Carla Cristina Bauermann Brasil
(Organizadora)

4

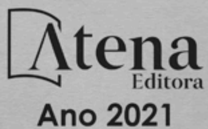
ALIMENTOS, NUTRIÇÃO E SAÚDE

Ano 2021

Carla Cristina Bauermann Brasil
(Organizadora)

4

ALIMENTOS, NUTRIÇÃO E SAÚDE



Ano 2021

Editora chefe
Profª Drª Antonella Carvalho de Oliveira
Assistentes editoriais
Natalia Oliveira
Flávia Roberta Barão
Bibliotecária
Janaina Ramos
Projeto gráfico
Natália Sandrini de Azevedo
Camila Alves de Cremo
Luiza Alves Batista
Maria Alice Pinheiro
Imagens da capa
iStock
Edição de arte
Luiza Alves Batista
Revisão
Os autores

2021 *by Atena Editora*
Copyright © Atena Editora
Copyright do Texto © 2021 Os autores
Copyright da Edição © 2021 Atena Editora
Direitos para esta edição cedidos à Atena Editora pelos autores.
Open access publication by Atena Editora

Todo o conteúdo deste livro está licenciado sob uma Licença de Atribuição *Creative Commons*. Atribuição-Não-Comercial-NãoDerivativos 4.0 Internacional (CC BY-NC-ND 4.0).

O conteúdo dos artigos e seus dados em sua forma, correção e confiabilidade são de responsabilidade exclusiva dos autores, inclusive não representam necessariamente a posição oficial da Atena Editora. Permitido o *download* da obra e o compartilhamento desde que sejam atribuídos créditos aos autores, mas sem a possibilidade de alterá-la de nenhuma forma ou utilizá-la para fins comerciais.

Todos os manuscritos foram previamente submetidos à avaliação cega pelos pares, membros do Conselho Editorial desta Editora, tendo sido aprovados para a publicação com base em critérios de neutralidade e imparcialidade acadêmica.

A Atena Editora é comprometida em garantir a integridade editorial em todas as etapas do processo de publicação, evitando plágio, dados ou resultados fraudulentos e impedindo que interesses financeiros comprometam os padrões éticos da publicação. Situações suspeitas de má conduta científica serão investigadas sob o mais alto padrão de rigor acadêmico e ético.

Conselho Editorial
Ciências Humanas e Sociais Aplicadas
Prof. Dr. Alexandre Jose Schumacher – Instituto Federal de Educação, Ciência e Tecnologia do Paraná
Prof. Dr. Américo Junior Nunes da Silva – Universidade do Estado da Bahia
Profª Drª Andréa Cristina Marques de Araújo – Universidade Fernando Pessoa
Prof. Dr. Antonio Carlos Frasson – Universidade Tecnológica Federal do Paraná
Prof. Dr. Antonio Gasparetto Júnior – Instituto Federal do Sudeste de Minas Gerais
Prof. Dr. Antonio Isidro-Filho – Universidade de Brasília

Ano 2021

Prof. Dr. Arnaldo Oliveira Souza Júnior – Universidade Federal do Piauí
Prof. Dr. Carlos Antonio de Souza Moraes – Universidade Federal Fluminense
Prof. Dr. Crisóstomo Lima do Nascimento – Universidade Federal Fluminense
Profª Drª Cristina Gaio – Universidade de Lisboa
Prof. Dr. Daniel Richard Sant'Ana – Universidade de Brasília
Prof. Dr. Deyvison de Lima Oliveira – Universidade Federal de Rondônia
Profª Drª Dilma Antunes Silva – Universidade Federal de São Paulo
Prof. Dr. Edvaldo Antunes de Farias – Universidade Estácio de Sá
Prof. Dr. Elson Ferreira Costa – Universidade do Estado do Pará
Prof. Dr. Eloi Martins Senhora – Universidade Federal de Roraima
Prof. Dr. Gustavo Henrique Cepolini Ferreira – Universidade Estadual de Montes Claros
Prof. Dr. Humberto Costa – Universidade Federal do Paraná
Profª Drª Ivone Goulart Lopes – Istituto Internazionele delle Figlie de Maria Ausiliatrice
Prof. Dr. Jadson Correia de Oliveira – Universidade Católica do Salvador
Prof. Dr. José Luis Montesillo-Cedillo – Universidad Autónoma del Estado de México
Prof. Dr. Julio Candido de Meirelles Junior – Universidade Federal Fluminense
Profª Drª Lina Maria Gonçalves – Universidade Federal do Tocantins
Prof. Dr. Luis Ricardo Fernandes da Costa – Universidade Estadual de Montes Claros
Profª Drª Natiéli Piovesan – Instituto Federal do Rio Grande do Norte
Prof. Dr. Marcelo Pereira da Silva – Pontifícia Universidade Católica de Campinas
Profª Drª Maria Luzia da Silva Santana – Universidade Federal de Mato Grosso do Sul
Prof. Dr. Miguel Rodrigues Netto – Universidade do Estado de Mato Grosso
Prof. Dr.Pablo Ricardo de Lima Falcão – Universidade de Pernambuco
Profª Drª Paola Andressa Scortegagna – Universidade Estadual de Ponta Grossa
Profª Drª Rita de Cássia da Silva Oliveira – Universidade Estadual de Ponta Grossa
Prof. Dr. Rui Maia Diamantino – Universidade Salvador
Prof. Dr. Saulo Cerqueira de Aguiar Soares – Universidade Federal do Piauí
Prof. Dr. Urandi João Rodrigues Junior – Universidade Federal do Oeste do Pará
Profª Drª Vanessa Bordin Viera – Universidade Federal de Campina Grande
Profª Drª Vanessa Ribeiro Simon Cavalcanti – Universidade Católica do Salvador
Prof. Dr. William Cleber Domingues Silva – Universidade Federal Rural do Rio de Janeiro
Prof. Dr. Willian Douglas Guilherme – Universidade Federal do Tocantins

Ciências Agrárias e Multidisciplinar
Prof. Dr. Alexandre Igor Azevedo Pereira – Instituto Federal Goiano
Prof. Dr. Arinaldo Pereira da Silva – Universidade Federal do Sul e Sudeste do Pará
Prof. Dr. Antonio Pasqualetto – Pontifícia Universidade Católica de Goiás
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. 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. Jael Soares Batista – Universidade Federal Rural do Semi-Árido
Prof. Dr. Jayme Augusto Peres – Universidade Estadual do Centro-Oeste
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. Ronilson Freitas de Souza – Universidade do Estado do Pará
Profª Drª Talita de Santos Matos – Universidade Federal Rural do Rio de Janeiro

Ano 2021

Prof. Dr. Tiago da Silva Teófilo – Universidade Federal Rural do Semi-Árido
Prof. Dr. Valdemar Antonio Paffaro Junior – Universidade Federal de Alfenas

Ciências Biológicas e da Saúde
Prof. Dr. André Ribeiro da Silva – Universidade de Brasília
Profª Drª Anelise Levay Murari – Universidade Federal de Pelotas
Prof. Dr. Benedito Rodrigues da Silva Neto – Universidade Federal de Goiás
Profª Drª Daniela Reis Joaquim de Freitas – Universidade Federal do Piauí
Profª Drª Débora Luana Ribeiro Pessoa – Universidade Federal do Maranhão
Prof. Dr. Douglas Siqueira de Almeida Chaves – Universidade Federal Rural do Rio de Janeiro
Prof. Dr. Edson da Silva – Universidade Federal dos Vales do Jequitinhonha e Mucuri
Profª Drª Elizabeth Cordeiro Fernandes – Faculdade Integrada Medicina
Profª Drª Eleuza Rodrigues Machado – Faculdade Anhanguera de Brasília
Profª Drª Elane Schwinden Prudêncio – Universidade Federal de Santa Catarina
Profª Drª Eysler Gonçalves Maia Brasil – Universidade da Integração Internacional da Lusofonia Afro-Brasileira
Prof. Dr. Ferlando Lima Santos – Universidade Federal do Recôncavo da Bahia
Profª Drª Fernanda Miguel de Andrade – Universidade Federal de Pernambuco
Prof. Dr. Fernando Mendes – Instituto Politécnico de Coimbra – Escola Superior de Saúde de Coimbra
Profª Drª Gabriela Vieira do Amaral – Universidade de Vassouras
Prof. Dr. Gianfábio Pimentel Franco – Universidade Federal de Santa Maria
Prof. Dr. Helio Franklin Rodrigues de Almeida – Universidade Federal de Rondônia
Profª Drª Iara Lúcia Tescarollo – Universidade São Francisco
Prof. Dr. Igor Luiz Vieira de Lima Santos – Universidade Federal de Campina Grande
Prof. Dr. Jefferson Thiago Souza – Universidade Estadual do Ceará
Prof. Dr. Jesus Rodrigues Lemos – Universidade Federal do Piauí
Prof. Dr. Jônatas de França Barros – Universidade Federal do Rio Grande do Norte
Prof. Dr. José Max Barbosa de Oliveira Junior – Universidade Federal do Oeste do Pará
Prof. Dr. Luís Paulo Souza e Souza – Universidade Federal do Amazonas
Profª Drª Magnólia de Araújo Campos – Universidade Federal de Campina Grande
Prof. Dr. Marcus Fernando da Silva Praxedes – Universidade Federal do Recôncavo da Bahia
Profª Drª Maria Tatiane Gonçalves Sá – Universidade do Estado do Pará
Profª Drª Mylena Andréa Oliveira Torres – Universidade Ceuma
Profª Drª Natiéli Piovesan – Instituto Federacl do Rio Grande do Norte
Prof. Dr. Paulo Inada – Universidade Estadual de Maringá
Prof. Dr. Rafael Henrique Silva – Hospital Universitário da Universidade Federal da Grande Dourados
Profª Drª Regiane Luz Carvalho – Centro Universitário das Faculdades Associadas de Ensino
Profª Drª Renata Mendes de Freitas – Universidade Federal de Juiz de Fora
Profª Drª Vanessa da Fontoura Custódio Monteiro – Universidade do Vale do Sapucaí
Profª Drª Vanessa Lima Gonçalves – Universidade Estadual de Ponta Grossa
Profª Drª Vanessa Bordin Viera – Universidade Federal de Campina Grande
Profª Drª Welma Emidio da Silva – Universidade Federal Rural de Pernambuco

Ciências Exatas e da Terra e Engenharias
Prof. Dr. Adélio Alcino Sampaio Castro Machado – Universidade do Porto
ProFª Drª Ana Grasielle Dionísio Corrêa – Universidade Presbiteriana Mackenzie
Prof. Dr. Carlos Eduardo Sanches de Andrade – Universidade Federal de Goiás
Profª Drª Carmen Lúcia Voigt – Universidade Norte do Paraná
Prof. Dr. Cleiseano Emanuel da Silva Paniagua – Instituto Federal de Educação, Ciência e Tecnologia de Goiás
Prof. Dr. Douglas Gonçalves da Silva – Universidade Estadual do Sudoeste da Bahia
Prof. Dr. Eloi Rufato Junior – Universidade Tecnológica Federal do Paraná
Profª Drª Érica de Melo Azevedo – Instituto Federal do Rio de Janeiro

