Atena Editora
Ano 2020

# Alimento, Nutrição e Saúde 4

Givanildo de Oliveira Santos
(Organizador)

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Atena Editora
**Ano 2020**

*Ciências Biológicas e da Saúde*

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Alimento, nutrição e saúde
4

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**Revisão:** Os Autores
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**Dados Internacionais de Catalogação na Publicação (CIP)**

A411 Alimento, nutrição e saúde 4 / Organizador Givanildo de Oliveira Santos. – Ponta Grossa - PR: Atena, 2020.

Formato: PDF
Requisitos de sistema: Adobe Acrobat Reader
Modo de acesso: World Wide Web
Inclui bibliografia
ISBN 978-65-5706-652-2
DOI 10.22533/at.ed.522200312

1. Alimentação sadia. 2. Saúde. 3. Nutrição. I. Santos, Givanildo de Oliveira (Organizador). II. Título.
CDD 613.2

**Elaborado por Bibliotecária Janaina Ramos – CRB-8/9166**

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## APRESENTAÇÃO

O presente livro “Alimento, Nutrição e Saúde 4” está composta por 17 capítulos com vasta abordagens temáticas. Durante o desenvolvimento dos capítulos desta obra, foram abordados assuntos interdisciplinar, na modalidade de artigos científicos, pesquisas e revisões de literatura capazes de corroborar com o desenvolvimento cientifico e acadêmico.

O objetivo central desta obra foi descrever as principais pesquisas realizadas em diferentes regiões e instituições de ensino no Brasil, dentre estas, cita-se: a caracterização físico-química de frutos, desenvolvimento de novos alimentos, analise sensorial, segurança alimentar, nutrição funcional, utilização de plantas medicinais com o objetivo de melhorar os teores de nutrientes e possíveis efeitos sobre o emagrecimento, análises físico-química e microbiológicas. São conteúdos atualizados, contribuindo para o desenvolvimento acadêmico, profissional e tecnológico.

A procura por alimentos que contribuem para o bem-estar e prevenção de patologias do indivíduo aumentou-se nos últimos anos. Deste modo, a tecnologia de alimentos deve acompanhar a área da nutrição com o objetivo de desenvolver novos produtos que atendam a este público. No entanto, é preocupante o grande número de pessoas que buscam realizar “dietas” sem devido acompanhamento profissional, colocando em risco a sua saúde.

O livro “Alimento, Nutrição e Saúde 4” descreve trabalhos científicos atualizados e interdisciplinar em alimentos, nutrição e saúde. Resultados de pesquisas com objetivo de oferecer melhores orientações nutricionais, e alimentos que possam contribuir para melhorar a qualidade de vida dos consumidores, obtendo uma alimentação saudável e prevenindo de possíveis patologias.

Desejo a todos (as) uma boa leitura.

Givanildo de Oliveira Santos

## SUMÁRIO

# CAPÍTULO 12

# NUTRITIONAL, BIOCHEMICAL AND SPERM PARAMETERS OF RATS SUBMITTED TO FOOD SUPPLEMENTATION WITH PERUVIAN MACA

*Data de aceite: 01/11/2020*
*Data de submissão: 18/09/2020*

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**ABSTRACT**: **INTRODUCTION:** Sexual problems are able to negatively affect interpersonal relationships, mood and well-being of the individual. Thus, the effectiveness of the treatment of sexual dysfunction can improve not only the sex, but the overall quality of life individuals. The Lepidium meyenii, popularly known as Maca has been used for centuries by the Peruvian population to increase vitality and treating infertility**. OBJECTIVE**: To evaluate the effects of the administration of diet enriched with processed yellow maca powder marketed in Brazil in sperm parameters of rats. **METHODS:** For in vivo evaluation, 21 male Wistar rats (Rattus norvegicus), with 45 days old, were divided into three groups (n = 7): control group (C) - control diet (AIN-93M); Group Maca 1 (M1) - Diet plus Control maca at the dose of 1g / kg; Group Maca 2 (M2) - Diet plus Control maca at a dose of 2g / kg. We used dried extract of maca powder. The experiment lasted 54 days, considering the inclusion of sperm maturation in the epididymis period. They were collected weekly data on household food consumption, body weight, water intake, urine output and fecal animals. At the end of the study were analyzed the morphology, motility and concentration of sperm and testosterone, as well as the weight of the seminal vesicle and fat epididimal. **RESULTS**:

not identified statistically significant differences between experimental groups for all variables. **CONCLUSION**: We conclude that the yellow Peruvian Maca marketed in Brazil had no effect on sperm parameters of rats.
**KEYWORDS**: Rats, Peruvian maca, nutrition, reproductive organs, spermatogenesis.

