



**Revista Agrarian**

ISSN: 1984-2538

## Organic Soybean Meal in Diet for Nile Tilapia

### *Farelo de Soja Orgânica na Alimentação de Tilápia do Nilo*

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Recebido em: 30/09/2017

Aceito em:06/05/2018

**Abstract:** Aiming to assess organic soybean meal (OSM) for Nile tilapia, two studies were carried out: Study I - digestibility of OSM compared to conventional soybean meal (CSM) for Nile tilapia; and Study II - effect of OSM and CSM, supplemented or not with fish meal (FM) on the performance of Nile tilapia fingerlings. The apparent digestibility coefficients of the protein were 88.24 and 88.10%, resulting in 39.91 and 39.87% digestible protein for OSM and CSM, respectively. The apparent digestibility coefficients of energy were 75.92 and 70.48%, resulting in 3579.46 and 3141.44 kcal kg<sup>-1</sup> of digestible energy for OSM and CSM, respectively. The diet containing CSM+FM presented the best results for final weight and weight gain. However, the highest levels of crude protein in the fish carcass were observed in the animals fed with the OSM + FM diet. The OSM diet presented the best results for total protein and albumin in the blood. The OSM diet has good apparent digestibility coefficients and its use in tilapia feeding presents a satisfactory productive performance and a higher deposition of protein in the carcass when supplemented with 20% of fish meal.

**Keywords:** digestibility, feeding management, hematology, organic fish feed, productive performance

**Resumo:** Objetivando avaliar a utilização de farelo de soja orgânico (FSO) para a tilápia do Nilo, foram realizados dois estudos: Estudo I - digestibilidade do FSO comparado ao farelo de soja convencional (FSC) para tilápia do Nilo; e Estudo II - efeito do FSO e FSC, suplementado ou não com farinha de peixe (FP) no desempenho de alevinos de tilápia do Nilo. Os coeficientes de digestibilidade aparente da proteína foram de 88,24 e 88,10%, resultando em 39,91 e 39,87% de proteína digestível para o FSO e FSC, respectivamente. Os coeficientes de digestibilidade aparente da energia foram de 75,92 e 70,48%, resultando em 3,579.46 e 3,141.44 kcal kg<sup>-1</sup> de energia digestível para o FSO e FSC, respectivamente. Os peixes alimentados com a ração contendo FSC + FP apresentaram os melhores resultados de peso final e ganho em peso. No entanto, os maiores níveis de proteína bruta na carcaça dos peixes foram observados nos animais alimentados com a ração FSO + FP. Os peixes alimentados com a ração FSO apresentaram os melhores resultados para a proteína total e albumina no sangue. O FSO apresenta bom coeficiente de digestibilidade aparente e seu uso na alimentação da tilápia apresenta desempenho produtivo satisfatório e maior deposição de proteína na carcaça quando suplementados com 20% de farinha de peixe.

**Palavras-chave:** alimentos orgânicos, digestibilidade, desempenho produtivo, hematologia, manejo alimentar





### Introduction

Organic farming uses practices that resemble the natural conditions of organisms, in this sense organic aquaculture differs from conventional aquaculture, as it is based on the concept of harmony with the environment (Boscolo et al., 2010). The water should be free of pesticides, and the fish fed with food of natural origin or organic feed (Boscolo et al., 2012), a major obstacle to organic aquaculture production (Feiden et al., 2010), promoting innovation and sustainability in the activity (Marco et al., 2017).

Organic aquaculture is still at the early stages in Brazil, however, in Paraná State, the development of tilapia culture based on agro-ecological standards was initiated a few years ago in a partnership between companies and producers who were familiar with this system (Boscolo et al., 2012). These producers were also interested in the verticalization of the production, from raw materials to animal production in an integrated manner and following agro-ecological principles (Lui et al., 2012).

The organic farming system is geared towards the improvement of natural life cycles while preserving nature (Feiden et al., 2010). A future expansion of organic aquaculture is expected, promotes innovation and sustainability in aquaculture (Marco et al., 2017).