Ano 2021

Ciências Biológicas e da Saúde

Prof. Dr. Fabrício Menezes Ramos – Instituto Federal do Pará
Profª Dra. Jéssica Verger Nardeli – Universidade Estadual Paulista Júlio de Mesquita Filho
Prof. Dr. Juliano Carlo Rufino de Freitas – Universidade Federal de Campina Grande
Profª Drª Luciana do Nascimento Mendes – Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Norte
Prof. Dr. Marcelo Marques – Universidade Estadual de Maringá
Prof. Dr. Marco Aurélio Kistemann Junior – Universidade Federal de Juiz de Fora
Profª Drª Neiva Maria de Almeida – Universidade Federal da Paraíba
Profª Drª Natiéli Piovesan – Instituto Federal do Rio Grande do Norte
Profª Drª Priscila Tessmer Scaglioni – Universidade Federal de Pelotas
Prof. Dr. Sidney Gonçalo de Lima – Universidade Federal do Piauí
Prof. Dr. Takeshy Tachizawa – Faculdade de Campo Limpo Paulista

Linguística, Letras e Artes
Profª Drª Adriana Demite Stephani – Universidade Federal do Tocantins
Profª Drª Angeli Rose do Nascimento – Universidade Federal do Estado do Rio de Janeiro
Profª Drª Carolina Fernandes da Silva Mandaji – Universidade Tecnológica Federal do Paraná
Profª Drª Denise Rocha – Universidade Federal do Ceará
Profª Drª Edna Alencar da Silva Rivera – Instituto Federal de São Paulo
Profª DrªFernanda Tonelli – Instituto Federal de São Paulo,
Prof. Dr. Fabiano Tadeu Grazioli – Universidade Regional Integrada do Alto Uruguai e das Missões
Prof. Dr. Gilmei Fleck – Universidade Estadual do Oeste do Paraná
Profª Drª Keyla Christina Almeida Portela – Instituto Federal de Educação, Ciência e Tecnologia do Paraná
Profª Drª Miranilde Oliveira Neves – Instituto de Educação, Ciência e Tecnologia do Pará
Profª Drª Sandra Regina Gardacho Pietrobon – Universidade Estadual do Centro-Oeste
Profª Drª Sheila Marta Carregosa Rocha – Universidade do Estado da Bahia

Ciências Biológicas e da Saúde

Ano 2021

Ciências Biológicas e da Saúde

Alimentos, nutrição e saúde 4

Diagramação: Maria Alice Pinheiro
Correção: Thiago Meijerink
Indexação: Gabriel Motomu Teshima
Revisão: Os autores
Organizadora: Carla Cristina Bauermann Brasil

Dados Internacionais de Catalogação na Publicação (CIP)

A411 Alimentos, nutrição e saúde 4 / Organizadora Carla Cristina Bauermann Brasil. – Ponta Grossa - PR: Atena, 2021.

Formato: PDF
Requisitos de sistema: Adobe Acrobat Reader
Modo de acesso: World Wide Web
Inclui bibliografia
ISBN 978-65-5983-402-0
DOI: https://doi.org/10.22533/at.ed.020212308

1. Nutrição. 2. Saúde. I. Brasil, Carla Cristina Bauermann (Organizadora). II. Título.

CDD 613

Elaborado por Bibliotecária Janaina Ramos – CRB-8/9166

Atena Editora
Ponta Grossa – Paraná – Brasil
Telefone: +55 (42) 3323-5493
www.atenaeditora.com.br
contato@atenaeditora.com.br

DECLARAÇÃO DOS AUTORES

Os autores desta obra: 1. Atestam não possuir qualquer interesse comercial que constitua um conflito de interesses em relação ao artigo científico publicado; 2. Declaram que participaram ativamente da construção dos respectivos manuscritos, preferencialmente na: a) Concepção do estudo, e/ou aquisição de dados, e/ou análise e interpretação de dados; b) Elaboração do artigo ou revisão com vistas a tornar o material intelectualmente relevante; c) Aprovação final do manuscrito para submissão.; 3. Certificam que os artigos científicos publicados estão completamente isentos de dados e/ou resultados fraudulentos; 4. Confirmam a citação e a referência correta de todos os dados e de interpretações de dados de outras pesquisas; 5. Reconhecem terem informado todas as fontes de financiamento recebidas para a consecução da pesquisa; 6. Autorizam a edição da obra, que incluem os registros de ficha catalográfica, ISBN, DOI e demais indexadores, projeto visual e criação de capa, diagramação de miolo, assim como lançamento e divulgação da mesma conforme critérios da Atena Editora.

Ano 2021

Ciências Biológicas e da Saúde

DECLARAÇÃO DA EDITORA

A Atena Editora declara, para os devidos fins de direito, que: 1. A presente publicação constitui apenas transferência temporária dos direitos autorais, direito sobre a publicação, inclusive não constitui responsabilidade solidária na criação dos manuscritos publicados, nos termos previstos na Lei sobre direitos autorais (Lei 9610/98), no art. 184 do Código penal e no art. 927 do Código Civil; 2. Autoriza e incentiva os autores a assinarem contratos com repositórios institucionais, com fins exclusivos de divulgação da obra, desde que com o devido reconhecimento de autoria e edição e sem qualquer finalidade comercial; 3. Todos os e-book são *open access, desta forma* não os comercializa em seu site, sites parceiros, plataformas de *e-commerce,* ou qualquer outro meio virtual ou físico, portanto, está isenta de repasses de direitos autorais aos autores; 4. Todos os membros do conselho editorial são doutores e vinculados a instituições de ensino superior públicas, conforme recomendação da CAPES para obtenção do Qualis livro; 5. Não cede, comercializa ou autoriza a utilização dos nomes e e-mails dos autores, bem como nenhum outro dado dos mesmos, para qualquer finalidade que não o escopo da divulgação desta obra.

Ano 2021

APRESENTAÇÃO

A presente obra ‘’Alimentos, Nutrição e Saúde’’ publicada no formato *e-book*, traduz o olhar multidisciplinar e intersetorial da Alimentação e Nutrição. Os volumes abordarão de forma categorizada e interdisciplinar trabalhos, pesquisas, relatos de casos e revisões que transitam nos diversos caminhos da Nutrição e Saúde. O principal objetivo desse *e-book* foi apresentar de forma categorizada e clara estudos desenvolvidos em diversas instituições de ensino e pesquisa do país em quatro volumes. Em todos esses trabalhos a linha condutora foi o aspecto relacionado à avaliação antropométrica da população brasileira; padrões alimentares; avaliações físico-químicas e sensoriais de alimentos e preparações, determinação e caracterização de alimentos e de compostos bioativos; desenvolvimento de novos produtos alimentícios e áreas correlatas.

Temas diversos e interessantes são, deste modo, discutidos nestes volumes com a proposta de fundamentar o conhecimento de acadêmicos, mestres e todos aqueles que de alguma forma se interessam pela área da Alimentação, Nutrição, Saúde e seus aspectos. A Nutrição é uma ciência relativamente nova, mas a dimensão de sua importância se traduz na amplitude de áreas com as quais dialoga. Portanto, possuir um material cientifico que demonstre com dados substanciais de regiões específicas do país é muito relevante, assim como abordar temas atuais e de interesse direto da sociedade. Deste modo a obra ‟Alimentos, Nutrição e Saúde’’ se constitui em uma interessante ferramenta para que o leitor, seja ele um profissional, acadêmico ou apenas um interessado pelo campo das ciências da nutrição, tenha acesso a um panorama do que tem sido construído na área em nosso país.

Uma ótima leitura a todos(as)!

Carla Cristina Bauermann Brasil

SUMÁRIO

CAPÍTULO 1 1

TEOR PROTEICO EM ALIMENTOS PLANT-BASED: ESTUDO DE CASO SOBRE CORRELAÇÕES ENTRE BACALHAU, HAMBÚRGUER E "LEITE" VEGETAIS

Yanni Sales Caruso

Luiz Eduardo R. de Carvalho

https://doi.org/10.22533/at.ed.0202123081

CAPÍTULO 2 9

COMPARAÇÃO DOS EFEITOS DA TECNOLOGIA ULTRAVIOLETA E TECNOLOGIA CONVENCIONAL EM ASPECTOS DE QUALIDADE DE FOLHAS DE COUVE

Sidnei Macedo Pereira Filho

Iasmim Pereira de Moraes

Leticia Cabrera Parra Bortoluzzi

Márcia Regina Ferreira Geraldo-Perdoncini

Stéphani Caroline Beneti

Roberto Ribeiro Neli

Leila Larissa Medeiros Marques

Fábio Henrique Poliseli-Scopel

https://doi.org/10.22533/at.ed.0202123082

CAPÍTULO 3 24

PRÁTICAS DE PROCESSO FERMENTATIVO EM AMBIENTE DOMÉSTICO PARA O ENSINO REMOTO EMERGENCIAL

Rosangela Maria Oliveira Marinho

Rute Chayenne Teixeira de Azevedo

Glinailzia Dodó da Silva

Daiane de Moura Araújo

Felipe Sousa da Silva

Sheyla Maria Barreto Amaral

Mayara Salgado Silva

https://doi.org/10.22533/at.ed.0202123083

CAPÍTULO 4 34

VALIDATION OF IC-ELISA: LOW-COST IMMUNOASSAY DEVELOPED FOR AFLATOXIN ANALYSIS IN EGG

Lívia Montanheiro Médici Zanin

Thaís Marques Amorim

Fernando de Godoi Silva

Fabiana Akemi Hirata Bae

Giovana dos Santos Marcolino

André Ribeiro da Silva

Mariana Ribeiro Benfatti

Angélica Tieme Ishikawa

Cássia Reika Takabayashi Yamashita

Daiane Dias Lopes

Elisabete Yurie Sataque Ono
Eiko Nakagawa Itano
Osamu Kawamura
Elisa Yoko Hirooka

https://doi.org/10.22533/at.ed.0202123084

CAPÍTULO 5...53

ASSESSMENT OF SAFETY, FUNCTIONAL AND TECHNOLOGICAL PROPERTIES OF LACTICASEIBACILLI AND LIMOSILACTOBACILLI BEFORE AND AFTER *IN VITRO* GASTROINTESTINAL TRANSIT

André Fioravante Guerra
Layse Ferreira de Brito
Karina Coelho Moreira da Silva
José Francisco Pereira Martins
Rosa Helena Luchese

https://doi.org/10.22533/at.ed.0202123085

CAPÍTULO 6...64

ASPETOS NUTRICIONAIS E PROPRIEDADES BIOLÓGICAS DAS SEMENTES DE PAPOILA E DE QUINOA

Ana Cristina Mendes Ferreira da Vinha
Carla Alexandra Lopes Andrade de Sousa e Silva
Carla Manuela Soares de Matos
Carla Maria Sanfins Guimarães Moutinho

https://doi.org/10.22533/at.ed.0202123086

CAPÍTULO 7...89

PROCESSAMENTO DE RIZÓFOROS COMO ESTRATÉGIA PARA O FOMENTO DO CULTIVO ECONÔMICO DE CARÁ-DE-ESPINHO (*Dioscorea chondrocarpa* GRISEB. - DIOSCOREACEAE)

Eleano Rodrigues da Silva
Ana Paula Mileo Guerra Carvalho
Sheila Barros Cabral de Araújo
Flávia de Carvalho Paiva Dias
Sonia Seba Alfaia
Robert Corrêa Rodrigues

https://doi.org/10.22533/at.ed.0202123087

CAPÍTULO 8...100

PRODUTIVIDADE E PADRÃO COMERCIAL DE CULTIVARES DE MAMOEIROS AVALIADOS NO AMAZONAS

Lucio Pereira Santos
Enilson de Barros Silva

https://doi.org/10.22533/at.ed.0202123088

CAPÍTULO 9..109

UTILIZAÇÃO DE MODELOS NÃO LINEARES NA DESCRIÇÃO DO CRESCIMENTO DE FRUTOS DE MANGA DA VARIEDADE PALMER

Felipe Augusto Fernandes
Isolina Aparecida Vilas Bôas
Henrique José de Paula Alves
Tales Jesus Fernandes

https://doi.org/10.22533/at.ed.0202123089

CAPÍTULO 10..117

SEGURANÇA ALIMENTAR E TOXICIDADE PRELIMINAR DO ARAÇÁ AMARELO (*Psidium cattleianum*)

Aiane Benevide Sereno
Luciana Gibbert
Marina Talamini Piltz de Andrade
Carla Dayane Pinto
Michelli Aparecida Bertolazo da Silva
Josiane de Fátima Gaspari Dias
Obdulio Gomes Miguel
Cláudia Carneiro Hecke Krüger
Iara José de Messias Reason

https://doi.org/10.22533/at.ed.02021230810

CAPÍTULO 11..129

DESENVOLVIMENTO E CONTROLE DE QUALIDADE DE PICLES DO PECÍOLO DA VITÓRIA-RÉGIA (POEPP.)

