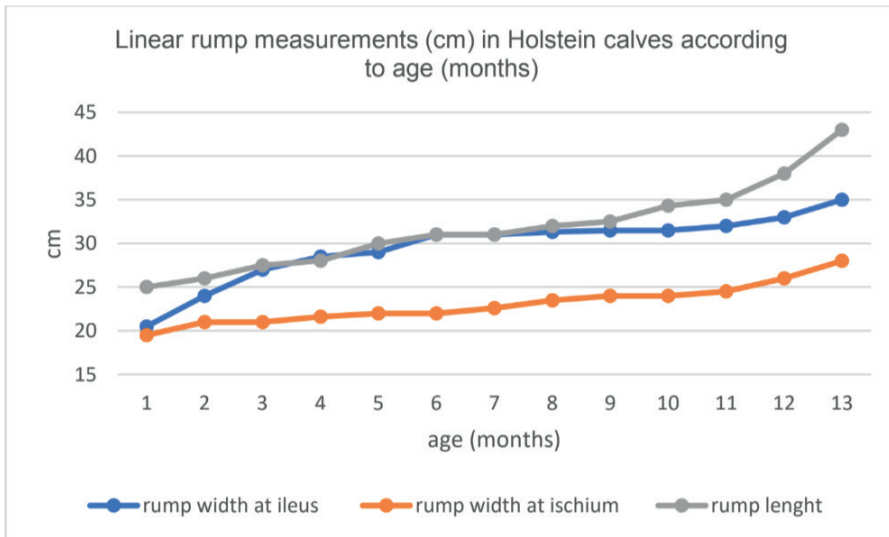


Figure 2 - Development of rump measurements in Holstein calves in São José dos Pinhais, PR, from March 2023 to March 2024. N = 46.

Source: Cardoso e Hartmann (2024)

The correlations calculated were: (a) withers height x rump length: $r = 0.91$; (b) rump width at ileus x body weight: $r = 0.86$; (c) rump width at ischium x body weight: $r = 0.96$; (d) rump width at ileus x rump width at ischium: $r = 0.84$; (e) rump length x rump width at ileus: $r = 0.84$ and (f) rump length x rump width at ischium: $r = 0.98$.

The correlation between body weight and withers height was: $r = 0.95$.

These correlations showed high values, indicating that, when selection occurs for one characteristic, there is simultaneously improvement of another among those studied.

Withers height and body weight

The results of the height and weight measurements can be seen in Figure 3, as well as the regression curves that allowed the development of the respective trend line equations, with a high degree of accuracy. A high correlation between height and birth weight until the age of 12 months was observed: $r = 0.96$.

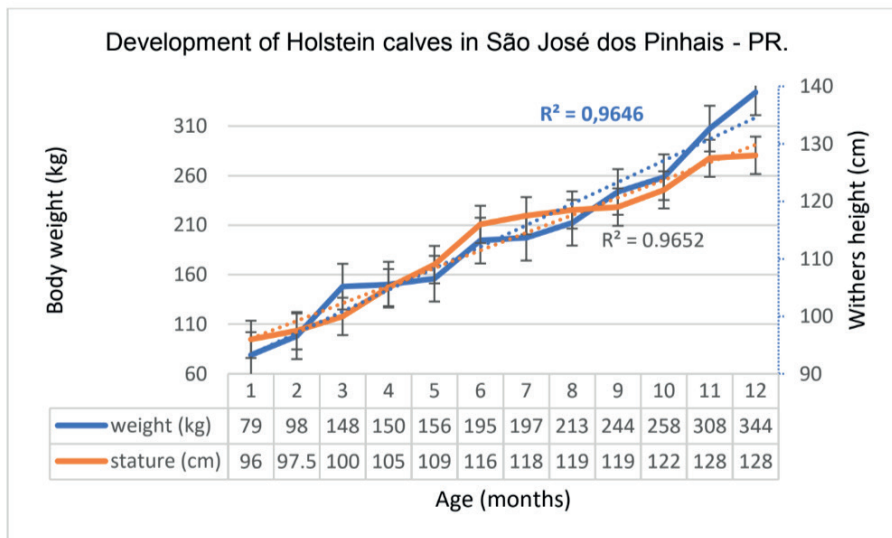


Figure 3: Body weight and withers height of Holstein calves in São José dos Pinhais, PR, Brazil. N = 46.