There are productive models than can be called agro-ecological, and organic systems stand out because of their differentiated production process offering safe and healthy products to consumers. Boscolo et al., (2013) verified the possibility of organic feeding in three species of fish, jundiá (*Rhamdia voulezi*), pacu (*Piaractus mesopotamicus*) and tilapia (*Oreochromis niloticus*), all showing good growth performance in this production system.

The Nile tilapia is a species characterized by being at a low trophic level, accepting plant origin feed, displaying easy sex manipulation through sexual reversal, tolerating intense productive management (high density and low oxygen level) with optimal growth performance, good fillet yield, excellent quality meat, and is greatly accepted in the consumer's market. These features have turned this species into one of the most produced in the world (Bomfim et al., 2008; Takishita et al., 2009).

Thus, this study aimed at assessing the apparent digestibility coefficients for organic soybean meal and its inclusion in the diet for Nile tilapia juveniles (*O. niloticus*).

### Materials and Methods

For the feed production, analyzes of chemical composition of the food tested were carried out for OSM and CSM (Table 1) using the methodology recommended by Institute Adolfo Lutz (1985).

**Table 1.** Centesimal composition of feed ingredients and diets for digestion studies

Parameters	Soybean meal	
	Organic	Conventional
Composition <sup>1</sup>		
Dry matter (%)	89.79 ± 0,15	91.68 ± 0,21
Crude protein (%)	45.23 ± 0,83	45.25 ± 0,58
Lipids (%)	9.41 ± 0,12	1.84 ± 0,04
Mineral matter (%)	5.34 ± 0,17	5.61 ± 0,09
Crude energy (kcal/kg)	4714.50 ± 132,02	4457.15 ± 98,87

<sup>1</sup>Analyses carried out in the Food Quality Laboratory - Unioeste, Toledo, PR.

A reference feed and two tests were prepared (Table 2). The test feed (containing organic and commercial soybean meal) included 70% of the reference feed and 30% of the test ingredient.

Chromic oxide<sup>III</sup> was used in the proportion of 0.1% as the inert indicator in the feed. The ingredients were ground in a mill hammer type with 0.5 mm pore sieve. The ground ingredients were weighted,



homogenized, and moistened for the preparation of feed, which were prepared in an extruded manner (Ex -Micro<sup>®</sup> extruder – ExTeec Company, Ribeirão Preto, Brazil).

Ninety Nile tilapias with initial average weight of  $184.16 \pm 22.79$ g were randomized and distributed in six 180 L-tanks cylinder-conical with constant aeration generated by a central air blower and thermostat water heating system. The experimental design was in Latin square

constituting two treatments, organic and conventional soybean meal and one reference feed.

The fish were fed five times a day (8:30, 12:00, 13:30, 15:30, and 16:00), until apparent satiety, during 60 days. The tanks were cleaned twice a day (7:30 and 17:00) and 50% of the water volume was replaced with clean water for the removal of feces and feed leftovers.

**Table 2.** Percentage and chemical composition of the basal diet (dry matter) for the digestibility study

Ingredients	Organic	Conventional
Organic corn	30.50	30.50
Organic wheat	28.00	28.00
Organic soybean meal	20.00	0
Conventional soybean meal	0	20.0
Fishmeal	20.00	20.00
Premix <sup>1</sup>	1.00	1.00
Dicalcium phosphate	0.10	0.10
Chromium oxide <sup>(III)</sup>	0.10	0.10
Salt	0.30	0.30
Total	100	100
Nutrients analyzed		
Dry matter (%)	94.61	93.90
Crude protein (%)	35.20	34.64
Crude energy (kcal/kg)	4721.50	4590.00
Lipids (%)	3.57	1.22
Mineral matter (%)	7.69	7.15

<sup>1</sup>Premix composition. Assurance levels per kilogram of product: Vit. A: 24,000 IU; Vit. D3: 6,000 IU; Vit. E: 300 mg; Vit. K3: 30 mg; Vit. B1: 40 mg; Vit. B2: 40 mg; Vit. B6: 35 mg; Vit. B12: 80 mg; Folic acid: 12 mg; Ca pantothenate: 100 mg; Vit. C: 600 mg; Biotin: 2 mg; Choline: 1,000 mg; Niacin: Iron: 200 mg; Copper: 35 mg; Manganese: 100 mg; Zinc: 240 mg; Iodine: 1.6 mg; Cobalt: 0.8 mg.

