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The Ability of Nile Tilapia to Regulate Protein and Energy Intake Evaluated by Carbon Relative Enrichment ($\delta^{13}C$)

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Abstract: The aim of this study was to assess the ability of Nile tilapia to balance its own diet, when two ingredient mixes were offered, using carbon stable isotopes. In order to accomplish that, 225 Nile tilapia juveniles (average initial weight 5.0 g ± 0.5 g) were distributed in five tanks, each containing a group of 45 fish. One group of fish were fed exclusively with a high protein mix (HPM; $\delta^{13}C$ = -22.62‰), the second one fed only with a low protein mix (LPM; $\delta^{13}C$ = -14.34‰). The other groups had free access to both mixes (free choice system). The fish from all tanks were fed four times a day. Muscle, liver and blood samples were collected at each five days (from 2 fish/tank/collection) for 86 days, except for the fish fed with LPM (fed for a 120 days period). The samples were analyzed in a mass spectrometer and proportions of the mixes consumed were estimated through its carbon isotope enrichment ($\delta^{13}C$). Energy intake slightly decreased after the 50th day and protein consumption increased after the same period. However, consumption did not present a clear pattern in relation to the individual weight, i.e., protein consumption patterns are mainly related to the age of the individuals and it is not clear if it is also correlated to their weight. Additionally, this technique allowed the observation of differences regarding consumption among the individuals from the experimental group.

Key words: Protein intake, energy intake, diet self-selection, carbon stable isotopes, Nile tilapia.

1. Introduction

Most animals obtain the necessary nutrients to their metabolism in several food items and the availability of nutrient sources does not match their physiological demands at different development phases [1]. The composition of the diets for the animals does not correspond to an exact proportion of the food items available around them [2]. Hence, animals present preferences when they need to obtain a balanced diet from several food options.

Studies of self-selection of macro nutrients performed with goldfish *Carassius auratus* [3], rainbow trout *Oncorhynchus mykiss* [4] and sea bass *Dicentrarchus labrax* [5, 6] affirm that these species had a good ability to balance their own diets. The act of choosing a food item is not only related to the organoleptic characteristics, but also can be related to the food color and post ingestion factors [5] and varies according to environmental conditions [7]. Most of the previous studies indicated that fish species are able to choose between food items and obtain a reasonable balance diet; however, those studies evaluate this ability through mean values of intake from a group.

The use of stable isotopes to estimate food intake was primarily performed in studies of bovine alimentary preferences. In those trials, the isotopic signature from the feces of the animals estimates the consumption of fodder plants. The utilization of this

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technique is based on the ratio between the heavier (^{14}C) and the lighter (^{13}C) isotope (isotopic ratio) [8, 9]. These atoms are discriminated in physical and chemical processes permitting their utilization as natural markers [10].

The isotopic composition of an animal tissue, or their feces, reflects the contribution of their alimentary sources. Therefore, the isotopic composition evaluation of ^{13}C of a specific animal tissue can indicate its previous diet. However, the fundamental condition to determine the contribution of either alimentary source is that it must present a distinguished isotopic signature. This is possible because plants, with different photosynthesis mechanisms C_3 and C_4 cycle, discriminate the atmospheric CO_2 according to the mass number of carbon (^{12}C or ^{13}C), which is reflected in different relative isotopic enrichment in the plant tissues [10].

Plants of both photosynthetic cycles are commonly used as ingredients to manufacture feeds, such as corn (C_4) and soybean (C_3). So, diets made out of most usual ingredients may present different isotopic signatures, and consequently, it is possible to trace matter from food items to its incorporation to different tissues [8, 9].

The alimentary behavior study, evaluating the ability of Nile tilapia to balance its own diet through free choice of food sources, would bring additional information about nutritional requirements of this species, including the correlation between the intake of protein and energetic food items. Aiming to achieve this information, the objective of this study was to evaluate the ability of Nile tilapia to balance its own diet when two ingredients mix through free choice of two mixes as nutrients sources.

2. Materials and Methods

2.1 Installations, Experimental Animals and Sample Collecting Procedures

In this trial, 225 Nile tilapia juvenile (average initial weight 5.0 g ± 0.5 g) were randomly distributed in five 1,000 L tanks (45 individuals per tank, uniform biomass). These tank were connected to a closed recirculating system equipped with a biological filter, circulating pump, supplemental aeration and electric water heaters (26.0 ± 0.5 °C). Prior to the experiment, the fish were acclimated for 25 days. In this period, the animals were fed with completed formulated feed in order to equalize the isotopic signature ($\delta^{13}C$) of their tissues.

