

Sunflower meal with and without phytase supplementation in diets for silver catfish (*Rhamdia quelen*) juveniles

Harina de girasol con y sin suplementación de fitasa en dietas para juveniles de bagre (barbudo, Col.; Rhamdia quelen)

Farelo de girassol com e sem suplementação de fitase em dietas para juvenis de jundiá (Rhamdia quelen)

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Abstract

Background: The cost of feed in fish farming can exceed 70% of the total costs. Thus, the feed industry have sought to formulate balanced diets including low-cost by-products. **Objective:** To evaluate sunflower meal as a partial substitute (0, 10, and 20%) for soybean meal, with or without phytase supplementation (1,500 FTU/kg) in isoproteic (27% crude protein (CP)) and isoenergetic diets (3,400 kcal/ED/kg of feed) for silver catfish (*Rhamdia quelen*) juveniles. **Methods:** A total of 360 fish (weight: 15.77 ± 0.56 g) were randomly distributed in 24 tanks in a completely randomized design following a factorial scheme (3x2) with 4 replicates. The fish were evaluated on the following variables after 90 days: productive performance, hematological variables, histology of intestinal tissue, bromatological composition of carcass, and composition of bone mineral matter. **Results:** 20% dietary inclusion of sunflower meal improved weight gain and protein efficiency. No difference was observed in the other treatments and no interactions were observed between the other factors. **Conclusion:** 20% sunflower meal can be included in the feed of silver catfish as a substitute for soybean meal. Phytase inclusion (1,500 FTU/kg) did not improve the nutritional value of the treatments.

Keywords: aquaculture; aquiculture; alternative feed; diet supplementation; enzyme; fish nutrition; phytase; productive performance; *Rhamdia quelen*; silver catfish; sunflower meal.

Resumen

Antecedentes: El costo de la alimentación en piscicultura pueden superar el 70% del costo total. Las industrias de alimentos para peces han intentado formular dietas balanceadas con subproductos de menor valor en el mercado para minimizar costos. **Objetivo:** Evaluar la harina de girasol como sucedáneo parcial (0, 10 y 20%) de la torta de soya, suplementada o no con fitasa (1.500 FTU/kg) en dietas isoprotéicas (27% de PB) e isoenergéticas 3400 kcal / ED / kg de ración) para bagre (barbudo, Col.; *Rhamdia quelen*). **Métodos:** Los peces, 360 juveniles ($15,77 \pm 0,56$ g), se distribuyeron aleatoriamente en 24 unidades experimentales, en un diseño completamente al azar, en esquema factorial (3x2) y 4 repeticiones. A los 90 días se evaluó: desempeño productivo, variables hematológicas, histología de tejido intestinal y composición bromatológica de la carcasa y de la materia mineral de los huesos. **Resultados:** La inclusión de 20% de harina de girasol mejoró la ganancia de peso de los peces y la tasa de eficiencia proteica de la ración, sin diferencia para las otras evaluaciones ni interacción entre los demás factores evaluados. **Conclusión:** La harina de girasol se puede utilizar en un 20% de la ración del bagre (barbudo, Col.) como sucedáneo a la harina de soja. La inclusión de fitasa (1.500 FTU/kg) no mejoró el valor nutritivo de estas raciones.

Palabras clave: acuicultura; acuicultura; bagre; barbudo; enzima; fitasa; harina de girasol; ingrediente alternativo; nutrición de peces; *Rhamdia quelen*; rendimiento productivo; suplementación dietaria; torta de girasol.

Resumo

Antecedentes: Os custos relativos a alimentação na piscicultura pode ultrapassar 70% do custo final, neste sentido, as indústrias de rações têm procurado formular dietas balanceadas com subprodutos de menor valor de mercado para minimizar estes custos. **Objetivo:** Esse estudo objetivou avaliar o farelo de girassol como sucedâneo parcial (0, 10 e 20%) do farelo de soja, suplementado ou não com a fitase (1.500 FTU/kg), em dietas isoprotéicas (27% de PB) e isoenergéticas (3400 kcal/ED/kg de ração) para o jundiá (*Rhamdia quelen*). **Métodos:** Os peixes, 360 juvenis ($15,77 \pm 0,56$ g) foram distribuídos aleatoriamente em 24 unidades experimentais, num delineamento inteiramente casualizado, em esquema fatorial (3x2) e 4 repetições. Após 90 dias foram avaliados: desempenho produtivo, variáveis hematológicas, histologia de tecido intestinal, composição bromatológica da carcaça e de matéria mineral dos ossos. **Resultados:** O emprego de 20% de farelo de girassol melhorou o ganho de peso dos peixes e a taxa de eficiência protéica da ração, sem diferença nas outras avaliações, ou para a interação entre os demais fatores avaliados. **Conclusão:** Pode-se concluir que o farelo de girassol pode ser utilizado em 20% da ração do jundiá, como sucedâneo ao farelo de soja e, que a inclusão de fitase (1,500 FTU/kg) não melhorou o valor nutritivo destas rações.

