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Research Articles

Redox balance and tissue development of juvenile *Piaractus mesopotamicus* subjected to high stocking density and fed dry diets containing nutraceutical food

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ABSTRACT. Colostrum is a source of molecules with nutraceutical features that can attenuate the consequences of adverse conditions, such as densification. Thus, the redox balance and tissue development of juvenile *Piaractus mesopotamicus* subjected to high stocking density and fed diets containing lyophilized bovine colostrum (LBC) were evaluated. Juveniles were distributed into 16 cages making 50 kg fish m^{-3} and fed diets containing 0, 10, 20 and 30% inclusion of LBC (n = 4). After 30 days, enteric, hepatic, muscular, renal and branchial tissue were sampled for the determination of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase activity, oxygen radical absorbance capacity (ORAC), lipid peroxidation and protein and nucleotides content. SOD activity and DNA content were higher in 20% LBC compared to the others in the enteric tissue ($P < 0.10$). ORAC value in erythrocytes was higher in 30% LBC than 0 and 10% LBC ($P < 0.10$). Juveniles fed 10% LBC showed higher lipid peroxidation in liver than 0 and 20% LBC ($P < 0.10$) and those fed 20% LBC showed lower protein/RNA ratio compared to the others ($P < 0.10$). Bovine colostrum determined a decrease in liver protein synthesis and increased in intestinal DNA content with an intake of 20% of this milk secretion. Additionally, LBC increased the protection of the enteric tissue of juveniles from superoxide radicals and blood antioxidant capacity. We conclude that bovine colostrum can be used as a nutraceutical food for fish, with positive effect in redox balance and tissue development in high concentrations of inclusion in the diet, 20 and 30%.

Keywords: *Piaractus mesopotamicus*; bovine colostrum; antioxidant; redox homeostasis; DNA; RNA; Neotropical fish

INTRODUCTION

Management practices of intensive fish farming such as transport, handling, biometrics, vaccination and temperature changes are conditions that can induce stress in fish. Acutely-induced stress causes an immunosuppressive effect, leading to large losses from illness. Chronically induced stress, when animals are kept for an extended period in inappropriate situations such as incorrect pH, low oxygenation and overpopulation, in addition to reduced resistance to pathogens, leads to decreased growth and reproductive failures (Schep *et al.*, 1999; Urbinati & Carneiro, 2004; Falcon *et al.*, 2008; Baldwin, 2010; Urbinati & Golçalves, 2010).

The release of catecholamines and corticosteroids is a mechanism involved in the physiological response to stress that affects oxygen transport, hydromineral balance and serum glycogen, besides being related to inhibition of growth, reproduction and immune response in fish (Diniz & Honorato, 2012). Another consequence of stress is the production of reactive oxygen species (ROS), generated by the incomplete reduction of oxygen (Berra *et al.*, 2006). Daily mitochondrial respiration, responsible for the generation of energy for the metabolic process under normal conditions, generates ROS as a byproduct. However, the increase in the metabolism under challenging conditions generates an imbalance in the production of ROS and antioxidants, which results in oxidative stress (Gutiérrez *et al.*, 2006). The ROS superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (HO•) due to unpaired electrons are extremely

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reactive and, besides the formation of lipid peroxidation of cell membranes, they attack DNA, RNA and proteins, also affecting enzymatic activity (Halliwell & Gutteridge, 1990; Nita & Grzybowski, 2016). The breakdown of cellular homeostasis may eventually lead to apoptosis or necrosis (Redza-Dutordoir & Averill-Bates, 2016).

The body has two groups of antioxidant factors to protect themselves against ROS, the enzymatic and non-enzymatic. In the first group, there are some enzymes such as catalase, glutathione peroxidase and superoxide dismutase. The glutathione peroxidase acts on peroxides in general, using glutathione as a cofactor, while superoxide dismutase catalyzes the dismutation of the superoxide radical anion (O_2) to hydrogen peroxide (H_2O_2) and O_2, and the catalase acts as a catalyst for the decomposition of H_2O_2 to H_2O and O_2. In the second group, vitamins A, C and E are considered highly efficient non-enzymatic antioxidants due to the high capacity to sequester free radicals in the body, besides being crucial for several chemical reactions that maintain welfare and good physiological condition of the body (Vasconcelos *et al*., 2007).