The feces collection was performed using the modified Guelph method advocated by Pezzato et al. (2002). The process of collecting feces started after the adaptation of fish in the tanks for seven days and the data of feces was obtained through eight collecting days. The feces samples were dried in a forced air oven at 55°C for 48 hours and ground before the analysis.

The water electrical conductivity ( $70.00 \pm 6.25 \mu\text{S cm}^{-1}$ ), pH ( $6.70 \pm 0.29$ ), and dissolved oxygen ( $7.56 \pm 0.92 \text{ mg.L}^{-1}$ ) were measured once a week using a digital portable potentiometer (Hanna Instruments<sup>®</sup>). The water temperature ( $27.36 \pm 1.32$  °C) was measured daily measured in the morning with a thermometer.

The feces samples were properly stored and frozen (-18°C) for further proximal composition analyses (crude protein, lipids, ash and dry matter) according to the methodology by Institute Adolfo Lutz (1985). The Chromic oxide<sup>III</sup> concentration was determined at the Animal Nutrition Laboratory (LANA) at Maringá State University (UEM). The analysis of the crude energy concentration was performed in the Fish Nutrition and Feeding Laboratory at Santa Cruz State University/Bahia using a calorimetric pump, IKA brand model C-200. The energy apparent digestibility coefficients and nutrients were determined in the feeds according to the formula described by Mukhopadhyay e Ray (1997).



Four diets were tested, formulated with 32% crude protein using energy sources such as corn, whole wheat, wheat bran, organic and conventional soybean meal, in combination or not with fish meal, the maximum inclusion of fish products was 20% according to Naturland Certifying Company (Table 3). Synthetic aminoacids were not added in the organic meal, following the current law. The diets were formulated to be isoenergetic, isoproteic (digestible nutrients), isocalcitic, and isophosphoric.

Four-hundred Nile tilapia juveniles with average weight of  $12.7 \pm 0.36$  g and average length of  $9.2 \pm 0.30$  cm initial were randomly distributed in

20 fiberglass tanks (500 L) connected to a water recirculation system with mechanical and biological filters with constant heating and oxygenation through electric heaters with a central air blower.

The ingredients were ground in a hammer mill with a 0.5 mm pore sieve and subsequently supplemented with micro-nutrients, oil, and water; the mixture was subjected to the extrusion process (Ex-Micro® - ExTeec Company, Ribeirão Preto, Brazil) with three mm in diameter. The fish were fed four times a day (8:00, 11:00, 14:00 and 17:00) until apparent satiety, for a period of 60 days.

**Table 3.** Percentage inclusion and chemical composition of the experimental diets (dry matter), to study productive performance

Ingredients (%)	Treatments			
	OSM	CSM	OSM + FM	CSM + FM
Corn	23.53	15.66	37.79	32.39
Soybean meal	62.14	61.75	30.78	31.36
Fish meal	-	-	20.00	20.00
Wheat bran <sup>1</sup>	10.00	10.00	10.00	10.00
Soybean oil	0.32	8.28	0.121	4.83
Vitamin and mineral supplement <sup>2</sup>	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.71	1.79	-	-
Calcitic limestone	1.00	0.92	-	-
Salt	0.30	0.30	0.30	0.30
Antifungal - calcium propionate <sup>3</sup>	-	0.10	-	0.10
Antioxidant – BHT <sup>3</sup>	-	0.02	-	0.02
DL-methionine <sup>3</sup>	-	0.18	-	-
Total	100	100	100	100
Nutrients (%)				
Starch	19.50	14.59	27.45	24.10
Calcium	1.02	1.02	1.02	1.02
Digestible energy (kcal/kg) <sup>1,4</sup>	3348	3348	3348	3348
Crude fiber	4.00	3.98	3.02	3.01
Total phosphorus	0.87	0.87	0.87	0.87
Fat	5.82	10.0	5.09	7.88
Lysine	1.94	1.91	1.90	1.91
Methionine	0.47	0.64	0.64	0.64
Digestible protein	30.00	30.00	30.00	30.00
Crude protein	32.00	32.00	32.00	32.00