Palavras chave: aquicultura; enzima; desempenho produtivo; farelo de girassol; fitase; ingrediente alternativo; jundiá; nutrição dos peixes; piscicultura; *Rhamdia quelen*.

Introduction

The rapid growth of industrial fish production in Brazil has motivated research focused on lowering production costs and reducing environmental impact. Feed is the highest cost in intensive fish farms, being the protein source the most costly ingredient of the diets (Munguti *et al.*, 2012; Kim *et al.*, 2019). Therefore, the feed industry has sought to obtain balanced diets with minimized costs and maximized quality to meet fish farmers expectations (Sanchez *et al.*, 2016).

The fish feed industry formulates diets according to the dietary habits of fish to meet its nutritional requirements at a low cost (Pezzato *et al.*, 2009). Feed manufacturers seek protein concentrates such as cotton, peanut, and sunflower meal to replace conventional animal meal and soybean meal. Sunflower meal is a byproduct of sunflower seed oil extraction. Sunflower is one of the world's most important oilseeds (FAO, 2012). Sunflower meal has high protein value, making it an ingredient that can be used in diet formulations (Shchekoldina and Aider, 2014). It has been tested in *Oreochromis niloticus* (Lira, 2014), *Salvelinus alpinus* (Smith *et al.*, 2018), *Cyprinus carpio* (Rahmdel *et al.*, 2018), and other species.

However, feeds of plant origin may contain antinutritional substances that decrease diet digestibility and can cause lesions in the gastrointestinal tract. This can reflect in poor growth, which has limited its incorporation in diets for monogastric animals (Hussain *et al.*, 2015; Biswas *et al.*, 2017). Phytate (phytic acid) is among these antinutritional agents. It is present in sunflower meal and decreases mineral availability, particularly phosphorus, which may impair bone mineralization and decrease protein, carbohydrate and lipid availability due to its ability to chelate phytate with other compounds (Vohra and Satyanarayan, 2002).

Mineral supplementation is necessary in diets formulated with plant-based ingredients because monogastric animals cannot synthesize phytase -the enzyme responsible for phytate

hydrolysis (Fortes-Silva *et al.*, 2011). Hence, this study evaluated sunflower meal as a partial substitute for soybean meal, with or without phytase supplementation, in diets for silver catfish (*Rhamdia quelen*) juveniles.

Materials and Methods

The experimental procedures were approved by the Animal Use Ethics Committee (AUEC), under protocol number 63/14, of Universidade Estadual do Oeste do Paraná (UNIOESTE). Fish spent seven days adjusting to the experimental diets prior to the 90 days experimental period. The study was conducted at Grupo de Estudos em Manejo da Aquicultura - GEMAQ of UNIOESTE - Toledo Campus- PR, Brazil.

Six isoproteic (27% CP) and isoenergetic (3400 kcal/ED/kg of feed) diets were formulated to meet the nutrient requirements of the fish (Freitas *et al.*, 2011). The diets contained 0, 10, and 20% sunflower meal with or without phytase supplementation (1,500 FTU/kg; Table 1). The ingredients were ground to 0.5 mm in a hammer mill. Then weighed, manually mixed, and extruded (Ex-Micro® Extruder). Pellets were dried in a forced air ventilation oven for 48 hours at 55 °C and stored under refrigeration (4°C). Phytase from *Aspergillus niger* (BASF-Natuphos®) was added to the diets according to the methodology of Rocha *et al.* (2008).

A completely randomized design based on a 3x2 factorial scheme was used. The first factor was the inclusion level of sunflower meal (0, 10, and 20%). The second factor was phytase supplementation (0 and 1,500 FTU/kg). A total of six treatments with four replicates each was obtained. Twenty-four 1 m³ tanks were placed inside a 200 m³ wooden tank for the experiments. Each experimental unit was composed of 15 fish per tank. A total of 360 silver catfish juveniles with 15.77 ± 0.56 g initial weight and 12.97 ± 1.66 cm length were used in the experiments.

Physical and chemical parameters of water were measured weekly using a multi-parameter

probe meter (YSI Professional Plus 6050000, YSI Incorporated 1725 Brannum Lane Yellow Springs, OH, USA). Water parameters were: pH (8.27 ± 0.31), dissolved oxygen (7.99 ± 1.15 mg/L), and electrical conductivity (41.95 ± 3.28 μ S/cm). Mean water temperature (25.50 ± 0.72 °C) was measured daily (morning and afternoon) using a mercury bulb thermometer. The mean values of the physical and chemical variables of water were within the optimal range for silver catfish cultivation (Gomes *et al.*, 2000).