After the acclimation period, the fish from one of the tanks were fed exclusively with a pelleted mix with low protein content (LPM) and relative carbon enrichment ($\delta^{13}C$) equal to -14.34‰ for 120 days. The fish from another tank were fed for 86 days with a pelleted ingredient mix containing a high protein level (HPM) and $\delta^{13}C$ = -22.62‰ (Table 1). These feeding procedures were necessary for nutrient consumption calculations using the simple isotopic dilution formula (Eq. 1), with two sources and one product. In this expression, the $\delta^{13}C$ values belong to the same tissues which avoid the isotopic fractioning between the diets and animal tissues and the different relations of carbon.

In the other three tanks (R1, R2 and R3), both LPM and HPM were offered to the fish, four times a day, for 86 days. In order to provide free access to both ingredient mixes, the feed were presented in submerse feeders made with PVC screen in a circular frame made with plastic tubes. In the feeding process, two feeders in each tank were submersed and the ingredient mixes were allocated in the top of them with a PVC tube. The animals were fed to apparent satiation in all groups, four times a day (8:00, 12:00, 14:00 and 17:00).

Tissues samples (blood, muscle and liver) were collected at the 1st, 5th, 10th, 15th, 21st, 25th, 31st, 35th, 40th, 45th, 50th, 56th, 61st, 66th, 71st, 76th and 86th day of the trial. In these collections the animals were randomly captured from the tanks, initially using two fish per tank/collection, except for the 15th, 16th

Table 1 Ingredients, composition and relative isotopic enrichment (‰ $\delta^{13}C$) of high protein mix (HPM) and low protein mix (LMP) used as food sources for Nile tilapia to obtain self regulated ingestion of energy and protein.

		Mix	
		LPM [a]	HPM [b]
Ingredient (%)	Fish meal	8.22	51.76
	Soybean meal	-	36.00
	Cotton meal	-	8.70
	Corn	65.17	-
	Corn starch	3.96	-
	Alginate	1.00	1.00
	Cellulose	4.25	1.42
	Corn oil	11.00	-
	Bicalcium phosphate	4.58	-
	Calcium carbonate	1.01	-
	DL-methyonine		0.31
	Vitamin C	0.09	0.09
	Sal	0.20	0.2
	Vitamin and mineral premix[c]	0.50	0.50
	BHT[d]	0.02	0.02
Nutrient	Crude protein (%)	12.19	52.69
	Digestible protein[e] (%)	11.25	46.71
	Gross energy (J/g)	18.41	17.64
	Digestible energy[e] (J/g)	13.94	11.93
	Ethyl ether extract (%)	12.57	7.66
Relative isotopic enrichment	(‰ $\delta^{13}C$)	-14.34	-22.62

[a] LPM = low protein mix; [b]HPM = high protein mix; [c]Vitamin and mineral premix—*Supre Mais*: guaranteed levels per kg of the product: Vitamins: A = 1,200,000 UI; D3 = 200,000 UI; E = 12,000 mg; K3 = 2,400 mg; B1 = 4,800 mg; B2 = 4,800 mg; B6 = 4,000 mg; B12 = 4,800 mg; folic acid = 1,200 mg; calcium pantothenate =12,000 mg; C = 48,000 mg; biotin = 48 mg; chlorine = 65,000 mg; = 24,000 mg; minerals: Fe = 10,000 mg; Cu = 600 mg; Mn = 4,000 mg; Zn = 0 mg; I = 20 mg; Co = 2 mg, Se = 20 mg;
[d]BHT = butyl hydroxyl toluene, antioxidant;
[e]Estimated values.

and 17th collections (the two first ones using one fish/tank and the last one six fish/tank). For the tissue sampling, the fish were anesthetized (benzocaine 1.0 g/15.0 L of water) and blood was taken from their caudal vein. The animals were then sacrificed in cold water (2 °C) in order to collect the liver and a section of their dorsal muscle (0.5 cm^3 approximately). All samples were frozen (-18 °C) until analysis procedures. Additionally, the same collections were done with the animals fed with LPM at the 120th day, in order to determine if the ^{13}C turnover of their tissues reached a plateau.

All experimental and animal care procedures were performed with the approval of the Animal Experimentation Ethics Committee from the College of Veterinary Medicine and Animal Science of São Paulo State University.

2.2 Isotopic Analysis

The samples of muscle, liver and blood were dried in an oven (55 °C) until a constant mass value (24 h). After drying process, the liver samples had the fat content removed with a Soxhlet system with ethyl ether [12]. Muscle samples were pulverized in a cryogenic mill (-195 °C). The samples of liver and blood were pulverized with mortar and pestle in order to minimize losses of material due to their small quantities after the drying process.

