INFLUENCE OF AHEMERAL PHOTOPERIOD ON BROMATOLOGICAL CHARACTERISTICS OF CARCASSES OF TAMBAQUI (*Colossoma macropomum*) JUVENILES

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_ ABSTRACT _

The aim of this study was to evaluate the influence of ahemeral photoperiod on the chemical composition of the carcasses of tambaqui (*Colossoma macropomum*). The experiment was conducted using 190 fish with average weight of $11.01\pm 2.08g$ and total length of 7.8 ± 0.18 cm, stored in sixteen bowls of 56 liters of water with daily renewal of 40-times volume. The water temperature was maintained at 29.1± 0.41°C and the stocking rate was equivalent to 2.75g/L. All tanks had constant aeration. Ahemeral photoperiods were maintained with the aid of timers. The following treatments were used: T1= 6 hours of light, T2= 12 hours of light, T3= 18 hours of light and

T4= 24 hours of light with four replications each. The juveniles were fed twice a day with commercial extruded feed (28% crude protein). The experiment lasted 64 days and the chemical composition of eviscerated and shedded carcasses were analysed at the beginning, at 32 days and at the end of it for the evaluation of crude protein, ether extract, ash and non-nitrogen extract in dry matter and in natural matter. Statistical analysis of variables was performed with the aid of SAEG application, version 9.1. For performing analysis of variance, Pearson correlations and regressions were used. There was no effect of ahemeral photoperiods (p<0.05) on variables.

KEYWORDS: aquaculture; bioclimatology; native fish.

RESUMO _

INFLUÊNCIA DO FOTOPERÍODO EMERAL SOBRE CARCTERISTICAS BROMATOLÓGICAS DA CARCAÇA DE JUVENIS DE TAMBAQUI (Colossoma macropomum)

O objetivo do presente estudo foi avaliar a influência do fotoperíodo emeral na composição bromatológica da carcaça de juvenis de tambaqui (*Colossoma macropomum*). O experimento foi realizado utilizando 190 peixes com peso médio de $11,01 \pm 2,08g$ e

comprimento total de 7,8 \pm 0,18cm, acondicionados em dezesseis aquários de 56 litros com renovação de 40 vezes o volume ao dia. A temperatura da água foi mantida em 29,1 \pm 0,41°C e a densidade de estocagem foi equivalente a 2,75 g/L, sendo que todos os aquários possuíam aeração constante. Os fotoperíodos emerais foram mantidos com auxílio de timers. Foram utilizados os seguintes tratamentos: T1=6 horas de luz, T2 = 12 horas de luz, T3 = 18 horas de luz e T4 = 24 horas de luz, com quatro repetições cada. Os juvenis foram alimentados duas vezes ao dia, com ração comercial extrusada (28% de proteína bruta). Ao iniciar o experimento, com 32 dias e ao final do período experimental, foram feitas análises da composição

bromatológica das carcaças evisceradas e descamadas, para avaliação da proteína bruta, estrato etéreo, matéria mineral e extrato não nitrogenado na matéria seca e na matéria natural. A análise estatística das variáveis foi realizada com auxílio do aplicativo SAEG, versão 9.1, para realização de análise de variância, regressões e correlações de Pearson. Não foi observado efeito dos fotoperíodos emerais (p<0,05) nas variáveis.

PALAVRAS-CHAVE: aquicultura; bioclimatologia; peixes nativos

INTRODUCTION

The volume of fish from aquaculture has been continuously growing over the past 10 years while the extraction activity has showed a decline in its production during the same time period (FAO, 2007). Aquaculture is an alternative to increase food production due to its contribution to world fish production. HUSS (1998) predicted that during the next years there will be an increased demand for fish in developing countries because it is an alternative food of high nutritional value with high digestibility protein and relatively low fat rates.

Amongst the factors related to the culture environment, the ahemeral photoperiod influences the development and survival in different ontogenic stages, because the light assists in feeding strategy as well as it stimulates the metabolic activities of several other fish species (REYNALTE-TATAJE, 2002). The photoperiod corresponds to a number of environmental stimuli and it is related to the duration of light along a day (BEZERRA et al., 2008). The intensity and the increase in the duration of this kind of light change with the seasons and the climate of the region (BROMAGE et al., 2001).

