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Microalgae *Schizochytrium* sp. in Feed for Piau *Leporinus friderici*

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Abstract: The objective of the study was to evaluate the growth of piau (*Leporinus friderici*) juveniles fed with diet supplemented with different levels of *Schizochytrium* sp. One hundred and forty juveniles of *L. friderici* were stocked in 20 aquariums (35 L) at the density of 0.2 fish L^{-1}, weighing and measuring 11.80±1.08 g and 9.68±0.31 cm, respectively. The feeds were prepared and supplemented with 0, 10, 20, 30 and 40 g of *Schizochytrium* sp. kg^{-1} of diet. On the 60th day, all juveniles were collected for measurement of the following parameters: Feed intake (g day^{-1}), weight (g), weight gain (g), food conversion, total length (cm), Specific Growth Rate (SGR) and Fulton's condition factor (K). Also, the whole-body composition was analyzed for dry matter, mineral matter, crude protein, lipids, calcium and phosphorus. A linear effect ($p<0.05$) was observed for weight gain, weight, biomass, feed intake, SGR and K when *Schizochytrium* sp. was included in the feed. The levels of crude protein, calcium and phosphorus in juveniles had a linear decreasing effect ($p<0.05$) with the increase of *Schizochytrium* sp. in the feeds. In brief, our results showed that *L. friderici* juveniles fed with artificial diets supplemented with *Schizochytrium* sp. had a better growth, with a significant influence on their body chemical profile.

Keywords: Algae, Body Chemical Composition, Fatty Acid Source, Thraustochytriaceae

Introduction

The species *Leporinus friderici*, Bloch 1794 have a great commercial value because of their well-appreciated meat (Nomura, 1984). Several types of research on incorporating essential nutrients in fish feeds are a new way to food enrichment for human consumption (Li *et al*., 2009; Qiao *et al*., 2014; Kousoulaki *et al*., 2015).

It is known that the fish body protein and lipid profile can be adjusted according to diet and inclusion of polyunsaturated acid (PUFA) source, such as Docosahexaenoic Acid (DHA), found in microalgae, being an alternative to changes organoleptic caractheristics and improve the growth (Lenihan-Geels *et al*., 2013; Martins *et al*., 2013).

A several species of microalgae, as *Schizochytrium* sp., are identified as rich in carbohydrates, proteins, lipids and nutritionally valuable components (Sarker *et al*., 2016; Sathasivam *et al*., 2017). A practical way of enriching diets for captive-bred fish is the inclusion of microalgae, which can modify the lipid and protein profile of the animal muscle composition (Richmond, 2004; Li *et al*., 2009; Qiao *et al*., 2014). Rsearches with freshwater fish shows increased lipids and protein profile after the inclusion of microalgae in feed, improving fish growth (Qiao *et al*., 2014; Sarker *et al*., 2016), been an alternative to increase productive yield.

Thus, the objective of this study was to evaluate the growth and the body chemical profile of *L. friderici* juveniles fed with diets supplemented with different levels of the microalgae *Schizochytrium* sp.

Material and Methods

The experiment was conducted indoors, in the city of Diamantina-MG, in Brazil (18°15' south latitude, 43°36' west longitude and 1.400 m above sea level), from September 12 to November 10 of 2015 (60 days). The research was carried out in accordance with the ethical standards and approved by the Ethics Committee on Animal Use (process n° 029/2016).

Prior to the experiment, fish were adapted to a trial for seven days, being fed a control artificial diet (without the inclusion of *Schizochytrium* sp.). Then, 140 juveniles were selected, weighed and measured (11.80±1.08 g and 9.68±0.31 cm, respectively). These juveniles were stocked in 20 aquariums (35 L) at a density of 0.2 fish L^{-1}, or seven fish per tank. These aquariums were provided with aeration and controlled temperature.

An artificial feed was prepared and supplemented with 0, 10, 20, 30 and 40 g of *Schizochytrium* sp. kg^{-1} of feed (Table 1). All the diets were extruded (INBRAMAQ MX40) and beads were produced with a mean diameter of 2 mm. Each treatment had four replicates, in a completely randomized design. The juveniles of *L. friderici* were fed *ad libitum* three times a day (10 am, 1 pm and 4 pm). The composition of the microalgae meal is shown in Table 2.