Fonte: Cardoso e Hartmann (2024)

The trend lines showed high data reliability, being respectively: $R^2 = 0.9646$ for body weight and $R^2 = 0.9652$ for withers height.

Average Daily Weight Gain (ADWG)

The average daily weight gain (ADWG) was calculated monthly, with the average weights, using the equation:

$$\text{ADWG} = \frac{\text{weight 2} - \text{weight 1}}{\text{days of interval}}$$

Thus, calves in the age group between birth and weaning (60 days) had a ADWG of 0.883 kg on average ($n = 46$). After weaning, a decrease in feed conversion and weight gain was observed, resulting in a ADWG of 0.806 kg, in the categories of calves and heifers from 61 days to 365 days of age. These values are adequate, because in this category special attention should be paid to avoid overweight, which would result in the accumulation of adipose tissue in the mammary gland.

DISCUSSION

The data showed a higher rate of weight gain in the period between birth and the age of six months. However, a subsequent deceleration in the growth rate is observed, probably due to behavioral factors intrinsic to the pre-puberty phase, negatively influencing dry matter intake. Management elements, such as feeding, disease and parasite control, and environmental conditions, have a direct influence.

The knowledge of growth curves helps genetic improvement programs for the selection of animals with precocity and higher expected yields (BERGAMASCO et al., 2001).

Management should be directed towards obtaining reproductive precocity to reduce costs on the farm. In addition, it is essential to consider the morphology of the female calves, especially the rump, which plays an important role in the ease of calving, postpartum recovery, and mobility of the animal. The ideal rump should be wide, long in the lateral and posterior view, gently joined to the loin, and the hip joint should be well separated, without fat accumulation (VALLOTO e PEDROSA, 2018). These morphological aspects are decisive to ensure not only the current performance of the cows, but also the longevity and future productivity in the dairy context.

Growth refers to an increase in linear size, weight, fat accumulation in tissues, and nitrogen and water retention. Growth is a highly complex and integrated process that involves increasing of the number and size of cells and the deposition of substances within them. It involves interaction between nutrients, environment, genotype, hormones and receptors for these hormones from different tissues (BORO et al., 2016).

Evaluating the peak of Luteinizing Hormone (LH), Getzewich (2005) concluded that puberty in Holstein heifers occurs around 11 months of age. It has been observed the anticipation of the exteriorization of heat in calves in recent years. This fact is relevant, and should be observed concomitantly with body development, especially in relation to weight, height, body length, and rump length and width, enabling heifers to enter at reproductive management without, however, hindering their growth (HARTMANN et al., 2023). Sexual maturity is highly dependent on growth rate, as it is a function of body weight rather than age (BORO et al., 2016).

Puberty in females is defined as the age at which there is ovarian cyclicity. The onset of puberty is characterized by an increase in the concentration of progesterone in the plasma, reaching levels above 1 ng/mL, which can occur from 6 to 10 months of age, depending on genetic and environmental factors (MADGWICK et al., 2005). Puberty occurs when the heifer's weight is between 40 and 50% of the adult body weight, regardless of age, but the onset of reproductive life should occur when the heifers reach 55-60% of the adult body weight. The growth rate should be maintained during gestation in such a way that heifers weigh 80 to 85% of mature body weight at first calving. Lower growth rate is associated with a late onset of puberty (BORO et al., 2016).

The mean values of weight gain in the prepubertal period found in the present study were 0.790 g/day (\pm 0.040), between 150 and 320 kg, in agreement with reports by Zanton and Heinrichs (2005). According to these authors, the average daily weight gain in prepubertal age is related to allometric growth of the mammary gland and, as a consequence, there is a maximization of milk production in the first lactation. On the other hand, high ADWG values, above those obtained, are harmful because they promote the reduction of somatotropin circulation, and result in a reduction in the development of the mammary gland.

FINAL THOUGHTS

The correct management of calves is an important element to ensure the best development of the animals and, consequently, the maximum productivity of the farm, considering that it is the category that will replace adult cows in the future. Dairy farms want to obtain animals with high milk productivity, so the main challenge lies in meeting several factors, including genetics, environment, nutrition and management, to achieve the expected goals.

