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RESEARCH ARTICLE

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Hematological parameters, liver integrity and growth of Nile tilapia fingerlings fed diets supplemented with propolis extract

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Abstract

Aim of study: To assess the effects of propolis extract supplementation in diets for Nile tilapia (*Oreochromis niloticus*) fingerlings on growth performance, hematological and histological parameters.

Area of study: The study was carried out in Paraná (Brazil).

Material and methods: The experimental design was based on six treatments including the control diet and propolis supplementation (2%, 4%, 6%, 8% and 10% in the diet). Three hundred Nile tilapia fingerlings, with an initial weight of 0.61± 0.02 g, were distributed in 30 plastic mesh hapas (0.15 m^3 each) arranged in a concrete tank of 25 m^3 of water volume. The duration of the experimental period was 90 days.

Main results: The increasing levels of propolis did not influence the growth performance and proximate composition of fishes. Red blood cells and hematological indices were not affected by propolis supplementation. However, total leukocytes and thrombocytes were higher in fish fed on propolis diets, being significant in fish fed 2% and 8%, and 6% and 8% supplemented diets groups, respectively.

Research highlights: The supplementation of propolis alcoholic extract in the range of 2, 4, 6 and 8% in the diet for Nile tilapia fingerlings promote healthier fish with increased immunity in the evaluated culture conditions.

Additional key words: additives; *Apis mellifera;* bee products; fish farm; productive performance

Abbreviations used: MCH (mean corpuscular hemoglobin); MCHC (mean corpuscular hemoglobin concentration); MCV (mean corpuscular volume).

Authors' contributions: Conception and design of study: GMRM and AF. Performance of the experiments: GMRM, DZD and FB. Laboratory analysis: GMRM, MLB and LAST. Writing of the paper: GMRM, DZD and MLR. Review of the manuscript: FB and AF.

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Introduction

Brazilian fish farming has increased significantly over the last few decades as the result of adequate climate, high availability of water, and excellent nutritional value of fish, in general, causing this market to emerge as one of the main activities of Brazilian agribusiness (Oliveira, 2015). One of the most cultivated species in the country is the Nile tilapia (*Oreochromis niloticus*), because it presents good adaptation to intensive culture, rapid growth, rusticity and excellent meat quality (Figueiredo-Júnior & Valente, 2008).

The presence of high storage densities, high concentrations of ammonia, inadequate feeding, mismanagement, and the presence of bacteria and ectoparasites are common factors that lead to increased stress and incidence of diseases (Karunasagar *et al.*, 1991) when intensive culturing practices are used, consequently affecting the growth and physiological performance of the fishes. The use of antibiotics to face possible diseases has been adopted, however, risks related to their use, such as cumulative effects on the environment and humans, and the development of resistant pathogens must be seriously considered (Holmstrom *et al.*, 2003).

One promising method for improving fish health performance and reducing the occurrence of diseases is the prophylactic administration of diets supplemented with immunostimulants (Robertsen, 1999) from natural

sources (Santos *et al.*, 2013) to enhance fish natural defense mechanisms. Some substances derived from natural sources have the added advantage of not harming either the environment or consumers (Sforcin, 2007).

Propolis is among the various phytotherapeutic sources (Talas & Gulhan, 2009) with potential to be used as a natural diet supplement. It is produced by *Apis mellifera* L. bees from resinous and balsamic material collected from leaf shoots and cracks in the bark of several plants (Burdock, 1998; Toreti *et al.*, 2013). Propolis has a complex chemical composition including compounds such as flavonoids (galangin, techtochrysin, pinocembrin, kaempferol, and quercetin), aromatic and phenolic acids (caffeic, ferulic, cinnamic, and coumaric), terpenoids, aldehydes, alcohols, aliphatic acids, and esters, amino acids, steroids, and sugars (Bankova *et al.*, 1995; Marcucci, 1996).

Propolis has been widely studied due to its antimicrobial activity (Sforcin *et al.*, 2000). In fish, the propolis extract has a growth-promoting and immunostimulatory effects, benefiting liver physiology and resistance to bacteria (Abd-El-Rhman, 2009; Meurer *et al.*, 2009b). In addition, anti-fungal (Uzel *et al.*, 2005), anti-inflammatory (Mirzoeva & Calder, 1996; Song *et al.*, 2002), immunostimulant (Burdock, 1998), immunomodulator (Fisher *et al.*, 2008), antiviral (Marcucci, 1996; Burdock, 1998), antioxidant (Cabral *et al.*, 2009), and cytostatic (Banskota *et al.*, 2001) properties have also been reported. Moreover, the scientific literature highlights several other activities such as hepatoprotective effects promoted by chemical compounds (Basnet *et al.*, 1996), and hypoglycemic (Matsui *et al.*, 2004) and hypotensive (Burdock, 1998) effects.

These biological and pharmacological properties make propolis an attractive supplement for nutritious diets, being hypothesized the benefit of diets containing propolis providing growth and fish homeostasis. Thus, this study evaluated the responses of tilapia fingerlings (*O. niloticus*) to the inclusion of propolis alcoholic extract in their diet through the analysis of growth performance, carcass proximate composition, blood parameters, and liver histopathological alterations.

Material and methods

This study was carried out at the State University of Western Paraná in Toledo Campus along with the Aquaculture Management Study Group (GEMAq). It was evaluated and approved by the Ethics Committee on Animal Use under Protocol number 03/15 - CEUA.

Experimental diets

Six isoenergetic (3083 kcal digestible energy/kg of ration) and isoproteic (29.73% crude protein) diets were formulated based on the nutrient and energy requirements for Nile tilapia (*O. niloticus*) according to Furuya *et al.* (2010). The experimental diets included a control diet without the addition of propolis extract (0%), and five treatments with different supplementation levels of alcoholic propolis extract (APE), 2%, 4%, 6%, 8% and 10% (Table 1).

The Laboratory of Research & Development of Drug Delivery Systems, Dept. of Pharmacy, State University of Maringa, Brazil, donated the ethanolic extract of green propolis. The extractive solution was prepared by turbo-extraction and using propolis/ethanol ratio of 30/70 (w/w) (Bruschi *et al.*, 2003a,b). Afterwards, the following characteristics of the extract were analyzed: pH (5.4 ± 0.00), density (0.87 ± 0.00 g mL^{-1}), dryness residue (16.93 ± 0.63%, w/w), and total polyphenol content (1.83 ± 0.09%, w/v). This propolis research was registered in Brazil with SISGEN No AC7A2F5. The polyphenol concentrations were 0.01%, 0.02%, 0.03%, 0.04% and 0.06% the levels of inclusion of propolis extract diets in this study. Quality assays were carried out according to Bruschi *et al.* (2003a).