## PARÂMETROS NUTRICIONAIS, BIOQUÍMICOS E ESPERMÁTICOS DE RATOS SUBMETIDOS A SUPLEMENTAÇÃO ALIMENTAR COM MACA PERUANA.

**RESUMO**: **Introdução**: Os problemas sexuais são capazes de afetar negativamente o relacionamento interpessoal, o humor e o bem estar do indivíduo. Deste modo, a efetividade do tratamento da disfunção sexual pode melhorar não apenas as relações sexuais, mas a qualidade de vida global do indivíduo. O Lepidium meyenii, popularmente conhecido como Maca, tem sido usado há séculos pela população do Peru para aumentar a vitalidade e tratar a infertilidade. **Objetivo**: Avaliar os efeitos da administração da dieta enriquecida com maca peruana amarela em pó desidratada, industrializada e distribuída no Brasil nos parâmetros espermáticos de ratos. **Métodos**: Para avaliação in vivo, 21 ratos machos Wistar (Rattus norvegicus), com 45 dias de vida, foram distribuídos em três grupos (n=7): Grupo Controle (C)- dieta controle (AIN-93M); Grupo Maca 1 (M1)- dieta controle acrescida de maca peruana na dose de 1g/kg de peso; Grupo Maca 2 (M2)- dieta controle acrescida de maca peruana na dose de 2g/kg de peso. Foi utilizado extrato seco em pó de maca peruana. O experimento teve duração de 54 dias, considerando a inclusão do período de maturação dos espermatozóides no epididimo. Foram coletados semanalmente os dados de consumo alimentar, peso corporal, ingestão hídrica, excreção urinária e fecal dos animais. Ao final do estudo foram analisadas a morfologia, a motilidade e a concentração de espermatozoides e de testosterona, bem como o peso da vesícula seminal e gordura epididimal. Resultados: Não se identificou diferenças estatisticamente significativas entre os grupos experimentais, para todas as variáveis estudadas. **Conclusão**: Conclui-se que a Maca peruana amarela comercializada no Brasil não apresentou efeitos nos parâmetros espermáticos de ratos.
**PALAVRAS - CHAVE**: Ratos, Maca Peruana, Nutrição, Órgãos Reprodutivos, Espermatogênese.

## 1 I INTRODUCTION

Originating from the central Andes of Peru, the *Lepidium meyenii* plant, commonly called Peruvian maca, consists of a tuber belonging to the Brassicaceae family and is grown at altitudes of 3’500 to 4’500 meters above sea level (WANG et al., 2007). Maca presents several varieties of hypocotyls, varying from white to black, which constitute the edible part of the plant and present various properties (VALERIO; GONZALE, 2005) . Dry hypocotyls are composed of 10.2% protein, 59% carbohydrate, 2.2% lipid and 8.5% fiber, which correspond to the primary metabolites and to the nutritional components. Secondary metabolites include macaridine, macaene, macamides and alkaloids, responsible for the biological and pharmaceutical properties of the plant (ZENG et al., 2000; GONZALES, 2012).

In recent years, the consumption of this vegetable, exported by Peru mainly in powder form, has boosted in many countries and is proposed in various processed varieties, such as micronized powder (powder, tablets), lyophilized or hydro-alcoholic extracts (XING-HAO et al., 2014). It is of great importance to point out that glucosinolates are equivalent to 1% of the entire fresh hypocotyl and are considered a fundamental criterion to judge the quality of the final product, since the ways of processing the hypocotyl produce the same glucosinolates present in the fresh hypocotyl, but in variable quantities (XING-HAO et al., 2014; LI et al., 2014).