The first mammalian lacteal secretion, colostrum, is a food rich in nutrients, among them, antioxidant factors (Boudry & Thewis, 2009; Pandey *et al.*, 2011). For mammals, the antioxidant properties of colostrum are as crucial as nutritional and immunological, and studies indicate that this potential against ROS may contribute to overcoming challenges, especially in the postnatal period, when the birth is associated with oxidative stress (Przybylska *et al.*, 2007). This secretion contains several molecules with antioxidant properties, among them the antioxidant enzymes, vitamins E and C, selenium and manganese, and has been considered as a nutraceutical food (Pandey *et al.*, 2011). Lactoferrin, also present in colostrum, besides being responsible for the defense against microorganisms and viral infections, it acts as a non-enzymatic antioxidant because it binds to the iron generated during the destruction process or cellular inflammation, minimizing the synthesis of hydroxyl radicals (Vasconcelos *et al*., 2007). Lactoperoxidase, in turn, has the primary function of catalyzing the oxidation of specific molecules in order to generate reactive products with high antimicrobial activity at the expense of H_2O_2 to H_2O decomposition (Przybylska *et al*., 2007). According to Seth & Das (2011), colostrum is also a rich source of glutathione, a powerful antioxidant that is often described as "the ultimate antioxidant." Due to antioxidant and nutritional properties, Ahmadi *et al.* (2011), consider colostrum a great natural food supplement. In fish, Sakai *et al.* (1993) observed that the oral supply of bovine lactoferrin to rainbow trout (*Oncorhynchus mykiss*) determines higher resistance against bacterial infection by the activation of phagocytes. Kumari *et al.* (2003) also report that the inclusion of bovine lactoferrin in the diet of Asian catfish (*Clarias batrachus*) is capable of increasing non-specific immunity and disease resistance, supporting the possible use of lactoferrin as an immunostimulant for catfish nursery. Falahatkar (2014) observed that bovine lactoferrin could suppress the stress response in *Siberian sturgeon*, being the 400 mg kg^{-1} the effective level. In Nile tilapia (*Oreochromis niloticus*), the inclusion of bovine lysozyme in the diet improved nonspecific immunity and resistance to diseases and decreased fish mortality after challenge with *Aeromonas hydrophila* (El-Ashram & El-Boshy, 2008). These studies reveal that the biologically active molecules present in bovine colostrum can influence the health of other species, including fish.

Strategies that attenuate the condition of stress in fish, besides contributing to better performance rates, has the potential to minimize the rate of morbidity and mortality. Thus, the present work evaluated the redox balance and tissue development of juvenile *Piaractus mesopotamicus*, subjected to high stocking density and fed a dry diet containing biologically active molecules from lacteal mammary secretion.

MATERIALS AND METHODS

Experimental diets

Isonitrogenated and isoenergetic experimental pelleted diets, considering 32% of crude protein and 4,000 kcal kg^{-1}, respectively, were formulated considering four levels of lyophilized bovine colostrum (LBC): 0, 10, 20 and 30% (Table 1). The bovine colostrum was obtained from Holstein cows of a commercial dairy farm. The first lacteal secretion after birth was collected, frozen at 20°C and lyophilized. The resulting powder was, then, analyzed for the bromotological composition (Table 2) and included in the diet. The diets were also evaluated for their antioxidant capacity by the Oxygen Radical Absorbance Capacity (ORAC) protocol according to Melo *et al.* (2015). The fluorescence decay was monitored for two hours at 37°C with a kinetic methodology using emission absorbance of 528 nm and excitation absorbance of 485 nm after incubation of samples with fluorescein solution (487 nM) and AAPH (2.2'-Azobis(2-amidinopropane) dihydrochloride, 76 mM) solution.