The $^{13}C/^{12}C$ ratios were determined using a low resolution mass spectrometer (DELTA S—Finnigan Mat), attached to an elemental analyzer (EA 1108 CHN). The values were expressed in $\delta^{13}C$ and calculated according to the equation $\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 10^3$, where $\delta^{13}C$ represents the carbon enrichment of the sample when related to the international standard (*Bellemnitella americana*) carbonated fossil from the Peede formation in the South of California State (USA) and *R* represents the isotopic ratio ($^{13}C/^{12}C$) from the sample and standard. The analytical precision of the equipment was 0.2‰.

2.3 Protein and Energy Intake Estimated through Carbon Stable Isotopes

The alimentary preference results were obtained through the simple isotopic dilution expression between two alimentary sources:

$$PC - HPM\ (\%) = (\delta_{source\ LPM} - \delta_{sample})/(\delta_{source\ LPM} - \delta_{source\ HPM}) \times 100 \quad (1)$$

$$PC - LPM\ (\%) = (\delta_{source\ HPM} - \delta_{sample})/(\delta_{source\ HPM} - \delta_{source\ LPM}) \times 100 \quad (2)$$

In these expressions the nomenclature means:

PC-HPM (%): proportion consumed of high protein mix by the experimental fish according to the relative carbon enrichment as determined by tissue analysis (blood, muscle or liver);

PC-LPM (%): proportion consumed of low protein mix by the experimental fish according to the relative carbon enrichment as determined by tissue analysis (blood, muscle or liver);

δ source HPM: relative isotopic enrichment from the analyzed tissues obtained from the group fed exclusively with HPM;

δ source LPM: relative isotopic enrichment from the analyzed tissues obtained from the group fed exclusively with LPM.

The results from Eqs. 1 and 2 were applied in the calculations of the estimated consumption of digestible protein (DP) and energy (DE) and its values were based on the centesimal composition of HPM and LPM, proposing the following equations:

$$DP\ (\%) = [(PC\text{-}HPM \times \%\ DP\ HPM) + (PC - LPM \times \%\ DP\ LPM)] \quad (3)$$

$$DE\ (J/g) = [(PC\text{-}HPM \times DE\ HPM) + (PC - LPM \times DE\ LPM)] \quad (4)$$

In these expressions the nomenclature means:

DP (%): estimated digestible protein consumed by the individuals;

DE: estimated digestible energy consumed by the individuals;

DP (%) HPM and DP (%) LPM: digestible protein content in HPM and LPM, respectively;

DE HPM and DE LPM: digestible energy content in HPM and LPM, respectively;

PC-HPM (%) and PC-LPM (%): same as expressions 1 and 2.

3. Results

Fig. 1 presents the change in the relative carbon enrichment at different tissues that received as food exclusively with HPM or LPM. The values used in the simple isotopic dilution expression (δ source LPM and