Dietary ingredients were individually ground in a hammer mill to prepare 0.5 mm diameter particles. The particles were weighed, mixed in the "V" equipment, and extruded (Ex-Micro® Extruder). The diets were dried in a forced circulation oven at 55ºC for 24 h and subsequently stored and protected from light in an airy location. The granulometry of the diets was adequate according to the fish growth phase. Fish were fed to apparent satiation four times a day (8 am, 11 am, 2 pm and 5 pm).

Animals and experimental design

Three hundred Nile tilapia fingerlings (*O. niloticus*), with an initial weight of 0.61± 0.02 g, were distributed in 30 plastic mesh hapas (experimental unit) with 0.15 m^3 of useful volume (40 cm × 40 cm × 70 cm in length, width, and depth respectively), arranged in a concrete tank with a capacity of 25 m^3 of constantly aerated water. Each experimental unit consisted of ten fish. The fish were evaluated during 90 days in a random experimental design based on feeding of six experimental diets previously described.

The water quality was monitored for temperature (24.07 ± 1.66°C), dissolved oxygen (5.84 ± 0.97 mg/L), pH (7.67 ± 0.69), and conductivity (91.03 ± 4.97 µS/cm) using multiparameter mater, YSI Professional model, YSI- EUA. The temperature was measured daily and other parameters were monitored weekly. The variables were in the optimal levels for *O. niloticus* growth (Ridha & Cruz, 2001).

Sample collection and growth performance evaluation

At the end of the experiment, all the fish were fasted for 12h to empty the gastrointestinal tract. Fish were

Table 1. Composition of the experimental diets with different levels of propolis supplementation

	Experimental diets					
	0	**2**	**4**	**6**	**8**	**10**
Ingredients [1]						
Soybean meal	278.0	278.0	279.4	279.4	280.8	281.5
Corn grain	256.3	252.5	248.8	248.8	241.3	237.5
Wheat bran	200.0	200.0	200.0	200.0	200.0	200.0
Poultry viscera flour	150.0	150.0	150.0	150.0	150.0	150.0
Rice grits	50.0	50.0	50.0	50.0	50.0	50.0
Fish flour	50.0	50.0	50.0	50.0	50.0	50.0
Premix [a]	5.0	5.0	5.0	5.0	5.0	5.0
Common salt	3.0	3.0	3.0	3.0	3.0	3.0
Dicalcium phosphate	2.8	2.8	2.8	2.9	2.9	2.9
Soy oil	1.2	2.2	3.2	4.2	5.2	6.2
Choline chloride	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin C	1.0	1.0	1.0	1.0	1.0	1.0
Calcium propionate	1.0	1.0	1.0	1.0	1.0	1.0
DL-methionine	0.2	0.2	0.2	0.2	0.2	0.2
BHT [b]	0.2	0.2	0.2	0.2	0.2	0.2
L-threonine	0.1	0.1	0.1	0.1	0.1	0.1
Propolis alcoholic extract	0.0	2.0	4.0	6.0	8.0	10.0
Total (g)	1000	1000	1000	1000	1000	1000
Nutrients (g/kg)						
Starch	282.6	282.6	282.6	282.6	282.6	282.6
Calcium	11.2	11.2	11.2	11.2	11.2	11.2
Digestible energy (kcal/kg)	3083.0	3083.0	3083.0	3083.0	3083.0	3083.0
Total phosphorus	10.0	10.0	10.0	10.0	10.0	10.0
Total lysine	16.4	16.4	16.4	16.4	16.4	16.4
Total methionine	5.2	5.2	5.2	5.2	5.2	5.2
Crude protein	297.3	297.3	297.3	297.3	297.3	297.3

[1] Composition values based on Boscolo *et al.* (2001) and Furuya *et al.* (2001). [a] Guaranteed levels per kg of product - Premix (DSM-Roche®): 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. [b] BHT, butyl hydroxy toluene.

subsequently anesthetized with benzocaine (100 mg/L) according to Gomes *et al.* (2001) for measurements of weight (g) and total length (cm). Growth performance was evaluated by estimation of the following parameters and indexes:

- Weight gain (WG) = Final body weight (g) – Initial body weight (g).
- Feed conversion rate (FCR) = Consumed diet (g) / Weight gain (g).
- Specific growth rate (SGR) = [(ln Final weight (g) – ln Initial weight (g)) / time] × 100.
- Protein efficiency ratio (PER) = (Weight gain (g) / Protein consumed (g)).
- Survival (SU) = (Final number of fish / Initial number of fish) × 100.
- Batch uniformity (BU) = (Number of fish with body weight within the mean / Total number of fish) × 100.

Three fish from each experimental unit were euthanized with benzocaine (250 mg/L) for the collection of visceral fat and liver. For hematological and proximate samples, three fish from each experimental unit were used for tissue collection. The following indexes were estimated:

- Hepatosomatic index (HSI) = [Liver weight (g) / Final body weight (g)] × 100.
- Viscerosomatic fat index (VFI) = [(Visceral fat weight (g) / Final body weight (g)] × 100.

Proximate composition

Three fish from each experimental unit were sent to the Laboratory of Food Quality (LQA) of the Aquaculture Management Study Group (GEMAq) for the analysis of proximate composition; these fish were frozen (-20°C) until analysis. Samples were subsequently ground, homogenized, and processed according to AOAC (2000) for the following analyses: moisture (pre-drying at 55ºC for 72 h followed by drying at 105ºC for 8 h), proteins (Kjeldahl method, Modle MA-036, Piracicaba-SP, Brazil), ethereal extract (Soxhlet extractor with petroleum ether as the solvent; Model TE-0,44, Piracicaba-SP, Brazil), and ash (calcination of samples at 550ºC for 6 h; Modle 2000B, Belo Horizonte-MG, Brazil).