The aquariums were cleaned three times a week (Monday, Wednesday and Friday) to remove waste. Weekly, water quality parameters were measured, such as temperature (°C), pH, dissolved oxygen (mg L^{-1}) and conductivity (μs cm^{-1}), using a multiparameter (U-50 Horiba). The concentrations of total ammonia, nitrite and nitrate (mg L^{-1}) were measured according to (APHA, 2012) (method 4500).

On the 60^{th} day of experiment, the juveniles were anesthetized with eugenol solution (120 mg L^{-1}), being measured for biomass (g), feed intake (g day^{-1}), weight (g), weight gain (g), feed conversion, total length (cm), standard length (cm), specific-growth rate (SGR = 100 $(ln Pt_f - ln Pt_i)\ \Delta t^{-1}$, considering Δt the duration in days between samplings, Pt_i the initial weight and Pt_f the final weight of each replicate) and Fulton's condition factor (K = weight x standard $length^{-3}$)*100). After the data were collected, the fish were euthanized with benzocaine hydrochloride as CONCEA (2013) recommendation.

Table 1: Composition and analysis of experimental diets (natural matter)

Ingredients (%)	Treatments (g kg^{-1}) 0	AS10	AS20	AS30	AS40
Soybean meal 45%	29.03	29.03	29.03	29.03	29.03
Corn grain	8.62	8.62	8.62	8.62	8.62
Rice bran	24.00	24.00	24.00	24.00	24.00
Gluten 60	23.79	23.79	23.79	23.79	23.79
Dicalcium phosphate	3.01	3.01	3.01	3.01	3.01
Calcitic limestone	1.00	1.00	1.00	1.00	1.00
Soy oil	5.00	4.50	4.00	3.50	3.00
Schizochytrium sp.[1]	0.00	1.00	2.00	3.00	4.00
Inert (Kaolin)	4.00	3.50	3.00	2.50	2.00
Vitamin and mineral premix[2]	0.50	0.50	0.50	0.50	0.50
L- lysin	0.48	0.48	0.48	0.48	0.48
Vitamin C	0.05	0.05	0.05	0.05	0.05
Common salt	0.50	0.50	0.50	0.50	0.50
Antioxidant	0.02	0.02	0.02	0.02	0.02
Calculated and analyzed composition					
Dry matter (%)	89.63	89.32	89.70	89.51	90.32
Crude protein (%)	32.48	32.77	32.51	32.14	32.17
Digestible energy (Kcal/Kg)	3100.00	3100.00	3100.00	3100.00	3100.00
Crude fiber (%)	3.91	3.91	3.91	3.91	3.91
Ethereal extract (%)	9.74	9.71	9.50	9.50	9.55
Total Calcium (%)	1.45	1.28	1.31	1.56	1.40
Total phosphorus (%)	1.64	1.61	1.66	1.69	1.69
Available phosphorus (%)	0.70	0.70	0.70	0.70	0.70
Total lysin (%)	1.60	1.60	1.60	1.60	1.60
Linoleic acid (%)	3.91	3.91	3.91	3.91	3.91

[1]*Schizochytrium* sp. Alltech Inc. [2]Vitamin and commercial mineral supplement for fish; guarantee levels (per kg of product): vit. A, 1.200.000 IU; vit. B1, 4.800 mg; vit. B12, 4.8 mg; vit. B2, 4.800 mg; vit. B6, 4.800 mg; vit. C, 48 g; vit. D3, 200.000 IU; vit. E, 1.200 mg; vit. K3, 2.400 mg; B.C. folic acid, 1.200 mg; biotin, 48 mg; calcium pantothenate, 12.000 mg; choline chloride, 108 g; niacin, 24.000 mg; selenium, 100 mg; iodine, 100 mg; cobalt, 10 mg; copper, 3.000 mg; iron, 50.000 mg; manganese, 20.000 mg; zinc, 30.000 mg; vehicle Q.S.P., 1.000 g; Antioxidant, 25 g. 3BHT-butylated hydroxytoluene

Table 2: Nutritional composition of microalgae *Schizochytrium* sp. meal

Nutritional composition	(%)
Dry matter	96.30
Crude protein	19.22
Ethereal extract	50.00
Crude fiber	0.90
Ashes	3.67
Phosphorus	0.47
Calcium	0.34
Fatty acids	(%)
Saturated	
Myristic (C14:0)	3,86
Palmitic (C16:0)	54,69
Margaric (C17:0)	0,63
Stearic (C18:0)	1,80
Arachidic (C20:0)	0,28
Monounsaturated	
Myristolic (C14:1n9)	1,60
Polyunsaturated	
Eicosapentaenoic - EPA (C20:5n3)	0,28
Erucic (C22:1n9)	0,53
Docosadienoico (C22:2n6)	0,43
Docosahexaenoico - DHA (C22:6n3)	27,20
Other fatty acids (1)	-
Unidentified	0,71
EPA + DHA	27,48