Values were compared with a calibration curve of Trolox (6-hydroxy-2.5, 7.8-tetramethylchroman-2-carboxylic acid) and are expressed as µmol of equivalent Trolox in mg of total solid. The relationship

Table 1. Composition of diets supplied to *Piaractus mesopotamicus* juvenile subjected to high stocking density. Guabi Nutrição Animal, Campinas, São Paulo (ingredient per kg). Vitamins: A, 2,500 UI; D3, 600,000 UI; E, 37,500 UI; K3, 3,750 mg; C, 50,000 mg; B1, 4,000 mg; B2, 4,000 mg; B6, 4,000 mg; B12, 4,000 mg; calcium pantothenate 12,000 mg; biotin 15 mg; acid folic 1,250 mg; niacin 22,500 mg. Mineral: Cu 2,500 mg; Zn 12,500 mg; I 375 mg; Se 87.5 mg; Co 125 mg; Mn 12,500 mg; Fe 15,000 mg; BHT 15,000 mg.

Ingredients (g kg^{-1})	0% LBC	10% LBC	20% LBC	30% LBC
Bovine colostrum	-	100	200	300
Albumin	202	183	120	58
Gelatin	150	95	79	60
Starch	466	456	435	425
Soy oil	80	53	37	14
Dicalcium phosphate	25	32	47	59
Cellulose	66	69	69	70
Tryptophan	-	0.4	1.3	2.2
BHT	0.2	0.2	0.2	0.2
Premix[1]	10	10	10	10
NaCl	1	1	1	1
Chemical composition (g kg^{-1})				
Dry matter	921	919	914	910
Crude protein	313	313	315	313
Crude fiber	4	7	10	8
Fat	90	72	65	56
Gross energy (MJ kg^{-1})	18.2	17.9	17.4	16.8

Table 2. Composition of lyophilized bovine colostrum supplied to *Piaractus mesopotamicus* juvenile subjected to high stocking density.

Chemical composition (g kg^{-1})	
Dry matter	962
Crude protein	684
Fat	112
Crude fiber	8
Gross energy (MJ kg^{-1})	23

between antioxidant capacity and percentage of bovine colostrum inclusion in the diet is shown in Figure 1.

High stocking density experimental design

Sixteen cages of 40 L (28×48×30 cm, each diet or treatment was performed in quadruplicate) were distributed into a tank with continuous water flow and aeration in a closed circulation system and controlled conditions of temperature, luminosity, pH and dissolved oxygen (24 ± 1°C; photoperiod of 12 h; pH 8.4 ± 0.3; and 57 ± 7% of dissolved oxygen). Juveniles *Piaractus mesopotamicus* (Holmberg, 1887) with an initial weight of 140 ± 9 g, were distributed into the cages until the density of 50 kg fish m^{-3}, a condition considered as stressful for this species according to Merola & de Souza (1988). Diets containing 0, 10, 20 or 30% of LBC were supplied twice daily until satiety.

After 30 experimental days, the survival rate was 100%, showing that all cages were maintained in the same stocking density all the period. Then, two fish from each cage were weighted and anesthetized with benzocaine (0.05 g L^{-1}), after food restriction of 24 h. Samples from blood and enteric, hepatic, muscle, gill and renal tissues were dissected. Blood samples were collected with disposable, sterile and heparinized polypropylene (3 mL) syringes, centrifuged at 3,000x *g* for 15 min. and the resulting plasma was stored at -80°C. The erythrocytes pellet was homogenized (1:6) with Triton-x (5 g L^{-1}) and the supernatant stored at -80°C. Tissue samples were immediately stored at -80°C. The Ethics Committee on Animal Use previously approved the procedures - CEUA of the "Luiz de Queiroz" College of Agriculture (ESALQ/USP), protocol Nº2015-13.

Evaluation of oxidative stress

Redox balance was evaluated in different tissues by determining the activity of antioxidant enzymes, oxygen radical absorbance capacity (ORAC) and lipid peroxidation. The activity of glutathione peroxidase was evaluated by the decay of the absorbance of samples incubated with 48 mM buffer phosphate, pH 7.0, 0.38 mM EDTA, 0.95 mM sodium azide, 1 mM glutathione, 0.12 mM nicotinamide adenine dinucleotide phosphate, 3.2 U of glutathione reductase, 0.02