OSM: Organic soybean meal; CSM: conventional soybean meal; OSM + FM: organic soybean meal + fish meal; CSM + FM: conventional soybean meal + fish meal. <sup>1</sup>Lui et al., (2012). <sup>2</sup>Premix composition. Assurance levels per kilogram of product: Vit. A : 24,000 IU; Vit. D3: 6,000 IU; Vit. E: 300 mg; Vit. K3: 30 mg; Vit. B1: 40 mg; Vit. B2: 40 mg; Vit. B6: 35 mg; Vit. B12: 80 mg; Folic acid: 12 mg; Ca pantothenate: 100 mg; Vit. C: 600 mg; Biotin: 2 mg; Choline: 1,000 mg; Niacin: Iron: 200 mg; Copper: 35 mg; Manganese: 100 mg; Zinc: 240 mg; Iodine: 1.6 mg; Cobalt: 0.8 mg. <sup>3</sup>Aminoacids were not added in the organic meal, following the current law. <sup>4</sup>Furuya (2010).



The water parameters, pH ( $6.56 \pm 0.09$ ), electrical conductivity ( $81.73 \pm 8.34 \mu\text{S}\cdot\text{cm}^{-1}$ ) and dissolved oxygen ( $4.70 \pm 0.53 \text{ mg}\cdot\text{L}^{-1}$ ) were measured once a week using the portable digital potentiometer (Hanna Instruments®). Water temperature ( $28.64 \pm 0.15 \text{ }^\circ\text{C}$ ) was daily checked with a thermometer in the morning.

The fish were fasted for 12 hours after the experimental period for evacuation of the digestive tract. The animals were subsequently anesthetized with eugenol solution ( $65 \text{ mg}\cdot\text{L}^{-1}$ ) according to Vidal et al. (2008), for individual measurements of weight (g), total length (cm), and blood analyses. Three fish per tank were euthanized in ice for the removal of visceral fat and liver. Three whole fish were used for the centesimal analysis; these animals were sent to the Quality Control Laboratory from the Aquaculture Management Study Group – GEMAQ. The centesimal composition was determined according to the methodology by Institute Adolfo Lutz (1985), for the analyses of proteins, fat, moisture, and mineral matter.

All fish were weighed and counted for the calculation of final weight, weight gain (final weight-initial weight), daily weight gain (weight gain/days of cultivation), final length, condition factor ( $((\text{final weight}/\text{final length}^3)*100)$ ), apparent feed conversion (ration intake/weight gain), food efficiency (weight gain/ration intake) and survival ( $((\text{final number of animals}/\text{initial number of animals})*100)$ ).

After being weighted, the animals were identified, immersed in ice, and sent to the Laboratory of Fish Technology. These fish were subsequently eviscerated for the removal of visceral fat and liver for the calculation of body yield ( $((\text{weight without viscera}*100)/\text{total weight})$ ), the hepatosomatic ratio ( $(\text{liver weight}*100)/\text{total weight}$ ), and visceral fat ( $((\text{visceral fat weight}*100)/\text{total weight})$ ). The fish were anesthetized with Eugenol® solution ( $65 \text{ mg}\cdot\text{L}^{-1}$ ) (Vidal et al., 2008) for the hematological and biochemical analyses. Blood samples were subsequently taken from three fish per treatment, in the volume of 2.0 ml, by vein puncture using heparinized syringes. 0.5 ml of blood were used for the erythrocyte count in Neubauer chamber

(Goldenfarb et al., 1971); the hematocrit was determined by the micro-hematocrit and the hemoglobin rate by the cyanomethaemoglobin (Collier, 1944).

The absolute hematimetric index was calculated according to mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) were calculated with the values obtained from the erythrocyte counts, hematocrit, and hemoglobin rate. Total protein, albumin, triglyceride, and cholesterol levels were evaluated in 1.5 ml of plasma in the biochemical analyses. The samples were centrifuged at 2,500 rpm for five minutes for these analyses. Specific commercial "kits" were used for each analysis (Gold Analisa® - Gold Analisa Diagnóstica LTDA Company, Belo Horizonte, Brazil) and the results were analyzed in a spectrophotometer, through the Enzymatic-Colorimetric.