The caproic fatty acids (C6:0), heptanoic (C7:0), caprylic (C8:0), nonanoic (C9:0), capric (C10:0), undecanoic (C11:0), lauric (C12:0), tridecanoic (C13:0), pentadecanoic acid (C15:0), (C18: 1n7), oleic (C18: 1n9), elaidic (C18: 1n9t), linoleic (C18: 2n6), linolelaic (C18:2n6t), α- linoleic, γ-linoleic, nonadecanoic (C19: 0), eicosenoic (C20: 1n9), eicosadienoic (C20: 2n6), eicosatrienoic (C20:3n6), homo-γ-linoleic, arachidonic (C20: 4n6), heneicosanoic (C21: 0), behenic (C22: 0), docosapentaenoic (C22:5n3), (C23: 0), lignoceric (C24: 0) and nerve (C24: 1n9) were detected at concentrations below 0.005%.

Fish whole-body and artificial diet proximate compositions were determined using the standard methods of AOAC (2012) (methods: 990.03 protein, 2003.05 lipids, 930.15 dry matter, 965.17 phosphorus and 968.08 calcium) at the Laboratory of Animal Nutrition of the Department of Animal Science. In the whole-body analysis, dry matter, mineral matter, crude protein, lipids, calcium and phosphorus contents were determined. All the analyses were carried out in duplicates.

Means and standard deviations were calculated for water quality parameters and characterization of the culture environment. To evaluate the effects of the inclusion of *Schizochytrium* sp. in the fish diets, the growth parameters and body chemical composition data were analyzed by ANOVA and linear regression, using the SigmaStat 3.5 software (Systat Software Inc.).

Results

The water quality parameters in the tanks were maintained constant throughout the entire experiment by controlled aeration and temperature (Table 3).

Table 3: Water quality parameters during the experimental period, 56 days, of *Leporinus. friderici* juveniles fed with different levels of *Schizochytrium* sp.

Parameters	Mean	CV (%)
Temperature (°C)	27.340	3.39
pH	7.340	2.36
Dissolved oxygen (mg L^{-1})	4.850	3.97
Total ammonia (mg L^{-1})	0.020	3.53
Nitrite (mg L^{-1})	0.001	2.98
Nitrate (mg L^{-1})	0.910	4.52
Conductivity (µSm cm^{-1})	14.100	3.58

Table 4: Performance of *Leporinus friderici* juveniles fed with diets supplemented with *Schizochytrium* sp., during 56 days

	Schizochytrium sp. inclusion level (g kg^{-1})						P-value[1,2]		
Parameters	0	10	20	30	40	CV (%)	L	Q	LF
Initial weight (g)	11.50	12.10	12.00	11.50	11.90	2.40	0.076	0.926	0.124
Weight gain (g)[1]	41.90	45.70	69.90	71.50	86.10	28.19	0.004	0.910	0.013
Weight (g)[2]	53.40	57.80	51.70	55.20	61.20	9.16	0.004	0.896	0.017
Total length (cm)	10.64	10.56	10.67	10.38	10.33	1.46	0.514	0.704	0.096
Biomass (g)[3]	256.80	357.50	476.20	545.20	665.60	34.63	0.003	0.975	0.132
Feed intake (g)[4]	61.40	68.90	89.50	87.00	96.50	18.37	0.001	0.264	1.340
Food conversion	2.30	1.60	1.30	1.20	1.10	32.32	0.097	0.824	0.123
Specific growth rate (day^{-1})[5]	3.40	4.10	6.10	6.30	7.80	32.10	0.003	0.919	0.010
Fulton's condition factor[6]	4.60	5.50	6.40	7.20	7.60	19.62	0.008	0.730	0.070

[1]L and Q – effects of linear and quadratic order concerning the inclusion of *Schizochytrium* sp. in the diet.
[2]LF – lack of fit
[3] $\hat{Y} = 41.54+1.108x$ ($r^2 = 0.952$)
[4] $\hat{Y} = 41.038+1.184x$ ($r^2 = 0.953$)
[5] $\hat{Y} = 257.76+10.35x$ ($r^2 = 0.995$)
[6] $\hat{Y} = 61.540+2.905x$ ($r^2 = 0.990$)
[7] $\hat{Y} = 3.47+1.212x$ ($r^2 = 0.953$)
[8] $\hat{Y} = 4.567+0.1068x$ ($r^2 = 0.998$)