In this context, monitoring and evaluating the morphological conditions of female calves becomes essential to achieve the desired results. In addition to contributing to the improvement of farm management, this practice provides valuable data that guides possible improvements or adjustments, providing a clear direction to improve herd performance.

CONCLUSION

Monitoring height and weight is recommended, as these are highly correlated phenotypic characteristics, with the aim of showing early sexual maturity at the appropriate weight. The high correlation observed between withers height and rump length ($r = 0.91$) and between rump length and rump width at ischium allows us to infer that the selection of female calves by height results in animals with longer and wider rumps. In adult cows, it is observed that this factor brings benefits to the greater length of the mammary gland and factors related to the ease of calving and development of the udder.

REFERENCES

- ARMELIN, I. C. A.; HARTMANN, W. Índices de crescimento em bezerras da raça Holandesa. *Revista Eletrônica Biotecnologia, Biotecnologia e Saúde*, Curitiba/PR, n. 30, maio-ago. 2021.
- AZEVEDO, S. R. A.; SILVA, J. C. S.; AZEVEDO, C. C. F. B. et al. Manejo alimentar de bezerras leiteiras. *Diversitas Journal*. Santana do Ipanema/AL. v. 1, n. 1, p.100-112, jan./abr. 2016.
- BERGAMASCO, A. F.; AQUINO, L. H.; MUNIZ, J. A. Ajuste de modelos não lineares a dados de crescimento de fêmeas da raça Holandesa. *Ciência Agrotécnica*, Lavras/MG, v. 25, n. 2, p. 235-241, 2001.
- BORO, P.; NAHA, B. C.; MADKAR, A. R. et al. The effects of heifer growth on milk production and efficiency. *International Journal of Science and Nature*. ISSN 2229 – 6441. v. 7 (2) 220-227. 2016.
- COELHO, S. G.; GONÇALVES, L. C.; COSTA, T. C.; FERREIRA, C. S. Alimentação de Bezerras Leiteiras. In: GONÇALVES, L. C.; BORGES, I.; FERREIRA, P. D. S. Alimentação de gado de leite. Belo Horizonte: FEPMVZ, 2009.
- COZLER, Y.; JURQUET, J.; BEDERE, N. Effects of feeding treatment on growth rate and performance of primiparous Holstein dairy heifers. *Peer Community in Animal Science*. 2019.

GETZEWICH, K. E. Hormonal regulation of the onset of puberty in purebred and crossbred Holstein and Jersey heifers. *Animal Science*, Virginia Polytechnic University Blacksburg, 2005.

HARTMANN, W.; YEGER, C.; FERREIRA, C. G. D. B.; RIBEIRO, S. R. B. Desenvolvimento ponderal de bezerras da raça Holandesa. *Zootecnia: tópicos atuais em pesquisa*. Editora Científica Digital. 2023. Doi: 10.37885/230713660.

MADGWICK, S.; EVANS, A.C.; BEARD, A. P. Treating heifers with GnRH from 4 to 8 weeks of age advanced growth and the age at puberty. *Theriogenology*, 63:2323- 2333. 2005.

OMSA. Organização Mundial de Sanidade Animal. Status do Brasil com relação à febre aftosa. Certificado. 2021.

SANTOS, G. T.; DAMASCENO, J. C.; MASSUDA, E. M.; CAVALIERI, F. L. B. Importância do manejo e considerações econômicas na criação de bezerras e novilhas. In: *II Sul- Leite: Simpósio sobre Sustentabilidade da Pecuária Leiteira na Região Sul do Brasil*. Edição Geraldo Tadeu dos Santos et al. Toledo/PR. Anais. Universidade Estadual de Maringá/CCA/DZO – NUPEL, 2002.

VALLOTO, A. A.; PEDROSA, V. B. Melhoramento genético em rebanhos leiteiros. Curitiba: SENAR - PR., 2018. 108 p.

WEICH, W.; BLOCK, E.; LITHERLAND, N.B. Extended negative dietary cation-anion difference feeding does not negatively affect postpartum performance of multiparous dairy cows. *Journal of Dairy Cows*. 96: 5780-5792, 2013.

ZANTON, G. I.; HEINRICHS, A. J. Meta-Analysis to Assess Effect of Prepubertal Average Daily Gain of Holstein Heifers on First-Lactation Production. *Journal of Dairy Science* 88:3860–3867. American Dairy Science Association, 2005.