The search for food supplements with high nutritional value intensified the consumption of Peruvian maca because of its energetic, antioxidant and hypocholesterolemic properties. In addition to standing out as an alternative for infertility treatment, it increases athletic performance (GRUNEWALD; BAILEY, 1993), shows favorable effects on the metabolic syndrome (VEČEŘA et al., 2007; GONZALES et al., 2002), present antioxidant properties (SANDOVAL et al., 2002), reduces the risk of hypertension, and has favorable effects on memory (GONZALES et al., 2002), and on sexual desire (ZENG et al., 2000; GONZALES et al., 2002.

The objective of the present study is to evaluate the consumption of Peruvian maca, in the dehydrated form, on the nutritional, biochemical and spermatic parameters of rats.

## 2 | MATERIAL AND METHODS

The study was developed at the Laboratories of Experimental Nutrition and of Reproductive Biotechnology of the University of « Vale do Itajaí » (Univali), under the approval No. 04/15 of the Ethics Committee on Animal Use (CEUA). The procedures adopted were carried out according to the Law No. 11.794 of 2008 for the use of rodents for scientific purposes and in accordance with the Guidelines for the Use of Animals of the National Council for the Control of Animal Experimentation (CONCEA) (MINISTÉRIO DA CIÊNCIA TEIM, 2015) and the International (NATIONAL RESEARCH COUNCIL et al., 2010).

Twenty-one adult male Wistar albino rats (*Rattus norvegicus*) with a mean initial weight of 220.57 g ± 19.66, were obtained from the Univali central animal facility. The animals were randomized in 3 groups of 7 animals: 1) Control Group (C): control diet (AIN - 93M), as recommended by the American Institute of Nutrition (NATIONAL RESEARCH COUNCIL et al., 2010), 2) Maca Group 1 (M1): control diet + Peruvian maca (1 g/kg animal weight), 3) Maca Group 2 (M2): control diet + Peruvian maca (2 g/kg animal weight). The suggested amount for human consumption is 23.33 g / day (1 tablespoon), which equals 1 g / kg of animal weight. The quantity of maca was recalculated weekly after weighing the animals. In this study, maca was used in the dried yellow variety, industrialized and distributed in Brazil (Jasmine, Curitiba, PR, Brazil). The nutritional composition, according to the product label,

in a portion of 100 g, has an energy value of 332 kcal, 70% carbohydrate, 12.8% protein, 0% fat, 5% fiber and 24 mg sodium.

The animals were kept in stainless steel metabolic cages in a closed room, under controlled temperature (22 ± 2 ºC), a ventilation system through insufflators and exhausts, and a photoperiod of 12 hours. During a period of adaptation to the environment of 10 days, the animals received commercial pelleted diet (Biolab®) and water ad libitum. Subsequently, the animals received their respective diets and unconditional water supply. Data on food consumption, body weight, water intake, urinary and fecal excretion of the animals were collected weekly. On the 54th day of the experiment, which outlasts spermatogenesis and epididymal maturation, the animals without previous fasting, were anesthetized by intraperitoneal injection (ketamine hydrochloride, FAGRA® 75 mg/kg (Hortolândia, SP, Brazil), xylazine hydrochloride ANASEDAN® 10 mg/kg (Ceva, Paulínia, SP, Brazil), acepromazine ACEPRAN® 5 mg/kg (Vetnil, Louveira, SP, Brazil)). After an opening of the abdominal-thoracic wall, euthanasia was performed by cardiac puncture of the right ventricle (17). The collected blood was centrifuged at 3'000 RPM (1000-1200 G) at room temperature for 15 minutes to obtain the serum.

## 2.1 Feed conversion ratio

The feed conversion ratio (FCR) was calculated for each animal at the end of the study as:

FCR (%) = (Final body weight - Initial body weight) / (weight of food consumed) x 100

## 2.2 Biochemical analysis

Serum glucose, lipid profile (total cholesterol, HDL-cholesterol, and triglycerides), liver profile (alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity), and renal profile (urea and creatinine) were determined using commercial kits for each substance according to the manufacturers' protocols. Readings were performed with an automated equipment (Cobas Mira Plus, Roche®). Testosterone was measured by chemiluminescence immunoassay.