Midori Nakamura Marques
Jaime Paiva Lopes Aguiar
Francisca das Chagas do Amaral Souza

https://doi.org/10.22533/at.ed.02021230811

CAPÍTULO 12..142

MYCOTOXINS, A PROBLEMATIC AFFECTING FOOD SAFETY IN FOOD INDUSTRY FOR PETS WORLDWIDE

Nadia Boncompagno
Gianni Galaverna
Andrea Astoreca

https://doi.org/10.22533/at.ed.02021230812

CAPÍTULO 13..155

ÁCIDOS GRAXOS TRANS: ORIGEM ANIMAL E INDUSTRIAL

Mahyara Markievicz Mancio Kus-Yamashita
Thaís Fukui de Sousa

https://doi.org/10.22533/at.ed.02021230813

CAPÍTULO 14..164

ANÁLISE PARASITOLÓGICA DE HORTALIÇAS COMERCIALIZADAS EM FEIRAS LIVRES DE SALVADOR-BAHIA

Rafael de Sá Barreto Leandro Cruz
Rebeca Bispo de Morais
Cássia Cristina Leal Borges
Paulo Leonardo Lima Ribeiro

https://doi.org/10.22533/at.ed.02021230814

CAPÍTULO 15..175

CONHECIMENTO DOS CLIENTES DE UM SUPERMERCADO SOBRE HIGIENIZAÇÃO DE HORTIFRUTIS

Licia Maria Amaral Albuquerque
Mirella Castro Dantas
Eliane Costa Souza

https://doi.org/10.22533/at.ed.02021230815

CAPÍTULO 16..183

AVALIAÇÃO QUANTITATIVA E QUALITATIVA DA ADEQUAÇÃO NUTRICIONAL DAS REFEIÇÕES OFERECIDAS AOS TRABALHADORES CONTEMPLADOS PELO PROGRAMA DE ALIMENTAÇÃO DO TRABALHADOR: UMA REVISÃO DE LITERATURA

Cibele Maria de Araújo Rocha
Yanna de Jesus Carneiro
Ariele Milet do Amaral Mercês

https://doi.org/10.22533/at.ed.02021230816

CAPÍTULO 17..197

AVALIAÇÃO DO ÍNDICE DE RESTO INGESTÃO E SOBRAS SUJAS EM UMA UNIDADE DE ALIMENTAÇÃO HOTELEIRA LOCALIZADA EM MACEIÓ/AL

Júlia Mayara Correia de Farias
Maria Carolina de Melo Lima
Carla Beatriz Martins da Silva
Maria Augusta Tenório Ferreira
Eliane Costa Souza

https://doi.org/10.22533/at.ed.02021230817

SOBRE O ORGANIZADORA 205

ÍNDICE REMISSIVO..206

CAPÍTULO 4

VALIDATION OF IC-ELISA: LOW-COST IMMUNOASSAY DEVELOPED FOR AFLATOXIN ANALYSIS IN EGG

Data de aceite: 01/08/2021

Data de submissão: 06/05/2021

Lívia Montanheiro Médici Zanin
Department of Food Science and Technology, State University of Londrina
Londrina, Paraná, Brasil
ID Lattes: 6295492943449035

Thaís Marques Amorim
Department of Food Science and Technology, State University of Londrina
Londrina, Paraná, Brasil
ID Lattes: 5825065399245544

Fernando de Godoi Silva
Department of Food Science and Technology, State University of Londrina
Londrina, Paraná, Brasil
ID Lattes: 4013372901815345

Fabiana Akemi Hirata Bae
Department of Food Science and Technology, State University of Londrina
Londrina, Paraná, Brasil
ID Lattes: 1585013910309012

Giovana dos Santos Marcolino
Department of Food Science and Technology, State University of Londrina
Londrina, Paraná, Brasil
ID Lattes: 8261021118222059

André Ribeiro da Silva
Department of Food Science and Technology, State University of Londrina
Londrina, Paraná, Brasil
ID Lattes: 3084223230359837

Mariana Ribeiro Benfatti
Department of Food Science and Technology, State University of Londrina
Londrina, Paraná, Brasil
ID Lattes: 6088928364865717

Angélica Tieme Ishikawa
Department of Food Science and Technology, State University of Londrina
Londrina, Paraná, Brasil
ID Lattes: 7792634769385318

Cássia Reika Takabayashi Yamashita
Department of Food Science and Technology, State University of Londrina
Londrina, Paraná, Brasil
D Lattes: 2697502551053565

Daiane Dias Lopes
Department of Food Science and Technology, State University of Londrina
Londrina, Paraná, Brasil
ID Lattes: 8800633109553726

Elisabete Yurie Sataque Ono
Department of Biochemistry and Biotechnology, State University of Londrina
Londrina, Paraná, Brasil
ID Lattes: 9080149399359784

Eiko Nakagawa Itano
Department of Biology, State University of Londrina
Londrina, Paraná, Brasil
ID Lattes: 0678087604864219

Osamu Kawamura
Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, Ikenobe, Miki-cho, Kita-gun, Kagawa, Japan

Elisa Yoko Hirooka
Department of Food Science and Technology, State University of Londrina
Londrina, Paraná, Brasil
ID Lattes: 8703225783188622

ABSTRACT: Aflatoxin is a hazardous toxic contaminant in food and feed, with frequent losses in animal production. A low cost indirect competitive ELISA (ic-ELISA) was developed using monoclonal antibody (mAb) produced *in vitro* by cultivation of hybridoma strain AF4 (highly specific mAb producer which react 100% with aflatoxin AFB_1). This immunoassay was validated as a promising rapid, sensitive and simple immunotechnique for aflatoxin screening in egg. The in-house validated ic-ELISA with limit of detection (LOD) and quantification (LOQ) of 0.35 μg kg^{-1} and 0.71 μg kg^{-1}, respectively, and R^2 >0.99 showed more sensitive than official high cost methods, and it also increased the detection pattern for lower level contaminated samples. The validated ic-ELISA used a very low concentration of main reagent, the mAb (1.5 mg mL^{-1}, titer of 1:30,000), reducing the cost, and it reached matrix interference lower than 10% for egg substrate. Mean recovery rate was 98% in egg, spiking AFB_1 from 1.0 to 5.0 μg kg^{-1} in samples. Precision was expressed by repeatability and intermediate precision, both with relative standard deviations below 15%. This intra laboratory validated assay was applied to evaluate aflatoxin contamination in egg of two large-scale local producers. AFB_1 was detected in 8 % samples from producer A with mean level of 0.84 μg kg^{-1}, while producer B showed contamination in 15 % samples, with mean level of 0.94 μg kg^{-1}. This monitoring reduced the analysis cost by approximately 70-folds, when compared with commercial kits. It also indicated low occurrence as well as low contamination level, without any visual deformation in egg, concerning quality evaluation. Such high sensitive immunoassay would be crucial to assure a wide-range aflatoxin control through reliable tracking in developing country, and successively provide risk-free egg for consumers.
KEYWORDS: immunoassay**,** monoclonal antibody, aflatoxin, poultry, validation.

VALIDAÇÃO DE IC-ELISA: DESENVOLVIMENTO DE IMUNOENSAIO ACESSÍVEL PARA ANÁLISE DE AFLATOXINA EM OVO

RESUMO: A aflatoxina é um contaminante tóxico perigoso em alimentos e rações, com perdas freqüentes na produção animal. Um ELISA competitivo indireto de baixo custo (ic-ELISA) foi desenvolvido usando anticorpo monoclonal (mAb) produzido *in vitro* pelo cultivo da cepa de hibridoma AF4 (produtor de mAb altamente específico que reage 100% com aflatoxina AFB1). Este imunoensaio foi validado como uma promissora imunotécnica rápida, sensível e simples para a triagem de aflatoxina em ovo. O ic-ELISA validado internamente com limite de detecção (LOD) e quantificação (LOQ) de 0,35 μg kg-1 e 0,71 μg kg-1, respectivamente, e R2> 0,99 mostrou-se mais sensível do que os métodos oficiais de alto custo, e também aumentou o padrão de detecção para amostras contaminadas de nível inferior. O ic-ELISA validado utilizou concentração baixíssima do reagente principal, o mAb (1,5 mg mL-1, título 1: 30.000), reduzindo o custo, e alcançou interferência da matriz inferior a 10% para o substrato de ovo. A taxa média de recuperação foi de 98% em ovo, aumentando AFB1 de 1,0 a 5,0 μg

kg-1 nas amostras. A precisão foi expressa por repetibilidade e precisão intermediária, ambas com desvios padrão relativos abaixo de 15%. Este ensaio validado intralaboratorialmente foi aplicado para avaliar a contaminação por aflatoxina em ovos de dois grandes produtores locais. AFB1 foi detectado em 8% das amostras do produtor A com nível médio de 0,84 μg kg-1, enquanto o produtor B apresentou contaminação em 15% das amostras, com nível médio de 0,94 μg kg-1. Esse monitoramento reduziu o custo da análise em aproximadamente 70 vezes, quando comparado aos kits comerciais. Indicou também baixa ocorrência e baixo nível de contaminação, sem qualquer deformação visual no ovo, no que se refere à avaliação da qualidade. Tal imunoensaio de alta sensibilidade seria crucial para garantir um controle de aflatoxina de amplo alcance por meio de rastreamento confiável em países em desenvolvimento e, sucessivamente, fornecer ovos sem risco para os consumidores.
PALAVRAS - CHAVE: Imunoensaio, anticorpo monoclonal, aflatoxina, aves, validação.

1 I INTRODUCTION

Chicken meat and egg are popular and economically accessible protein rich animal source, comparable with beef and pork. The mean chicken consumption is 30 kg per capita worldwide, while the United States, Brazil and Argentina exceed the intake of 40 kg per capita (AVISITE, 2017). A consensus established low effect of dietary egg cholesterol in serum LDL-cholesterol and cardiovascular disease, stimulating its intake as a rich source of valuable proteins, unsaturated fats, fat-soluble vitamins (mainly A & E), vitamin B12 and antioxidants, with approximate world consumption of 230 units per capita / year (ABPA, 2021; GRIFFIN, 2016).