Hematologic evaluations

Blood samples were collected by caudal puncture using a 1.0 mL syringe with anticoagulant (10% EDTA) from three fish in each experimental unit. Total erythrocytes were counted using the Neubauer chamber and Hayen solution. The hemoglobin rate was calculated by Collier´s (1944) method, the hematocrit percentage was calculated by Goldenfarb *et al.*´s (1971) method. Hematimetric indexes, such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Winthrobe (1933):

$$MCV\ (fL) = (hematocrit \times 10)/\ erythrocytes$$

$$MCH\ (g\ dL^{-1}) = (hemoglobin \times 10)/\ erythrocytes$$

$$MCHC\ (\%) = (hemoglobin \times 100)/\ hematocrit$$

Blood smears were prepared on frosted glass slides, air dried. These slides were read in an optical microscope at 100x magnification using immersion oil; the total count of leukocytes and thrombocytes was performed by the indirect method of Ranzani-Paiva *et al.* (2013). The staining of blood extensions was performed according to Rosenfeld (1947).

The leukocyte differential counting consisted in determining the ratio (as a percentage) between the different leukocyte forms: lymphocytes, neutrophils and monocytes. One hundred leukocytes were counted on the smear area by traversing the entire material and moving the slide in a "zig-zag" motion.

Histology of the liver

Two livers from each replicate (10 fish per treatment) were washed with distilled water and samples from the medial portion of the left lobe were fixed in aqueous Bouin solution and transferred to vials containing 70% alcohol (Tokumaru *et al.*, 1968). This material was dehydrated through an increasing alcohol series, diaphanized in xylol, and embedded in paraffin to obtain 6-µm-thick semi seriate cross sections using a rotary microtome (Microm HM 340 E Thermo Scientific, Germany).

These cuts were mounted on slides, stained with hematoxylin and eosin for general morphological analysis and determination of the number of hepatocytes per area (counting area: 2048.1532 μm^2). The histological analyses of the liver were performed using images captured with an optical microscope (P1 Olympus BX 50, Manila, Philippines) coupled to a camera (Olympus PMC 35 B, Berlin, Germany) and used a 40x objective; 10 images were taken per slide (30 images/animal), totaling 300 images/treatment. These measurements were performed using the Image-Pro Plus® image analysis software (vers 4.5, Media Cybernetics, USA). These analyses were performed at the Histology Laboratory of State University of Western Paraná, Unioeste, Campus Toledo, PR, Brazil.

Statistical analyses

The data were submitted to normality tests (Shapiro-Wilk) and homoscedasticity, and subsequent analysis of variance. The Tukey's test (5%) was applied when the ANOVA was significant for the variables' means ($p<0.05$). The Bernet index was performed through the non-parametric Kruskal-Walis analysis. These analyses were all conducted on Statistica 7.1® program.

Results

Growth performances and proximate composition

Fish fed with the 10% propolis alcohol extract diet showed significantly the worst feed conversion and survival ($p<0.05$) compared to values observed in fish fed with the other experimental and control diets (Table 2). On the other hand, the increasing levels of propolis in the diets studied for Nile tilapia fingerlings did not influence the final weight, weight gain, final length, specific growth rate, visceral fat, hepatosomatic index, protein efficiency rate and batch uniformity (Table 2). Proximate composition did not show significant differences between treatments (Table 3).

Hematological parameters

The different levels of propolis alcoholic extract in the diet did not show influence ($p>0.05$) on the hematological parameters of the red blood cells represented by mean erythrocyte values of 1.7 10^6 µL, hematocrit with a percentage of 29 to 31%, and hemoglobin between 6.6

Table 2. Productive performance of Nile tilapia (*Oreochromis niloticus*) fingerlings fed with increasing levels of propolis extract in the diet

Parameters [1]	Experimental diets (%)					
	0	2	4	6	8	10
FW (g)	28.98±3.92	33.95±1.99	32.88±3.09	30.57±2.51	28.49±3.25	30.94±3.83
WG (g)	28.31±3.36	32.35±2.70	32.01±3.39	29.96±2.50	27.89±3.25	29.64±4.07
FL (cm)	10.96±0.36	11.28±0.43	11.40±0.44	10.94±0.39	10.88±0.36	11.16±0.34
FCR	1.18±0.22a	1.25±0.24a	1.18±0.14a	1.13±0.10a	1.16±0.11a	1.57±0.22b
SGR (%)	1.76±0.03	1.85±0.09	1.89±0.07	1.84±0.09	1.77±0.12	1.85±0.17
VFI (%)	1.90±0.37	2.28±0.31	2.55±0.45	2.23±0.42	2.77±0.60	2.83±0.95
PER (%)	5.06±0.83	4.32±0.33	4.47±0.62	5.75±1.49	4.99±0.66	4.69±1.38
SU (%)	82.50±2.58ab	80±8.16ab	96.60±5.77a	84.00±11.40ab	95.00±15.00a	62.50±9.57b
BU (%)	69.95±12.29	65.77±4.24	68.10±5.67	80.47±6.42	65.85±12.37	80.64±8.30
HSI (%)	2.18±0.36	2.11±0.67	2.48±0.49	2.60±0.36	2.88±0.43	2.62±0.38

[1] FW: final weight, WG: weight gain FL: final length, FCR: feed conversion rate, SGR: specific growth rate, VFI: viscerosomatic fat index, PER: protein efficiency ratio, SU: survival, BU: batch uniformity, HSI: hepatosomatic index. Values are presented as mean ± standard deviation. Different letters indicate significant differences among treatments, Tukey's test (p<0.05).

and 7.1 g/dL. The hematimetric indexes showed values of MCV above 171 fL, MCH averages between 39 and 48 µg, and MCHC between 22.1 and 24.08 g/dL (Table 4).

Total leukocytes and total thrombocytes levels were increased with propolis supplementation in diet. According to leukocytes (p<0.05) fish fed with 2% and 8% of propolis extract showed significant higher values (56.226 and 55.169 µL, respectively). Total thrombocytes showed the highest significant values (p<0.05) in fish fed with 6% and 8% of propolis extract (56.954 and 56.138 µL, respectively). The mean values of lymphocytes were 94.18±0.66%, the percentage of neutrophils were 2.89±0.52% and monocytes were 0.99±0.22%; in these parameters no significant difference (p>0.05) was observed between treatments.

Liver histology

The macroscopic evaluation of the liver showed well-developed organs, homogeneous, with a slightly brownish reddish-brown color and absence of striations or whitish lesions. The microscopic qualitative evaluation of hepatocytes showed hepatic parenchyma composed of hepatocytes arranged in continuous cords permeated by sinusoids. Typical hepatocytes were characterized with round nuclei of central position, with evident nucleoli and slightly acidophilic and vacuolated cytoplasm. In all treatments containing propolis as well as in the control diet.