It is known that the photoperiod influences the growth and reproductive cycle of tilapia (BROMAGE et al., 2001). However, there is little information on the effects of environmental factors on the chemical composition of tropical fish, such as tambaqui, at different stages of production.

Knowledge of the chemical composition of fish is necessary for a more effective introduction

in the market, allowing competition with other widely consumed sources of animal protein, as beef, pork and poultry (BELLO & RIVAS, 1992). This knowledge will also be used to evaluate the efficiency of nutrients transfer from food to fish and the most appropriate management strategies to improve carcass composition.

The analysis of the chemical parameters in confined fish has been widely used to assess the health status of animals under intensive production system (TAVARES-DIAS et al., 2001), assisting in the diagnosis, prevention and control of stress-related diseases (CHAGAS et al., 2002).

The objective of this study was to evaluate the existence or not of an effect of ahemeral photoperiod on the chemical composition of the carcass of tambaqui juneviles.

MATERIAL AND METHODS

The experiment was conducted at the Aquaculture Division of the Universidade Estadual do Norte Fluminense (UENF/RJ), located in the Agricultural State School Antônio Sarlo in Campos dos Goytacazes – RJ, during the period from May 4th to July 7th, 2006, totaling 64 days of experiment.

We used 190 tambaqui juveniles (Colossoma macropomum) from Piabanha Project located in Itaocara, municipality of Rio de Janeiro, aged approximately 60 days and presenting initial body weight of 11.01 ± 2.08 g, distributed in 16 tanks with approximate measures of 30 x 60 x 50 cm

(width x length x height), using a total volume of 56 liters and useful volume of 40L each, with initial density equivalent to 2.75 g/L tank. At the beginning of the experiment, the initial lot of 30 fish was slaughtered for chemical analysis of the carcasses.

The juveniles went through a process of adaptation to the experimental routine for 12 days, being this period divided in seven days in the aquaculture laboratory and five days in the experimental aquaria, where the replacement of any animal which might present any kind of irregular behavior that could compromise the final data of the experiment was carried out. After the beginning of the trial period, no juvenile was replaced in the experimental units. The fish were fed twice a day.

The aquarium and the ten tambaqui fish, together, formed an experimental unit. The experimental units were distributed in a completely randomized design with four treatments and four replications each, totaling 16 experimental units. They were isolated from any light which did not come from their respective treatments, by using black tarpaulin to prevent the incidence of light during the dark period of each treatment, guaranteeing that the supply of light would only be made during the periods established for each treatment.

Dissolved oxygen (mg/L), pH, temperature (°C), electric conductivity (μ S) and light intensity (μ mol m⁻² s⁻¹) were the parameters measured after the meals (at 8:20 a.m. and 1:20 p.m.). Oxygenation levels were maintained with the help of aerators and measured by an oximeter. The pH, temperature, electrical conductivity and light intensity were measured, respectively, via pH-meter, digital thermometer, digital conductivity meter and digital photometer.

A closed and continuous recirculating system was used during the experiment, containing a box for water filtration (through physical and biological processes), a deposit box (where the water was maintained to return to experimental units), submersible pumps for the return of water from the system and two 300W thermostats to maintain the temperature during the experiment. The systems had supply and drainage of water system with a renewal of 40 times the total volume of the tank per day. The water flow was constant in order to maintain high oxygen content, eliminating the stool and preventing plankton formation.

Feeding was carried out using a commercial diet containing 28% CP and 3,100 kcal DE/kg. Feed was provided ad libitum at 8:20 a.m. and 1:20 p.m. After 15 minutes, the remains were removed. In aquariums where there was no leftover, feeding was repeated until leftovers could be found.

Blood samples were collected for blood glucose measurement, in order to verify the presence of stress indicators in fish and to perform chemical analyses of carcasses eviscerated and scaled, to check possible effects caused by different photoperiods on the chemical composition of carcasses of 30 tambaqui fish.

Blood samples were taken at three occasions: at the beginning of the experiment, at 32 days and at the end (64 days). Of the five fish removed from each experimental unit for chemical analysis at 32 and 64 days, three were used for blood collection, which was performed with the aid of 3.0 mL syringes with 25/7 needles. The set of syringes and needles was washed with potassium fluoride in order to prevent blood clotting and glycolysis.