The significance of the effects was verified by the Analysis of Variance (ANOVA) and the test of comparison of means, Tukey test, with a significant p-value  $<0.05$ , through statistical program Statistica version 8.0 (Statsoft, Tulsa, USA) checking the assumptions of normality and homogeneity. The results were expressed as means with standard deviation, the experiments were performed with four replicates.

## Results

There were no significant differences ( $p>0.05$ ) between the digestible protein parameters and the apparent energy and protein digestibility coefficients. However, the result of digestible energy was higher ( $p<0.05$ ) for organic soybean meal (Table 4).

The best results on final weight, weight gain, and daily weight gain, were observed in the fish fed with the CSM + FM ration; this result differed ( $p < 0.05$ ) from the fish fed with OSM + FM (Table 5).

For a centesimal composition of the fish, only one crude protein showed variations ( $p<0.05$ ) as a function of the dietary ingredient, with the highest value found for animals fed with organic soybean meal plus fish meal, and the lowest was obtained in



the animals that received conventional soybean meal without inclusion of fish meal (Table 6).

Hematological parameters presented differences ( $p < 0.05$ ) for hemoglobin, concentration

of mean corpuscular hemoglobin (MCH), cholesterol, albumin and total proteins, as can be seen in Table 7.

**Table 4.** Apparent digestibility coefficients (ADC) of protein and energy

Apparent digestibility coefficients	Soybean meal		f-value
	Organic	Conventional	
Protein digestibility coefficient (%)	88.24 ± 5.24	88.10 ± 2.62	0,002 <sup>ns</sup>
Energy digestibility coefficient (kcal.kg <sup>-1</sup> )	75.92 ± 4.03	70.48 ± 2.13	5,699 <sup>ns</sup>
Digestible protein (%)	39.91 ± 2.48	39.87 ± 1.19	0,001 <sup>ns</sup>
Digestible energy (kcal.kg <sup>-1</sup> )	3579.46 ± 190.09	3141.44 ± 9.95	16,997*

\* Significant at the 95% confidence level; the treatments differ statistically. <sup>ns</sup> not significant at the 95% confidence level; the treatments are statistically similar.

**Table 5.** Productive performance in Nile tilapia fed with OSM and CSM, supplemented or not with FM

Parameters	Treatments				f-value
	OSM	CSM	OSM+FM	OSM+FM	
Initial weight (g)	12.60 ± 0.18	12.70 ± 0.44	12.56 ± 0.49	12.63 ± 0.41	0.230 <sup>ns</sup>
Final weight (g)	99.87 ± 4.54 <sup>d</sup>	110.30 ± 3.26 <sup>c</sup>	125.28 ± 1.63 <sup>b</sup>	134.55 ± 5.32 <sup>a</sup>	77.907*
Weight gain (g)	87.20 ± 4.65 <sup>d</sup>	97.60 ± 2.84 <sup>c</sup>	112.72 ± 1.82 <sup>b</sup>	121.92 ± 5.34 <sup>a</sup>	81.169*
Survival (%)	97.50 ± 5.00	97.50 ± 2.89	97.50 ± 2.89	98.75 ± 2.50	0.111 <sup>ns</sup>
Final length (cm)	16.42 ± 0.17 <sup>b</sup>	17.33 ± 0.10 <sup>ab</sup>	19.53 ± 2.46 <sup>a</sup>	18.71 ± 0.11 <sup>ab</sup>	5.148*
Food conversion	1.30 ± 0.01 <sup>cd</sup>	1.25 ± 0.004 <sup>bc</sup>	1.15 <sup>b</sup> ± 0.02 <sup>a</sup>	1.06 ± 0.10 <sup>a</sup>	14.409*
Food efficiency	0.77 ± 0.01 <sup>bc</sup>	0.80 ± 0.03 <sup>bc</sup>	0.87 ± 0.02 <sup>ab</sup>	0.95 ± 0.10 <sup>a</sup>	13.372*
Condition factor	2.26 ± 0.07	2.12 ± 0.04	1.78 ± 0.51	2.05 ± 0.05	2.619 <sup>ns</sup>
Eviscerated yield (%)	88.21 ± 1.06	88.69 ± 2.00	87.67 ± 0.87	87.53 ± 1.03	0.595 <sup>ns</sup>
Visceral fat (%)	2.08 ± 0.63	1.99 ± 0.19	2.54 ± 0.50	2.97 ± 0.32	1.632 <sup>ns</sup>
Hepatosomatic index (%)	1.08 ± 0.37	1.16 ± 0.59	1.55 ± 0.48	1.64 ± 0.28	1.327 <sup>ns</sup>