However, higher number of hepatocytes was observed in fish fed 2% and 6% propolis extract diets (p<0.05) compared to those fed control diet (Fig. 1).

Discussion

Growth performance

An increasing number of studies have evaluated the effects of non-conventional products on animal nutrition. The use of propolis as an additive has been of great interest because its complex composition may yield improved results in productive performance and animal health (Abd-El-Rhman, 2009; Velotto *et al.*, 2010; Sforcin & Bankova, 2011; Abbas *et al.*, 2012; De la Cruz-Cervantes *et al.*, 2018).

Table 3. Proximate composition of Nile tilapia (*Oreochromis niloticus*) fingerlings fed with increasing levels of propolis extract. Values are means ± standard deviation

Parameters (%)	Experimental diets (%)					
	0	2	4	6	8	10
Moisture	81.52±0.20	81.53±0.77	81.19±0.85	80.78±1.42	81.01±1.37	81.47±0.73
Protein	11.58±0.86	11.48±0.90	11.68±0.79	11.93±0.83	11.73±1.12	11.28±0.98
Ethereal extract	4.91±0.21	4.79±0.12	5.07±0.41	4.96±0.34	4.92±0.70	4.85±0.21
Ash	2.85±0.28	2.83±0.30	2.84±0.19	3.01±0.27	2.94±0.14	2.88±0.28

Table 4. Hematological parameters, mean values of white blood cells and thrombocytes of tilapia (*Oreochromis niloticus*) fingerlings fed with diets containing increasing levels of alcoholic propolis extract

Parameters [1]	Experimental diets (%)					
	0	2	4	6	8	10
RBC (10^6 µL)	1.70±0.14	1.66±0.07	1.71±0.11	1.65±0.12	1.66±0.12	1.68±0.11
Hematocrit (%)	29.86±3.29	30.16±2.65	31.73±2.57	29.0±2.50	31.13±4.27	31.58±2.67
Hemoglobin (g/dL)	7.14±0.87	6.87±0.80	6.78±1.95	6.63±1.54	6.74±1.93	6.85±1.53
MCV (fL)	176.44±22.26	181.97±18.40	174.34±19.05	172.33±17.24	173.42±28.78	188.98±21.62
MCH (µg)	42.20±6.00	40.95±4.70	40.86±10.57	39.47±9.74	40.27±12.24	47.97±10.20
MCHC (g/dL)	24.08±3.17	23.46±3.79	22.36±6.00	22.13±6.01	22.50±9.48	22.77±6.09
Leukocytes (µL)	27256±14742b	56226±24815a	50849±14306ab	52558±19826ab	55169±16118a	45495±19353ab
Thrombocytes (µL)	29189±15515b	42556±15560ab	39680±12991b	56954±15724a	56139±15661a	39261±6492b
Lymphocytes (%)	93.42±4.03	94.83±2.63	93.50±5.26	94.85±3.18	93.85±3.23	94.63±2.62
Neutrophils (%)	3.00±1.91	2.66±2.42	3,14±2,54	2.00±1.82	3.57±2.22	3.00±2.33
Monocytes (%)	1.33±1.03	1.16±0.83	0.88±0.75	1.00±0.57	0.71±0.48	0.88±0.75

[1] RBC: red blood cells, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration. Values are presented as mean ± standard deviation (p<0.05). Different letters indicate significant differences among treatments, Tukey's test (p<0.05).

Deng *et al.* (2011) reported different results using an alcoholic propolis extract supplemented in the diet of rainbow trout (Oncorhynchus mykiss); these authors observed a significant improvement in growth rates, food efficiency, and protein efficiency that promoted improvement in performance. Meurer *et al.* (2009b) evaluated increasing levels of brown propolis extract used as growth promoters in Nile tilapia (*O. niloticus*) and reported improved results in final weight, weight gain, and feed conversion using between 1.83 and 2.74 g of brown propolis extract/kg of diet.

In this study, final weight, weight gain, final length, specific growth rate, and protein efficiency in tilapia fingerlings showed no significant differences between propolis alcoholic extract diet supplementation and the control. Santos *et al.* (2013) found similar results using residues of red propolis extract in the concentrations of 0.5, 1.0, and 1.5% for Nile tilapia fingerlings. Uczay *et al.* (2014) did not observe a significant difference in final length, and specific growth rate in silver catfish juveniles (*Rhamdia quelen*) fed with 0.5, 1.0, 1.5, and 2.0% propolis. Similarly, the use of 0.1, 0.2, 0.3 and 0.4% of alcoholic propolis extract in the diet of the common carp (*Cyprinus carpio*) did not result in differences in final weight and specific growth rate compared to the control (Uczay *et al.*, 2011).

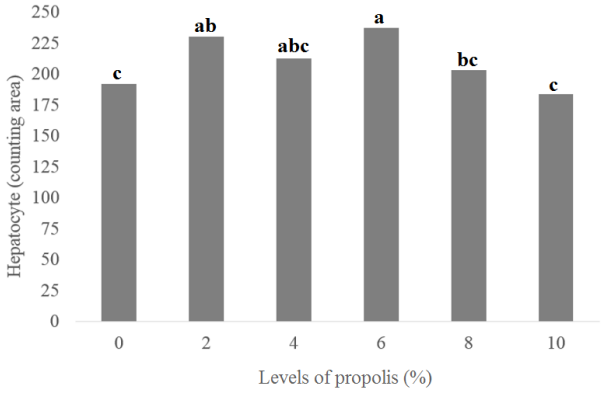

Figure 1. Hepatocytes in the hepatic tissue of Nile tilapia (*Oreochromis niloticus*) fingerlings fed with diets containing increasing levels of alcoholic propolis extract. Counting area: 2048.1532 µm^2. Different letters above the histogram bars indicate significant differences between treatments by the Tukey's test (p<0.05%)

According to Santos *et al.* (2013), the fish digestive system is developing during the initial phase of life and has small amounts of microorganisms compared to adult fish. Therefore, the capacity of propolis to modulate the quality of the intestinal microbiota and promote the growth of beneficial microorganisms, influencing the expression of its effect on the absorption of nutrients available in diets (Guo *et al.*, 2004), is still inconsistent.