Fish were anesthetized with a solution containing 65ppm eugenol. Blood collection was made by means of caudal vein puncture, taking a minimum volume of 0.4 ml of blood per fish. The blood was transferred from the syringe to test tubes which were centrifuged at a speed of 3500rpm for five minutes. The plasma was removed from the samples and was centrifuged again at the same speed and time for total elimination of waste.

After blood collection, the fish (five juveniles at 32 days and five juveniles at 64 days from each experimental unit) were slaughtered by heat shock, packed in plastic bags properly identified and stored in the freezer. After the experiment, the fish were thawed and the scales and viscera were removed. The carcasses were weighed, crushed in a small industrial processor and weighed again. The mass obtained was frozen and, after 24 hours of freezing, it was taken to the lyophilizer to remove moisture.

After a period of 48 hours the samples were weighed on digital scales accurate to 0.1 and gound

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in a ball mill. After processing the samples, they were analyzed by adapting the methodology described by SILVA & QUEIROZ (2000) for analysis of ether extract, crude protein and mineral matter. Non-nitrogen extract, dry matter, samples unit rate and blood glucose were also quantified.

All analyses were performed at the Laboratory of Animal Science and Animal Nutrition (LZNA) of UENF-CCTA. With the values obtained in previous analyses, crude protein retention efficiency (CPRE) and protein efficiency ratio (PER) were calculated.

For statistical analysis of the experiment we used the statistical program SAEG, version 9.1. Analysis of variance, multiple and simple linear regressions and Pearson correlations were carried out with the data obtained through the analyses made in LZNA.

RESULTS AND DISCUSSION

The properties related to water quality – dissolved oxygen, temperature, pH and electrical conductivity, measured during the experimental period – are shown in Table 1.

Table 1:Mean values of the physical-chemical properties of the water in the experiment

Properties		Treatment (hL)				
		6	12	18	24	
Dissolved oxygen (mg/L)		5.9	5.8	56	5.7	
pH		6.17	6.08	5.72	5.76	
Eletric conductivity (µS)		366	396	543	561	
T 4 90	\mathbf{M}	29.5	29,8	29.1	29.4	
Temperature °C	Α	29	28.9	28.6	28.7	

During the experimental period, the average content of dissolved oxygen was 5.75 mg / L. According to ARAUJO-LIMA & GOMES (2005), tambaqui fish has its normal growth in oxygen levels above 3.0 mg / L; therefore, the dissolved oxygen values obtained in the experiment described herein fully meet the requirement of tambaqui. Likewise, the average pH was maintained within the standards recommended by ARIDE et al. (2004) between 4.0 and 6.5. The temperature of the experimental tanks was adequate for tropical climate species, with average of 29.45°C in the morning and 28.8°C in the afternoon.

The values of electrical conductivity of the water also remained within ranges suitable for the cultivation of tropical fish. These values are very close to the ones found by POLESE et al. (2010), juveniles who studied of (Piaractus values mesopotamicus). The highest of this parameter is probably associated with the presence of calcium ions in the water supply of the experimental units. Therefore, it can be stated that these water quality parameters do not interfere with the present results.

The results observed in the experiment on the chemical composition of the carcasses of tambaqui fish showed a tendency to increase the percentage of fat in the carcass at the expense of the percentage of protein regarding the values achieved at the beginning and at the end of the experimental period (Table 2).

Table 2: Chemical composition of dry matter in the eviscerated and scaled carcass of tambaqui juveniles submitted to different photoperiods at the beginning, on the 32nd and 64th day of experiment

		Treatment							
Variables			6	1	2	1	8	2	.4
		32°	64°	32°	64°	32°	64°	32°	64°
DM (%)	28.58	29,63	32.79	28,99	33,37	27,78	32,7	29,23	33.1
CP(%)	64,97	48.78	44.51	49.42	44.27	51.85	43.12	49.64	43.04
EE(%)	10.35	32,06	36.12	31.36	36,12	27.59	36.72	30.65	35.66
MM(%)	13,42	14.06	15,21	13.94	14.89	14.71	14.73	13,88	15,08
NNE(%)	11,26	5.11	4.16	5,28	4.72	5.85	5.44	5.83	6,22

(DM%) - dry matter percentage in the carcass; (CP%) - crude protein percentage in the carcass; (EE%) - ether extract percentage in the carcass; <math>(MM%) - mineral matter percentage in the carcass; and <math>(NNE%) - non-nitrogen extract percentage in the carcass.