At the end of the experimental period fish were fasted for 24 hours and subsequently anesthetized with benzocaine (100 mg/L). Weight and length were measured, and the following variables were calculated: survival (S); weight gain (WG) = final weight (g) - initial weight (g); specific growth rate (SGR) = $\{[(\text{Ln}(\text{final weight (g)}) - \text{Ln}(\text{initial weight (g)}))/\text{time (days)}] \times 100\}$; apparent feed conversion (AFC) = feed intake (g)/weight gain (g); and proteic efficiency rate (PER) = protein intake (g)/weight gain (g).

Table 1. Calculated and analyzed ingredient and nutrient composition of the experimental diets.

| Inclusion level of sunflower meal | 0% | | | 10% | | | 20% | | |
|---|-----------------|-------|-------|--------------|-------|-------|-----|--|--|
| | Without phytase | | | With phytase | | | | | |
| Ingredient (%) | | | | | | | | | |
| Soybean meal (42% CP) | 47.10 | 41.05 | 35.00 | 47.10 | 41.05 | 35.00 | | | |
| Sunflower meal | 0.00 | 10.00 | 20.00 | 0.00 | 10.00 | 20.00 | | | |
| Corn - meal | 35.15 | 30.66 | 26.16 | 35.15 | 30.66 | 26.16 | | | |
| Fish meal | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | | | |
| Mineral and vitamin supplement ¹ | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | | | |
| L-threonine | 0.41 | 0.39 | 0.38 | 0.41 | 0.39 | 0.38 | | | |
| DL-methionine | 0.35 | 0.30 | 0.24 | 0.35 | 0.30 | 0.24 | | | |
| Choline chloride | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | | | |
| Vitamin C ² | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | | | |
| Calcium propionate | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | | | |
| Butylhydroxy toluene | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | | | |
| Soy oil | 0 | 0.95 | 1.89 | 0 | 0.95 | 1.89 | | | |
| Lysine 78,5% ³ | 0.68 | 0.34 | 0.00 | 0.68 | 0.34 | 0.00 | | | |
| Phytase (Natuphos®) | 0.00 | 0.00 | 0.00 | 0.015 | 0.015 | 0.015 | | | |
| Common salt | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | | | |
| Calculated nutrients (%) | | | | | | | | | |
| Starch | 27.86 | 24.29 | 20.73 | 27.86 | 24.29 | 20.73 | | | |
| Calcium | 0.83 | 0.85 | 0.87 | 0.83 | 0.85 | 0.87 | | | |
| Digestible energy (Kcal/kg) | 3400 | 3400 | 3400 | 3400 | 3400 | 3400 | | | |
| Available phosphorus | 0.48 | 0.46 | 0.45 | 0.48 | 0.46 | 0.45 | | | |
| Total lysine | 2.58 | 2.25 | 1.93 | 2.58 | 2.25 | 1.93 | | | |
| Methionine + Cystine | 1.46 | 1.46 | 1.46 | 1.46 | 1.46 | 1.46 | | | |
| Digestible protein | 27.70 | 27.34 | 26.98 | 27.70 | 27.34 | 26.98 | | | |
| Threonine | 1.73 | 1.73 | 1.73 | 1.73 | 1.73 | 1.73 | | | |
| Tryptophan | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | | | |

| Analyzed nutrients (%) | | | | | | |
|---|-------------------|-------------------|-----------------|-------|-------|-------|
| Humidity | 9.69 | 8.60 | 8.39 | 8.80 | 8.49 | 8.13 |
| Ether extract | 3.25 | 3.24 | 3.87 | 3.27 | 3.12 | 3.32 |
| Crude protein | 36.39 | 36.63 | 36.63 | 36.81 | 36.81 | 36.04 |
| Crude fiber | 5.81 | 6.64 | 6.97 | 5.75 | 6.88 | 6.47 |
| Nutritional composition of sunflower meal | | | | | | |
| Humidity (%) | Ether extract (%) | Crude protein (%) | Crude fiber (%) | | | |
| 9.57 | 0.10 | 41.98 | 3.786 | | | |

¹Guaranteed levels per kilogram of product: Vit. A, 1,750,000 IU; Vit. D3, 375,000 IU; Vit. E, 20,000 IU; Vit. K3, 500 mg; Vit. B1, 2,000 mg; Vit. B2, 2,500 mg; Vit. B6, 2,500 mg; Vit. B12, 5,000 mg; Folic acid, 625 mg; Calcium pantothenate, 7,500 mg; Vit. C, 37,500 mg; Biotin, 50 mg; Inositol, 12,500 mg; Niacin, 8,750 mg; Choline, 100,000 mg; Co, 50 mg; Cu, 1,250 mg; Fe, 15,000 mg; I, 100 mg; Mn, 3,750 mg; Se, 75 mg; Zn, 17,500 mg. ²Nutron -Vitamin C polyphosphate with 35.0% activity. ³Synthetic amino acids supplied by Ajinomoto do Brasil.