Table 5: Body chemical analysis of *Leporinus friderici* juveniles fed with diets supplemented with *Schizochytrium* sp., during 56 days

	Treatments (g kg^{-1})					
Nutrients	Control	AS10	AS20	AS30	AS40	CV (%)
DM (%)*[1]	89.9±0.13	90.5±0.68	90.9±0.69	91.1±0.14	91.3±0.26	1.32
MM (%)	13.61±0.01	14.41±0.01	15.61±0.13	13.87±0.01	12.84±0.01	7.33
L (%)*[2]	19.8±0.20	19.9±0.26	19.9±0.13	20.0±2.07	22.1±2.43	5.32
CP (%)*[3]	56.7±0.02	60.7±0.02	59.9±0.02	60.7±0.01	56.8±0.04	1.12
Ca (%)*[4]	4.85±0.001	4.44±0.001	4.47±0.001	4.34±0.001	4.25±0.001	5.13
P (%)	7.73±0.07	7.74±0.03	7.75±0.03	7.03±0.02	7.31±0.01	4.36

DM – Dry Matter. MM – Mineral Matter; L – Lipids; CP – Crude Protein; Ca – Calcium; P – Phosphorous;
[1]Linear effect: $Y = -0.900+0.00355x$; $R^2 = 0.274$
[2]Linear effect: $Y = 1.787+0.00780x$; $R^2 = 0.971$
[3]Quadratic effect: $Y = 56.621+0.2846x-0.0079x^2$; $R^2 = 0.794$
[4]Linear effect: $Y = 4.86-0.013x$ ($R^2 = 0.802$)

An increasing linear effect ($p<0.05$) was observed for weight gain, weight, biomass, feed intake, SGR and K (Table 4) when *Schizochytrium* sp. was included in the artificial diets of *L. friderici* juveniles.

No differences ($p>0.05$) were observed for dry matter, mineral matter and lipids of *L. friderici* juveniles (Table 5). Still, with the increase of *Schizochytrium* sp. in the diets, crude protein, calcium and phosphorus levels in juveniles had a linear decreasing effect ($p<0.05$).

Discussion

The water quality parameters were maintained constant throughout the experiment period, falling within the ranges recommended for tropical species (Boyd and Tucker, 2014) and for *Leporinus* species (Sipaúba Tavares and Magalhães-Santeiro, 2013), thus not compromising the development of *L. friderici* juveniles.

Weight gain, weight, biomass, feed intake, SGR and K increased as the amount of *Schizochytrium* sp. included in the diets of *L. friderici* juveniles was raised. Freshwater fishes inhabit environments that are poor in polyunsaturated fatty acids, mainly docosahexaenoic acid; therefore, through an evolutionary pressure, they are able to retain the content produced endogenously (Tocher, 2010), which explains the efficient assimilation of *Schizochytrium* sp. in this experiment.

Algae inclusion in feeds had no influence on juvenile total length and food conversion. Sarker *et al*. (2016) also observed a low feed conversion in tilapia fed the same algae species, but with no differences in total length. Likewise, river prawns are known to have a better feed conversion when supplemented with *Schizochytrium* sp., but again with no effect on total length (Kangpanich and Senanan, 2015).

The microalgae *Schizochytrium* sp. can enhance the efficiency of nutrient absorption by the gastrointestinal tract since its content of fatty acids improves digestion, which has contributed to *L. friderici* juvenile growth. Similarly, Sarker *et al*. (2016) noted that tilapia fed diets supplied with *Schizochytrium* sp. had an improvement in weight gain and weight. Likewise, Hoestenberghe *et al*. (2016) also observed a higher weight gain in jade perch juveniles (*Scortum barcoo*). According to Li *et al*. (2009), the addition of 1.0 g kg^{-1} of dried microalgae (*Schizochytrium* sp.) in the diet of channel catfish (*Ictalurus punctatus*) promoted weight gain when compared to control artificial diets (without the microalgae). Moreover, (Li *et al*., 2009; Santos *et al*., 2015) reported increases in weight gain and biomass when *Schizochytrium* sp. was added in the diets of Nile tilapia (*Oreochromis niloticus*) and catfish (*I. punctatus*), respectively.