The contradictory results described for growth performance using alcoholic propolis may be related to the complexity of its chemical composition. Although propolis is classified as an opotherapeutic substance,in the case of substances obtained from glands, other organs, tissues, and animal secretions, the composition is determined by the individual plant source and the effects of seasonality which influence the synthesis and concentrations of bioactive compounds and, therefore, potentially affect activity (Marcucci, 1996).

Another important factor related to the response of productive performance in this study is the absence of

challenge. Specifically, the favorable environmental conditions used in this study ensured the fish's well-being. Some studies on non-ruminant animals demonstrated that growth promoters such as prebiotics show improved results when challenged (Meurer *et al.*, 2009a). The effects of crude propolis and propolis extract on the diet of tilapia juveniles infected by *Aeromonas hydrophila* were evaluated by Abd-El-Rhman (2009). Their results showed improved productive performance, immunity, and resistance to bacteria with the use of alcoholic propolis extract followed by crude propolis when compared to the control group.

According to Bae *et al.* (2012), the inclusion of propolis levels above 10% in the diet of juvenile *Anguilla japonica* may negatively affect fish physiology. In this study levels assayed were up to 10% (10 g/kg). In fact, the lowest survival rate (62.5%) was observed in fish fed with 10% of propolis extract suggesting that this concentration did not favor productive performance and fish health. This would explain the increased food conversion values in this experimental group (Table 2) probably due to the lower fish survival rate associated the feeding *at libitum* in this experiment.

As reported, propolis contains several chemical substances that are responsible for its therapeutic activities. Nevertheless, some compounds can provide negative side effects. According to Ramos & Miranda (2007), propolis-induced cinnamic acid derivatives can lead to hypersensitivity and intoxication in sensitive organisms. Therefore, the use of propolis alcoholic extract at concentrations of 10% in the 90-day period influenced the physiological response of tilapia fingerlings in this study, suggesting that this concentration must be used with caution in order to maintain the optimal supplementation level at this stage of development.

Proximate composition

In this study, the proximate composition was not influenced by the different concentrations of alcoholic propolis extract in the diet (Table 3). Uczay *et al.* (2014) found decreased values of fat in carcass in silver catfish (R. quelen) juveniles fed on diets supplemented with propolis (0.5, 1.0, 1.5, and 2.0%). The presence or absence of specific components (Haard, 1992) could explain the lack of differences under different propolis supplementation when compared with other studies (Meurer *et al.*, 2009b; Uczay *et al.*, 2011, 2014; Santos *et al.*, 2013). Although we did not find any difference in proximate composition, Bae *et al.* (2012) stated that propolis has the ability to improve the proximate composition in fish but the active ingredient and the mechanism involved in this beneficial effect are still unknown.

Hematological parameters

The effect of propolis as an additive in animal nutrition showed varied responses to its biological and pharmacological activities. According to Arauco *et al.* (2007) it was observed an immunostimulating effect when using low concentrations of propolis in diets; however, Kashkooli *et al.* (2011) found no effect of propolis when used in the long-term diet and Talas & Gulhan (2009) found that in high concentrations propolis has a negative effect on hematological variables.

The evaluation of hematological parameters is important for understanding the homeostatic and pathological conditions in fish (Barton & Iwama, 1991; Ranzani-Paiva *et al.*, 2004). The hematological parameters evaluated in this study (Table 4) verified the influence of the different levels of propolis ($p<0.05$). However, our results are within the recommended reference for the species. The percentages of hematocrit, hemoglobin and concentrations of erythrocytes observed in this study are in good agreement with those described in literature (Tavares-Dias & De-Moraes, 2007; Tavares-Dias *et al.*, 2009).

Hematimetric indexes are used in the evaluation and classification of anemia, in general. It is a condition in which the blood's ability to transport oxygen to tissues is reduced (Ranzani-Paiva *et al.*, 2013). The values obtained for MCV, MCH, and MCHC (Table 4) were not altered ($p>0.05$) by the concentration of propolis in the diet. These results are in agreement with the variation considered normal for this species as proposed by Hrubec *et al.* (2000), and the use of propolis alcohol extracts up to 10% may be indicated, as observed in this study.

In general, the levels of alcoholic propolis extract provided an increase in total leukocytes and thrombocytes (Table 4), despite results within the range of variation for tilapias (Hrubec *et al.*, 2000). Leukocytes together with thrombocytes form a cellular population of organic defense (Tavares-Dias *et al.*, 2002) and present important phagocytic function in the regulation of the immune system (Harikrishnan *et al.*, 2011). Increased levels of leukocytes and thrombocytes in the vascular blood of fish could be an indicative of a rapid renewal and an increased protection against different pathogenic microorganisms (Ranzani-Paiva *et al.*, 2004).

The analysis of leukocyte differentiation showed no significant differences between treatments (Table 4), which consequently justify the absence of pathological and stressful agents during the experiment. These results are in line with those previously reported by Ledic-Neto *et al.* (2014), who did not find significant differences in monocytes and neutrophils with the addition of 2% propolis in the diet for tilapia fed during 15 and 21 days.

The efficacy of the heterogeneous composition of propolis that has flavonoids as the main group of compounds responsible for therapeutic activities (Marcucci, 1996)

can interfere in several physiological processes and act as immunostimulants in fish. Such compounds, in turn, may increase resistance to infectious diseases by enhancing nonspecific defense mechanisms (Zhang *et al.*, 2009), which are normally identified by their ability to activate leukocytes in *in vitro* tests (Bricknell & Dalmo, 2005; Mouriño *et al.*, 2012).

Histological alterations

The macro and microscopic evaluations of the hepatic histology observed in the present study are in line with those described by Vicentini *et al.* (2005), allowing the use of the structural pattern for the species for characterization of the liver histology of the Nile tilapias in this study.

Fish liver, as well as that of other vertebrates, is responsible for the animal's physiology as well as the execution of many vital activities. Fish liver is formed by hepatocytes, which are cells with high mitotic potential (Costa *et al.*, 2012), that function in metabolism of proteins, lipids, and carbohydrates, storage of some nutrients, hematopoiesis, antibody production (Takashima & Hibiya, 1995) and the capacity of biotransformation and excretion of xenobionts (Bernet *et al.*, 1999).

The liver analysis in this study identified a higher number of hepatocytes in fish fed with 2%, 6% and 8 % propolis extract compared to those in the control, 4% and 10 % in the diet (Fig. 1). This result suggests that these levels of propolis promote a possible hepatoprotective activity as demonstrated by Bhadauria *et al.* (2008) in studies with rats.