OSM: organic soybean meal; CSM: conventional soybean meal; OSM + FM: organic soybean meal + fishmeal; CSM + FM: conventional soybean meal + fishmeal; <sup>ns</sup> Not significant. \* Significant at the 95% confidence level; the treatments differ statistically. <sup>ns</sup> not significant at the 95% confidence level; the treatments are statistically similar.

**Table 6.** Chemical composition of whole tilapias fed with OSM and CSM, supplemented or not with FM

Parameters	Feed				f-value
	OSM	CSM	OSM+FM	CSM+FM	
Moisture (%)	72.19 ± 1,47	74.28 ± 3,28	72.88 ± 1,27	72.41 ± 0,85	0.890 <sup>ns</sup>
Crude protein (%)	15.65 ± 0,55 <sup>ab</sup>	14.74 ± 1,89 <sup>b</sup>	17.19 ± 0,70 <sup>a</sup>	16.84 ± 0,51 <sup>ab</sup>	3.924*
Lipids (%)	9.22 ± 0,21	7.95 ± 1,28	8.54 ± 1,10	8.27 ± 0,27	1.018 <sup>ns</sup>
Mineral matter (%)	3.07 ± 0,63	2.88 ± 0,39	2.76 ± 0,28	5.07 ± 3,93	1.113 <sup>ns</sup>

OSM: organic soybean meal; CSM: conventional soybean meal; OSM + FM: organic soybean meal + fishmeal; CSM + FM: conventional soybean meal + fishmeal. <sup>ns</sup> not significant. \*significant at the 95% confidence level; the treatments differ statistically. <sup>ns</sup> not significant at the 95% confidence level; the treatments are statistically similar.



**Table 7.** Blood parameters in tilapias fed with OSM and CSM, supplemented or not with FM

Parameters	Treatments				f-value
	OSM	CSM	OSM+FM	CSM+FM	
Hematocrit (%)	38.37 ± 0.25	38.12 ± 0.48	38.87 ± 0.95	40.12 ± 0.25	2.988 <sup>ns</sup>
Erythrocytes (10 <sup>6</sup> /μl)	1.88 ± 0.12	1.84 ± 0.05	1.99 ± 0.20	1.86 ± 0.34	0.913 <sup>ns</sup>
Hemoglobin (g/dL)	7.45 ± 0.48 <sup>c</sup>	7.61 ± 0.17 <sup>bc</sup>	8.70 ± 0.48 <sup>ab</sup>	9.12 ± 0.66 <sup>a</sup>	8.183 <sup>*</sup>
MCV (μ <sup>3</sup> )	204.76 ± 14.12	207.43 ± 4.99	199.98 ± 17.82	219.31 ± 33.08	1.557 <sup>ns</sup>
MCH (pg)	39.80 ± 4.57	41.43 ± 0.57	44.05 ± 6.53	49.61 ± 6.25	2.457 <sup>ns</sup>
MCHC (%)	19.41 ± 1.28 <sup>b</sup>	19.98 ± 0.35 <sup>ab</sup>	22.41 ± 1.72 <sup>a</sup>	22.73 ± 1.51 <sup>a</sup>	5.239 <sup>*</sup>
Cholesterol (mg/dL)	159.36 ± 12.05 <sup>b</sup>	110.70 ± 9.27 <sup>a</sup>	129.69 ± 19.43 <sup>ab</sup>	139.71 ± 21.73 <sup>ab</sup>	4.128 <sup>*</sup>
Triglycerides (mg/dL)	261.00 ± 52.52	180.61 ± 27.6	190.99 ± 33.35	184.37 ± 32.92	2.285 <sup>ns</sup>
Albumin (mg/dL)	1.61 ± 0.10 <sup>a</sup>	1.33 ± 0.12 <sup>ab</sup>	1.26 ± 0.09 <sup>b</sup>	1.25 ± 0.02 <sup>b</sup>	5.742 <sup>*</sup>
Total protein (mg/dL)	4.49 ± 0.12 <sup>a</sup>	4.13 ± 0.06 <sup>ab</sup>	4.08 ± 0.07 <sup>b</sup>	4.12 ± 0.12 <sup>ab</sup>	3.527 <sup>*</sup>