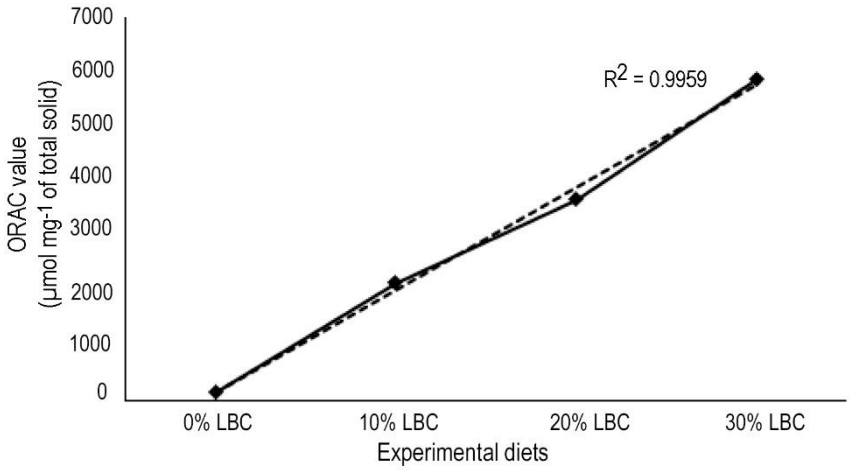

Figure 1. Relationship between antioxidant capacity and percentage of bovine colostrum inclusion in the diet supplied to pacu juvenile subjected to high stocking density. 0, 10, 20 and 30% LBC isonitrogenated (32% of crude protein) and isoenergetic (4,000 kcal kg^{-1}) experimental pelleted diets formulated considering four level of lyophilized bovine colostrum (LBC) inclusion 0, 10, 20 and 30%, respectively.

mM DL-dithiothreitol and 0.0007% hydrogen peroxide was monitored for five minutes at 340 nm (Wendel *et al.*, 1981). Values are expressed as U per mg of total protein for tissues or mg of hemoglobin for blood, being one U the amount of enzyme that catalyzes the oxidation of one µmol of reduced glutathione to oxidized glutathione per minute at 37°C, pH 7.0. The superoxide dismutase activity was determined after incubation of samples with hypoxanthine and nitrobluetetrazolium salt and registration of color development inhibition by the samples compared against a blank (McCord & Fridovich, 1969). The three types of SOD (Cu/Zn, Mn and FeSOD) were determined and values are expressed as unit U per mg of total protein for tissues or mg of hemoglobin for blood, being one U the amount of enzyme needed to inhibit 50% dismutation of the superoxide radical. Catalase activity was measured after the formation of foam resulted from the incubation of samples with triton-x (1%) and hydrogen peroxide (30%) in cylindrical tubes. The height of the foam was compared to a calibration curve according to Iwase *et al.* (2013). The values of catalase activity are expressed as U per mg of total protein for tissues or mg of hemoglobin for blood, being one U of catalase responsible for the consumption of one µmol of H_2O_2 per minute.

Samples were also evaluated by the Oxygen Radical Absorbance Capacity (ORAC) protocol according to Melo *et al.* (2015), as described above. Values are expressed as µmol of equivalent Trolox mL^{-1}. The formation of Malondialdehyde (MDA) was determined in tissues after incubation of samples with a solution containing 15% of trichloroacetic acid and 0.375% thiobarbituric acid. The solution was boiled for 15 min, centrifuged for 10 min at 3,500 rpm and the absorbance at 532 nm was compared with a calibration curve of MDA. Values are expressed as nmol of MDA per g of total protein for tissues or g of hemoglobin for blood.

Evaluation of tissue development

Tissue development was accessed by the determination of protein and nucleotide content. Tissue samples (1 g) were homogenized in distilled water (1:10) using ultra-turrax (Polytron®-Kinematica GnbH). The concentration of total protein in the homogenate was then determined by the method of Lowry *et al.* (1951). Quantification of DNA was determined according to the protocol established by Labarca & Paigen (1980). DNA was extracted with perchloric acid (PCA), and content in the precipitated determined with Hoechst dye (bis-benzamide H33258, Sigma-Aldrich Co.). Calf thymus DNA (Sigma-Aldrich Co.) was used for the standard curve. The results are shown as mg of DNA g^{-1} of tissue. The RNA present in the supernatant from the protocol described above was determined at 260 nm with a spectrophotometer. The results are shown as mg RNA g^{-1} tissue. Relations between protein/DNA, protein/RNA and RNA/DNA were also calculated.

