Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
https://periodicos.ufersa.edu.br/index.php/caatinga

ISSN 0100-316X (impresso)
ISSN 1983-2125 (online)
http://dx.doi.org/10.1590/1983-21252019v32n124rc

METHIONINE PLUS CYSTINE TO LYSINE RATIO IN DIETS FOR TAMBAQUI JUVENILES[1]

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ABSTRACT – The objective of this study was to determine the appropriate ratio of methionine plus cystine to lysine in rations for tambaqui (*Colossoma macropomum*) juveniles, with initial weights of 0.28 ± 0.08 g and 0.94 ± 0.33 g. A randomized block design consisting of six treatments, five replicates in two blocks, and 20 fish per replicate was implemented. Six isolisinic (1.45%), isoenergetic, isophosphoric, and isocalcic diets, containing different ratios of methionine plus cystine to digestible lysine (50%, 55%, 60%, 65%, 70%, and 75%) were used. Performance and feed efficiency variables, including body composition (moisture, protein, and fat), daily protein deposition rate, daily fat deposition rate, and nitrogen retention efficiency, were evaluated. The specific growth rate and feed conversion presented quadratic effects, with maximum effects from the digestible methionine plus cystine to lysine ratio of 64.4% and 64.8%, respectively. The increase in digestible methionine plus cystine intake resulted in a linear reduction in body fat content with the elevation of the digestible methionine plus cystine to lysine ratio. The digestible methionine plus cystine to lysine ratio recommended in rations for tambaqui fingerlings is 64.8%.

Keywords: Industrial amino acids. Protein nutrition. Ideal protein.

RELAÇÃO DA METIONINA MAIS CISTINA COM A LISINA EM RAÇÕES PARA JUVENIS DE TAMBAQUI

RESUMO – Objetivou-se determinar a relação da metionina mais cistina com a lisina em rações para juvenis de tambaqui (*Colossoma macropomum*). Utilizou-se 600 juvenis de tambaqui com pesos iniciais de 0,28 ± 0,08 g e 0,94 ± 0,33 g em delineamento em blocos ao acaso, com seis tratamentos, cinco repetições em dois blocos, e vinte peixes por repetição, durante 45 dias. Os tratamentos foram constituídos de seis rações isolisínicas digestíveis (1,45%), isoenergéticas, isofosfóricas e isocálcicas, contendo diferentes relações de metionina mais cistina com a lisina digestível (50%, 55%, 60%, 65%, 70% e 75%). Avaliaram-se variáveis de desempenho e eficiência alimentar, composição corporal (umidade, proteína e gordura), deposições diárias de proteína e gordura corporais e a eficiência de retenção de nitrogênio. A taxa de crescimento específico e a conversão alimentar apresentaram efeitos quadráticos, com efeitos máximos na relação metionina mais cistina:lisina digestível de 64,4% e 64,8%, respectivamente. O aumento no consumo de metionina mais cistina digestível proporcionou redução linear no teor de gordura corporal com a elevação da relação metionina mais cistina:lisina digestível. As demais variáveis não foram influenciadas pelos tratamentos. A relação metionina mais cistina com a lisina digestível recomendada nas rações para juvenis de tambaqui é de 64,8%.

Palavras-Chave: Aminoácidos industriais. Nutrição proteica. Proteína ideal.

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[1]Received for publication in 11/29/2017; accepted in 01/24/2019.
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INTRODUCTION

The tambaqui (*Colossoma macropomum*) is a species native to the Amazon and Orinoco rivers. It is an important species in pisciculture due to its fast growth, omnivorous alimentary habit, acceptance of artificial feeding, and adaptation to captivity (MEDONÇA et al., 2012). However, there is a lack of information on their nutritional requirements (DAIRIKI; SILVA, 2011; GUIMARÃES; MARTINS,2015).

The determination of the requirements for each amino acid is necessary for the formulation of minimum cost rations, which favor a greater efficiency of protein retention, productivity, and support capacity of the aquaculture production systems (MARCOULI; ALEXIS; ANDRIOPOULOU, 2006).

Methionine is considered the first diet limiting amino acid, based on soybean meal and corn (FURUYA et al., 2001; FURUYA et al., 2004; BOMFIM et al., 2008b; ROSTAGNO et al., 2017). Methionine is a sulfur amino acid, essential for the growth of fish, which initiates protein synthesis and has important physiological functions (TESHIMA et al., 2002; ALAM et al., 2005). The use of methionine deficient diets may result in a low survival rate. Additionally, common side effects of methionine deficiency are the appearance of cataracts, reduction in dietary efficiency, reduction in weight gain, and a higher propensity to fungi attack (KELLY et al., 2006; TANG et al., 2009).

Methionine supplementation may improve hepatosomatic activity, as well as intestinal glutamyltransferase and creatinase enzymes (ESPE et al., 2008; TANG et al., 2009). However, the determination of methionine levels should be linked to those of cystine (methionine plus cystine), a non-essential amino acid, which, at suboptimal levels, is synthesized from methionine (LEWIS, 2003; BOMFIM et al., 2008b).