The hematological indexes (Ranzani-Paiva *et al.*, 2013) were evaluated in three fish per replicate. After anesthesia with benzocaine (100 mg/L), blood was collected by puncturing the caudal vessel using syringes with needles internally moistened with 3% EDTA. The percentage of micro-hematocrits, hemoglobin levels, and erythrocyte counts were determined in a Neubauer chamber. These data were used to calculate mean corpuscular volume ($MCV = Ht \times 10/Er$) and mean corpuscular hemoglobin concentration ($MCHC = Hb \times 100/Ht$).

Leukocyte counts, total thrombocyte counts, and leukocyte differentiation in lymphocytes, neutrophils, monocytes, SGC (special granulocytic cell), and immature lymphocytes (defense cells) were determined in blood extensions (Ranzani-Paiva *et al.*, 2013). The readings were obtained by microscopy using 100 x magnification. Leukocytes and thrombocytes were counted by the indirect method: total leukocytes (μL) = [(number of leukocytes counted in the extension \times number of erythrocytes in the Neubauer chamber)/2000]; and total thrombocytes (μL) = [(number of thrombocytes counted in the extension \times number of erythrocytes in the Neubauer chamber)/2000]. One hundred cells were counted for the determination of leukocyte differentiation by establishing the percentage of each cellular component of interest.

Fish were euthanized after blood collection by means of a benzocaine overdose (200 mg/L) and subsequently eviscerated for removal and weighing of the liver and visceral fat. The following somatic indexes were calculated from the liver and visceral fat weights: visceral somatic fat index ($VSFI\% = [(visceral\ fat\ weight\ (g)/body\ weight\ (g))] \times 100$), and hepatosomatic index ($HI\% = [(liver\ weight\ (g)/body\ weight\ (g))] \times 100$).

Histological analysis of intestinal villi was performed on portions of the fish midgut. Sections were harvested and fixed in alfac solution for six hours, then washed and preserved with 70% alcohol to remove the fixative. Subsequently, these samples were dehydrated in increasing concentrations of ethanol, clarified in xylol, and embedded in paraffin to obtain histological sections. The histochemical analysis was performed on 7 μm paraffin sections fixed on a slide and stained using the HE and PAS + alcian blue (AB) technique. These samples were analyzed using a light microscope equipped with a camera; villi height and total height (distance from the apex to the beginning of the muscular layer, and from the apex to the end of the serosa, respectively), villi width, and epithelium thickness were measured.

After evisceration, carcasses were frozen to determine centesimal composition (moisture, mineral matter, crude protein, and ether extract)

according to the AOAC (2000). Carcasses of three other fish per treatment were frozen for the determination of bone mineral composition; the submandibular bone and all bones of the vertebral column were ground and degreased with petroleum ether for the analysis of calcium, phosphorus, magnesium, manganese, and zinc retention (Furuya *et al.*, 2001). Bone mineral concentration was assessed with nitro-perchloric digestion for quantification of calcium, copper, phosphorus, magnesium, manganese and zinc, determined by Flame Absorption Spectrometry (FAAS).

The data were analyzed by homogeneity and normality tests. When assumptions were met, data were submitted to variance analysis in a factorial scheme (Two-Way) at a 5% ($P < 0.05$) significance level to check for a possible interaction

between the two factors (replacement of soybean meal with sunflower meal and enzyme supplementation). The mean comparison test was performed only for significant interactions excluding isolated effects. The Tukey's test ($p < 0.05$) was used in the Statistica 7.1® program to compare means that showed significant differences.

Results

The average performance values of fish fed the experimental rations are shown in Table 2. The use of 20% sunflower meal in the diet improved ($P < 0.05$) weight gain and the feed's protein efficiency rate. It should be emphasized that, at the levels tested, this diet did not show negative impacts ($P > 0.05$) on feed conversion or other performance indexes.