## 2.3 Sperm parameters analysis

For each animal, both vas deferens and tails of the epididymides were dissected and placed for 10 minutes at 37 ºC in a buffered medium (GV-HEPES, INGAMED®, Maringá, PR, Brazil) for dispersion and capacitation of the spermatozoa (PADMANABHAN et al., 2008). A 20 µL aliquot of the dispersion medium was then placed between slide and coverslip for determining the percentage of motile sperm at 200x magnification (GUPTA et al., 2013). For sperm morphology smears were air-dried, stained using a kit (Panótico, NewProv®, Pinhais, PR, Brazil) and observed under 1000x magnification under oil. Spermatozoa were considered normal or abnormal (without hook, banana-shaped, triangular or amorphous

heads) (GUPTA et al., 2013). One hundred spermatozoa were evaluated per slide. Sperm concentrations were measured using a counting chamber (Makler®, Spectrun, SP, Brazil) with a bright field microscope under 100x magnification.

## 2.4 Histology of the testes

The left testes of the animals (N=21) were immersed and fixed in a 10% formaldehyde solution for 2 days. The pieces were then dehydrated in an ethyl alcohol series of 70%, 90% and 100% for a total period of 24 hours. Subsequently, pieces were brightened in xylol and embedded in paraffin. The 1:5 semi-seriate sections were stained with Hematoxylin-Eosin ( FRANÇA , 1991; COSTA; ENEZES; PAULA, 2007). Two tubules of each 3 sections of all slides were chosen on the basis of their circular contour, excluding elliptic or elongated tubules, and were photographed at a magnification of 400x.

The morphometric measurements and evaluation of the seminiferous tubules and germinal epithelium were performed using the open source image processing program ImageJ (https://imagej.net). Measurements were performed as shown in Figure 1. Pictures (400x) of the seminiferous tubules were calibrated with a micrometrical scale and submitted to ImageJ. The external perimeter of the seminiferous tubule was identified and the corresponding surface (EA) calculated, then likewise the perimeter of the lumen and its surface (IA) were determined. The surface of the germinal epithelium (GE) was calculated by the difference (EA - IA). The diameters (D) of the seminiferous tubule were calculated from the EA value using the following formula:

$D = 2 \times (EA \times \pi)0.5$ , where $\pi=3.14$

The thickness of the active germinative layer was measured using a micrometrical reticule incorporated into the ocular at a 200x magnification.

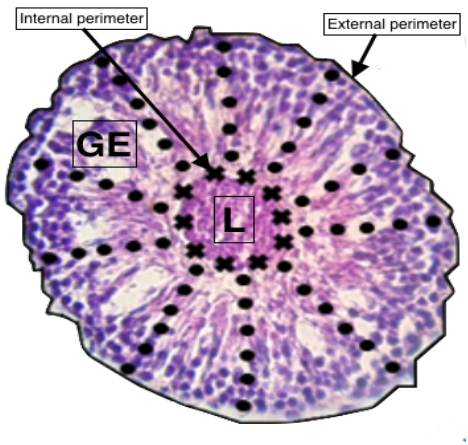


Figure 1. Representation of a seminiferous tubule and of the measurement performed with ImageJ. External perimeter = boundary of the seminiferous tubule; internal perimeter =

boundary of the lumen (L); entire germinative epithelium = zone between the external and internal perimeters.

## 2.5 Statistical analysis

Statistical analysis was performed using GraphPad Instat software, version 3.0. Data were submitted to an analysis of variance (ANOVA), two-tailed, with Tukey-Kramer post-test for comparison of the means between groups. Differences were considered significant at $p < 0.05$.

## 3 I RESULTS

The mean FCR values, calculated for each group (C, M1, and M2), are presented in Figure 2.

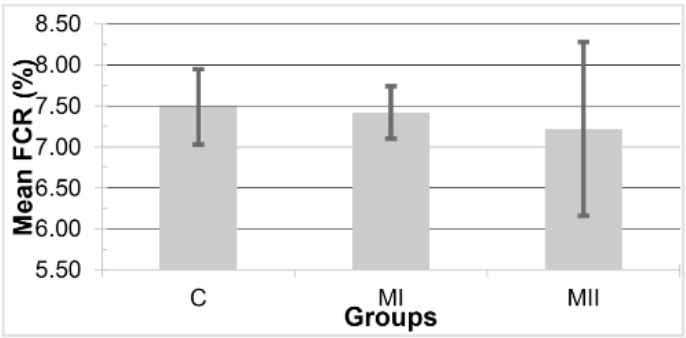


Figure 2. Mean feed conversion ratio (FCR) in the control group (C) and maca-treated groups (MI, MII) at the end of the 7 week study period. FCR is calculated as (weight gain/ food consumption) in %. Error bars indicate the standard deviation of the mean. There was no statistical difference between groups (p=0.75).