OSM: organic soybean meal; CSM: conventional soybean meal; OSM + FM: organic soybean meal + fishmeal; CSM + FM: conventional soybean meal + fishmeal. MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: corpuscular hemoglobin concentration: not significant;

<sup>ns</sup>not significant. Significant at the 95% confidence level; the treatments differ statistically. <sup>ns</sup>not significant at the 95% confidence level; the treatments are statistically similar.

## Discussion

The chemical composition of organic and conventional soybean meal (Table 1), showed higher values of dry matter and mineral matter for the conventional meal. For the lipid contents in the diet, the highest values were for organic food. These variations are related to the method of extraction of soybean oil. Organic soy flour results from the extraction of oil by pressing and a greater amount of fat is retained in the food; Conventional soy flour results from the oil extracted with hexane, resulting in a low amount of fat in the food. The ADC protein values observed were close to those reported in other studies evaluating the digestibility of CSBM in tilapia (Pezzato et al., 2002).

The apparent digestibility coefficient for the soybean meal energy was higher than the conventional one observed in the present study due to the oil extraction method as described by Dallagnol (2010), which evaluated the digestibility of soybean oil produced in different methods of processing and verified that the digestible energy values were 3.614 kcal.kg<sup>-1</sup>, for the extruded and pressed soybean meal, and 3.070 kcal.kg<sup>-1</sup> for the conventional soybean meal (obtained through hexane); these differences are related to the levels of fat retained in the food, which were 8.35 and

1.25%, respectively. Therefore, the differences between the CSM and OSM rations are related to the method of food production since the oil retention is superior to the OSM.

The determination of the ADC energy and nutrients in organic-certified ingredients is incipient in fish, making comparisons difficult and limited to values identified in conventional feeds. The results in this study demonstrated that the use of the OSM ration led to the highest percentages of digestible energy because this feed presented higher lipid content in its composition, and thus, the tilapias featured better use of lipids as an energy source. Data on digestibility of nutrients in fish is important to the formulation of feed designed to obtain enhanced performance in animals and support the definition of guidelines and regulation on the release of residues into the aquatic environments.

Some nutrients might be unbalanced when using protein-based feed based on soybean meal. This food is low in methionine and according to Furuya, (2010), soy contains approximately 0.50% methionine, being the most limiting amino acid; its requirement is 0.54% in the Nile tilapia feed (Furuya et al., 2004; Bomfim, 2013), and this may be triggering the lower performance of fish in organic and conventional soybean treatments, since amino acid synthesis may be limited by methionine deficiency, and according to Boscolo et al. (2005)



meals in which the soybean meal is the main source of protein should be supplemented with synthetic methionine aiming at better productive performance.

Another factor that may lead to the lower performance of organic fish is that due to the soybean meal process, this leads to higher amounts of antinutritional factors in the feed, reducing protein digestion (Marco et al., 2017) and the consequence is the lower growth of fish.

Nevertheless, an improvement in the results from the use of organic feed was observed when fish meal is added to the feed, mainly because the dietary methionine requirement is provided (0.64%) (Table 2). This is possible because fish meal presents an average of 1.15% methionine and 92.51% digestibility (Boscolo et al., 2008), which features it as a quality and important ingredient in feed, also verified by Boscolo et al. (2010) who observed higher growth in tilapia when 16% of fish meal was fed with organic feeds, possibly due to the increase of methionine. Tusche et al. (2011) also report that trout growth is higher with higher amounts of fish meal than when replacing this source of protein with an organic one of plant origin, and stresses that it may be due to the palatabilizing role of fish meal.