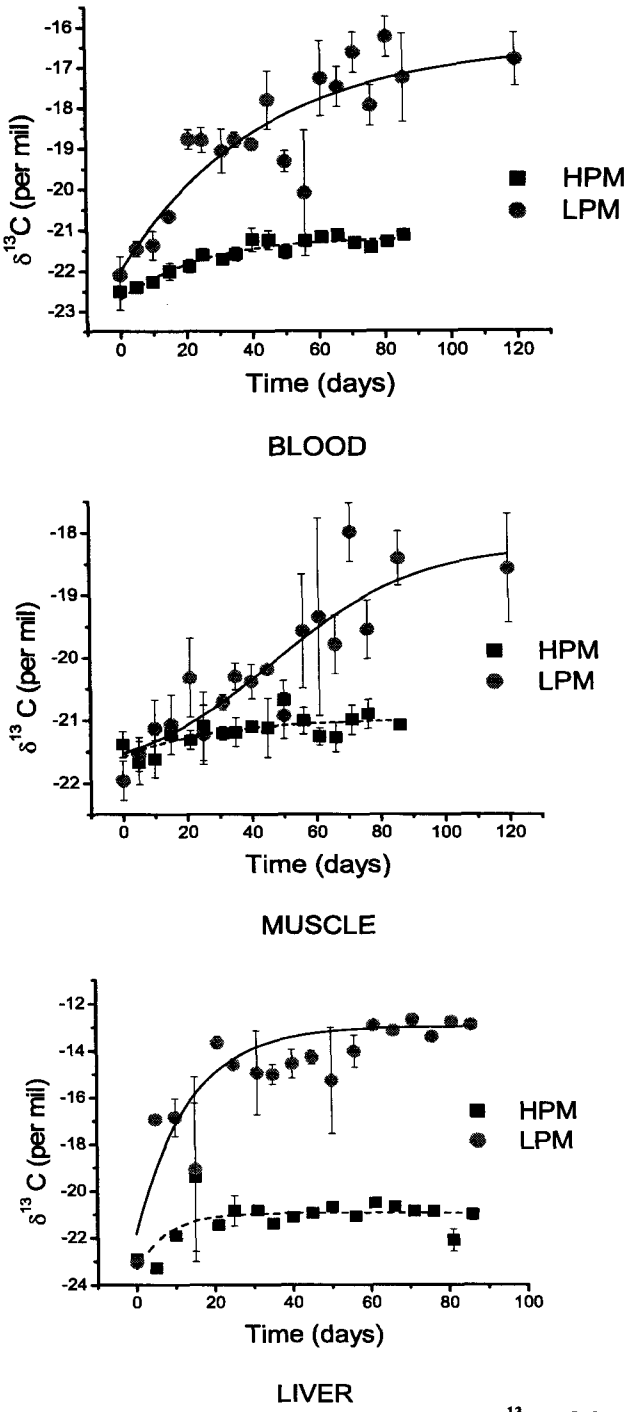

Fig. 1 Relative carbon enrichment exchange ($\delta^{13}C$) of the tissues (liver, muscle and blood) of Nile tilapia fed with a mixes either with a high protein or high energy content related to the experimental period.

δ source HPM) were obtained from the isotopic enrichment of blood, liver and muscle from fish exposed to the treatment through 120 days for LPM

(blood and muscle) and 86 days for HPM and liver samples of LPM (Table 2).

The isotopic signature (^{13}C) among liver, muscle and blood required different periods to reach a plateau. HPM stabilized $\delta^{13}C$ more rapidly due to its more similar isotopic signature to the initial isotopic signature of the tissues (C_3 photosynthetic cycle) and higher protein content (Tables 1 and 2).

LPM had a $\delta^{13}C$ distant from the initial isotopic signature of the individuals and closer to plants with a C_4 photosynthetic cycle, additionally, it also had lower protein content. These factors were responsible for the longer period of time that these tissues required to reach the isotopic signal of the diets. The liver of the animals fed with LPM showed a faster turnover when compared with the other tissues, even with low protein content.

The curves of consumption as estimated by $\delta^{13}C$ presented a positive exponential shape for energy and negative exponential shape for protein (Fig. 2). These curves where were obtained from the $\delta^{13}C$ of the tissues and the chart represents the $\delta^{13}C$ exchange in each tissue in relation to the experimental days. This does not necessarily indicate an exponential shape on the consumption of protein and energy by the fish, because not all the carbon atoms of the analyzed tissues had yet been substituted for the atoms from HPM or LPM.

However, Table 3 presents protein and energy intake data after the 50th experimental day. At that time, the animals had been fed through self feeding system for enough time to turnover most of the carbon atoms of the cells at the analyzed tissues. Through these results was observed a decrease on the consumption of DP and an increased on the intake of ED.

Individual weight (not considering time/age) when related with protein or energy intake did not present any defined shape. According to these results, the consumption of these nutrients is more related with age than to weight (Fig. 3).

4. Discussion

The $\delta^{13}C$ values from the tissues of the animals fed exclusively with either HPM or LPM (Fig. 1) required different periods to reach an isotopic plateau among the analyzed tissues. Consequently, these values presented different half life expressions (T). It was expected that the time expended for carbon exchange from the fish tissues fed with LPM, in which the $\delta^{13}C$ was more similar to plants with C_4 photosynthetic cycle and more distant to the initial $\delta^{13}C$ of the experimental fish, would present higher T values principally due to the low protein level of the LPM.

Table 2 Isotopic relative enrichment ($\delta^{13}C$) and carbon turnover expressions of muscle, blood and liver of Nile tilapia, fed either high protein mix (HPM) or low protein mix (LPM).

Tissue	Food source	Expression[a]	$\delta^{13}C_{source}$[b]	Half-life (days)[c]
Muscle	LPM	$\delta^{13}C(t) = \left\{-18.28‰ + \left[\frac{-3.37‰}{1 + e^{(t-47.0661)/11.4462}}\right]\right\}$	-17.38‰	47
	HPM	$\delta^{13}C(t) = -20.99 - 0.60\, e^{(-0.0366\, t)}$	-21.17‰	19
Blood	LPM	$\delta^{13}C(t) = -16.39 - 5.64\, e^{(-0.0230\, t)}$	-15.91‰	30
	HPM	$\delta^{13}C(t) = -21.18 - 1.53\, e^{(-0.0410\, t)}$	-21.32‰	17
Liver	LPM	$\delta^{13}C(t) = -12.96 - 8.89\, e^{(-0.0741\, t)}$	-12.86‰	9
	HPM	$\delta^{13}C(t) = -20.95 - 2.37\, e^{(-0.1284\, t)}$	-21.34‰	5

[a]In the expressions, $\delta^{13}C$ (t) means: isotopic relative enrichment at time (t); [b]$\delta^{13}C_{source}$: isotopic relative enrichment of the respective analyzed tissue of the fish fed either HPM or LPM at the end of the experimental period; [c]Half-life (T): time taken to replace half of the carbon atoms, according to the equation $T = \ln 2/k$, k representing the isotopic exchange constant in unities of $time^{-1}$.