There were no statistically significant differences between groups (p=0.75). Water intake, fecal excretion, and body weight were also similar in the three groups (data not shown). The daily urinary excretions (mL/day) of the animals during the whole study period is shown in Table 1. There were no statistically significant differences between groups, although, at week 7, groups M1 and M2 presented slightly lower mean values than group C (p=0.08).

| Week Number | C | M1 | M2 | p |
|---|---|---|---|---|
| 1 | 15.14 ± 11.88 | 16.83 ± 10.96 | 19.86 ± 20.38 | 0.84 |
| 2 | 18.29 ± 12.45 | 16.51 ± 8.86 | 20.26 ± 21.64 | 0.90 |
| 3 | 15.49 ± 8.83 | 16.17 ± 9.23 | 12.57 ± 4.08 | 0.66 |
| 4 | 10.71 ± 10.01 | 16.06 ± 9.29 | 8.17 ± 3.62 | 0.21 |
| 5 | 15.71 ± 7.48 | 17.00 ± 6.11 | 12.29 ± 7.57 | 0.45 |
| 6 | 14.57 ± 8.02 | 20.57 ± 13.25 | 14.86 ± 5.76 | 0.43 |
| 7 | 20.64 ± 5.04 | 14.07 ± 3.17 | 15.43 ± 7.07 | 0.08 |

Table 1. Means ± standard deviations of urinary excretion (mL/day) of the animals in the control (C) and the maca-treated groups (M1, M2) throughout the study.

The biochemical parameters (glucose, triglycerides, cholesterol, HDL, ALP, creatinine, urea, aspartate-transaminase (AST), and alanine-transaminase (ALT) are presented as means ± SD in Table 2. The three groups did not show any statistically significant differences for these parameters.

| Biochemical parameters | C | M1 | M2 | p |
|---|---|---|---|---|
| Glucose (mg/dL) | 347.00 ± 41.46 | 381.14 ± 75.67 | 371.43 ± 44.20 | 0.55 |
| Triglycerides (mg/dL) | 112.33 ± 43.60 | 141.29 ± 58.02 | 138.00 ± 35.41 | 0.50 |
| Cholesterol (mg/dL) | 59.83 ± 15.29 | 70.29 ± 16.76 | 61.71 ± 10.06 | 0.38 |
| HDL (mg/dL) | 31.17 ± 7.65 | 34.57 ± 6.92 | 30.29 ± 5.06 | 0.46 |
| ALP (mg/dL) | 77.67 ± 18.22 | 73.00 ± 18.35 | 59.43 ± 12.42 | 0.14 |
| Creatinine (mg/dL) | 0.65 ± 0.10 | 0.69 ± 0.07 | 0.64 ± 0.08 | 0.41 |
| Urea (mg/dL) | 25.67 ± 5.16 | 27.14 ± 4.05 | 28.57 ± 3.10 | 0.47 |
| AST (U/L) | 78.67 ± 17.59 | 97.86 ± 35.61 | 83.86 ± 16.75 | 0.38 |
| ALT (U/L) | 30.83 ± 12.98 | 32.29 ± 22.18 | 23.29 ± 6.94 | 0.52 |

Table 2. Means ± standard deviations of the biochemical parameters of the animals in the control (C) and the maca-treated groups (M1, M2) at the end of the study.

The relative weights of the testes, seminal vesicles and epididymal fats of the animals at the end of the experiment are presented in Table 3. No statistical differences were observed between groups.

| Organs | C | M1 | M2 | p |
|---|---|---|---|---|
| Testes | 0.81 ± 0.10 | 0.80 ± 0.07 | 0.84 ± 0.14 | 0.82 |
| Seminal vesicles | 0.32 ± 0.08 | 0.30 ± 0.05 | 0.31 ± 0.06 | 0.83 |
| Epididymal fats | 2.34 ± 0.71 | 2.28 ± 0.72 | 2.41 ± 0.96 | 0.96 |

Table 3. Means ± standard deviations of the relative body weights (%) of various organs in the control (C) and the maca-treated groups (M1, M2) at the end of the study. The relative weights were calculated by dividing the weight of the organs in grams by the body weight of each animal on the day of collection and multiplying the result by 100.