The limitation of these inclusions is mainly related to the amino acid profile of this ingredient, however, the soybean meal great plant protein ingredient to be used in feed for fish (Lovell, 1988; Meurer et al. 2008). In addition, there is a higher fiber content in the diets without the inclusion of fish meal and this may cause a higher speed in which the feed goes through the gastrointestinal tract, which consequently reduces the absorption time of the food due to less exposure to the digestive enzymes (Krogdahl et al., 2005), which can also be verified by higher feed efficiency when diets with the inclusion of fishmeal are used.

Boscolo et al. (2005) reported that the tilapia meal could be included in up to 15% in the feed, in a study with *Leporinus macrocephalus* fingerlings. According to Feiden et al. (2005), the meal produced from tilapia filleting residues is an important food to be used in feeding fish because,

beyond its low cost, it does not hamper the development of animals.

The OSM + FM ration provided greater carcass deposition of protein compared to the CSM ration. A CSM diet obtained the lowest result of body protein due to the fact of processing soybean meal, but also contributes to a lower protein absorption and consequent lower body deposition, compared to other feeds. This result might have been influenced by the fact that the fish meal improved the amino acids profile such as lysine, which is the main limiting amino acid in fish feeds and instrumental to the weight gain in tilapias (Meurer et al., 2008). The other components did not present significant differences, as expected, since the feeds were prepared to be isoproteic and isoenergetic.

The hematological parameters did not differ between treatments. The erythrogram is a red series where the red blood cells present a pink tinge, are devoid of nucleus, and show dimensions similar to the ones in healthy fish. The erythrocyte related parameters were similar to those observed in jundiá (*R. quelen*) fed with different protein levels (from 1.98 to 2.21 x 10<sup>6</sup>) (Higuchi et al., 2011).

Chagas and Val (2003) observed that decreased hematocrit could characterize anemia in fish submitted to food deprivation when evaluating hematological parameters in tambaqui (*Colossoma macropomum*). The best result was verified using the CSM + FM feed, not differing from OSM + FM; the lowest result was using the OSM feed, not differing from CSM and COM. The blood characteristics vary between pelagic and migratory species as a function of the swimming effects and eating habits because carnivorous fish present higher levels of hemoglobin and erythrocytes compared to omnivores and herbivores. This difference is correlated to diets, cultivation phase, and environmental conditions.

Hence, hematological data have been used in various studies in which the health and nutritional status are evaluated in fish; however, normal hematological values for healthy fish are still not well established. This complicates the identification of physiological changes derived from nutrition and factors that interfere in the hematopoiesis (Genovez,



2014). Hemoglobin values below the ideal for the species can indicate anemia, and, therefore, the variation in this value observed between species leads to erroneous interpretations.

The mean corpuscular volume and mean corpuscular hemoglobin did not show significant differences when comparing the use of different feeds. Values close to those observed in this study were reported by Chagas and Val (2003) for mean corpuscular volume and mean corpuscular hemoglobin. The use of the OSM + FM and CSM + FM feeds led to the best results for mean corpuscular hemoglobin concentration, which were similar to the values reported by Carvalho et al. (2009). However, it can be stated that by adding organic meals in the diet does not interfere with the hematological levels.

The use of the OSM feed negatively influenced the cholesterol levels, due to the method of extraction of the soybean meal, because there is a greater concentration of oil in the organic meal than in the conventional one as described in (Table 1) and positively influence the total albumin and protein levels, however, differences between OSM and CSM were not observed. No significant difference was observed for triglycerides. Higuchi et al. (2011) detected greater values than those observed in this study in which the total albumin and proteins levels in jundiá (*R. quelen*) fed with different protein levels ranged from 3.32 to 4.42, and from 6.92 to 5.78 g.dL<sup>-1</sup>, respectively. Biochemical analyses in the blood aimed at evaluating its chemical components. Cholesterol is a vitamin and hormone precursor, essential in the processes of reproduction and maintenance of the animal health.

### Conclusion

The organic soybean meal presents great apparent digestibility coefficient and its use in feeds for tilapia delivers satisfactory productive performance and greater carcass protein deposition when the feeds are supplemented with 20% fishmeal and compared to the use of conventional feeds.

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