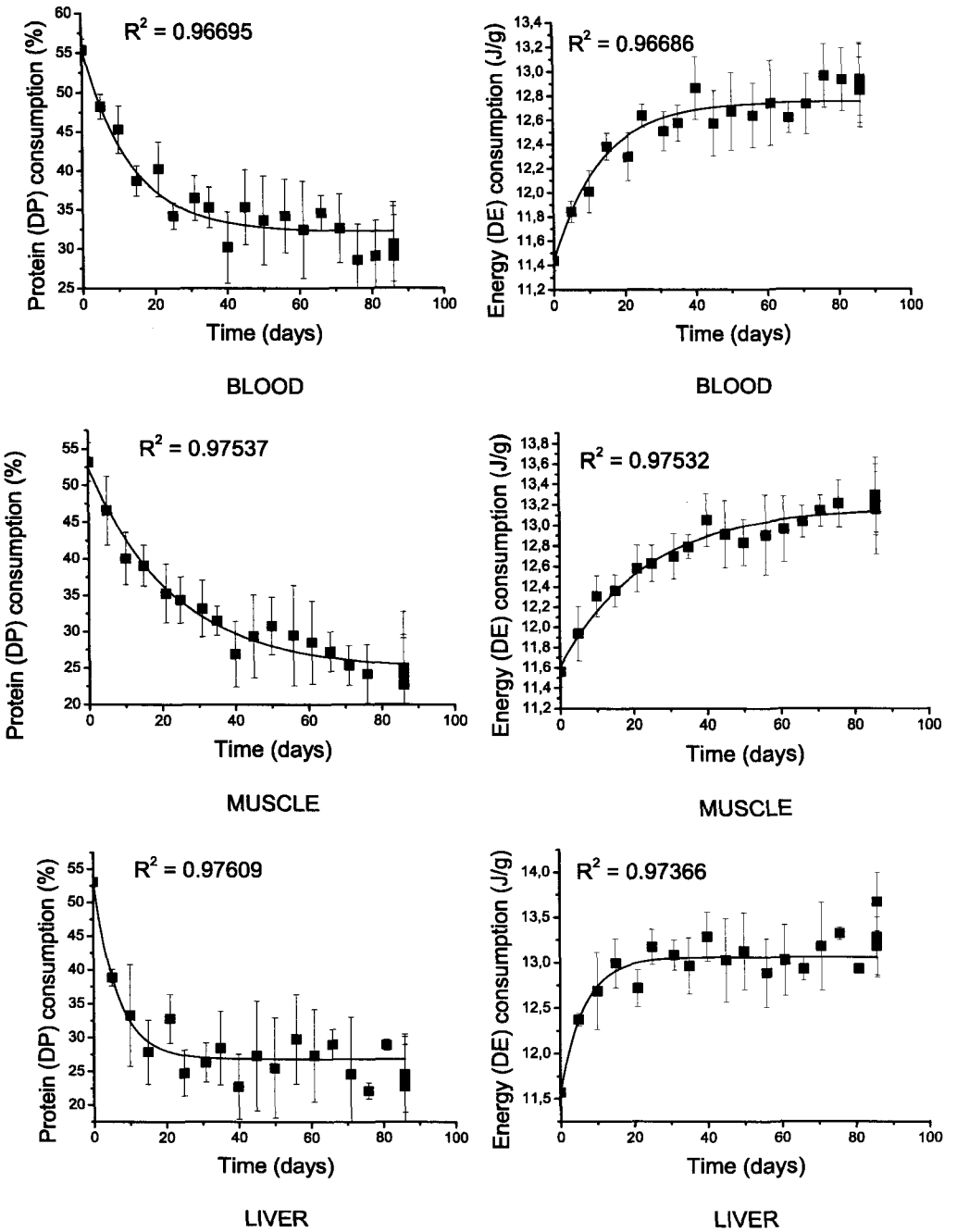

Fig. 2 Consumption of protein (DP) and energy (DE) estimated by the relative carbon enrichment ($\delta^{13}C$) from the blood, muscle and liver of Nile tilapia related to time.