Statistical analyses

After confirmed for normal distribution with the Shapiro-Wilk test and homoscedasticity, data were evaluated as a randomized experimental design with four treatments and submitted to the analysis of variance, using the "General Linear Model" procedure by SAS software (SAS Institute Inc., 1999). Diffe-

rences between means were evaluated by Duncan´s test, considering alpha of 0.10. Final weight was analyzed as co-variable (158 ± 27 g) but was not significant.

RESULTS

Redox balance

No differences were observed for glutathione peroxidase and catalase values in the blood and hepatic, renal, muscle, enteric and gill tissues ($P > 0.10$) of juveniles (Table 3). SOD activity was higher ($P = 0.04$) in the 20% LBC group compared to the other groups in the enteric tissue. ORAC value in erythrocytes was higher in juveniles fed 30% LBC than 0 and 10% LBC ($P = 0.06$). Considering $P = 0.07$, TBARS value in the liver of juveniles fed 10% LBC was higher than juveniles fed 0 and 20% LBC.

Tissue development

The results for protein and nucleotide content in the enteric, hepatic, muscular, renal and gill tissues of juveniles are shown in Table 4. Effect of diet was observed for the DNA content in the enteric tissue; juveniles fed 20% LBC showed a higher value than the other groups ($P < 0.10$). Juveniles fed 20% LBC also showed lower protein/RNA ratio in the liver than juveniles fed 0% LBC and 30% LBC ($P < 0.05$).

DISCUSSION

The present investigation evaluated the possibility of used bovine colostrum as nutraceutical food, which attenuates the adverse effects of high stocking density in redox balance and tissue development. Colostrum is a lacteal secretion rich in molecules that have biological activity against inflammation, microbes and reactive oxygen species (Boudry & Thewis, 2009; Pandey *et al.*, 2011). In this work, colostrum was included in the diets in the lyophilized form to preserve the biological activity of molecules, and the pelletizing process was maintained at a temperature below protein breakdown (56°C). Diet processing care resulted in a high determination index ($R^2 = 0.99$) between antioxidant capacity and inclusion of colostrum in the diet, indicating that there was a high possibility of cellular protection with the ingestion of these diets. In the mammal, in addition to coming into an environment with a much higher concentration of oxygen than the intrauterine environment after birth, the antioxidant defenses of the newborn are not entirely developed, conditions that can lead to oxidative stress (Przybylska *et al.*, 2007). Thereby, colostrum consumption is crucial for antioxidant defense, since this first milk secretion is much higher in antioxidant capacity and radical scavenging activity than subsequent lacteal secretions (Zarban *et al.*, 2009).

Effect of colostrum ingestion in redox balance was observed to superoxide dismutase activity in intestinal tissue. Moretti *et al.* (2017) also observed an increase of SOD activity in the intestine of *Piaractus mesopotamicus* fed with 20% of LBC inclusion in the diet. The authors suggest a positive effect of bovine colostrum intake in enteric cell protection. Kwon *et al.* (2010) showed that bovine colostrum also increases SOD, GPx and CAT activity of rats subjected to intestinal ischemia/reperfusion. Tang *et al.* (2013) evaluating the effects of dietary copper on the antioxidant defense of the intestine of young grass carp *Ctenopharyngodon idella*, suggest an improvement of intestinal health with the increase in the activity of antioxidant enzymes and decrease in lipid peroxidation and protein oxidation. Oxidative stress is related to an imbalance between the antioxidant defenses and the generation of free radicals (Gutiérrez *et al.*, 2006). In the present work, lipid peroxidation was not changed while SOD activity increased in the intestine, leading us to suggest that juvenile fed 20% LBC were in a better redox balance than juveniles fed 0, 10 and 30% LBC, since these juveniles have more ability to scavenge the oxygen radicals. Pontin (2018) observed the decreased rate of apoptosis in the intestinal tissue of *P. mesopotamicus* receiving diets with a high concentration of bovine colostrum and subjected to high stocking density. The authors suggest that growth and antioxidant factors present in the diets containing colostrum may be responsible for this result. The positive relationship between ORAC value and level of colostrum inclusion in the diets corroborates this hypothesis. Although the activity of antioxidant enzymes, and TBARS values, did not change in blood, the antioxidant capacity (ORAC value) in juveniles, fed with 30% LBC, was higher in relation to 0 and 10% LBC, indicating a positive influence of dietary intake with the nutraceutical feeding on the redox balance of juveniles subjected to high stock densities. Linear regression between diets and ORAC values was observed ($P < 0.05$), showing an $R^2 = 0.99$.