Data on motility, sperm concentration, sperm morphology and serum testosterone concentration are presented in Table 4.

| Parameters | C | M1 | M2 | p |
|---|---|---|---|---|
| Sperm motility (%) | 64.8 ± 9.5 | 73.6 ± 10.9 | 75.1 ± 14.0 | 0.27 |
| Sperm concentrations (x$10^6$/mL) | 6.6 ± 2.8 | 7.4 ± 1.2 | 7.6 ± 2.9 | 0.52 |
| Sperm normal morphology (%) | 99.2 ± 1.6 | 99.4 ± 0.5 | 97.4 ± 2.1 | 0.06 |
| Testosterone (ng/dL) | 175.2 ± 37.1 | 171.3 ± 52.5 | 192.3 ± 62.2 | 0.73 |

Table 4. Means ± standard deviations of the sperm parameters and blood testosterone in the control (C) and the maca-treated groups (M1, M2).

Sperm motility in Groups M1 (73.6 ± 10.9 %) and M2 (75.1 ± 14.0 %) was slightly higher than that of the control group C (64.8 ± 9.5 %), but these differences did not reach statistical significance (p = 0.3). Similarly, the sperm concentrations of spermatozoa were higher in Groups M1 (7.4±1.2) and M2 (7.6±2.9), than in Group C (6.6±2.8), but here again the difference was not significant (p=0.5). Sperm normal morphology of Group M2 showed a non-significant (p=0.06) tendency to lower mean values when compared to Groups C and M1. The serum testosterone was equal in the three groups.

The morphometric parameters of the histological sections, measured with ImageJ, are presented in Table 5.

| Area (μm²) | C | M1 | M2 |
|---|---|---|---|
| Seminiferous tubule | 40811 ± 5418 (916) [a] | 41274 ± 4625 (714) [b] | 38171 ± 4823 (744) [a,b] |
| Lumen | 3080 ± 1032 (174) [c,d] | 3903 ± 1764 (272) [c] | 3710 ± 872 (135) [d] |
| Entire germinative epithelium | 37732 ± 5308 (897) [e] | 37371 ± 4446 (686) [f] | 34461 ± 4656 (718) [e,f] |

Table 5. Means ± standard deviations (standard error of the mean) of the seminiferous tubules morphometry in the control (C) and the maca-treated groups (M1, M2). Morphometric determinations were performed using the ImageJ software (see figure 1).

Superscripts indicate the following p values: a=0.013, b=0.001, c=0.008, d= 0.002, e=0.005, f=0.002. Columns without corresponding superscripts are not statistically different.

The mean surfaces (±SEM) of the seminiferous tubules were significantly greater (p = 0.01) in groups C (40'811 ± 916 $\mu m^2$) and M1 (41'274 ± 714 $\mu m^2$), than in group M2 (38'171 ± 744 $\mu m^2$). The mean surfaces of the lumen area were higher (p=0.02) in Groups M1 (3'903 ± 272 $\mu m^2$) and M2 (3'710 ± 135 $\mu m^2$) than in group C (3'080 ± 174 $\mu m^2$). Consequently, the area located between the tubule perimeter and the lumen, corresponding to the entire germinative epithelium, was significantly lower in group M2 in respect to group C (p = 0.005) and group M1 (p = 0.002).

The mean diameter of the seminiferous tubules and the mean thickness of active germinative layer are shown in Table 6.

| Morphometric parameters | C | M1 | M2 |
|---|---|---|---|
| Diameter (µm) | 228 ± 15 [a] | 230 ± 12 [b] | 220+14 [a,b] |
| Epithelium thickness (µm) | 107.6 ± 14.9 [d,e] | 96.6 ± 6.8 [d,f] | 87.8 ± 7.0 [e,f] |

Table 6. Means ± standard deviations of the seminiferous tubules diameter for the control (C) and the maca-treated groups (M1, M2).

Superscripts indicate the following p values: a=0.013, b=0.001, d,e=0.001. Columns without corresponding superscripts are not statistically different.