Brazil is the leading exporter as well as the second chicken meat producer worldwide, where the egg provider trading may be an emerging opportunity. Such a perspective should be matched with control program targeted on animal health, adequate production, and monitoring of hazardous residual contaminants.

Aflatoxins (AF), with emphasis in $AFB_{1,}$ have been the warning toxic contaminant in food and feed, where the International Agency for Research on Cancer classified as Group 1 - carcinogenic to human (D’MELLO; MACDONALD, 1997; IARC, 2002; SU, 2020). The AFB_1, AFB_1-exo-8,9-epoxide (AFBO) and other metabolites also affect the epigenetic mechanisms including the DNA methylation, histone modifications, and subsequent gene expression. Chronic AFs exposure leads to formation of reactive AFBO metabolites that could activate and de-activates the various epigenetic mechanisms, leading to development of cancer (BBOSA et al., 2013).

Analysis of aflatoxin is performed mostly by high-performance liquid chromatography (HPLC) and its improved ultra-version (U-HPLC) coupled with detectors, such as ultraviolet (UV), fluorescence (FLR) and mass spectrometry (MS), and advancing to multimycotoxin analysis (AMIRKHIZI et al., 2015; IQBAL et al., 2014; LI et al., 2015; SOLFRIZZO et al., 2018; TURNER; SUBRAHMANYAM; PILETSKY, 2009). Although of sensitivity and accuracy,

the high cost as well as extensive clean-up required for matrix interference reduction, use of harmful and toxic organic solvents have been the topic in concern in widespread use in developing countries.

The rapid detection based on highly specific monoclonal antibodies (mAb) emerged as an alternative which combines sensitivity and simplicity, with advantage of using non-toxic buffer, it dispenses extensive clean-up, and highly trained analytical staffs (TURNER; SUBRAHMANYAM; PILETSKY, 2009; WACOO et al., 2014). Current international approach in integrated proficiency of mycotoxin Check Sample Survey showed progressive use of ELISA as a suitable method shared by industry and laboratories. As example, an interlaboratory validation test involving forty countries indicated choice of ic-ELISA in 54 % (AF) and 61 % (fumonisin) of analysis, when compared with reference techniques – HPLC, UPLC, MS (AVDIC; KOGLER, 2018).

Indirect competitive ELISA has been the choice in mycotoxin detection focused on immunochemistry matched with chromatography; such a local low cost was developed and applied in intensive multi-year mycotoxin monitoring, outbreaks and natural contamination survey since 1980s (HIROOKA et al., 2015). Years of corn (*Zea mays* L.) monitoring in leading exporter Paraná state, Brazil indicated high fumonisin occurrence with 98%, but low frequency of AF with 11 %, which was prevailed in central-western Paraná (ONO et al., 2001). The mean of 1.25 $\mu g\ kg^{-1}$ of total AF in corn and feed for broilers indicated that general contamination was lower than Brazilian guideline for animal feeding (BAGATIN et al., 2016). Although of meaningfully low AF frequency in corn of Northern Paraná, chicken feed collected from crop year 2010 showed a total of 88 % of the feed contaminated with mean of 8.4 $\mu g\ kg^{-1}$ (ROSSI et al., 2012). The increasing food consumption point-out the relevance of AF control even though of low level due to cumulative effect, amount of food-intake and exposure time (MARIN et al., 2013).

The disadvantage of mAb based reagents for AF control is the high cost of commercial kits and immunoaffinity columns for routine analysis (TURNER; SUBRAHMANYAM; PILETSKY, 2009). Another limitation is that commercial kits were developed regarding mycotoxins analysis in cereals, but not for complex matrices such as meat and egg. In order to solve these limitations, the study aims to standardize and validate a low cost quantitative ic-ELISA for AF analysis targeted on chicken egg, using mAb with high cross-reaction with AFB_1 and aflatoxicol (AFL), a reversible stored metabolite derived from cytoplasmic reductase system. Its application is tested to evaluate AF level in egg, and its relation with physical quality parameters is matched.

2 I MATERIALS AND METHODS

2.1 Materials

The AF4 hybridoma cell line secreting specific anti-AFB_1 mAb (IgG_1 lambda isotype) was generated by cell fusion of myeloma cell line SP2/0-AG14 and activated mouse BALB/c spleenic cell at the Kagawa University, Japan (KAWAMURA et al., 1988); the mAb was produced in a joint research at the State University of Londrina, Brazil. This highly specific mAb cross-reacted with AFB_1 (100%) and AFL (122% with AFL I, natural isomer [1S]; 14.1% with AFL II, natural isomer [1R]), but it showed very low cross-reactivity against AFB_2 (2.3%), AFG_1 (3.4%), AFG_2 (2.4%), AFM_1 (4.5%) and AFQ_1 (10.8%).

Egg samples from two large-scale producers, A and B, both in Northern Paraná State, Brazil were weekly collected from April to August 2016 in a total of 20 samplings (50 eggs each in farm A, and 25 each in farm B per day). The eggs were weighed, then hatched for white and yolk weighing, and the shell thickness was measured. Each sample consisted of a pool of five homogenized eggs, resulting in 300 samples, and maintained at 4 ° C until the analysis.

AFB_1 standard solution was obtained from Sigma-Aldrich Co. (St Louis, Missouri, USA). Methanol, sodium chloride and other salts were purchased from J.T.Baker (New Jersey, USA).

2.2 Production of monoclonal antibody (mAb)

The mAb anti-AF was produced *in vitro* culturing hybridoma AF.4 in RPMI medium (Roswell Park Memorial Institute Medium, Gibco Co., Waltham, USA) + 10% fetal bovine serum (Gibco Co., Waltham, USA), followed by gradual adaptation in H-SFM medium (Hybridoma Serum Free Medium, Gibco Co., Waltham, USA), i.e., RPMI:H-SFM (75:25, v/v) to 100% H-SFM. The supernatant with mAb was filtered and precipitate with $(NH_4)_2SO_4$ at 50% saturation (243 g/L) for purification. The precipitated was dissolved in 0.1 M PBS pH 7.3 and dialyzed against PBS (4 ˚C, 64 h). Then 0.02% Sodium azide (Sigma-Aldrich, St. Louis, USA) was added into dialyzed mAb,, aliquoted (1 mL), and stored at -20 ˚C until use. The mAb concentration (IgG) was determined at 280 nm (Biochrom® Anthos Zenyth 200, USA), using absorption coefficient (E280) of 1.35 for IgG (HARLOW; LANE, 2014a). Polyacrylamide gel electrophoresis (5% acrylamide, stacking gel; 10%, running gel) was carried out for mAb purity checking. An aliquot of 4.5 µl of mAb corresponding 2.5 µg of protein was diluted in 3-fold concentrated sample buffer, boiled (3 min, water bath), then applied onto gel together with 5 µl marker standard (Benchmark Protein Ladder, Invitrogen, Carlsbad, USA). After 2 h running under 80V, the gel was stained with Coomassie blue solution (Coomassie Brilliant Blue R, Sigma, St. Louis, Mo, USA) decolorized with acetic acid: methanol: water (1: 4: 5), and distinct protein bands were visualized (LAEMMLI, 1970).

2.3 Aflatoxin extraction

An aliquot of 5 g of egg sample was shaken with 25 mL methanol : water (70 : 30, v/v) and 0.5 g sodium chloride for 30 min at 150 rpm. The extract was filtered (45 µm pore size filter, Macherey-Nagel, Germany), and 500 µL of filtrate was dried at 40˚C under nitrogen flow and diluted in PBS: methanol (9:1, v/v).

2.4 ic-ELISA procedure

Polystyrene microtiter plate wells (Corning, New York, USA) were coated with 50 µL of AFB_1-BSA (Sigma-Aldrich, St. Louis, USA) in PBS (0.015 M, pH 7.3) at 4 ˚C for 24 h. The microplate was washed three times after each incubation step with PBST (PBS + 0.05% Tween). The non-specific binding in wells were blocked with 150 µL of 0.1% BSA (Sigma-Aldrich, St. Louis, USA) in PBS at 25 ˚C for 1 h. The microplate was washed, and 50 µL of AFB_1 standards (0.05 – 5.0 ng mL^{-1}) or 50 µL of sample were added with 50 µL of anti-AFB_1 mAb, and incubated at 25 ˚C for 1 h. After washing, 50 µL of horseradish peroxidase labelled goat anti-mouse IgG (anti-IgG-HRP, from Sigma-Aldrich, St. Louis, USA) were added, and incubated at 25 ˚C for 1 h. The microplate was again washed, and 100 µL of substrate (3,3',5,5'-tetramethylbenzidine / $H_2O_{2,}$ Sigma-Aldrich, St. Louis, USA) were added. The reaction was stopped after 20 min by adding 50 µL of 1 M H_2SO_4, and absorbance was read at 450 nm (ELISA microplate reader, Biochrom® Anthos Zenyth 200, USA). The mean absorbance was calculated from the individual absorbance obtained in triplicate wells, and the results were expressed as percentage of binding:

$$\text{Binding (\%)} = (A^+/A^-) \times 100,$$

where A^+ is the mean absorbance in the presence of aflatoxin standard or sample, and A^- is the mean absorbance in their absence.

2.5 Intra-laboratory validation

In-house validation of icELISA was carried out evaluating specificity, linearity, accuracy, precision, detection (LOD) and quantification limit (LOQ), according the specifications of Brazilian and international guidelines [ANVISA, 2021; EUROPEAN COMMISSION (EC) 401, 2006; EUROPEAN COMMISSION (EU) 519, 2014; INMETRO, 2007, 2017; MAPA, 2015]. Specificity was evaluated by interference of non-contaminated matrix (egg) diluted from 2 to 15-fold. Matrix interference was evaluated comparing the standard curve prepared in PBS: methanol (9:1, v/v) with curve prepared in matrix extract. The linearity was expressed according to the linear regression analysis of seven calibration curves of AFB_1 from 0.05 to 5.0 ng mL^{-1}. Accuracy and precision (repeatability and intermediate precision) were based on relative standard deviations (RSD %) of the AFB_1 recovery tests. Uncontaminated egg samples were artificially added with AFB_1 at 1.0, 2.0 and 5.0 μg kg^{-1} for egg, the mixture was vortexed for 1 min, and stored at 8 ˚C for 24 h before extraction. Accuracy was assessed

by AFB_1 recovery from three determinations (three extractions) in triplicate. Repeatability was evaluated by one determination of each analyzed concentration in seven replicates in the same day. Intermediate precision was evaluated by three determinations of each analyzed concentration in seven replicates on three different days. The LOD and LOQ were calculated, respectively, as 3.143-fold and 5-fold the standard deviation of absorbance of seven replicates of uncontaminated sample in three different days. Blank reaction was carried out in three replicate in each analysis, to check that any solvent, reagent, or instrumentation caused some detectable positive biases in toxin concentration.

2.6 Egg quality

Egg was weighed in a 0.001 g precision analytical balance (Shimadzu AW 220, Philippines) to obtain the mean weight (pool of five eggs). The egg yolks of each sample were weighed; the shells were washed and dried at room temperature to obtain their weight and from the weight, the percentage of shell in the egg was calculated. The weight of egg white was obtained by difference between the mass of whole egg and sum of masses of yolk and shell. The thickness of dried shell was measured at two distinct points using manual micrometer (Mitutoyo Micrometer 25 mm, Japan).