Crude protein values verified in this study, in spite of declining between the beginning and the end, are close to the results found by TERRAZAS et al. (2002), who they studied fish of the same species and obtained values from 44.08 to 45.65% of CP in the carcasses of juveniles. An increase in the percentage of crude protein was noticed when considering studies that evaluated only the chemical composition of the fillet, such as LANNA et al. (2004), which showed an average of 87.86% crude protein in dry matter of the fillet of juvenile Nile tilapia, and SANTOS et al. (2000) and SANTOS et al. (2001), who worked with (Hoplias lacerdae) fillet and obtained around 81.34% crude protein in dry matter. Despite the variation in these studies, both final results were well above those found in this work.

The lowest crude protein values found in this study are due to the fact that when analyzing a carcass, a set of tissues (bone, cartilage, muscle, etc.) is analyzed, which results in a lower percentage of crude protein.

Opposite results regarding ether extract were observed in this study because the values found were higher than the ones obtained by SANTOS et al. (2006), who found values of 5.81 and 1.21 for dry matter and natural matter, respectively; MACEDO-VIEGAS et al. (2002) worked with trout and obtained values from 7.34 to 5.41%; and CAULA et al. (2008) analyzed the fillet of different fish species and found values between 1.0 and 4.6. However, when comparing the results obtained in this work with others who examined fish carcasses, it may be observed that the values are very similar to the ones verified by TERRAZAS et al. (2003), who obtained values ranging from 40.67 to 44.75, MARENGONI & SANTOS (2006), who evaluated tilapia from different fee-fishing farms, obtaining a result between 30.68 and 35.65% for males and 20.90 and 38.59 for females, and ITUASSÚ et al. (2004), who, working with tambaqui under food restriction, found values of crude protein in dry matter of the carcass in a range from 55.50 to 58.73%, and ether extract in a range from 19.50 to 23.37%.

The change between the values obtained at the beginning and end of this work for the nutritive variables was also observed. This difference is due to the growth of the animal and possibly to the feed, which is not specific for the species, causing an accumulation of fat in the carcass because of the protein:energy imbalance, and, consequently, protein reduction and fat increase, which is not connected or not influenced by the different treatments, since statistical analysis showed no significant difference between treatments.

Table 4 shows the values of blood glucose of protein efficiency ratio (PER) and the crude protein retention efficiency (CPRE). These ratios use values obtained from the biometric and chemical analyses which are transformed into data for evaluating and quantifying the use of nitrogen supplied in the diet, correlated with the production of animal protein.

The variables in Table 3 showed no regression equations with reliable degree of significance. However, the values obtained in this experiment for these same variables are close to the ones obtained in other experiments, as in the research by MUÑOZ-RAMÍREZ & CARNEIRO (2002), who obtained PER values ranging between 2.27 and 1.76% and CPRE values between 29.81 and 38.56.

Table 3: Mean values of protein efficiency ratio (PER), crude protein retention efficiency (CPRE) and glycemia levels

				Tr	eatments			
Variables	6	ih	12	2h	1	Sh	2	4h
	32	64	32	64	32	64	32	64
CPRE (%)	34	27.2	35	28.5	34.3	26.6	39.1	24,3
PER(%)	1.54	2.01	1.57	2.02	1.57	1.98	1.45	1.83
Glycemia (mg/dL)	98.75	129.63	80.15	143	72.3	111	64,78	100.13

LANNA et al. (2004), on the other hand, obtained higher values than those verified in this work for PER, between 3.29 and 4.37 g, when working with tilapia juvenile using different sources of oils and fiber in the diet. FERNANDES et al. (2001), working with different levels of protein for (Piaractus mesopotamicus) fingerlings, found values of PER equal to 3.23, 3.13 and 2.92% for the respective amounts of dietary CP of 22, 26 and 30%.