Effect of bovine colostrum ingestion in tissue development was also observed in enteric tissue, again indicating a positive effect of the diet containing 20% of LBC. Total protein content, DNA and RNA concentrations are indicators of cellular activity of fish tissues that are under the influence of growth rate and cellular metabolism (Gwak *et al.*, 2003; Chícharo & Chícharo, 2008; Sivaraman *et al.*, 2009). Nordi *et al.* (2017) evaluating the effect of bovine colostrum in the

Table 3. Redox balance in different tissues of *Piaractus mesopotamicus* juveniles juvenile fed with 0, 10, 20 and 30% of lyophilized bovine colostrum and subjected to high stocking density (50 kg fish m^{-3}), mean ± standard error. [a,b]Means with different letters in the line differ by Duncan test, $P < 0.10$. [1]Treatments: 0% LBC; 10% LBC; 20% LBC; 30% LBC - juvenile fed diets containing 0, 10, 20 and 30% of lyophilized bovine colostrum, respectively; [2]Values are expressed as U per mg of total protein for tissues or mg of hemoglobin for blood; [4]Values are expressed as µmol of equivalent Trolox per mg of total protein for tissues or mg of hemoglobin for blood. [5]Values are expressed as nmol of MDA per g of total protein for tissues or mmol per g of hemoglobin for blood.

Oxidative stress parameters	Treatments[1]				*P*-value
	0% LBC	10% LBC	20% LBC	30% LBC	
Glutathione peroxidase[2]					
Blood	1.8 ± 0.2	1.4 ± 0.3	1.2 ± 0.2	1.2 ± 0.4	0.88
Intestine	6.9 ± 0.5	7.0 ± 0.9	5.7 ± 1.4	5.0 ± 0.2	0.34
Liver	0.6 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.47
Muscle	6.0 ± 0.5	6.2 ± 0.3	5.3 ± 0.3	6.0 ± 0.4	0.47
Kidney	4.6 ± 1.5	3.9 ± 0.6	3.0 ± 0.8	4.2 ± 1.1	0.81
Gills	3.9 ± 0.4	3.2 ± 0.2	3.7 ± 0.5	3.6 ± 0.5	0.62
Superoxide dismutase[2]					
Blood	5.7 ± 0.9	6.5 ± 1.5	9.2 ± 3.6	6.5 ± 1.5	0.18
Intestine	417.8 ± 74.4b	354.5 ± 57.2b	751.9 ± 132.0a	448.1 ± 83.6b	0.04
Liver	42.3 ± 6.7	23.5 ± 6.5	27.2 ± 4.8	47.0 ± 9.4	0.23
Muscle	30.3 ± 3.7	39.6 ± 5.0	36.7 ± 3.0	31.5 ± 2.5	0.28
Kidney	18.3 ± 3.8	27.6 ± 3.8	23.8 ± 1.8	22.9 ± 1.7	0.22
Gills	35.1 ± 5.0	26.3 ± 3.8	26.3 ± 7.9	29.1 ± 2.8	0.57
Catalase[2]					
Blood	2.9 ± 0.1	3.2 ± 0.1	3.0 ± 0.2	2.9 ± 0.1	0.30
Intestine	113 ± 11	104 ± 9	98 ± 14	102 ± 11	0.81
Liver	7 ± 3	4 ± 1	4 ± 1	7 ± 1	0.31
Muscle	25 ± 3	20 ± 2	25 ± 4	26 ± 4	0.61
Kidney	10 ± 1	11 ± 1	11 ± 1	14 ± 1	0.26
Gills	5 ± 1	3 ± 1	3 ± 1	3 ± 1	0.17
ORAC[3]					
Blood	237 ± 12b	216 ± 37b	264 ± 18ab	324 ± 32a	0.06
Intestine	1,318 ± 164	1,450 ± 166	1,221 ± 196	1,268 ± 194	0.82
Liver	107 ± 11	99 ± 9	103 ± 13	95 ± 9	0.87
Muscle	71 ± 5	76 ± 8	86 ± 9	74 ± 3	0.42
Kidney	60 ± 5	54 ± 4	55 ± 4	51 ± 5	0.55
Gills	206 ± 26	231 ± 21	220 ± 22	234 ± 24	0.82
TBARS[4]					
Blood	15 ± 1	14 ± 1	15 ± 1	15 ± 1	0.97
Intestine	33 ± 10	55 ± 25	72 ± 26	48 ± 26	0.68
Liver	10 ± 2b	22 ± 4a	7.7 ± 4b	14 ± 4ab	0.07
Muscle	5.8 ± 0.1	6.4 ± 0.3	5.7 ± 0.1	6.2 ± 0.3	0.19
Kidney	1,478 ± 97	1,626 ± 97	1,687 ± 183	1,706 ± 129	0.62
Gills	1,057 ± 343	1,470 ± 90	1,181 ± 353	1,362 ± 188	0.70