Table 2. Growth performance of silver catfish juveniles fed the experimental diets for 90 days.

| Variables* | Phytase | Sunflower Meal (%) | | | F ¹⁰ | p-value | |
|-----------------------|----------------|--------------------|---------------|---------------|-----------------|------------------|-----------------|
| | | 0 | 10 | 20 | | AL ¹¹ | I ¹² |
| WG ¹ (g) | A ⁸ | 69.60 ± 0.94AB | 53.84 ± 0.94B | 71.78 ± 3.41A | Ns | 0.018 | Ns |
| | P ⁹ | 63.58 ± 7.73AB | 58.29 ± 3.41B | 69.08 ± 5.02A | | | |
| AFC ² | A | 1.32 ± 0.60 | 1.53 ± 0.57 | 1.31 ± 0.50 | Ns | Ns | Ns |
| | P | 1.29 ± 0.23 | 1.47 ± 0.52 | 1.41 ± 0.20 | | | |
| SGR ³ (%) | A | 1.52 ± 0.18 | 1.43 ± 0.16 | 1.66 ± 0.08 | Ns | Ns | Ns |
| | P | 1.47 ± 0.12 | 1.30 ± 0.34 | 1.62 ± 0.12 | | | |
| PER ⁴ (%) | A | 3.37 ± 0.17B | 3.00 ± 0.31B | 4.21 ± 0.25A | Ns | 0.01 | Ns |
| | P | 3.22 ± 0.22B | 2.64 ± 0.11B | 3.67 ± 0.16A | | | |
| HI ⁵ (%) | A | 2.03 ± 0.36 | 1.66 ± 0.60 | 1.65 ± 0.64 | Ns | Ns | Ns |
| | P | 2.02 ± 0.74 | 1.78 ± 0.43 | 1.49 ± 0.63 | | | |
| VSFI ⁶ (%) | A | 0.91 ± 0.53 | 0.92 ± 0.44 | 0.96 ± 0.74 | Ns | Ns | Ns |
| | P | 1.60 ± 1.12 | 1.36 ± 1.01 | 1.24 ± 0.85 | | | |
| S ⁷ (%) | A | 83.33 ± 11.11 | 94.44 ± 3.70 | 94.44 ± 3.70 | Ns | Ns | Ns |
| | P | 75.00 ± 5.55 | 77.77 ± 12.96 | 97.22 ± 3.70 | | | |

*The Tukey's test was performed only for significant interactions. ¹Weight gain; ²Apparent food conversion; ³Specific growth rate; ⁴Protein efficiency rate; ⁵Hepatosomatic index; ⁶Viscero-somatic fat index; ⁷Survival; ⁸Absent; ⁹Present; ¹⁰Phytase; ¹¹Feed; ¹²Interaction.

The histological measurements (μm) of villi height and width and epithelium thickness after 90 days.

of treatment are shown in Table 3. The histological analysis results showed no alterations ($P > 0.05$) of the intestinal epithelium

Table 3. Histological measurements (μm) villi height and width and epithelium thickness of the intestinal tissue of silver catfish juveniles fed the experimental diets for 90 days.

| Variables* | Phytase | Sunflower Meal (%) | | | p-value | | |
|--|----------------|--------------------|--------------|--------------|----------------|-----------------|----------------|
| | | 0 | 10 | 20 | F ³ | AL ⁴ | I ⁵ |
| Villus height (μm) | A ¹ | 224.5352.07 | 243.11 60.93 | 237.52 85.10 | Ns | Ns | Ns |
| | P ² | 239.8260.07 | 249.2643.13 | 253.9478.87 | | | |
| Villus width (μm) | A | 38.6114.39 | 38.45 8.36 | 36.75 5.33 | Ns | Ns | Ns |
| | P | 45.268.36 | 42.5411.19 | 33.279.05 | | | |
| Epithelium thickness (μm) | A | 35.46 6.31 | 30.94 11.74 | 37.034.78 | Ns | Ns | Ns |
| | P | 30.9413.07 | 33.065.70 | 40.3116.77 | | | |

*The Tukey's test was performed only for significant interactions. ¹Abscent; ²Present; ³Phytase; ⁴Feed; ⁵Interaction.

Table 4 shows the results of hematological and biochemical parameters in fish fed with the different rations after 90 experimental days. No differences were observed

in the evaluated hematological and biochemical parameters ($P > 0.05$) between fish fed with rations containing soybean meal or sunflower meal, with or without phytase.