The histopathological changes observed are part of a spontaneous tissue regenerative process (Baldisserotto, 2013). The qualitative variation of fish hepatocytes showed subtle changes in the liver structure, with mildly acidophilic cytoplasm and central nuclei, indicating normal metabolic activity (Bernet *et al.*, 1999), with no significant liver changes in the concentrations of propolis extract used. These results are similar to those found by Honorato *et al.* (2014) in tilapia fed with diets containing biological fish silage and in traíras (*Hoplias malabaricus*) exposed to methyl mercury (Mela *et al.*, 2007).

Conclusion

The use of propolis alcoholic extract in the range of 2% to 8% provided an increase in the number of leukocytes, total thrombocytes, and hepatocytes, suggesting that inclusion within this range leads to healthier fish with increased immunity in the evaluated culture conditions. Therefore, the inclusion of up to 8% of propolis in the diet for Nile tilapia fingerlings is recommended.

References

Abbas AA, El-Asely AM, Kandiel MMM, 2012. Effects of dietary propolis and pollen on growth performance, fecundity and some hematological parameters of *Oreochromis niloticus*. Turk J Fish Aquat Sci 12: 851-859. https://doi.org/10.4194/1303-2712-v12_4_13

Abd-El-Rhman, AMM, 2009. Antagonism of *Aeromonas hydrophila* by propolis and its effect on the performance of Nile tilapia, *Oreochromis niloticus*. Fish Shellfish Immun 27: 454-459. https://doi.org/10.1016/j.fsi.2009.06.015

AOAC, 2000. Official methods of analysis. Association of Official Analytical Chemists, 17th ed. Gaithersburg, MD, USA.

Arauco LRR, Stéfani MV, Nakaghi LSO, 2007. Efeito do extrato hidroalcoólico de própolis no desempenho e na composição leucocitária do sangue de girinos de rãtouro (*Rana catesbeiana*). Acta Sci Anim Sci 29: 227-234. https://doi.org/10.4025/actascianimsci.v29i2.240

Bae JY, Park GH, Lee JY, Okorie OE, Bai SC, 2012. Effects of dietary propolis supplementation on growth performance, immune responses, disease resistance and body composition of juvenile eel, *Anguilla japonica.* Aquacult Int 20: 513-523. https://doi.org/10.1007/s10499-011-9482-4

Baldisserotto B, 2013. Fisiologia de peixes aplicada à piscicultura, 3rd ed. UFSM, Brazil.

Bankova V, Christov R, Kujumgiev A, Marcucci MC, Popov S, 1995. Chemical composition and antibacterial activity of Brazilian propolis. J Biosci 50: 167-172. https://doi.org/10.1515/znc-1995-3-402

Banskota AH, Tezuka Y, Kadota S, 2001. Recent progress in pharmacological research of propolis. Phytother Res 15: 561-571. https://doi.org/10.1002/ptr.1029

Barton BA, Iwama GK, 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu Rev Fish Dis 1: 3-26. https://doi.org/10.1016/0959-8030(91)90019-G

Basnet P, Matsushige K, Hase K, Kadota S, Namba T, 1996. Four di-O-caffeoyl quinic acid derivatives from propolis. Potent hepatoprotective activity in experimental liver injury models. Biol Pharma Bull 19: 1479-1484. https://doi.org/10.1248/bpb.19.1479

Bernet D, Schmidt H, Meier W, Burkhardt-Holm P, Wahli T, 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. J Fish Dis 22: 25-34. https://doi.org/10.1046/j.1365-2761.1999.00134.x

Bhadauria M, Nirala SK, Shukla S, 2008. Multiple treatment of propolis extract ameliorates carbon tetrachloride induced liver injury in rats. Food Chem Toxicol 46: 2703-2712. https://doi.org/10.1016/j.fct.2008.04.025

Boscolo WR, Hayashi C, Meurer F, Soares MS, 2001. Farinhas de peixe, carne e ossos, vísceras e crisálida como atractantes em dietas para alevinos de

Tilápia do Nilo (*Oreochromis niloticus*). R Bras Zootec 30: 1397-1402. https://doi.org/10.1590/S1516-35982001000600002

Bricknell I, Dalmo R, 2005. The use of immunostimulants in fish larval aquaculture. Fish Shellfish Immun 19: 457-472. https://doi.org/10.1016/j.fsi.2005.03.008

Bruschi ML, Franco SL, Gremião MPD, 2003a. Application of an hplc method for analysis of propolis extract. J Liq Cromatogr Relat Technol 26: 2399-2409. https://doi.org/10.1081/JLC-120023254

Bruschi ML, Cardoso MLC, Lucchesi MB, Gremião MP, 2003b. Gelatin microparticles containing propolis obtained by spray-drying technique: preparation and characterization. Int J Pharm 264: 45-55. https://doi.org/10.1016/S0378-5173(03)00386-7

Burdock GA, 1998. Review of the biological properties and toxicity of bee propolis (propolis). Food Chem Toxicol 36: 347-363. https://doi.org/10.1016/S0278-6915(97)00145-2

Cabral ISR, Oldoni TLC, Prado A, Bezerra RMN, Alencar SM de, Ikegaki M, Rosalen PL, 2009. Composição fenólica, atividade antibacteriana e antioxidante da própolis vermelha brasileira. Quím Nova 32: 1523-1527. https://doi.org/10.1590/S0100-40422009000600031

Collier HB, 1944. Standardization of blood haemoglobin determinations. Can Med Assoc J 50: 550-552.

Costa RFR, Santos IF, Santana AP, Tortelly R, Nascimento ER, Fukuda RT, *et al.*, 2012. Caracterização das lesões por Cysticercus bovis, na inspeção post mortem de bovinos, pelos exames macroscópico, histopatológico e pela reação em cadeia da polimerase (PCR). Pesq Vet Bras 32: 477-484. https://doi.org/10.1590/S0100-736X2012000600002

De la Cruz-Cervantes JA, Benavides-González F, Sánchez-Martínez JG, Vázquez-Sauceda M de la L, Ruiz-Uribe AJ, 2018. Propolis in Aquaculture: A review of its potential. Rev Fish Sci Aquac 26: 337-349. https://doi.org/10.1080/23308249.2018.1424798

Deng J, An Q, Bi B, Wang Q, Kong L, Tao L, Zhang X, 2011. Effect of ethanolic extract of propolis on growth performance and plasma biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). Fish Physiol Biochem 37: 959-967. https://doi.org/10.1007/s10695-011-9493-0

Figueiredo-Junior CA, Valente ASJ, 2008. Cultivo de tilápias no Brasil: Origens e cenário atual. Congr Soc Bras de Economia, Administração e Sociologia Rural; Acre, Brasil, pp: 1-9.