2.7 Statistical analysis

The specificity data was subjected to one-way ANOVA followed by Tukey test using Statistica v. 7 (StatSoft®, USA). The AF contamination data and egg quality parameters were submitted to Wilcoxon test using software RStudio (1.0.153). The significance was accepted at $p < 0.05$.

3 | RESULTS AND DISCUSSION

3.1 Anti-aflatoxin monoclonal antibody (MAb)

The cross-reactivity of mAb against AF analogues was evaluated when hybridoma AF4 strain was generated (KAWAMURA et al., 1988). The high cross-reactivity of mAb generated by AF4, i.e. 100% with AFB_1 and 122 with AFL 122%, could be regarded as an advantageous profile concerning AF detection skill in biological materials. AFL is the main adduct derived from reductase action on AFB_1 cyclo-pentenone carbonyl both *in vitro* and *in vivo.* Such an interconversion in several avian and mammalian species plays AFB_1 reservoir role in tissue (NAKAZATO et al., 1990; WONG et al., 1979).

The protein concentration in mAb batch (IgG) was 1.5 mg mL^{-1}, reaching a total of 508.6 mg of purified anti-AF mAb. The electrophoresis showed band of approximately 25 kDa, and another of 50 kDa (Fig. 1). These two bands were derived from breakdown of IgG glycoprotein tetramer of approx.150 kDa molecular mass in two 25 kDa light chains and two 50 kDa heavy chains (HARLOW; LANE, 2014b). Another band corresponding to 75 kDa

indicated an incomplete breakdown, i.e., remaining of light-heavy chain bound. Such bands corresponding IgG light (25 kDa) and heavy (50 kDa) chains demonstrated the successful production and purification of mAb.

3.2 ic-ELISA procedure

Different dilutions were tested to determine the titers of mAb (0.5 mg mL^{-1}) and anti-IgG-HRP (0.005 mg mL^{-1}) for use in ic-ELISA (Fig. 2A). The mAb diluted (1: 10^1 to 1: 10^5) was tested with 1: 2,000; 1: 4,000 and 1: 6,000 diluted anti-IgG-HRP. The parameter used to define the titers was IC 50 (Inhibition Concentration at 50%), normally used to express the sensitivity of immunoassay, in which the best accuracy for immunoassay was defined by dilution of mAb required to achieve 50% of binding when compared with initial concentration (DRESSER, 1986). The mAb diluted at 1: 10,000 was selected for ic-ELISA in this study, as its binding percentage was approx. 50% in all anti-IgG-HRP dilutions tested, with 62%, 51% and 49% binding for anti-IgG-HRP at 1: 2,000, 1: 4,000 and 1: 6,000, respectively. The optimized anti-IgG-HRP dilution was 1: 6,000, with mean absorbance value lower than 0.9 to avoid deviations in Beer-Lambert Law (SKOOG; WEST; HOLLER, 1992).

In comparison, an indirect ELISA optimization study for AFB_1 in cereals testing dilutions of commercial mAb reached the optimum in 1: 5,000 (LÚCIO; PINTO; MARRIE, 2007). The mAb (1.5 mg ml^{-1}) applied for egg analysis in this study could be diluted at 1: 30,000. High dilutions of highly specific mAb reduced the amount of reagents, and further reduction of analysis cost in approx.70-fold, when compared with commercial kit (Table 1). Such viable alternative assay would achieve a wide AF screening, reducing losses in animal production, as well as to provide hazard-free egg. Brazil is promising country as egg provider in globalized world.

3.3 Intra-laboratory validation of ic-ELISA

Intra-laboratory validation (Table 2) was based on specificity, linearity, accuracy, precision, detection limit (*LOD*) and quantification limit (*LOQ*). In addition, the matrix interference (specificity) is an important parameter to be evaluated, especially in food with complex matrix as egg with diversified constituents and high fat content. The matrix interference may cause false-positives in immunoassay, which is one of main restriction in ic-ELISA; such reaction is observed as decrease in color intensity, with fault in interpretation, suggesting toxin presence. The enzyme activity is inhibited by interfering components of matrix, and / or it affects the accurate toxin (antigen) – antibody reaction (LEE et al., 2004).

In order to minimize the interference, specificity was evaluated carrying out the dilution of uncontaminated egg in a factor of 2 to 15-fold (data not shown). Egg diluted at 2 and 5-fold showed any significant difference, with mean interference values of 3.4 % ± 1.2 and 2.5 % ± 1.0, respectively; however, the factor of 10-fold increased the interference (5.8 ± 3.9), maybe due to overcoming of dilution-point for protective components, and damaging

the protein structure (mAb). Therefore, the procedure with dilution factor of 2-fold, low matrix interference (3.4 %), lower RSD (35 %) and better AFB_1 recovery (99 %) was selected for further assay ($p > 0.05$).

The linearity was evaluated with aflatoxin standard curve ranging from 0.05 to 5.0 ng ml^{-1}, which was prepared in egg extract diluted at 2-fold, and compared with a standard curve in PBS : methanol (9 : 1, v/v) (Fig. 2B). A one-way ANOVA was followed by Tukey test between curves; no difference was shown among them ($p > 0.05$). Furthermore, all the points were superimposed, indicating that dilution of egg in a factor of 2-fold eliminated the matrix interference; i.e., 2-fold diluted egg samples can be analyzed by standard curve prepared without matrix. A linear range was obtained from 0.05 to 5.0 ng ml^{-1} of AFB_1, which was expressed by regression equation $y = -14.85 \ln(x) + 47.131$; the R^2 was 0.9922 ($p < 0.05$), i.e. higher than the minimal acceptance of 0.99 (INMETRO, 2017).

Table 2 showed the recovery and precision rates of AFB_1 in egg. Egg samples added with 1.0, 2.0 and 5.0 $\mu g\ kg^{-1}$ of AFB_1 showed recovery rates of 96, 101 and 99 %, respectively, indicating that every data was within the recommended value of 70 – 110 % of recovery, which is required for samples contaminated within 1 – 10 $\mu g\ kg^{-1}$ (EC 401/2006). LEE et al. (2016) studied aflatoxins in egg by HPLC coupled to FLR, and showed recovery rates of 109, 118 and 102 % using same AFB_1 concentrations of our study: 1.0, 2.0 and 5.0 $\mu g\ kg^{-1}$, respectively; such data ratified that ic-ELISA can reach recovery levels equal to and even better than those obtained by the recommended standard technique (HPLC), such as the rate for 2 $\mu g\ kg^{-1}$ that in our study is within the recommended by European Community, but exceeds the rate of 110 %, when analyzed by chromatography.

The precision of the method was evaluated by repeatability and intermediate precision, based on relative standard deviations (RSD %) of the aflatoxin recovery tests (Table 2). The mean repeatability rate was 12 % for egg, and the mean intermediate precision rate was 15 %, with all results are within the recommended by local and international regulations. A study for detection of AFB_1 in chicken liver and egg by HPLC-UV (AMIRKHIZI et al., 2015) reported repeatability rate (4 %) and intermediate precision rate (8 %). IQBAL et al. (2014) analyzing AF in chicken meat and egg by HPLC-FLR reported repeatability rate of 9 % and intermediate precision rate of 10 %.

The LOD of this method was 0.35 $\mu g\ kg^{-}1$, and LOQ of 0.71 $\mu g\ kg^{-1}$ for egg (Table 3). AMIRKHIZI et al. (2015) reported the LOD for chicken liver and egg lower than obtained in this study (0.08 $\mu g\ kg^{-1}$) by HPLC-UV. However, ANFOSSI et al. (2015) obtained LOD of 3.0 $\mu g\ kg^{-1}$ for detection of AFB_1 in egg by competitive direct ELISA), which was higher than our method. The LOQ of this method was 1.5 $\mu g\ kg^{-1}$ for liver and 0.7 $\mu g\ kg^{-1}$ for egg; these limits were lower than the LOD reported for detection of aflatoxin in egg by UPLC-MS, which was equal to 3.0 $\mu g\ kg^{-1}$ (LI et al., 2015).

The Table 3 shows the effectiveness of developed icELISA, when compared with commercial ELISA kits showing LOD of 0.5 to 1.00 $\mu g\ kg^{-1}$ and LOQ of 2.50 $\mu g\ kg^{-1}$ (DEEB;

AMAN; EL-HAWARY, 2017; TULAYAKUL et al., 2018; PEREIRA ET AL. 2020; GONZÁLEZ-PEÑAS, 2020). The intra-laboratory validation of ic-ELISA demonstrated that the developed immunoassay reached levels of specificity, sensitivity and precision similar to, and in some cases, even better than those obtained by the standard technique (HPLC). This advantage, associated with a reduction in cost by approx. 70-fold, when compared with commercial kits indicated promising application in AF screening in egg, where the consumption is increasing. Furthermore, aflatoxicosis is usually non-identified due to failure in feed sampling, mycotoxin analysis, or the symptoms in animals begin when the feed is no more available for analysis.

3.4 Aflatoxin contamination and its relation with egg quality

Natural AF monitoring in egg from two large-scale producers was carried out to evaluate its applicability, aiming to support the productive sector and further application in agroindustry. Egg is formed by incorporing secreted albumin around yolk, with higher contamination probability than other meat tissues.

It is important to point-out critical strategy for control, although the level of AF contamination in crude materials (maize and feed) in this producing region was low; the positivity may eventually be high, as verified in a study, where 92 % of laying hen feed from crop year 2010 were contaminated with aflatoxin, with a mean of 19.8 µg kg^{-1} (ROSSI et al., 2012; HIROOKA et al., 2015).

Table 4 shows the physical data of egg quality where there was no difference concerning egg shell thickness and percentage of shell among farms. The thickness was 0.370 mm ± 0.04 and 0.373 mm ± 0.04 in samples from farm A and B, respectively; percentage of shell was 10.4 % ± 0.86 and 10.2 % ± 0.71 for farm A and B, respectively. The shell expresses 9 to 14 % of egg weight, and thickness & percentage is directly affected by hen's age, where the shell weight remains fairly constant, while egg weight increases along age (CARVALHO; FERNANDES, 2013). There was a difference in the weight of whole egg, and in the weights of the yolk and white among farms ($p < 0.05$). The mean weight in whole egg was 56.24 g ± 3.33 (farm A) and 58.85 g ± 3.40 (farm B), while the mean weight for yolk was 16.72 g ± 1.41 (farm A) and 17.85 g ± 1.42 (farm B); the mean weight for white was 33.68 g ± 2.35 (farm A) and 34.98 g ± 2.93 (farm B). Such differences may be due to the lineages of laying hens; wideely disseminated lineage *Hisex White* in Farm A, while *Nick Chick* in farm B consisted a lineage more resistant to conditions of creation.

Table 5 shows AFB_1 contamination in egg; 8 % of samples in farm A were positive for AFB_1 (mean, 0.84 ± 0.11 μg kg^{-1}; range, 0.70 – 1.12 μg kg^{-1}); the positivity in farm B was 15 % (mean, 0.94 ± 0.22 μg kg^{-1}; range, 0.70 – 1.29 μg kg^{-1}), but without difference among two farms ($p < 0.05$). Anfossi et al. (2015) didn't detected aflatoxin by direct competitive ELISA (LOD, 0.3 μg kg^{-1}), when analyzed 50 egg samples in large & small distributors in northwestern Italy, January to March, 2014. Amirkhizi et al. (2015) analyzed the occurrence of aflatoxin by HPLC-UV in 150 egg samples, collected randomly in the Tabriz-Iran market;

the contamination was similar with this study (Table 5 & 7), but with higher percentage of positive samples. The authors found AFB_1 in 58% of samples from farm (mean, 0.74 ± 0.04 µg kg^{-1}) and domestic eggs (0.94 ± 0.07 *µ*g kg^{-1}), attributing such high contamination percentage to low quality of feed.