In the concept of ideal protein, the other essential amino acids are related as a function of lysine. Thus, recommendations have been made for experiments aiming to determine the ratio of a certain essential amino acid to lysine. For lysine, levels should be fixed at values considered suboptimal, to ensure that it is the second limiting amino acid of the basal diet. The other essential amino acids should be in amounts, or amino acid:lysine ratios, higher than the requirement values reported in the literature (BOMFIM et al., 2008a; BOMFIM et al., 2008b).

Considering the lack of information on the requirement of methionine plus cystine in the diets of tambaqui juveniles, based on the ideal protein concept, the present study was carried out to determine the ratio of methionine plus cystine to lysine in rations for tambaqui juveniles.

MATERIAL AND METHODS

The experiment was conducted according to the ethical standards of animal research, after being approved by the Ethics Committee on Animal Use of the Federal University of Maranhão (Protocolo Nº: 23115004063/2012-95). The experimental period lasted for a duration of 45 days at the Aquatic Organism Food and Nutrition Laboratory in the Agricultural and Environmental Sciences Center at the Federal University of Maranhão (UFMA), in Chapadinha, Maranhão.

We used 600 juvenile tambaqui of two different initial weights, 0.28 ± 0.08 g L^{-1} and 0.94 ± 0.33 g L^{-1}, in an experiment with a completely randomized block design (criterion as a function of the initial mean weight), composed of six treatments, with five replicates in two blocks, and 20 fish per replicate.

The treatments were composed of a basal ration supplemented with six levels of DL-methionine, resulting in six isonutritional experimental rations containing different ratios of methionine plus cystine (lysine ratios of 50%, 55%, 60%, 65%, 70%, and 75%). The digestible lysine content used in the experimental rations was fixed at 1.45%, which is considered suboptimal in relation to the recommended minimum level (1.73%) in the rations for tambaqui at this stage of life (SILVA, 2016), according to the methods recommended by Boisen (2003). The threonine:lysine and tryptophan:lysine ratios, as well as the other essential amino acids with lysine, were maintained at least three points higher than those estimated by Takishita (2012) and Furuya et al. (2010), respectively (Tables 1 and 2).

Table 1. Composition of the experimental rations (natural matter).

Ingredients (%)	Methionine ratio plus cystine:lysine digestible (%)					
	50	55	60	65	70	75
Soybean meal (45%)	50.08	50.08	50.08	50.08	50.08	50.08
Corn	30.16	30.16	30.16	30.16	30.16	30.16
Wheat bran	8.92	8.92	8.92	8.92	8.92	8.92
Corn starch	0.18	0.14	0.11	0.07	0.04	0.00
Soy oil	5.63	5.60	5.56	5.52	5.48	5.44
L – Lysine – HCl	0.19	0.19	0.19	0.19	0.19	0.19
DL – Methionine	0.000	0.07	0.15	0.22	0.30	0.37
L – Threonine	0.42	0.42	0.42	0.42	0.42	0.42
Dicalcium phosphate	3.36	3.36	3.36	3.36	3.36	3.36
Vitamin and mineral premix [1]	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin C [2]	0.05	0.05	0.05	0.05	0.05	0.05
Antioxidant (BHT)	0.02	0.02	0.02	0.02	0.02	0.02

[1]Vitamin and mineral supplement (5 kg t^{-1}), with guarantee levels per kilogram of product: Vit. A, 1,200,000 UI; Vit. D_3, 200,000 UI; Vit. E, 1,200 mg; Vit. K_3, 2,400 mg; Vit. B_1, 4,800 mg; Vit. B_2, 4,800 mg; Vit. B_6, 4,800 mg; Vit. B_{12}, 4,800 mg; Vit. C, 48 g; folic acid, 1,200 mg; Ca pantothenate, 12,000 mg; biotin, 48 mg; choline chloride, 108 g; niacin, 24,000 mg; Fe, 50,000 mg; Cu, 3,000 mg; Mn, 20,000 mg; Zn, 30,000 mg; I, 100 mg; Co, 10 mg; Se, 100 mg. [2] Vit. C: Calcium L-ascorbic acid 2-monophosphate, 42% of active principle.

The juveniles were kept in 30 polyethylene boxes of 1,000 L, arranged in a closed water circulation system and equipped with individual supply, drainage, and aeration systems. The supply water from the aquariums came from an artesian well. The temperature of the water was measured daily at 07h30 and 05h30, with the help of a mercury bulb thermometer, and graduated from 0 to 50 °C. The pH, dissolved oxygen, and ammonia in the water were monitored every seven days using a pH-meter, oximeter, and commercial colorimetric kit to test for toxic ammonia, respectively.

Table 2. Nutritional composition of the experimental rations (natural matter)[1].