Table 4. Hematological and biochemical parameters of silver catfish juveniles fed with experimental diets for 90 days.

| Variable* | Phytase | Sunflower meal (%) | | | p-value | | |
|--|----------------|----------------------|----------------------|----------------------|----------------|-----------------|----------------|
| | | 0 | 10 | 20 | F ⁶ | AL ⁷ | I ⁸ |
| Erythrocytes ($\times 10^6 \mu\text{L}^{-1}$) | A ⁴ | 2.18 \pm 0.28 | 2.11 \pm 0.13 | 2.08 \pm 0.19 | Ns | Ns | Ns |
| | P ⁵ | 2.23 \pm 0.19 | 2.12 \pm 0.34 | 2.15 \pm 0.31 | | | |
| Hematocrit (%) | A | 34.28 \pm 4.46 | 36.33 \pm 5.57 | 34.12 \pm 4.12 | Ns | Ns | Ns |
| | P | 34.12 \pm 2.47 | 33.70 \pm 3.65 | 31.71 \pm 3.5 | | | |
| Hemoglobin (g dL ⁻¹) | A | 6.10 \pm 0.75 | 6.22 \pm 1.40 | 5.60 \pm 4.05 | Ns | Ns | Ns |
| | P | 5.82 \pm 1.14 | 5.47 \pm 1.09 | 4.72 \pm 1.10 | | | |
| MCV (fL) ¹ | A | 158.60 \pm 27.19 | 173.20 \pm 33.13 | 165.82 \pm 30.99 | Ns | Ns | Ns |
| | P | 153.82 \pm 19.9 | 162.07 \pm 28.99 | 149.30 \pm 22.19 | | | |
| MCHC (%) ² | A | 17.88 \pm 1.69 | 17.44 \pm 4.27 | 16.32 \pm 1.62 | Ns | Ns | Ns |
| | P | 17.08 \pm 3.17 | 16.29 \pm 3.09 | 14.90 \pm 3.23 | | | |
| Leucocytes (μL^{-1}) | A | 45733.23 \pm 10249 | 39732.02 \pm 19690 | 39211.13 \pm 15501 | Ns | Ns | Ns |
| | P | 43319.06 \pm 11201 | 39013.59 \pm 15545 | 44680.18 \pm 15679 | | | |

| Variable* | Phytase | Sunflower meal (%) | | | p-value | | |
|---------------------------------------|---------|--------------------|----------------|----------------|----------------|-----------------|----------------|
| | | 0 | 10 | 20 | F ⁶ | AL ⁷ | I ⁸ |
| Trombocytes (μL^{-1}) | A | 55375.10±19702 | 58519.21±15172 | 68195.78±32126 | Ns | Ns | Ns |
| | P | 62373.10±11554 | 64021.90±27862 | 70144.45±23246 | | | |
| Lymphocytes (%) | A | 80.67± 6.63 | 82.00±9.50 | 87.50±7.50 | Ns | Ns | Ns |
| | P | 79.17±8.79 | 89.83±7.46 | 78.50±9.42 | | | |
| Neutrophils (%) | A | 15.00±4.39 | 12.40±7.30 | 5.40±4.09 | Ns | Ns | Ns |
| | P | 15.60±6.26 | 6.40±2.88 | 12.0±5.33 | | | |
| Monocytes (%) | A | 2.67±1.21 | 2.67±2.33 | 2.33±1.50 | Ns | Ns | Ns |
| | P | 1.17±0.75 | 0.83 ±0.98 | 4.33±2.80 | | | |
| SGC (%) ³ | A | 1.0±1.26 | 0.17±0.41 | 0.33±0.81 | Ns | Ns | Ns |
| | P | 1.33±2.33 | 0.83±1.33 | 0.33±0.52 | | | |
| Immature Lymphocytes (%) | A | 1.00±1.26 | 0.67±0.82 | 2.00±3.94 | Ns | Ns | Ns |
| | P | 1.16±1.47 | 0.16±0.40 | 2.66±1.21 | | | |

*The Tukey's test was performed only for significant interactions. ¹MCV = mean corpuscular volume; ²MCHC = mean corpuscular hemoglobin concentration, ³SGC: Special granulocytic cells, ⁴Ausent, ⁵Present, ⁶Phytase, ⁷Feed, ⁸Interaction.

The centesimal composition values (natural matter) showed that fish fed the different diets had similar values of moisture, mineral matter, crude protein, and ether extract (Table 5).