The different half life showed by each tissue corresponded to their different metabolism velocity. From the tissues analyzed, the liver had the most rapid carbon exchange in its cells. These results agree with studies performed with other animal species in which liver had lower half life values followed by blood and then the muscle [12, 13].

In order to estimate the consumption of the mixes in this trial, it was necessary to know the influence of the diets $\delta^{13}C$ in the tissues separately. This procedure allowed consideration of the effects of isotopic fragmentation caused by digestion and metabolism in the conversion of the matter from the food into matter of the animal tissues [14].

Table 3 Protein and energy self-regulated ingestion based on carbon relative enrichment ($\delta^{13}C$) data obtained in different Nile tilapia tissues.

Time (days)	Weight (g)	Tissue	$\delta^{13}C$ (‰)[a]	HPM (%)[b]	LPM (%)[c]	DP (%)[d]	DE (J/g)[e]
		Muscle	-19.47 ± 0.42	55.1 ± 11.2	44.9 ± 11.2	30.8 ±4.0	12.83 ± 0.23
50	112.5 ± 41.0	Blood	-19.33 ± 0.86	63.1 ± 15.9	36.9 ± 15.9	37.8 ± 6.5	12.67 ± 0.32
		Liver	-16.24 ± 1.8	39.9 ± 21.2	60.1 ± 21.2	25.4 ± 7.5	13.13 ± 0.43
		Muscle	-19.33 ± 0.74	51.4 ± 19.4	48.6 ± 19.4	29.5 ± 6.9	12.90 ± 0.39
56	123.4 ± 30.1	Blood	-19.41 ± 0.72	64.8 ± 13.3	35.2 ± 13.3	38.4 ± 5.4	12.64 ± 0.27
		Liver	-17.27 ± 1.60	51.9 ± 18.8	48.1 ± 18.8	29.7 ± 6.7	12.90 ± 0.38
		Muscle	-19.23 ± 0.61	48.7 ± 16.1	51.3 ± 16.1	28.5 ± 5.7	12.96 ± 0.32
61	161.2 ± 50.1	Blood	-19.14 ± 0.94	59.8 ± 17.4	40.2 ± 17.4	36.4 ± 7.1	12.74 ± 0.35
		Liver	-16.68 ± 1.65	45.0 ± 19.4	55.0 ± 19.4	27.2 ± 6.9	13.03 ± 0.39
		Muscle	-19.09 ± 0.29	45.0 ± 7.6	55.0 ± 7.6	27.2 ± 2.7	13.03 ± 0.15
66	194.5 ± 18.8	Blood	-19.47 ± 0.35	65.8 ± 6.4	34.2 ± 6.4	38.8 ± 2.6	12.61 ± 0.13
		Liver	-17.08 ± 0.53	49.8 ± 6.3	50.2 ± 6.3	28.9 ± 2.2	12.94 ± 0.13
		Muscle	-18.89 ± 0.29	39.8 ± 7.7	60.2 ± 7.7	25.4 ± 2.7	13.14 ± 0.15
71	191.6 ± 12.1	Blood	-19.17 ± 0.67	60.2 ± 12.4	30.8 ± 12.4	36.6 ± 5.0	12.73 ± 0.25
		Liver	-16.02 ± 2.04	37.29 ± 24.1	62.7 ± 24.1	24.5 ± 8.5	13.19 ± 0.48
		Muscle	-18.76 ± 0.43	36.5 ± 11.4	63.5 ± 11.4	24.2 ± 4.0	13.20 ± 0.23
76	177.5 ± 40.5	Blood	-18.55 ± 0.70	48.7 ± 13.0	51.3 ± 13.0	31.9 ± 5.2	12.96 ± 0.26
		Liver	-15.42 ± 0.30	30.2 ± 3.5	69.8 ± 3.5	22.0 ± 1.2	13.33 ± 0.07
		Muscle	-18.74 ± 0.70	35.8 ± 18.5	64.2 ± 18.5	24.0 ± 6.6	13.22 ± 0.39
86	251.8 ± 64.3	Blood	-18.75 ± 0.82	52.5 ± 15.1	47.5 ± 15.1	33.5 ± 6.1	12.88 ± 0.30
		Liver	-15.76 ± 1.54	34.2 ± 18.1	65.8 ± 18.1	23.4 ± 6.4	13.25 ± 0.36

[a]$\delta^{13}C$ (‰): carbon relative enrichment; [b]HPM (%): high protein mix (HPM) ingestion estimated by simple isotopic dilution equation through carbon relative enrichment of muscle, blood and liver; [c]LPM (%): low protein mix (HPM) ingestion estimated by simple isotopic dilution equation through carbon relative enrichment of muscle, blood and liver; [d]PD (%): digestible protein ingestion estimated thought HPM (%) and LPM (%) values and their respective percentage of digestible protein, dry matter based;[e]DE(J/g): digestible energy ingestion estimated thought HPM (%) and LPM (%) values and their respective quantity of digestible energy, dry matter based.