Fisher G, Hübner S, Vargas G, Vidor T, 2008. Imunomodulação pela própolis. Arq Inst Biol 75: 247-253. https://doi.org/10.1590/1808-1657v75p2472008

Furuya WM, Pezzato LE, Pezzato AC, Barros MM, Miranda EC, 2001. Coeficientes de digestibilidade e valores de aminoácidos digestíveis de alguns ingredientes para Tilápia (*Oreochromis niloticus*). Rev Bras Zoot 30: 1143-1149. https://doi.org/10.1590/S1516-35982001000500002

Furuya WM, Pezzato LE, Barros MM, Boscolo WR, Cyrino JEP, Furuya VRB, Feiden A, 2010. Tabelas brasileiras para a nutrição de tilápias. Editora Toledo: GFM.

Goldenfarb PB, Bowyer FP, Hall E, Brosious E, 1971. Reproducibility in the Hematology Laboratory: The microhematocrit determination. Am J Clin Pathol 56: 35-39. https://doi.org/10.1093/ajcp/56.1.35

Gomes LC, Chippari-Gomes AR, Lopes NP, Roubach R, Araujo-Lima CARM, 2001. Efficacy of benzocaine as an anesthetic in juvenile tambaqui *Colossoma macropomum*. J World Aquacult Soc 32: 426-431. https://doi.org/10.1111/j.1749-7345.2001.tb00470.x

Guo FC, Williams B, Kwakkel RP, Li HS, Li XP, Luo JY, *et al.*, 2004. Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. Poult Sci J 83: 175-182. https://doi.org/10.1093/ps/83.2.175

Haard NF, 1992. Control of chemical composition and food quality attributes of cultured fish. Food Res Int 25: 289-307. https://doi.org/10.1016/0963-9969(92)90126-P

Harikrishnan R, Kim MC, Kim JS, Han YJ, Jang IS, Balasundaram C, Heo MS, 2011. Immunomodulatory effect of sodium alginate enriched diet in kelp grouper *Epinephelus brneus* against Streptococcus iniae. Fish Shellfish Immun 30: 543-549. https://doi.org/10.1016/j.fsi.2010.11.023

Holmstrom K, Graslund S, Wahlstrom A, Poungshompoo S, Bengtsson BE, Kautsky N, 2003. Antibiotic use in shrimp farming and implications for environmental impacts and human health. Int J Food Sci Tech 38: 255-266. https://doi.org/10.1046/j.1365-2621.2003.00671.x

Honorato CA, Cruz CDA, Carneiro DJ, Machado MRF, Nascimento CA, Saturnino KC, 2014. Histologia do fígado de tilápia do Nilo (*Oreochromis niloticus*) alimentados com dietas contendo silagem biológica de pescado. Pesqu Vet Bras 34: 64-68. https://doi.org/10.1590/S0100-736X2014001300012

Hrubec TC, Cardinale JL, Smith SA, 2000. Hematology and plasma chemistry reference intervals for cultured tilapia (Oreochromis hybrid). Vet Clin Path 29: 7-12. https://doi.org/10.1111/j.1939-165X.2000.tb00389.x

Karunasagar I, Rosalind G, Karunasagar I, 1991. Immunological response of the Indian major carps to *Aeromonas hydrophil*a vaccine. J Fish Dis 14: 413-417. https://doi.org/10.1111/j.1365-2761.1991.tb00841.x

Kashkooli OB, Dorcheha EE, Mahboobi-Soofiani N, Nasrollah AS, 2011. Ecotoxicology and environmental safety long-term effects of propolis on serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). Ecotoxicol Environ Saf 74: 315-318. https://doi.org/10.1016/j.ecoenv.2010.10.014

Ledic-Neto J, Dotta G, Garcia P, Brum A, Gonçalves ELT, Martins ML, 2014. Haematology and melanoma crophage centers of Nile tilapia fed supplemented diet with propolis. Acta Sci Biol Sci 36: 263-269. https://doi.org/10.4025/actascibiolsci.v36i3.22024

Marcucci MC, 1996. Propriedades biológicas e terapêuticas dos constituintes químicos da própolis. Quím Nova 19: 529-536.

Matsui T, Ebuchi S, Fujise T, Abesundara KJM, Doi S, Yamada H, *et al.*, 2004. Antihyperglycemic effects of water-soluble fraction of Brazilian propolis and its bioactive constituent, 3,4,5-tri-O-caffeoylquinic acid. Biol Pharm Bull 27: 1797-1803. https://doi.org/10.1248/bpb.27.1797

Mela M, Randi MAF, Ventura DF, Carvalho CEV, Pelletier E, Oliveira Ribeiro CA, 2007. Effects of dietary methylmercury on liver and kidney histology in the neotropical fish *Hoplias malabaricus*. Ecotox Environ Safe 68: 426-435. https://doi.org/10.1016/j.ecoenv.2006.11.013

Meurer F, Silva MS, Costa MM, Colpini LMS, Mascioli A dos S, 2009a. Probiótico com levedura na alimentação da tilápia do Nilo, durante o período de reversão sexual, cultivada em água de tanque de cultivo. Rev Bras Saúde Prod Anim 10: 406-416.

Meurer F, Matiuzzi MC, Barros DAD, Oliveira STL, Barros PSP, 2009b. Brown propolis extract in feed as a growth promoter of Nile tilapia (*Oreochromis niloticus,* Linnaeus 1758) fingerlings. Aquac Res 40: 603-608. https://doi.org/10.1111/j.1365-2109.2008.02139.x

Mirzoeva OK, Calder PC, 1996. The effect of propolis and its components on eicosanoid production during the inflammatory response. Prostag Leukotr Ess 55: 441-449. https://doi.org/10.1016/S0952-3278(96)90129-5

Mouriño JLP, Do Nascimento Vieira F, Jatobá AB, Da Silva BC, Jesus GFA, Seiffert WQ, Martins ML, 2012. Effect of dietary supplementation of inulin and W. cibaria on haemato-immunological parameters of hybrid surubim (Pseudoplatystoma sp). Aquac Nutr 18: 73-80. https://doi.org/10.1111/j.1365-2095.2011.00879.x

Oliveira RC, 2015. O panorama da aqüicultura no Brasil: A prática com foco na sustentabilidade. Revinter 2: 71-89. https://doi.org/10.22280/revintervol2ed1.18

Ramos AFN, Miranda JL, 2007. Propolis: a review of its anti-inflammatory and healing actions. J Venom Anim Toxins incl Trop Dis 13: 697-710. https://doi.org/10.1590/S1678-91992007000400002

Ranzani-Paiva MJT, Takemoto RM, Lizama MAP, 2004. Sanidade de organismos aquáticos, 1st ed. Livraria Varela, São Paulo.