DENIS MEDINA GUEDES: Engenheiro Agrícola e Ambiental formado pela Universidade Federal de Viçosa (UFV), com mestrado em Engenharia Agrícola na área de mecanização agrícola pela mesma instituição. Possui experiência no desenvolvimento de equipamentos agrícolas, como secadores, fornalhas e transportadores, tendo atuado no mercado de trabalho como supervisor de projetos em uma indústria de Máquinas e equipamentos agrícolas. Também atuou como professor substituto na UFV Campus de Florestal e como professor efetivo e coordenador do curso de Tecnologia em Gestão Ambiental no Instituto Federal de Educação, Ciência e Tecnologia do Mato Grosso campus Sorriso. Atualmente é professor efetivo na UFV campus Florestal, onde já coordenou os cursos de Agronomia e Técnico em Agropecuária. Atua principalmente nas áreas de topografia e geoprocessamento, agricultura de precisão e secagem e armazenagem de produtos agrícolas.

LEONARDO FRANÇA DA SILVA: Engenheiro Agrônomo pela Universidade Federal De Minas Gerais (UFMG). Engenheiro Segurança do Trabalho, especialista em Engenharia de Produção. Mestre em Agronomia pela Universidade Estadual Paulista -UNESP. Doutor em Engenharia Agrícola (Construções Rurais e Ambiência) pela Universidade Federal de Viçosa. Atualmente atua como membro como membro colaborador dos grupos de pesquisa vinculado ao CNPq: Núcleo em Ambiência e Engenharia de Sistemas Agroindustriais - AMBIAGRO- UFV, Ergonomia e segurança industrial, Ergonomia e Segurança do Trabalho, Segurança e Saúde do Trabalho, Ergonomia Florestal - LABOERGO - UFV . Atuou como Professor Substituto de Magistério Superior na Universidade Federal de Viçosa, campus Florestal, lecionando as disciplinas de Desenho Técnico e Construções Rurais. Possui experiência nas áreas de Engenharia agrícola, com ênfase em Engenharia de Construções Rurais, Desenho técnico e Assistido por computador, Sustentabilidade em sistemas de produção (Agrícola / Animal), Segurança do trabalho e Ergonomia, Desenvolvimento rural, Energia renováveis na agricultura.

JÉSSICA MANSUR SIQUEIRA FURTADO CRUSOÉ: Zootecnista formada pela Universidade Federal de Viçosa (UFV), com mestrado e doutorado em Zootecnia na área de nutrição e produção de animais monogástricos pela mesma instituição. Atualmente atua como professora substituta na UFV – Campus de Florestal, é coordenadora da Unidade de Ensino, Pesquisa e Extensão – Aves e Suínos da UFV e atua também como instrutora do Sistema FAEMG Senar Minas nas áreas de avicultura e suinocultura. Possui experiência em produção, nutrição e alimentação de aves e suínos, bioclimatologia, avicultura com foco em produção de ovos em sistemas alternativos, nutrição e alimentação de poedeiras e desenvolvimento sustentável da avicultura e suinocultura familiar.

A

- Acetamiprid 20, 26
- Agricultura celular 174
- Análise do solo 80, 129, 130
- Annonaceae 10
- Antioxidant activity 34, 35, 36, 38, 39, 40
- Apparent efficient recuperation of nitrogen absorption 124
- Atividade biogênica 80

B

- Bioinsumo 53, 54, 65
- Biopesticide 28
- Brackish water 110, 111, 112, 115, 116

C

- Calcium 36, 75, 222, 223, 224, 225, 226, 227, 228, 229, 231, 232, 233, 234, 235, 236, 237, 238, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 254, 261, 263, 264, 266, 270, 271, 272, 273, 274, 275
- Calcium sulfate 222, 223, 224, 228, 247
- Carne cultivada 174, 188
- Catecolato 54, 55, 56, 63
- Chlorophyll contents 41, 42, 45, 47, 49, 50
- Class A Pan 1, 2, 3, 4, 8
- Coração 96, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220
- Corn 120, 124, 136, 137, 260, 261, 262, 265, 271
- Crop management 223, 227, 248, 256, 271
- Culex pipiens 19, 20, 22, 23, 24, 25, 27, 28, 29, 30, 31, 33