indicators of cellular activity, did not observe changes in protein, DNA and RNA content in the intestine and liver of *P. mesopotamicus*. Pauletti *et al.* (2007), in turn, reported an increase in DNA concentration in the muscle of *Pseudoplatystoma fasciatum* fed with the same lacteal secretion. The inclusion of 20% of LBC in the diet of juvenile *P. mesopotamicus* increased DNA content in intestinal tissue, indicating an increase in cell density which may be a result of an increase of cell proliferation or decrease, in agreement with the observations made by Pontin (2018), in apoptosis rate. Juveniles fed 20% LBC also showed a lower liver protein/RNA ratio than the other groups. This variable indicates an endogenous synthesis of protein resulted from translation, suggesting that colostrum decreased the liver production of proteins.

The nutraceutical proprieties of bovine colostrum have been investigated in different species (King *et al.*,

Table 4. Tissue development of *Piaractus mesopotamicus* juveniles fed with 0, 10, 20 and 30% of lyophilized bovine colostrum and subjected to high stocking density (50 kg fish m^{-3}), mean ± standard error. [*a,b]Means with different letters in the line differ by Duncan test, $P < 0.10$. [1]Treatments: 0% LBC; 10% LBC; 20% LBC; 30% LBC-juvenile fed diets containing 0, 10, 20 and 30% of lyophilized bovine colostrum, respectively.

Parameters	Treatments[1]				*P*-value
	0% LBC	10% LBC	20% LBC	30% LBC	
DNA, mg g of tissue^{-1}					
Intestine	0.8 ± 0.1b	0.6 ± 0.1b	1.3 ± 0.2a	0.7 ± 0.2b	0.04
Liver	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.1	0.7 ± 0.1	0.96
Muscle	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.80
Kidney	5.2 ± 0.3	4.4 ± 0.5	5.7 ± 0.3	5.1 ± 0.5	0.25
Gills	0.20 ± 0.03	0.23 ± 0.02	0.21 ± 0.03	0.23 ± 0.02	0.67
RNA, mg g of tissue^{-1}					
Intestine	5.9 ± 2.0	3.4 ± 1.3	6.6 ± 1.5	6.3 ± 1.5	0.51
Liver	13 ± 1	14 ± 2	17 ± 2	13 ± 1	0.38
Muscle	3.1 ± 0.4	3.9 ± 0.3	3.4 ± 0.6	2.8 ± 0.6	0.51
Kidney	37 ± 6	34 ± 3	42 ± 2	36 ± 7	0.67
Gills	30 ± 2	32 ± 1	27 ± 2	30 ± 3	0.49
Protein, mg g of tissue^{-1}					
Intestine	160 ± 11	152 ± 11	157 ± 3	179 ± 5	0.18
Liver	249 ± 15	285 ± 26	248 ± 25	251 ± 4	0.49
Muscle	149 ± 6	157 ± 13	163 ± 7	153 ± 10	0.74
Kidney	200 ± 13	217 ± 5	208 ± 26	209 ± 13	0.89
Gills	220 ± 13	213 ± 2	208 ± 15	218 ± 6	0.85
Protein/DNA					
Intestine	245 ± 64	286 ± 37	165 ± 48	293 ± 70	0.21
Liver	577 ± 171	806 ± 244	386 ± 56	432 ± 117	0.40
Muscle	248 ± 8	265 ± 19	272 ± 18	242 ± 15	0.51
Kidney	39 ± 4	44 ± 6	37 ± 6	41 ± 4	0.79
Gills	1,220 ± 284	958 ± 100	1,061 ± 98	953 ± 96	0.58
Protein/RNA					
Intestine	121± 52	117 ± 58	31 ± 6	41 ± 11	0.27
Liver	19 ± 1a	23 ± 4a	12 ± 1b	20 ± 1a	0.01
Muscle	56 ± 12	41 ± 6	60 ± 21	80 ± 31	0.64
Kidney	5.7 ± 0.8	6.6 ± 0.6	5.0 ± 0.8	6.3 ± 1.1	0.56
Gills	7.3 ± 0.7	6.7 ± 0.2	7.7 ± 0.3	7.4 ± 0.8	0.68
RNA/DNA					
Intestine	11 ± 5	7 ± 4	7 ± 3	8 ± 2	0.88
Liver	32 ± 10	31 ± 9	48 ± 20	22 ± 5	0.60
Muscle	5.1 ± 0.7	6.7 ± 0.6	5.6 ± 1.1	4.3 ± 1.1	0.35
Kidney	7.3 ± 1.3	7.6 ± 1.3	7.4 ± 0.2	7.1 ± 1.4	0.99
Gills	167 ± 22	146 ± 18	139 ± 13	135 ± 17	0.61