The diameters ± SEM of the seminiferous tubules were significantly lower (p=0.01) in group M2 (220 ± 2 $\mu m$) than in group C (228 ± 3) and group M1 (230 ± 2). Similarly, there was a significant decrease (p=0.001) in the thickness of the active germinative epithelium layer from 107 $\mu m$ in group C down to 88 $\mu m$ in group M2.

Examples of histological cross sections of the seminiferous tubules of Groups C, M1, and M2 are shown in Figure 3. Besides the morphometric differences indicated in Table 5 and Table 6, no structural differences were observed, as well as no testicular degeneration, germinative cell spacing, desquamation, the formation of vacuoles or inflammatory infiltrates.

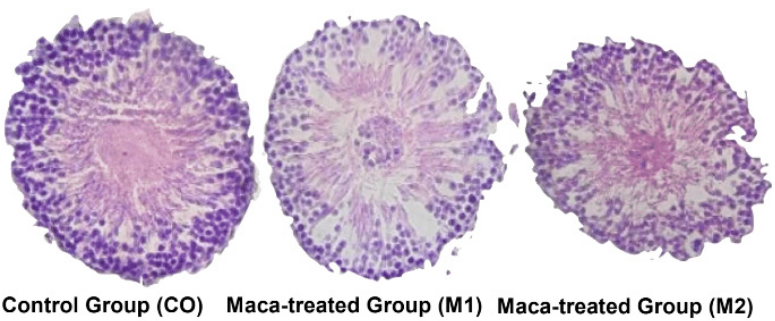


Figura 3. Examples of seminiferous tubules in the Control group (CO) and maca-treated groups (M1, M2).

## 4 | DISCUSSION

Yellow Peruvian maca added as a powder to the diet of the animals had no effect on their nutritional profile, as indicated by the absence of significant differences for FCR and fecal excretions between the control and treated groups after 54 days (Figure 2). Peruvian maca did not influence either the water intake and urinary excretion in respect to the control (Table 1). Likewise, weights of the testicles, seminal vesicles, and epididymal fats did not differ between groups (Table 3). These observations are in partial agreement with others studies on rats (CHUNG; RUBIO; GONZALES, 2005; GONZALES et al., 2006), in which various aqueous extracts of maca were administered in short (7 days) and long term (42 days) study. When using a high amount of the yellow extract (5 g per kg body weight) during 7 days, Chung et al. (2005) found an increase in body weight and a reduction in the weight of seminal vesicles, which correlated with lower serum testosterone concentrations. On the contrary, Gonzalez et al. (2006), comparing short and long term treatments with 3 different ecotypes of aqueous extracts (red, yellow and black), found no effects on the weights of the kidneys, liver, spleen, heart and the seminal vesicles.

In the present study, no significant differences in serum glucose concentrations were found between the control and the treated groups (Table 2). This result corroborates a study by Choi et al. (2012), who evaluated the effect of the liposoluble extract of yellow maca on the serum biochemical parameters of rats. Similarly, total cholesterol, HDL-cholesterol, and triglycerides serum levels were not statistically different between the control and the treated groups (Table 2). The hypocholesterolemic capacity attributed to maca, which is related to the bioactive compounds sitosterol present in this plant (SUGANO; MORIOKA; IKED, 1997; DINI et al., 1994), could not be confirmed in our animals who were fed with normal diet. Yet, in rats in which hypertriglyceridemia was induced with high sucrose diet (VEČEŘA et al., 2007), Peruvian maca was able to reduce significantly the concentration of total cholesterol. Maca did not influence the renal profile of the animals in our study, as determined by the creatinine and urea concentrations (Table 2). This observation is in agreement with a study on 557 human individuals, aged between 35 and 75 years, in which renal function, creatinine, and uric acid concentrations were similar between consumers and non-users of maca (GONZALES, 2010).