Ranzani-Paiva MJT, Pádua SB, Tavares-Dias M, Egami MI, 2013. Métodos para análise hematológica de peixes, 1st ed. Eduem, Maringá. https://doi.org/10.7476/9788576286530

Ridha MT, Cruz EM, 2001. Effect of biofilter media on water quality and biological performance of the Nile tilapia *Oreochromis niloticus* L. reared in a simple recirculating system. Aquacult Eng 24: 157-166. https://doi.org/10.1016/S0144-8609(01)00060-7

Robertsen B, 1999. Modulation of the non-specific defence of fish by structurally conserved microbial polymers. Fish Shellfish Immun 9: 269-290. https://doi.org/10.1006/fsim.1998.0186

Rosenfeld G, 1947. Método rápido de coloração de esfregaços de sangue: noções práticas sobre corantes pancromáticos e estudo de diversos fatores. Mem Inst Butantan 20: 315-328.

Santos EL, Silva FCBDA, Pontes EDAC, Lira RC, Cavalcanti MCA, 2013. Resíduo do processamento do extrato de própolis vermelha em ração comercial para alevinos de Tilápia do Nilo (*Oreochromis niloticus*). Comun Sci 4: 179-185.

Sforcin JM, 2007. Propolis and the immune system: A review. J Ethnopharmacol 113: 1-14. https://doi.org/10.1016/j.jep.2007.05.012

Sforcin JM, Bankova V, 2011. Propolis: Is there a potential for the development of new drugs? J Ethnopharmacol 133: 253-260. https://doi.org/10.1016/j.jep.2010.10.032

Sforcin JM, Fernandes A, Lopes CA, Bankova V, Funari SR, 2000. Seasonal effect on Brazilian propolis antibacterial activity. J Ethnopharmacol 73: 243-249. https://doi.org/10.1016/S0378-8741(00)00320-2

Song YS, Park EH, Hur GM, Ryu YS, Lee YS, Lee JY, *et al.*, 2002. Caffeic acid phenethyl ester inhibits nitric oxide synthase gene expression and enzyme activity. Cancer Lett 175: 53-61. https://doi.org/10.1016/S0304-3835(01)00787-X

Takashima F, Hibiya T, 1995. An atlas of fish histology: normal and pathological features, 2nd ed. Kodansha, Tokyo.

Talas ZS, Gulhan MF, 2009. Effects of various propolis concentrations on biochemical and hematological parameters of rainbow trout (Oncorhynchus mykiss). Ecotox Environ Safe 72: 1994-1998. https://doi.org/10.1016/j.ecoenv.2009.04.011

Tavares-Dias M, De-Moraes FR, 2007. Leukocyte and thrombocyte reference values for channel catfish (Ictalurus punctatus Raf), with an assessment of morphologic, cytochemical, and ultrastructural features. Vet Clin Path 36: 49-54. https://doi.org/10.1111/j.1939-165X.2007.tb00181.x

Tavares-Dias M, Mataqueiro MI, Perecin D, 2002. Total leukocyte counts in fishes by direct or indirect methods. Bol Inst Pesca 28: 155-161.

Tavares-Dias M, Ishikawa MM, Martins ML, Satake F, Hisano H, Pádua SB, *et al.*, 2009. Hematologia: Ferramenta para o monitoramento do estado de saúde de peixes em cultivo. In: Tópicos especiais em saúde

e criação animal; Neto AS *et al.*, eds.. Pedro & João Editores, São Carlos, p. 38.

Tokumaru M, Godinho H, Ferri A, 1968. Fixação de tecidos de peixes de água doce. Braz J Vet Res Anim Sci 7: 841-848. https://doi.org/10.11606/issn.2318-5066.v7i4p841-848

Toreti VC, Sato HH, Pastore GM, Park YK, 2013. Recent progress of propolis for its biological and chemical compositions and its botanical origin. Evid Based Complementary Altern Med 2013: 1-13. https://doi.org/10.1155/2013/697390

Uczay J, Lazzari R, Pianesso D, Adorian TJ, Mombach PI, Decarli JÁ, 2011. Evaluación del propóleo como promotor de crecimiento em la carpa común (*Cyprinus carpio*). Rev Cient-Fac Cien V 21: 408-413.

Uczay J, Pianesso D, Adorian TJ, Mombach PI, Coldebella IJ, Lazzari R, 2014. Própolis em dietas para o jundiá (Teleostei, Pimelodidae). Bioscience 30: 1912-1918.

Uzel A, Sorkun K, Öncag Ö, Cogulu D, Gencay Ö, Salih B, 2005. Chemical compositions and antimicrobial activities of four different Anatolian propolis samples. Microbiol Res 160: 189-195. https://doi.org/10.1016/j.micres.2005.01.002

Velotto S, Vitale C, Varricchio E, Crasto A 2010. Effect of propolis on the fish muscular development and histomorphometrical characteristics. Acta Vet Brno 79: 543-550. https://doi.org/10.2754/avb201079040543

Vicentini C, Franceschini-Vicentini I, Bombonato M, Bertolucci B, Lima S, Santos A, 2005. Morphological study of the liver in the Teleost *Oreochromis niloticus*. Int J Morphol 23: 211-216. https://doi.org/10.4067/S0717-95022005000300003

Winthrobe M, 1933. Variations in the size and hemoglobin content of erythrocytes in the blood of various vertebrates. Folia Haematol 51: 32-49.

Zhang G, Gong S, Yu D, Yuan H, 2009. Propolis and Herba Epimedii extracts enhance the non-specific immune response and disease resistance of Chinese sucker, Myxocyprinus asiaticus. Fish Shellfish Immun 26: 467-472. https://doi.org/10.1016/j.fsi.2009.01.011