Table 5. Centesimal composition (natural matter) of silver catfish juveniles fed with experimental diets for 90 days.

| Variables* | Phytase | Sunflower Meal (%) | | | p-value | | |
|--------------------|----------------|--------------------|--------------|--------------|----------------|-----------------|----------------|
| | | 0 | 10 | 20 | F ³ | AL ⁴ | I ⁵ |
| Humidity (%) | A ¹ | 71.72 ± 1.76 | 69.56 ± 1.45 | 67.98 ± 1.41 | Ns | Ns | Ns |
| | P ² | 70.08 ± 1.59 | 68.41 ± 2.69 | 69.42 ± 1.20 | | | |
| Protein (%) | A | 18.80 ± 1.68 | 19.82 ± 0.77 | 19.49 ± 0.79 | Ns | Ns | Ns |
| | P | 18.94 ± 0.58 | 18.95 ± 0.82 | 18.75 ± 0.82 | | | |
| Ether extract (%) | A | 5.83 ± 1.75 | 6.09 ± 0.64 | 7.18 ± 1.01 | Ns | Ns | Ns |
| | P | 7.13 ± 0.70 | 6.41 ± 0.36 | 7.53 ± 0.24 | | | |
| Mineral matter (%) | A | 4.44 ± 0.81 | 4.02 ± 1.28 | 4.60 ± 0.29 | Ns | Ns | Ns |
| | P | 3.99 ± 0.81 | 4.03 ± 0.71 | 4.22 ± 0.02 | | | |

*The Tukey's test was performed only for significant interactions. ¹Absent; ²Present; ³Phytase; ⁴Feed; ⁵Interaction.

The bone mineral composition (mg/g) of fish after the experimental period is shown in Table 6. These results showed that the diets did not influence this parameter.

Table 6. Bone mineral composition (mg/g) of silver catfish juveniles fed the experimental rations for 90 days.

| Variable* | Phytase | Sunflower meal (%) | | | p-value | | |
|------------------------|----------------|--------------------|------------|-------------|----------------|-----------------|-----------------|
| | | 0 | 10 | 20 | F ⁸ | AL ⁹ | I ¹⁰ |
| Ca (mg/g) ¹ | A ⁶ | 74.6518.75 | 78.15 4.98 | 91.53 6.97 | Ns | Ns | Ns |
| | P ⁷ | 80.143.18 | 83.053.54 | 82.7711.15 | | | |
| Mg (mg/g) ² | A | 0.91 0.27 | 1.00 0.09 | 1.08 0.04 | Ns | Ns | Ns |
| | P | 0.990.07 | 1.070.18 | 1.010.13 | | | |
| Mn (mg/g) ³ | A | 0.09 0.04 | 0.10 0.03 | 0.07 0.02 | Ns | Ns | Ns |
| | P | 0.120.02 | 0.080.03 | 0.100.03 | | | |
| P (mg/g) ⁴ | A | 94.60 8.06 | 98.65 7.00 | 112.14 9.58 | Ns | Ns | Ns |
| | P | 93.139.26 | 103.770.98 | 96.4415.53 | | | |
| Zn (mg/g) ⁵ | A | 0.06 0.02 | 0.07 0.01 | 0.05 0.01 | Ns | Ns | Ns |
| | P | 0.070.01 | 0.060.02 | 0.060.02 | | | |

*The Tukey's test was performed only for significant interactions. ¹Calcium; ²Magnesium; ³Manganese; ⁴Phosphorus; ⁵Zinc; ⁶Absent; ⁷Present; ⁸Phytase; ⁹Feed; ¹⁰Interaction.

Discussion

The results showed that sunflower meal may be an effective supplement in diets formulated for silver catfish and that the addition of phytase does not have a positive effect ($P>0.05$) on production performance, blood parameters, carcass chemical composition, and bone composition. Soybean meal is the standard ingredient in the formulation of animal feed because of its high protein concentration. Sunflower meal has higher methionine values when compared to soybean meal (0.91% x 0.55%); however, soybean meal has higher values for the other amino acids essential to fish (Lira, 2014; Furuya *et al.*, 2001). The results of this study showed that even when differences in amino acid composition were considered, inclusion of 20% sunflower meal in the feed as a replacement for soybean meal did not impair growth performance. These results are in agreement with other studies using *Tilapia rendalli*, *Salvelinus alpinus*, *Cyprinus carpio* in which 20% sunflower meal inclusion did not affect fish performance (Olvera-Novoa *et al.*, 2002; Smith *et al.*, 2017; Rahmdel *et al.*, 2018). On the other hand, fish fed 10% sunflower meal resulted in lower weight gain. These results confirm the evidence presented by Lira (2014), who verified lower performance of Nile tilapia

fed diets containing 7.5 and 11.25% sunflower meal. Furthermore, fish fed 15% of this ingredient had better weight gain (Lira, 2014).