Our study showed no statistical differences between the control and the treated groups in respect to sperm motility, concentration, and morphology (Table 4). Still, both motility and sperm concentrations presented higher values at the highest dose of maca tested (M2). The absence of statical difference might be due to the small number of treated animals or alternatively, to the yellow maca variety used, which may induce a distinct biological response from the black ecotype, for which enhancements of epididymal sperm counts and motility were reported (GONZALES et al., 2006). It is also still unclear whether longer treatment periods might also favorably affect these variables in our case. Gonzales et

al. (2010) tested the aqueous extract of yellow maca in the prevention of testicular disorders induced by high altitudes. The rats were separated into 4 groups, with 2 groups being maintained at sea level and 2 groups at high altitude (4'340 m). Peruvian maca was given in the amount of 666.6 mg/day for 21 days. There was an increase in the sperm counts of the epididymis in the maca group at high altitude of 39.7% and it was verified that the inclusion of maca in the diet prevented the interruption of spermatogenesis induced by altitude. An aqueous extract of maca was also shown to protect rats from the deleterious effects of lead acetate on spermatogenesis ( RUBIO et al., 2006). In a study on supplementation of dry extract of Peruvian maca for 23 weeks on the quality of bovine semen, Clément et al. (2010) observed an improvement of sperm concentration, but no alterations in the mating behavior. In our study, the M2 group presented lower mean values of normal spermatozoa compared to Groups C and M1 ($p = 0.06$). This observation, which might be confirmed statistically by increasing the dose or the length of maca supplementation and the number of treated individuals, is contrary to what was found in human infertile males, for whom maca treatment tended to increase the percentage of normal spermatozoa ( TANCARA et al., 2010).

In our study, the levels of serum testosterone were not altered by maca consumption (Table 4). Similar results were observed in healthy humans subjects, in which the consumption of maca compared to a placebo had no effect on the serum levels of various endocrine and sex hormones, such as LH, FSH, PRL, T, E2 (GONZALEZ et al., 2003). Furthermore, the effect of maca on the libido was independent of the serum concentrations of testosterone, estradiol or anxiety state (GONZALES et al., 2002).

The morphometric parameters (mean surfaces of the seminiferous tubule, of the lumen, and of the germinative epithelium), determined with ImageJ (Table 5), and the mean diameter and mean thickness of the active cell layer of the germinative epithelium (Table 6), showed that maca in group M2 tended to induce a reduction of the tubule surface, an increase of the lumen area and a reduction of the epithelium thickness. These observations indicate that maca interacts directly or indirectly with the testicular structure and function, albeit no significant changes in sperm production and quality are observed. Structural changes in the seminiferous tubules have been shown to occur in diabetic rats, in which seminiferous tubule area and epithelium thickness were lower than in healthy animals (TRINDADE et al, 2013). Furthermore, the diameter of the seminiferous tubules and of the lumen are known to exhibit substantial differences throughout the testis according to the spermatogenic cycle (WING; CHRISTENSEN, 1982), whereas the thickness of the germinative epithelium may vary without affecting negatively the spermatogenic process (franca ET AL., 2000). In elderly men suffering from advanced prostate cancer and submitted to hormone treatment and radiotherapy, a reduction of the seminiferous tubule diameter and of the thickness of the germinative epithelium was observed principally as a result of germ cell loss ( MARTELO, 2013). It is recognized that a decrease of the thickness of the germinative epithelium accompanies testicular degeneration in various species (

BEZERRA et al., 2008 ; CAVALCANTE et al., 2014). Such observation was also reported in sheep, fed with alfalfa hay, a herbaceous plant rich in mineral and proteins, in which tubular diameter and the total length of the seminiferous tubules were preserved, whereas the thickness of the germinative epithelium decreased as alfalfa concentrations were increasing ( VÊNANCIO, 2015).

In the present study, a testicular degeneration, germinative cell spacing, desquamation, formation of vacuoles and inflammatory infiltrates, cannot explain the reduction of the seminiferous tubule diameter. It can be explained by a decreased thickness of the germinative epithelium, an observation that seems more attributable to sexual inactivity and rest than to a loss in germ cell activity, as sperm concentration at the highest maca treatment (M2) tend to be slightly higher than the control (Table 4).

## 5 I CONCLUSION

The dehydrated maca yellow powder, industrialized and distributed in Brazil, showed little effects on the biochemical and reproductive parameters of albino Wistar rats after a 54 day treatment period. This finding may be related to the fact that maca was added to the diet as a solid paste. Aqueous extracts of maca submitted to high temperatures allow a better extraction of glucosinolates, phenolic compounds and other secondary metabolites of Peruvian maca and might therefore be a better way of administering this compounds.

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