D

- DPPH 34, 35, 37, 38
- Durum wheat 41, 42, 50, 51, 52

E

- Eco-friendly agriculture 223
- Ecotoxicity 20

Efficient food production 223

Espécie bovina 206

Essential oils 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38

F

Feto 89, 90, 92, 93, 94, 95, 96, 98, 99, 100, 103

Fibra natural 195

G

Grain yield 41, 42, 45, 46, 49, 50, 51, 227, 233, 234, 237, 238, 239, 240, 241, 242, 243, 247, 249, 252, 262, 269

Growth curve 276, 278

H

Heifers 139, 141, 142, 276, 277, 278, 279, 281, 282, 283, 284

Herd monitoring 276

Hidroxamato 54, 55, 56

Hybrids 124

I

Inhibition 35, 37, 38, 115, 116, 149

Inovação 137, 174

Intestinal health 68, 71, 74, 75, 76

M

Manejo sustentável 80

Manure 86, 110, 111, 117, 237, 267, 269, 273, 274

Material compósito 195, 196, 198, 204

Microorganismos 53, 54, 55, 56, 57, 58, 60, 61, 62, 64, 65, 68, 81

N

Nitrogen fertilization 124, 136

Nutrientes 53, 56, 64, 68, 86, 89, 90, 91, 92, 93, 94, 95, 96, 98, 99, 100, 102, 103, 122, 205, 211, 212

O

Opuntia stricta Haw 111

Origanum majorana 27, 28, 29, 30, 31, 32, 33

Ovariectomy 139, 142, 143

P

- Pan coefficient 1, 2, 3, 4, 6, 8
 Patologias 205, 206, 207, 213, 219
 Placenta 89, 90, 93, 94, 95, 98, 99, 101, 102, 103, 106, 109
 Plant nutrition 120, 223, 236, 246, 259, 261, 262
 Porcas 89, 92, 96, 99, 100, 101, 102, 103, 104
 Porta-enxerto 10, 11
 Probiotic 68
 Propagação 10, 17
 Propiedade física 80
 Proteína alternativa 174

R

- Reference evapotranspiration 1, 2, 3, 4, 9
 Reflectance 41, 42, 45, 47, 48, 49, 50, 51, 52
 Risks and benefits 139
 Rustile 19, 20, 21, 22, 25

S

- Semi-arid 41, 42, 50, 51, 114, 122, 166
 Semiarid 25, 111, 112, 113, 114, 115, 117, 118, 120, 121
 Senescence 41, 42, 43, 44, 45, 47, 48, 49, 50, 51, 181
 Sideróforo 53, 54, 55, 56
 Soil parameters 223, 226, 246, 254
 Sulfur 222, 223, 224, 225, 230, 232, 233, 238, 240, 241, 242, 243, 244, 245, 246, 247, 249, 250, 251, 252, 254, 259, 269
 Sustentabilidade 174, 195, 284, 285
 Symbiotic 68, 70

T

- Thapsia garganica 34, 35, 36, 37, 38, 39, 40

V

- Vascularização 93, 95, 96, 206, 207, 219

Y


Yield 30, 32, 41, 42, 45, 46, 47, 49, 50, 51, 52, 105, 113, 115, 119, 120, 124, 136, 137, 139, 155, 222, 225, 226, 227, 229, 230, 233, 234, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 247, 249, 250, 251, 252, 256, 257, 258, 259, 260, 261, 262, 263, 264, 266, 269, 270, 271, 272, 273, 274

AGRICULTURAL SCIENCES UNVEILED:

EXPLORING THE DYNAMICS OF FARMING AND SUSTAINABILITY

 www.atenaeditora.com.br

 contato@atenaeditora.com.br

 [@atenaeditora](https://www.instagram.com/atenaeditora)

 www.facebook.com/atenaeditora.com.br

AGRICULTURAL SCIENCES UNVEILED:

EXPLORING THE DYNAMICS OF FARMING AND SUSTAINABILITY

 www.atenaeditora.com.br

 contato@atenaeditora.com.br

 [@atenaeditora](https://www.instagram.com/atenaeditora)

 www.facebook.com/atenaeditora.com.br