Decrease in protein consumption and slight increase in the energy consumption was already reported for Nile tilapia indicating that individuals weighting below 0.51 g should be offered a diet with 40% of protein but required to 30% protein for larger fish (96.0 g-264.0 g) [15]. Another study indicates that protein demand of 5 g tilapia individuals were approximately 28%; however, increasing the protein content to 34% improved performance [16]. A review on nutrient requirements of tilapia appointed a digestible protein demand of approximately 30% for individuals weighting 50 g [17]. Results from the current study, as well as the previous studies, also demonstrated a reduction in protein consumption along the period, which correlated with the average weight of the group.

On the other hand, another study found a higher protein (45%) and energy (16.74 J/g) demand for this species to obtain maximum performance. However, their conclusions related to energy demand did not consider the variations when considering average growth [18]. An evaluation of energetic metabolism of the same species on growth, concluded that the metabolic energy utilization remains constant even with excess in food availability, pointing energy intake as an important role in food intake for Nile tilapia [19].

In conventional studies of protein and energy requirements, the evaluation of a diet is exclusively related to performance criteria, ignoring the physiological variability and behavioral of the individuals from an experimental group of fish. The protein demand estimative can be easily overestimated

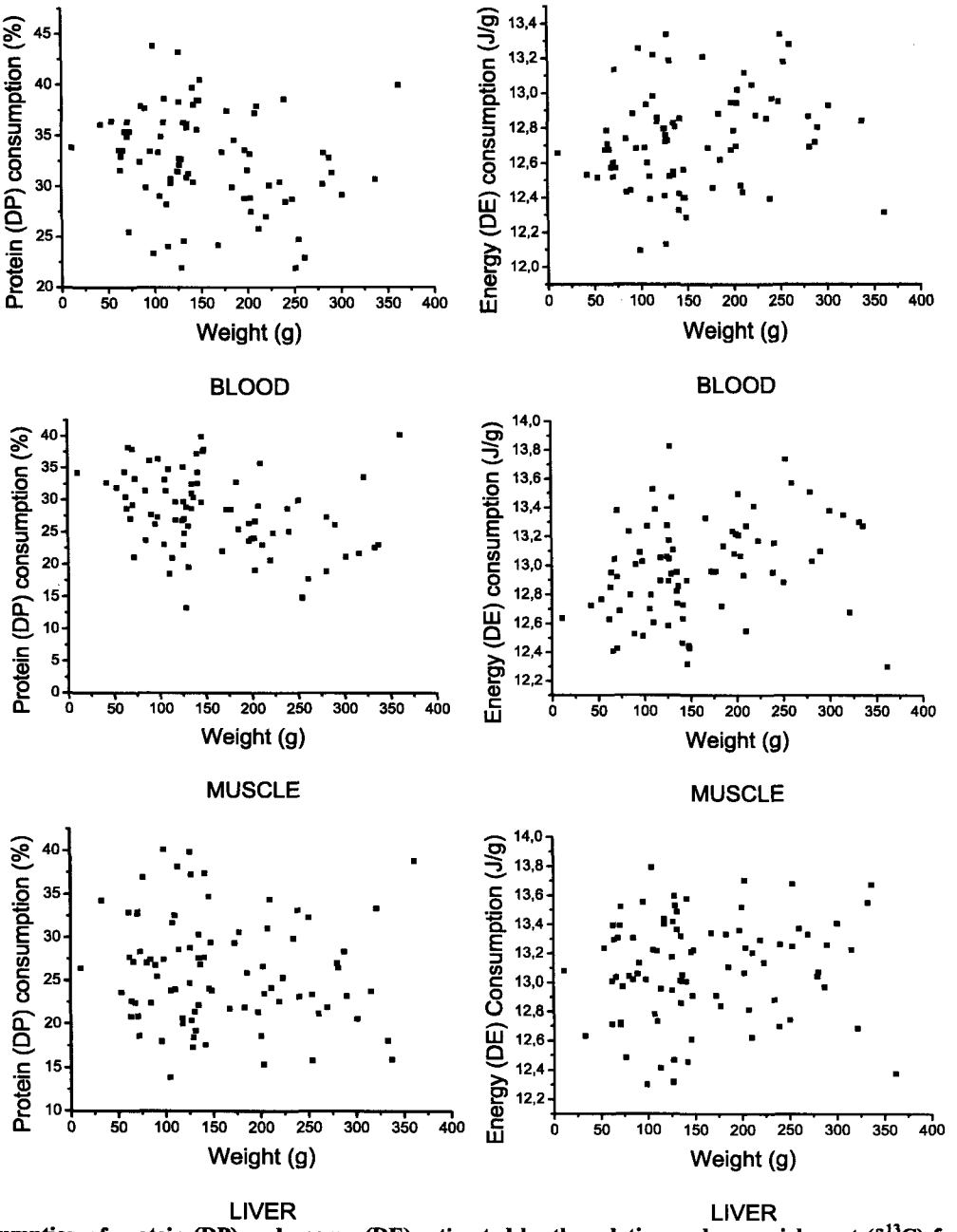

Fig. 3 Consumption of protein (DP) and energy (DE) estimated by the relative carbon enrichment ($\delta^{13}C$) from the blood, muscle and liver of Nile tilapia related individual body weight.

due to the ability of fish to use protein as an energy source, when compared to mammals and birds [1]. Additionally, to obtain optimal quantities of nutrients in the experimental feed, the level of inclusion needs to be pre-established, limiting the results to the level of inclusion closer to an ideal level, but only among the treatments tested.

Several studies [3, 4, 6] evaluated the ability of different species to regulate their food consumption and concluded that those species are able to do so, based on the protein and energy of the food items. Another study affirmed that sea bass was able to balance its diet when carbohydrates, protein and lipids were offered separately; and this ability arises from

the association of sensorial information and post-digestion consequences [5]. In the present study, the results indicated that Nile tilapia was also able to make this regulation, but some factors contributed to a high individual variation on the choice of HPM and LPM. The variation is most likely due to the interactions of dominance and territoriality among the individuals, typical behavioral characteristics of this species.

Previous studies [20, 21] also concluded that tilapia was able to regulate their protein consumption through a free choice of food items system using self feeders. Their results indicated that this species adjust its consumption to a 24% and 45.2% protein level, respectively. These levels are different from the results of the present study and protein requirement values found in conventional protein demand essays. Additionally, those results represented only an average of feed consumption; therefore, it was not possible to observe differences on intake among the individuals from the experimental group in both previous studies.