Table 6 compared egg quality (weights, shell percentage & thickness) and AFB_1 contamination in both farms. There was no difference concerning weights of whole egg, yolk and white, nor between the percentage or thickness of shell in eggs with AFB_1 contaminated and uncontaminated samples ($p > 0.05$). Jia et al. (2016), comparing egg quality with AF levels, found a decrease in egg shell thickness in samples contaminated with 0.02 µg kg^{-1} of AFB_1 + 0.11 µg kg^{-1} of AFB_2, but without difference in yolk weight & color, egg white height, shape index, egg shell color and haugh unit ($p < 0.05$). Although of higher level of contamination, the mixing of five eggs to make a single sample probably may have minimized the variability in eggs of this study (Table 6). Nevertheless, AF can reduce shell thickness due to poor calcium and phosphorus absorption, interference in vitamin D_3 metabolism or reducing parathyroid hormone level (YILDIRIM et al., 2011).

Table 7 detailed the quality parameters and contamination of AFB_1 in positive egg samples; there was no difference ($p < 0.05$) in aflatoxin level when hen of farm A had been fed with corn of crop year 2015 (0.84 ± 0.12 µg kg^{-1}) and 2016 (0.83 ± 0.04 µg kg^{-1}), as well as in farm B (1.00 ± 0.23 µg kg^{-1}, 2015 crop; 0.76 ± 0.07 µg kg^{-1}, 2016 crop). However, 56% of positive egg samples were collected in April 2016 in farm A; similar percentage occurred in farm B, with 53% of positive samples in same month. Such contamination in April may be due to the high temperature and younger age of hens; such temperature, as well the humidity can favor the fungal growth, toxin production, and younger animals are more susceptible to aflatoxin contamination (HUFF et al., 1986).

4 I CONCLUSION

The ic-ELISA developed with mAb from hybridoma strain AF4 can be a reliable in-house rapid assay with cost reduced in 70-fold, with advantageous specificity for analysis of biological material (egg), proving a promising application *in loco*. The AFB_1 screening in egg indicated low incidence and contamination level, without affecting the egg quality, which contributes in value aggregation of egg as safe ingredient in food industry.

ACKNOWLEDGMENTS

The authors thank the NANOBIO/CAPES Foundation (the Co-ordination for Formation of High Level Professionals) - Ministry of Education, the CNPq (the Brazilian Government Organization for fellowship to Brazilian researchers) - Ministry of Science & Technology, and the Parana State Fund/SETI for the financial support through projects. The authors also thank the CNPq for the productivity and Initiation in Science fellowships

granted to Brazilian researchers.

REFERENCES

ABPA. **Relatório Anual 2021**. São Paulo: Associação Brasileira de Proteína Animal, 2011.

AMIRKHIZI, B.; AREFHOSSEINI, S. R.; ANSARIN, M.; NEMATI, M. Aflatoxin B 1 in eggs and chicken livers by dispersive liquid–liquid microextraction and HPLC. **Food Additives & Contaminants: Part B**, London, v. 8, n. 4, p. 245–249, 2015. DOI: 10.1080/19393210.2015.1067649.

ANFOSSI, L.; DI NARDO, F.; GIOVANNOLI, C.; PASSINI, C.; BAGGIANI, C. Enzyme immunoassay for monitoring aflatoxins in eggs. **Food Control**, Amsterdam, v. 57, p. 115–121, 2015. DOI: 10.1016/j.foodcont.2015.04.013.

ANVISA. INSTRUÇÃO NORMATIVA - IN N° 88 de 26 de março de 2021 estabelece os limites máximos tolerados (LMT) de contaminantes em alimentos. **Diário Oficial da União**, Brasília, 2021. Setion 1, n. 61, p. 225–235.

AVDIC, D.; KOGLER, E. **Aflatoxins/Fumonisins in Corn:** Final Report. Tulln: Romers Lab Check Sample Survey, 2018.

AVISITE. **Consumo mundial de carne de frango em 2017, pelo USDA**. 2017. Accessed 01 oct. 2018. Available from: https://www.agrolink.com.br/noticias/consumo-mundial-de-carne-de-frango-em-2017--pelo-usda_399366.html.

BAGATIN, A. K.; HASHIMOTO, E. H.; HIROOKA, E. Y.; KAWAMURA, O. The contamination with aflatoxins in Brazilian corn and feed for chickens and the risk evaluation. **Tech. Bull. Fac. Agr. Kagawa Univ.**, Miki, v. 68, n. 121, p. 25–31, 2016.

BBOSA, G. S.; KITYA, D.; ODDA, J.; OGWAL-OKENG, J. Aflatoxins metabolism, effects on epigenetic mechanisms and their role in carcinogenesis. **Health**, Wuhan, v. 05, n. 10, p. 14–34, 2013. DOI: 10.4236/health.2013.510A1003.

CARVALHO, L. S. S.; FERNANDES, E. A. Formação e qualidade da casca de ovos de reprodutoras e poedeiras comerciais. **Medicina Veterinaria (Brazil)**, Recife, v. 7, n. 1, p. 35–44, 2013.

DEEB, Azza M.; AMAN, Ibrahim M.; EL-HAWARY, Ibrahim. Determination of Aflatoxin M 1 and B1 in Egyptian Raw Milk , Soft Cheese and Table Eggs using ELISA. **International Journal of Innovative Reserarch in Science & Engineering**, Bangalore, v. 4, n. 11, p. 154–159, 2017.

D'MELLO, J. P. F.; MACDONALD, A. M. C. Mycotoxins. **Animal Feed Science and Technology**, Amsterdam, v. 69, n. 1–3, p. 155–166, 1997. DOI: 10.1016/S0377-8401(97)81630-6.

DRESSER, D. W. Immunization of experimental animals. *In*: WEIR, D. M.; HERZENBERG, L. A.; BLACKWELL, C.; HERZENBERG, L. A. (org.). **Handbook of Experimental Immunology**. 4. ed. Oxford; Boston: Blackwell Scientific Publications, 1986. v. 1, p. 8.1-8.21.

EUROPEAN COMMISSION. Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. **Official Journal of the European Union**, 2006. Section 401, p. 12–34.

EUROPEAN COMMISSION. Commission Regulation (EU) No 519/2014 of 16 May 2014 amending Regulation (EC) No 401/2006 as regards methods of sampling of large lots, spices and food supplements, performance criteria for T-2, HT-2 toxin and citrinin and screening methods of analysis T. **Official Journal of the European Union**, 2014. Section 519, p. 29–43.

GRIFFIN, B. A. Eggs: good or bad? **Proceedings of the Nutrition Society**, Cambridge, v. 75, n. 3, p. 259–264, 2016. DOI: 10.1017/S0029665116000215.

HARLOW, E.; LANE, D. Protein Techniques. *In*: GREENFIELD, E. A. (org.). **Antibodies**: a laboratory manual. 2. ed. New York: Cold Spring Harbor Laboratory Press, 2014. a. cap. 19, p. 777–798.

HARLOW, E.; LANE, D. The antibody molecules. *In*: GREENFIELD, E. A. (org.). **Antibodies**: a laboratory manual. 2. ed. New York: Cold Spring Harbor Laboratory Press, 2014. b. cap. 2, p. 9–20.

HIROOKA, E. Y. et al. Safety and Quality in the Agricultural Product Chain in Brazil. *In*: EISSA, Ayman Hafiz Amer (org.). **Food Production and Industry**. London: InTech, 2015. cap. 5, p. 137–165. DOI: 10.5772/60593.

HUFF, W. E.; KUBENA, L. F.; HARVEY, R. B.; CORRIER, D. E.; MOLLENHAUER, H. H. Progression of Aflatoxicosis in Broiler Chickens. **Poultry Science**, Amsterdam, v. 65, n. 10, p. 1891–1899, 1986. DOI: 10.3382/ps.0651891.

IARC. Aflatoxins. *In*: **IARC monographs on the evaluation of carcinogenic risks to humans**: some traditional herbal medicines, some mycotoxins, naphthalene and styrene. Vol. 82. Lyon: IARCPress, 2002. p. 170–300.

INMETRO. **DOQ-CGCRE-008**: Orientação sobre validação de métodos de ensaios químicos. Rev. 02. Brasília: Instituto Nacional de Metrologia, Normalização e Qualidade Industrial, 2007. 25 p.

INMETRO. **DOQ-CGCRE-008**: Orientação sobre validação de métodos analíticos. Rev. 06. Brasília: Instituto Nacional de Metrologia, Normalização e Qualidade Industrial, 2017. 30 p.

IQBAL, S. Z.; NISAR, S.; ASI, M. R.; JINAP, S. Natural incidence of aflatoxins, ochratoxin A and zearalenone in chicken meat and eggs. **Food Control**, Amsterdam, v. 43, p. 98–103, 2014. DOI: 10.1016/j.foodcont.2014.02.046.

JIA, R.; MA, Q.; FAN, Y.; JI, C.; ZHANG, J.; LIU, T.; ZHAO, L. The toxic effects of combined aflatoxins and zearalenone in naturally contaminated diets on laying performance, egg quality and mycotoxins residues in eggs of layers and the protective effect of *Bacillus subtilis* biodegradation product. **Food and Chemical Toxicology**, Amsterdam, v. 90, p. 142–150, 2016. DOI: 10.1016/j.fct.2016.02.010.

KAWAMURA, O.; NAGAYAMA, S.; SATO, S.; OHTANI, K.; UENO, I.; UENO, Y. A monoclonal antibody-based enzyme-linked immunosorbent assay of aflatoxin B1 in peanut products. **Mycotoxin research**, Basingstoke, v. 4, n. 2, p. 75–88, 1988. DOI: 10.1007/BF03192102.

LAEMMLI, U. K. Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. **Nature**, Basingstoke, v. 227, n. 5259, p. 680–685, 1970. DOI: 10.1038/227680a0.

LEE, M.; SEO, D. J.; JEON, S. B.; OK, H. E.; JUNG, H.; CHOI, C.; CHUN, H. S. Detection of Foodborne Pathogens and Mycotoxins in Eggs and Chicken Feeds from Farms to Retail Markets. **Korean Journal for Food Science of Animal Resources**, Seoul, v. 36, n. 4, p. 463–468, 2016. DOI: 10.5851/kosfa.2016.36.4.463.

LEE, N. A.; WANG, S.; ALLAN, R. D.; KENNEDY, I. R. A Rapid Aflatoxin B 1 ELISA: Development and Validation with Reduced Matrix Effects for Peanuts, Corn, Pistachio, and Soybeans. **Journal of Agricultural and Food Chemistry**, Washington, D.C., v. 52, n. 10, p. 2746–2755, 2004. DOI: 10.1021/jf0354038.

LI, Y.; WEN, S.; CHEN, Z.; XIAO, Z.; MA, M. Ultra-high performance liquid chromatography tandem mass spectrometry for simultaneous analysis of aflatoxins B1, G1, B2, G2, zearalenone and its metabolites in eggs using a QuEChERS-based extraction procedure. **Analytical Methods**, London, v. 7, n. 10, p. 4145–4151, 2015. DOI: 10.1039/C5AY00124B.