These lower PER values in the present study may be related to the stress measured at low levels in the first blood collection for the fish that remained in longer photoperiods. However, the result of the second collection also indicated high blood glucose values, which can characterize stressed animals, for all treatments.

When the fish is in a disadvantage situation, blood glucose level increases, as shown by MARTINS et al. (2002), who submitted the hybrid tambacu to consecutive stress stimuli. These authors observed an initial value of blood glucose equal to 71.00 mg / dL at time zero, reaching values of blood glucose equal to 148.35 mg / dL two hours after stimulation, demonstrating an acute stress.

BRANDÃO et al. (2004) quantified the level of blood glucose of tambaqui fish during a density experiment and obtained values for blood glucose between 51 and 65 mg / dL. These results do not show signs of stress regarding the densities used in the study.

CHAGAS et al. (2002), testing stocking densities of tambaqui fish, showed the following blood glucose values: 61.80, 67.30 and 63.67 mg / dL for their respective stocking densities of 25, 50 and 75 fish / m^3 , which did not demonstrate the presence of stressors during the experiment, considering these values are within the range prescribed for blood glucose values of the species.

GOMES et al. (2001) quantified the blood glucose values for tambaqui fish in a state of rest reaching values between 50 and 70 mg / dL. Such values are close to those obtained in the longest photoperiods of the experiment described herein (18 and 24 hours of daylight), indicating no stress in these treatments until 32 days.

The stress demonstrated by the blood glucose level can explain the low values found for PER and CPRE, because stress decreases metabolism efficiency, increase the food passage rate, and decreases the time of diet digestion, impairing the utilization of food for the development of the animal.

Table 4: Matrix for Pearson correlation for the following variables: blood glucose (GLUC), crude protein retention efficiency (CPRE), ether extract in dry matter (EEDM), crude protein dry matter (CPDM), crude protein in natural matter (CPNM), ashes in natural matter (MMNM) and non-nitrogen extract in dry matter (NNEDM)

Variable	Variable	Correlation	Significance -	
GLUC	NNEDM	-0.6688	0.0023	
	NNENM	-0.6665	0.0024	
CPRE	PER	0.8498	0.0001	
FEDM	CPDM	-0.8679	0.0001	
EEDM	CPNM	-0.8548	0.0001	
CPDM	PER	-0.2935	0.1350	
СРОМ	EENM	-0.8656	0.0001	
CPNM	EEDM	-0.8548	0.0001	
	EENM	-0.8003	0.0001	
MMNM	NNEDM	-0.6320	0.0043	
IVENINE	NNENM	-0.6115	0.0059	
NNEDM	MMDM	-0.6911	0.0015	
	MMNM	-0.6320	0.0043	

The values found in the correlation between CP and EE show that these variables have opposite directions on the chemical composition of carcasses evaluated during the experiment, and the greater the percentage of one variable in the carcass, the lower the other. However, no correlations were observed between CP and other variables chemically analyzed as CP and MM, CP and DM and CP and NNE.

Nevertheless, a negative and over 60% correlation was observed a between blood glucose and non-nitrogen extract in the carcass. A correlation between two variables is expected, because, according to SILVEIRA et al. (2009), in fish as it occurs in mammals, glucose is the body's main energy source. When there is an excess of blood glucose, it is transformed into glycogen and stored in the liver and muscles. To maintain energy homeostasis during a period of stress (food deprivation, territorial dispute, escape, etc.), glycogen is mobilized and converted into glucose,

maintaining blood glucose level, and thus avoiding the lack of energy for cells of the animal body (SILVEIRA et al, 2009

In the stress situation observed in the experiment by blood glucose levels of juveniles, one can say that the metabolism was probably impaired and, consequently, the maintenance of the metabolic reserves of glycogen of juveniles was activated, causing the blood glucose to increase, confirming the existence of an inverse correlation between the glycemia factors and the non-nitrogen extract in the carcass.

CONCLUSION

The chemical composition of tambaqui juvenile (Colossoma macropomum), the protein efficiency ratio and the ratio of crude protein retention efficiency were not affected by the ahemeral photoperiod during the experimental period.

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