In trials to evaluate nutritional demand from pre-determined values, the objective is to pursue the best performance of the individuals when fed with different quantities of a particular nutrient. In the present study, fishes were under a free-choice alimentary regime, where they regulated their ingestion of energy and protein. However, their intake regulations were not necessarily to obtain a better growth rate, despite their compatible performance when compared to conventional feeding strategies.

Territorialism and interactions of dominance and submission are typical characteristics of cichlids, the group in which tilapia is included [20]. This author also stated that the animals of this group of fish are more aggressive in situations of intermediary availability of food. In environments with food restriction, or food excess, intra-specific conflicts are reduced. Intermediary amount of feed offer is a condition to obtain optimized feeding procedures, when captive fish is raised for economical purposes. It leads to more significant competition among the individuals of a group. Additionally, little is known about individual variability regarding the metabolic mechanisms of fish, making average consumption data from a group easier to be interpreted when evaluating fish intake though free-choice alimentary systems.

As the fish from this study needed to choose between the mixes to obtain a balanced diet, the results lead to believe that the individuals of tilapia do not present the same ability to properly choose food items at confined environments. Therefore, these choices had a significant influence on behavioral characteristics principally territorialism and competition for food resources. The results of this study indicate that Nile tilapia had the ability to obtain a complete diet that complete the requirements for satisfactory growth, but there is a significant variability among the individuals. Consequently, this is a significant variability in performance.

In previous free-choice studies performed with other species [3, 5-7] and Nile tilapia [20, 21], the intake estimations were based on mean values of apparent consumption observed through demand feeders. These authors found that the fish were able to self-regulate their consumption of protein and energy and obtain a diet optimized for growth and health conditions.

The results of this study did not conflict with the previously mentioned ones, however, current observations indicate that not all individuals of a confined group of fish have the same skills or opportunity to obtain a complete diet when choosing between food items is necessary. So, the estimative of protein and energy intake from a Nile tilapia group through carbon stable isotopes presented similar results when compared to other techniques, however, this method allowed observations of behavioral diversity among individuals of a particular group.

Additionally, it was also possible to observe that

protein and energy consumption when related to animal weight, as opposed to when related to age, did not present any tendency or pattern. These results conflict with nutrient previous demand essays [16, 22-24] in which the results of protein and energy requirement were linked to fish weight. According to current results, protein and energy intake was mainly related to age and it is not clear if this is also true when related to weight.

5. Conclusions

Nile tilapia is able to balance its own energy and protein intake but this ability is not uniform in a group of confined fish.

Protein and energy intake is more related to the age of the fish than to its weight.

Acknowledgments

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