Gonçalves *et al.* (2004) determined the nutritive value of sunflower meal in a study with Nile tilapia and found no effect of phytase supplementation on protein digestibility, although digestible energy increased 230 kcal/kg compared to diets without phytase. The limited use of sunflower meal is due to its high fiber content and presence of anti-nutritional factors, although these are minimized during the oil extraction processing (Bergamim *et al.*, 2013; González-Pérez & Vereijken, 2007). Reduction in growth and worsening of feed conversion were evidenced in studies with inclusion levels higher than 20% due to proteolytic enzyme suppression caused by antinutritional factors present in sunflower meal (Lin *et al.*, 2010; Lovatto *et al.*, 2014; Rahmdel *et al.*, 2018)

The inclusion of sunflower meal resulted in increased crude fiber levels. In spite of this increase, fish fed these diets showed no alterations in intestinal histology. Although considered an omnivorous species, the silver catfish has a carnivorous tendency: its intestine is short, and its protein requirement is higher

than that of other omnivorous species (Meyer and Fracalossi, 2004). According to Rodrigues *et al.* (2012), silver catfish demonstrates a physiological adaptation capacity without performance impairment when fed fiber-rich diets. This may explain the similarity in intestinal epithelium measurements in fish fed the different diets in the present study.

It is noteworthy that all blood hematological and biochemical values observed in the present study are in agreement with the literature. The hematocrit and hemoglobin values are up to 43% and 8.7 g dl⁻¹, respectively (Borges *et al.*, 2004); MCV values varied from 139.0 to 241.9 fL (Tavares-Dias & Moraes, 2004). However, according to Tavares-Dias & Moraes (2004), the number of erythrocytes was above the reference range for the species (1.42 to 1.95) while MCHC was below the value observed for the species, which can vary between 22.7 and 32.9% (Tavares-Dias & Moraes, 2004). These alterations can be related to the physical and chemical characteristics of water, which are often extreme in culture environments and may alter hematological variables (Costa *et al.*, 2004; Carvalho & Fernandes, 2006). Fish size can also influence these parameters because fish release metabolic residues proportional to their body size (Tavares-Dias *et al.*, 2000; Tavares-Dias *et al.*, 2001). Nevertheless, hemoglobin present in erythrocytes is only accumulated with cell maturation, which explains the high number of erythrocytes and low CHCM (Tavares-Dias & Moraes, 2004). Leukocytes, total thrombocytes, lymphocytes, SGC, and immature lymphocytes in fish fed the different diets were similar (Table 4). The number of total leukocytes and thrombocytes, SGC, and immature lymphocytes were within the normal ranges for the species (Tavares-Dias & Moraes, 2004). The diets did not alter neutrophil values, which were within the normal range (from 1.8 to 30.0%, according to Tavares-Dias *et al.*, 2002), although total leukocytes (between 2,960 and 49,800 ul⁻¹), SGC (from 0 to 6%), and lymphocyte percentages (78.0 to 89.83%) were higher than indicated for the species (from 58.0 to 68.1%) (Tavares-Dias and Moraes, 2004). Inclusion of

sunflower meal or phytase supplementation did not influence the monocytes values. Although blood parameters may be influenced by many factors, it should be noted that the diets did not influence these results. According to Tavares-Dias *et al.* (2002), the diet is directly correlated with responses to external stimuli or exposures in the environment. Conversely, erythrocyte parameters remained within the normal ranges indicating no dietary effects on blood variables.

Carcass composition indicates that the inclusion of up to 20% sunflower meal, independent of phytase supplementation, does not affect protein synthesis or lipid deposition, demonstrating that this ingredient can partially replace soybean meal in diets formulated for this species. These results confirm those reported by Olvera-Novoa *et al.* (2002) in a study with sunflower meal substituted for fish meal in the diet for *Tilapia rendalli*.

Bone mineral composition may vary with age, size, and reproductive stage of animals. The presence of anti-nutritional factors, such as phytic acid, which is common in plant-based foods, affect nutrient digestibility, especially that of minerals such as phosphorus, decreasing its absorption by up to 70% (Rocha *et al.*, 2008; Pontes *et al.*, 2015). Phytase supplementation in the present study did not improve mineral utilization. This can be explained by the fact that phosphorus requirements for growth, nutrient utilization, and bone mineral deposition were met by the experimental diets without phytase supplementation.

In conclusion, 20% sunflower meal can be used in diets formulated for silver catfish, and phytase addition does not have a positive effect on performance, blood parameters, carcass or bone composition.

Declarations

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

Author contributions

Kátia A Weiler: Responsible for the conception of the study, data collection and article writing; Jhonis E Pessini: data collection, writing, review and editing of the article; Milena S S Sanchez: Data collection, writing, review and editing of the article; Mariana L Rodrigues: Data collection, writing, review and editing of the article; Wilson R Boscolo: responsible for the experimental design, managed the project, review and editing of the article. Luiz E Pezzato: Responsible for the experimental design and revised and edited the article. Fábio Bittencourt: collected the data, review and editing of the article. Altevir Signor: Responsible for the experimental design, managed the project, review and editing of the article.

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