2005; Huguet *et al*., 2006; Lima *et al*., 2009; Rodrigues *et al*., 2010; Bodammer *et al*., 2011). In fish, the ingestion of this lacteal secretion leads to changes in the intestinal distribution of goblet cells (Cruz *et al.*, 2017); intestinal activity of alkaline phosphatase (Moretti *et al.*, 2014) and superoxide dismutase (Moretti *et al.*, 2017); intestinal morphometric features (Rodrigues *et al.*, 2010); decrease in intestinal apoptosis rate (Pontin, 2018) an increase in muscle DNA content (Pauletti *et al*., 2007). Beyond that, specific compounds of colostrum also demonstrate a positive effect on the immune system and growth. Bovine lactoferrin, for example, determines higher resistance against bacterial infection in the rainbow trout *Oncorhynchus mykiss* (Sakai *et al.*, 1993) and increases non-specific immunity and disease resistance in Asian catfish *Clarias batrachus* (Kumari *et al.*, 2003). Falahatkar (2014), in turn, investigating the effect of bovine lactoferrin against stress, observed decreasing levels of cortisol and lactate in juvenile *Siberian sturgeon* fed bovine lactoferrin and subjected to two minutes of air exposure. The authors suggest that bovine lactoferrin may be suitable to suppress the cortisol response to stress and improve the physiological condition of *S.*

sturgeon, being the 400 mg kg^{-1} the effective level. Bovine lysozyme also promoted the improvement of nonspecific immunity and resistance to disease in Nile tilapia (*Oreochromis niloticus*) challenged with *Aeromonas hydrophila* (El-Ashram & El-Boshy, 2008). These results with those presented in the present study indicate the potential that the biologically active molecules present in bovine colostrum have to interfere with animal health and welfare.

CONCLUSIONS

Considering the present results, we conclude that the effect of colostrum ingestion in juvenile development is limited to enteric and hepatic tissue, determining the decrease in liver protein synthesis and increase in intestinal DNA content with the intermediate (20%) intake of this milk secretion. Additionally, the inclusion of bovine colostrum in the diet of juvenile *P. mesopotamicus* had the potential to protect the enteric tissue from superoxide radicals, acting locally and possibly against the intracellular actions of this oxygenated radical. Bovine colostrum also increased the antioxidant protection in the blood of juveniles stored at high density. Taken all together, we conclude that bovine colostrum can be used as a nutraceutical food for *P. mesopotamicus*, with positive effect in redox balance and tissue development in high concentrations of inclusion in the diet, 20 and 30%.

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