The dietary inclusion of *Schizochytrium* sp. microalgae increased feed intake of *L. friderici* juveniles, as observed for Atlantic salmon juveniles (*Salmo salar*) by Kousoulaki *et al*. (2015). Conversely, tilapia juveniles had a decrease in feed intake with an increasing inclusion of the microalgae (Sarker *et al*., 2016). As in this study, channel catfish had a greater feed intake when *Schizochytrium* sp. was included in the feed (Li *et al*., 2009).

The SGR of *L. friderici* juveniles increased as the levels of microalgae was raised in the diets, as already observed for sea cucumbers and prawns fed diets supplied with *Schizochytrium* sp. (Kangpanich and Senanan, 2015; Md *et al*., 2017). Yet, for freshwater fishes, the addition of *Schizochytrium* sp. in the diet had no influence on SGR (Sarker *et al*., 2016; Qiao *et al*., 2014); yet the use of microalgae oil (*Crypthecodinium cohnii* and *Schizochytrium* sp.) in diets reduced SGR in gilthead sea bream (*Sparus aurata*) (Ganuza *et al*., 2008). Despite these reports, SGR studies in fish fed microalgae are still scarce.

Fulton's condition factor (K) is the ratio between body weight and length; it expresses the degree of well-being and feeding of fish in a previous season (N'da *et al*., 2016). This factor remained similar among the additional levels of *Schizochytrium* sp. in the diet, showing a linear effect, thus indicating that fish well-being was increased with the inclusion of this organism.

The values observed in this study were higher than those reported in the literature (Guidelli *et al*., 2011; Nascimento *et al*., 2012), suggesting that the culture conditions were adequate for *L. friderici* juveniles. According to Adite *et al*. (2017), relatively high condition factors, indicated by K factor, promoted a perfect establishment of *Chrysichthys nigrodigitatus* in the aquatic environment they were growing.

The *L. friderici* juveniles have an improved dry matter and lipids levels in the whole-body when the supplementation of *Schizochytrium* sp. was increased and the level of protein was greater with the inclusion between 10 to 30 g of *Schizochytrium* sp. kg^{-1} in the feed. The increase of the corporal dry matter and protein, with the elevation of the levels of *Schizochytrium* sp. was directly related to *L. friderici* growth, with the increase in weight gain, biomass and SGR.

Sarker *et al*. (2016) also observed higher levels of body protein in juveniles of tilapia and consequently an increase in weight gain after feeding with *Schizochytrium* sp. The level of body protein also increased in juveniles of *Paralichthys olivaceus* fed with diets enriched with *Schizochytrium* sp. and *Nannochloropsis* sp. (Qiao *et al*., 2014). Juveniles of the channel catfish fed with diets enriched with *Schizochytrium* sp. had no significant difference in the contents of protein, lipids and moisture in fillet (Li *et al*., 2009), may be associated with the food habit of the species. Microalgae are a source of protein and lipids (Fleurence, 1999; Guccione *et al*., 2014) for fish species.

The increase of the body protein content in *L. friderici*, since proteins possess excellent amino acid scores and digestibility characteristics for humans, is a productive advantage and the enrichment of fish meat with lipids sources, as DHA, makes it a functional food for human health.

Contents of calcium in the body chemical profile of *L. friderici* decreased linearly with the inclusion of *Schizochytrium* sp. in the diet. Perhaps, the saponification reaction (Lehninger *et al*., 2008) between fatty acids in microalgae and this mineral in the gut of the juvenile impairing the digestion and metabolism of this mineral, hence causing the lower quantities found in the fish body composition.

Conclusion

Juveniles of *Leporinus friderici* fed with artificial diets supplemented with increasing levels of *Schizochytrium* sp. have better growth and changes in it the body chemical profile.

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Author's Contributions

Prates, A.D.S.: Was the one who carried out the experimental part of the experiment, as well as the writing of his dissertation of Master.

Schorer, M.: Is the corresponding author, and was responsible for the new writing, research, English translation and corrections suggested by the reviewers.

Moura, G.S. and E.A. Lanna: Were the ones that gave the idea of this study bringing the microalga to our laboratory.

Gustavo F. Castro: Assisted in the laboratory analyzes of biotechnology.

Pedreira, M.M.: Was the supervisor of the dissertation and Master, and assisted in the writing and review of the article.

Ethics

The authors will address any ethical issues that may arise after the publication of this manuscript.

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