LÚCIO, C. H.; PINTO, N. F. J.; MARRIE, I. E. **Otimização do método de Elisa indireto não-competitivo para detecção e quantificação de aflatoxina B1 em cereais**. Sete Lagoas: MAPA/EMBRAPA, 2007. 7 p. (Comunicado Técnico 152, ISSN 1679-0162).

MAPA. **Manual de garantia da qualidade analítica**: áreas de identidade e qualidade de alimentos e de insumos / Ministério da Agricultura Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília: MAPA/ACS, 2015. 51 p.

MARIN, S.; RAMOS, A. J.; CANO-SANCHO, G.; SANCHIS, V. Mycotoxins: Occurrence, toxicology, and exposure assessment. **Food and Chemical Toxicology**, Amsterdam, v. 60, p. 218–237, 2013. DOI: 10.1016/j.fct.2013.07.047.

MUÑOZ-SOLANO, B.; GONZÁLEZ-PEÑAS, E. Mycotoxin Determination in Animal Feed: An LC-FLD Method for Simultaneous Quantification of Aflatoxins, Ochratoxins and Zearelanone in This Matrix. **Toxins**, Basel, v. 12, n. 6, p. 374, 2020. DOI: 10.3390/toxins12060374.

NAKAZATO, M.; MOROZUMI, S.; SAITO, K.; FUJINUMA, K.; NISHIMA, T.; KASAI, N. Interconversion of aflatoxin B1 and aflatoxicol by several fungi. **Applied and Environmental Microbiology**, Washington, D.C., v. 56, n. 5, p. 1465–1470, 1990. DOI: 10.1128/AEM.56.5.1465-1470.1990.

ONO, E. Y. S.; ONO, M. A.; FUNO, F. Y.; MEDINA, A. E.; OLIVEIRA, T. C. R. M.; KAWAMURA, O.; UENO, Y.; HIROOKA, E. Y. Evaluation of fumonisin-aflatoxin co-occurrence in Brazilian corn hybrids by ELISA. **Food Additives & Contaminants**, London, v. 18, n. 8, p. 719–729, 2001. DOI: 10.1080/02652030118906.

PEREIRA, C. S.; CUNHA, S. C.; FERNANDES, J. O. Validation of an Enzyme-Linked Immunosorbent Assay (ELISA) Test Kit for Determination of Aflatoxin B1 in Corn Feed and Comparison with Liquid-Chromatography Tandem Mass Spectrometry (LC-MS/MS) Method. **Food Analytical Methods**, Basingstoke, v. 13, n. 9, p. 1806–1816, 2020. DOI: 10.1007/s12161-020-01805-4.

ROSSI, C. N.; TAKABAYASHI, C. R.; ONO, M. A.; SAITO, G. H.; ITANO, E. N.; KAWAMURA, O.; HIROOKA, E. Y.; ONO, E. Y. S. Immunoassay based on monoclonal antibody for aflatoxin detection in poultry feed. **Food Chemistry**, Amsterdam, v. 132, n. 4, p. 2211–2216, 2012. DOI: 10.1016/j.foodchem.2011.12.067.

SKOOG, D. A.; WEST, D. M.; HOLLER, F. J. An Introduction to Spectroscopic Methods. *In*: **Fundamentals of Analytical Chemistry**. 6. ed. Florida: Saunders College Publishing, 1992. p. 508–535.

SOLFRIZZO, M.; GAMBACORTA, L.; BIBI, R.; CIRIACI, M.; PAOLONI, A.; PECORELLI, I. Multimycotoxin Analysis by LC-MS/MS in Cereal Food and Feed: Comparison of Different Approaches for Extraction, Purification, and Calibration. **Journal of AOAC INTERNATIONAL**, Oxford, v. 101, n. 3, p. 647–657, 2018. DOI: 10.5740/jaoacint.17-0339.

SU, Q.-Y. The Toxification and Detoxification Mechanisms of Aflatoxin B1 in Human: An Update. *In*: LONG, X.-D. (org.). **Aflatoxin B1 Occurrence, Detection and Toxicological Effects**. London: IntechOpen, 2020. cap.11, p. 1–17. DOI: 10.5772/intechopen.89221.

TULAYAKUL, P.; MINGKHWAN, R.; HANANANTACHAI, H.; NETVICHIAN, R.; KHAODHIAR, S.; SONGSERM, T. Heavy Metal (Cd and Pb) and Aflatoxin Contamination in Tissues and Eggs from Free Grazing Ducks and Their Environment in Central Thailand. **Biological Trace Element Research**, Lynnfield, Oxford, v. 186, n. 2, p. 514–520, 2018. DOI: 10.1007/s12011-018-1321-2.

TURNER, N. W.; SUBRAHMANYAM, S.; PILETSKY, S. A. Analytical methods for determination of mycotoxins: A review. **Analytica Chimica Acta**, Amsterdam, v. 632, n. 2, p. 168–180, 2009. DOI: 10.1016/j.aca.2008.11.010.

WACOO, A. P.; WENDIRO, D.; VUZI, P. C.; HAWUMBA, J. F. Methods for Detection of Aflatoxins in Agricultural Food Crops. **Journal of Applied Chemistry**, London, v. 2014, p. 1–15, 2014. DOI: 10.1155/2014/706291.

WONG, Z. A.; DECAD, G. M.; BYARD, J. L.; HSIEH, D. P. H. Conversion of aflatoxicol to aflatoxin B1 in rats in vivo and in primary hepatocyte culture. **Food and Cosmetics Toxicology**, Amsterdam, v. 17, n. 5, p. 481–486, 1979. DOI: 10.1016/0015-6264(79)90007-5.

YILDIRIM, E.; YALÇINKAYA, L.; KANBUR, M.; ÇINAR, M.; ORUÇ, E. Effects of yeast glucomannan on performance, some biochemical parameters and pathological changes in experimental aflatoxicosis in broiler chickens. **Revue de Medecine Veterinaire**, Lyon, v. 162, n. 8–9, p. 413–420, 2011.

FIGURES AND TABLE LIST

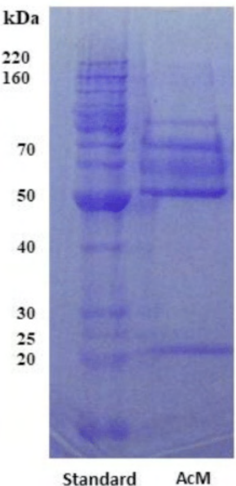


Fig. 1. Electrophoresis of anti-AFB$_1$ mAb (IgG$_1$ lambda isotype) secreted by hybridoma strain AF4.

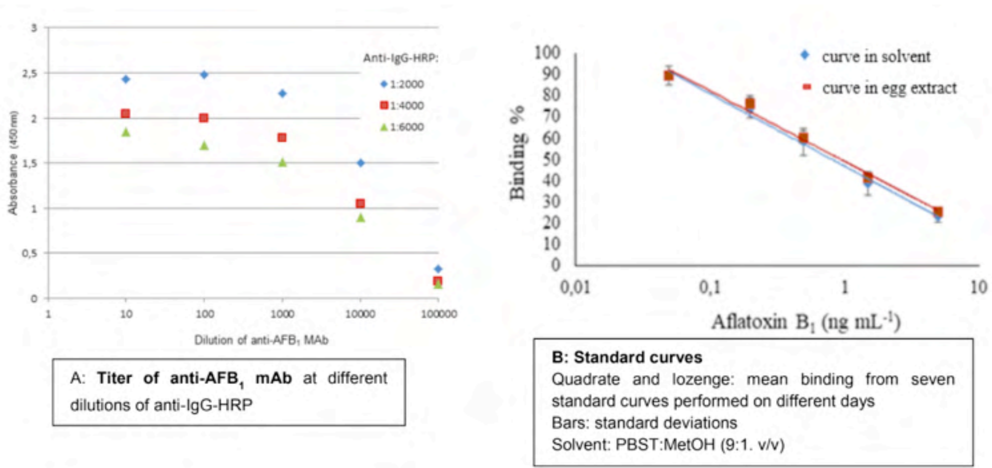


Fig. 2. Anti-AFB$_1$ mAb titer & anti-IgG-HRP, and standard curves for detection of aflatoxin by ic-ELISA.

Reagent	Developed ic-Elisa[1]	Commercial kit[1]
AFB_1-BSA	US$ 0.22	
BSA 0.1%	US$ 0.14	
MAb anti-AFB_1	-[2]	-
Anti-IgG-HRP	US$ 0.29	
Microplate (96 wells)	US$ 2.86	
Total value	US$ 3.51	US$ 230.52

Table 1. Cost comparison: ic-ELISA developed for aflatoxin analysis in egg and commercial kit in different matrixes.

[1]Cost for microplate with 96 wells

[2] Value considered insignificant to be below US$ 0.01

Dollar quotation: US$1.00 = R$ 3.80 (april / 2019)

RECOVERY[1]			PRECISION			
			Repeatability[2]		Intermediate Precision[3]	
AFB_1 spiked (μg kg^{-1})	Mean* ± SD (μg kg^{-1})	Mean Recovery (%)	RSD (%)	Mean (%)	RSD (%)	Mean (%)
1.0	0.9 ± 0.12	96	9.5	12	14.7	15
2.0	1.9 ± 0.03	101	13.0		14.8	
5.0	4.9 ± 0.25	99	13.5		14.8	

Table 2. Recovery and precision of aflatoxin B_1 in artificially contaminated egg by ic-ELISA.

[1] Data expressed as average ± SD of three determinations analyzed in seven replicates

[2] Data expressed as the relative standard deviation (RSD) of one determination analyzed in seven replicates for each concentration

[3] Data expressed as the relative standard deviation (RSD) of three determinations analyzed in seven replicates for each concentration in different days

Matrix	Hybridome/year	Aflatoxin, our data*			Aflatoxin data in publications**			
		Method	(µg kg-1)		Method	(µg kg-1)		Reference
			LOD	LOQ		LOD	LOQ	
Egg	AF4/2017	icElisa	0.35	0.71	Commercial ELISA kit	1.00	N.M.	DEEB; AMAN; EL-HAWARY, 2017
	AF4/2019	icElisa	0.02	0.04	Commercial ELISA kit	0.50	1.00	TULAYAKUL et al., 2018
Feed	AF2/2012 Broiler	icElisa	1.25	1.43	Commercial ELISA kit	1.10	2.50	PEREIRA; CUNHA; FERNANDES, 2020
	AF2/2012 Laying hen	icElisa	0.04	0.05	HPLC	2.00	N.M.	MUÑOZ-SOLANO; GONZÁLEZ-PEÑAS, 2020
Cereal	- /2020	LC MS/MS multmycotoxins	0.30	0.70	LC MS/MS multmycotoxins	0.10	0.70	SOLFRIZZO et al., 2018

Table 3. Developed ic-ELISA for aflatoxin detection in egg and feed, in comparison with other methods and reports.

*LOD: 0.017 ng/g and LOQ: 0.027 ng/g in current AFB_1 analysis, corresponding to pg level of sensitivity. *ic-ELISA was developed using mAb produced by hybridoma AF4 with high specificity and reactivity to AFB_1 and Aflatoxicol (*in vivo* interconverting AFB_1 metabolite), but low cross-reactivity with other analogs.

**Such publications evaluated Aflatoxin in individual matrix, i.e. aflatoxin was monitored in egg, feed or corn, but did not consider the whole egg chain: from supplies to produced eggs.

N.M. (Not mentioned).

TLC (Thin Layer Chromatography)

HPLC-FLD (High Performance Liquid Chromatography with Fluorescence Detector)

LC-MS/MS (Liquid Chromatography tandem Mass Spectrometry)

Farm		WEIGHT (g)			SHELL	
		Egg	Yolk	White	Percentage (%)	Thickness (mm)
A	Mean	$56.24^b \pm 3.33$	$16.72^b \pm 1.41$	$33.68^b \pm 2.35$	$10.4^a \pm 0.86$	$0.370^a \pm 0.04$
	Range	45.32 - 64.99	13.61 - 21.24	26.13 - 43.85	7.9 - 13.8	0.301 - 0.491
B	Mean	$58.85^a \pm 3.40$	$17.85^a \pm 1.42$	$34.98^a \pm 2.93$	$10.2^a \pm 0.71$	$0.373^a \pm 0.04$
	Range	47.04 - 65.30	14.19 - 20.32	31.44 - 40.57	8.2 - 12.6	0.327 - 0.492

Table 4. Physical parameters concerning quality in egg sampled in Northern Parana state.

Means followed by different letters in the same column differed by Wilcoxon test ($p < 0.05$)

Farm	Number of Samples	Positive sample (%)	Mean (µg kg-1)	Range (µg kg-1)
A	200	8	$0.84^a \pm 0.11$	0.70 – 1.12
B	100	15	$0.94^a \pm 0.22$	0.70 – 1.29

Table 5. Aflatoxin B_1 contamination in egg by ic-ELISA, Northern Parana state.

Means followed by different letters in the same column differed by the Wilcoxon test ($p < 0.05$)

Farm	Aflatoxin	WEIGHT (g)			SHELL	
		Egg	Yolk	White	Percentage (%)	Thickness (mm)
A	Detected (n = 16)	$56.24^a \pm 3.33$	$16.12^a \pm 1.09$	$33.43^a \pm 1.72$	$10.61^a \pm 0.72$	$0.39^a \pm 0.05$
	Non-Detected (n = 184)	$56.32^a \pm 3.41$	$16.78^a \pm 1.42$	$33.70^a \pm 2.40$	$10.38^a \pm 0.87$	$0.37^a \pm 0,04$
B	Detected (n = 15)	$57.52^a \pm 4.37$	$17.56^a \pm 1.51$	$33.89^a \pm 3.42$	$10.54^a \pm 0.86$	$0.39^a \pm 0.06$
	Non-Detected (n = 85)	$59.06^a \pm 3.18$	$17.88^a \pm 1.41$	$35.17^a \pm 2.83$	$10.18^a \pm 0.68$	$0.37^a \pm 0.04$

Table 6. Weights, shell percentage & thickness, and AFB_1 detected / non-detected egg samples.

Samples >LOD (0.35 μg kg^{-1}) were considered positive

Means followed by different letters in the same column differed by Wilcoxon test ($p < 0.05$)

SAMPLING (2016)	LAYING HEN			EGG					Aflatoxin (µg kg^{-1}) Mean	
	Feed Corn crop**	T°CShed	Hen Age, weeks	Weight (g) Total	Yolk	White	%	Shell Thickness (mm)	Sampling	Corn crop**
Farm A		31	30	54.028	15.071	33,687	9.8	0.4095	0.77	
		28	31	53.045	15.143	32.291	10.6	0.4315	0.86	
		28	31	53.092	14.380	33.101	10.6	0.4276	0.81	
		28	31	52.343	15.032	31.789	10.6	0.4423	0.85	
Abril	2015	29	32	60.158	15.662	38.337	10.2	0.3720	0.73	0.84^a
		23	33	54.944	16.456	32.611	10.7	0.3786	0.80	
		23	33	56.491	15.626	33.948	12.2	0.3598	1.01	
		23	33	55.784	17.026	31.727	12.6	0.3711	0.72	
May	2015	19	34	57.474	17.402	34.017	10.5	0.3565	0.88	
		21	37	53.852	16.381	31.876	10.4	0.3400	0.78	
June	2015	22	39	54.604	16.417	32.482	10.4	0.3231	1.12	
		22	39	54.674	16.122	32.820	10.5	0.3164	0.93	
July	2016	26	42	55.720	17.011	32.844	10.5	0.3738	0.84	
		26	42	60.313	17.665	36.340	10.5	0.3584	0.78	0.83^a
August	2016	29	47	56.257	18.221	32.266	10.3	0.3213	0.86	
Farm B		31	33	53.609	14.967	33.184	10.2	0.4923	1.26	
		31	34	57.442	17.079	34.665	9.9	0.4849	1.21	
		31	34	59.345	16.625	36.736	10.1	0.4850	1.29	
April	2015	31	34	57.983	16.361	35.806	10.0	0.4852	1.23	
		30	36	62.284	20.302	35.847	9.8	0.3547	0.71	
		30	36	55.019	16.197	33.100	10.4	0.3599	1.03	1.00^a
		23	37	54.724	16.621	31.511	12.0	0.3664	0.98	
		23	37	54.595	16.587	31.637	11.7	0.3735	1.07	
May	2015	21	41	55.899	16.742	33.464	10.2	0.3642	0.76	
June	2015	21	42	47.037	17.408	23.705	12.6	0.3356	0.77	
		22	44	56.756	17.873	33.344	9.8	0.3431	0.75	
		26	46	61.040	19.015	35.390	10.4	0.3673	0.72	
July	2016	23	47	62.904	19.608	36.919	10.1	0.3401	0.86	
		24	49	60.154	18.987	34.773	10.6	0.3610	0.74	0.76^a
		24	49	63.966	19.063	38.289	10.3	0.3582	0.70	

Table 7. Aflatoxin positive samples*: quality parameters versus contamination in egg.

* Samples >LOD (0.35 μg kg^{-1}) were considered positive; for calculation of means, those >LOQ (0.71 μg kg^{-1}) were considered

** AFLA mean in egg from laying hen eating feed prepared with corn harvested in each corresponding crop year

Means followed by different letters in the same column differed by the Wilcoxon test ($p < 0.05$)

ÍNDICE REMISSIVO

A

Adaptabilidade 100

Adequação nutricional 13, 183, 185

Alimentação 9, 13, 9, 10, 11, 22, 68, 69, 70, 71, 72, 73, 76, 78, 83, 117, 125, 130, 142, 156, 159, 160, 165, 172, 176, 181, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205

Alimentação saudável 10, 72, 176, 181, 183, 194

Alimentos Funcionais 64, 66, 78, 79, 83, 87

Alimentos para animais de estimação 142, 143

Alimentos saudáveis 130, 173, 175, 176, 183

Araçá Amarelo 12, 117, 118, 119, 120, 121, 122, 123, 124, 125

Artemia salina 118, 120, 122, 123, 124, 125, 126, 127

Avaliação Sensorial 129, 132, 138

B

Bebidas Lácteas 1, 6

Benefícios 25, 54, 65, 66, 68, 70, 75, 78, 110, 165, 178, 194

C

Cará Gigante 90

Carica papaya 100, 101

Compostos bioativos 69, 74

Conservação de alimentos 26, 90

Contaminação 11, 36, 76, 129, 143, 164, 166, 167, 168, 169, 171, 176, 180

Couve 10, 9, 10, 11, 12, 17, 18, 19, 21, 22, 23, 165

Culinária 90

Curvas de crescimento 110, 115

D

Desperdício 64, 119, 197, 198, 200, 201, 202, 203, 204

Doenças de origem alimentar 175, 176, 178, 179, 180

E

Ensino Remoto Emergencial 10, 24, 25, 32

Estrutura Subterrânea 90

F

Fermentação 24, 25, 26, 27, 28, 29, 31, 32, 33

G

Gorduras Trans 155, 159, 160, 162

H

Hidrogenação 155, 157, 158

Higiene 21, 22, 175, 203, 205

Hortaliças 13, 9, 10, 11, 17, 22, 135, 137, 141, 164, 165, 166, 167, 169, 170, 171, 172, 173, 175, 176, 177, 181, 193

I

Industrial 12, 25, 46, 64, 65, 85, 86, 92, 119, 142, 151, 155, 156, 157, 159, 203

Infecção 164, 180

L

Lactobacilos 54

LED 10, 11, 12, 15, 16, 17, 18, 19, 20, 53

M

Manga Palmer 110

Metabolismo 25, 26, 31, 64, 66, 69, 70, 73, 79, 155, 157, 158

Micotoxinas 142, 143, 152

Modelagem 110

N

Novas tecnologias 10

P

Parasito 164

Picles 12, 129, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140

Planejamento de cardápio 183

Plant-Based 10, 1, 2, 3, 5, 6, 7, 83, 85

Probiótico 33, 54

Propriedades Biológicas 11, 64

Psidium Cattleianum 12, 117, 118, 119, 121, 122, 124, 125, 126, 127, 128

Q

Qualidade 10, 12, 1, 3, 9, 10, 11, 22, 33, 36, 45, 46, 47, 66, 78, 89, 91, 94, 95, 96, 100, 101, 102, 114, 124, 127, 129, 137, 140, 143, 165, 166, 170, 171, 172, 173, 176, 178, 184, 186, 187, 188, 189, 192, 195, 196, 199, 203, 205

R

Ruminantes 155, 156, 157, 158, 159, 161, 162

S

Sanitização 10, 11, 12, 14, 15, 16, 18, 19, 20, 22, 93, 170, 171, 173, 182

Saúde 2, 9, 4, 11, 18, 21, 22, 25, 54, 64, 65, 66, 68, 69, 70, 71, 73, 75, 77, 78, 79, 97, 110, 120, 124, 125, 142, 155, 156, 159, 160, 161, 162, 164, 165, 167, 169, 171, 172, 173, 175, 177, 178, 179, 180, 181, 183, 184, 185, 186, 187, 188, 191, 193, 194, 195, 196, 201

Segurança Alimentar 12, 20, 33, 79, 98, 117, 118, 120, 124, 142, 143, 181, 184, 195, 205

Sementes de papoila 11, 64, 67, 68, 69, 70, 71, 76, 77, 78

Sementes de quinoa 64, 71, 75, 77

Sensorial 25, 33, 61, 128, 129, 132, 133, 137, 138, 140, 141, 202, 205

Serviços de alimentação 160, 172, 190, 197, 202, 203, 205

T

Teor Proteico 10, 1, 6, 7, 72

Toxicidade 12, 75, 117, 118, 120, 122, 123, 124, 125, 126, 127

Tubérculo 90

U

Ultravioleta 10, 9, 10, 11, 14, 15, 17, 18, 19, 20, 21

V

Variabilidade Genética 100

Vegan 1, 2

Vegetais 10, 1, 2, 3, 6, 7, 17, 18, 20, 65, 77, 155, 157, 158, 164, 165, 170, 181

Vida de prateleira 11, 119, 129, 133, 134, 136

Vitória-Régia 12, 129, 130, 131, 133

www.atenaeditora.com.br

contato@atenaeditora.com.br

@atenaeditora

www.facebook.com/atenaeditora.com.br

ALIMENTOS, NUTRIÇÃO E SAÚDE 4

Ano 2021

www.atenaeditora.com.br
contato@atenaeditora.com.br
@atenaeditora
www.facebook.com/atenaeditora.com.br

Alimentos, Nutrição e Saúde 4

Ano 2021