Crude protein (%)	26.90	26.94	26.99	27.03	27.07	27.12
Digestible protein (%) [3]	24.43	24.47	24.51	24.56	24.60	24.64
Digestible energy (kcal kg^{-1}) [3]	3000.0	3000.0	3000.0	3000.0	3000.0	3000.0
Ether extract (%)	7.86	7.82	7.78	7.74	7.70	7.67
Crude fiber (%)	3.98	3.98	3.98	3.98	3.98	3.98
Total calcium (%)	0.96	0.96	0.96	0.96	0.96	0.96
Phosphorus available (%) [2]	0.70	0.70	0.70	0.70	0.70	0.70
Total lysine (%)	1.67	1.67	1.67	1.67	1.67	1.67
Digestible lysine (%) [2]	1.45	1.45	1.45	1.45	1.45	1.45
Total Met. + Cyst. (%)	0.79	0.87	0.94	1.01	1.09	1.16
Digestible Met. + Cyst. (%) [2]	0.73	0.80	0.87	0.94	1.06	1.09
Digestible threonine (%) [2]	1.32	1.32	1.32	1.32	1.32	1.32
Digestible tryptophan (%) [2]	0.33	0.33	0.33	0.33	0.33	0.33
Ratio Met. + Cyst./Lysine Digest.	50.0	55.0	60.0	65.0	70.0	75.0

[1]Based on the values proposed by Rostagno et al. (2011). [2] Industrial amino acid digestibility coefficients proposed by Rostagno et al. (2011) and the amino acids and availability of phosphorus of corn, soybean meal, and wheat bran proposed by Furuya et al. (2010). [3] Digestibility coefficients proposed by Furuya et al. (2010) for the Nile tilapia.

The ingredients of the experimental rations were mixed, moistened in water, heated to about 50 ° C, and pelletized using a meat grinder (C.A.F. Meat grinder; model 98 STI). Then, the rations were dried, crushed, and sieved. The final pellets varied from 2 to 4 mm in diameter.

The rations were offered daily in six meals (08h00, 10h00, 12h00, 14h00, 16h00, and 18h00). At every meal, the rations were offered in small quantities, with successive passes to apparent satiation, thus avoiding the over- or underdelivering of feed. The cleaning of the boxes was done daily by siphoning, always after the measuring of the water temperature (07h30 and 17h30).

All fish were weighed at the beginning and end of the experiment. At the beginning of the experiment, 50 fish were stunned and euthanized in water with ice, then frozen for further determination of the initial body composition. At the end of the experiment, after 24 h of fasting, all fish from each experimental unit were stunned, euthanized, weighed, and frozen to determine the final body composition.

After freezing, the fish (carcasses and viscera) were oven dried, pre-degreased, ground in the ball mill, and packed in laboratory containers. The samples were analyzed for their body composition (moisture - MC, protein - BP, and body fat - BF contents) according to the procedures described by Detmann et al. (2012). The laboratory analyses were carried out at the Animal Nutrition Laboratory of the Federal University of Maranhão - UFMA and at the Laboratory of Food Analysis of the Brazilian Agricultural Research Corporation (Embrapa Meio-Norte).

The following variables were evaluated: weight gain (WG), specific growth rate (SGR), feed consumption (FC), consumption of digestible methionine plus cystine (CDMC), feed conversion (FC), protein efficiency for weight gain (PEG), rate of daily body protein (BPD), rate of daily fat deposition (BFD), chemical body composition (body moisture - BM, protein - BP, and body fat - BF contents), and nitrogen retention efficiency (NRE). The variables of performance, body deposition, and food efficiency were calculated according to the following equations:

FC (g) = Feed consumed during the experimental period

WG (g) = Final mean weight - initial mean weight

$$\text{FC}\left(\text{g g}^{-1}\right) = \frac{\text{Consumption of feed (g)}}{\text{Weight gain (g)}}$$

$$\text{SGR}\left(\text{\% day}^{-1}\right) = \frac{[(\text{Natural logarithm of final weight (g)} - \text{natural logarithm of initial weight (g)}) \times 100]}{\text{Experimental period (days)}}$$

$$\text{CDMC (mg)} = \frac{[\text{Consumption of feed (mg)} \times \text{content of digestible methionine plus cystine in the ration (\%)}]}{100}$$

$$\text{PEG}\left(\text{g g}^{-1}\right) = \frac{\text{Weight gain (g)}}{\text{Consumption of crude protein (g)}}$$

$$\text{BPD}\left(\text{mg day}^{-1}\right) = \frac{\{[(\text{\% Final body protein, \%} \times \text{final weight, mg}) - (\text{initial body protein, \%} \times \text{initial weight, mg})] / 100\}}{\text{Experimental period (days)}}$$

$$\text{BFD}\left(\text{mg day}^{-1}\right) = \frac{\{[(\text{Final body fat, \%} \times \text{final weight, mg}) - (\text{initial body fat, \% x initial weight, mg})] / 100\}}{\text{Experimental period (days)}}$$

$$\text{NRE (\%)} = \frac{\{[\text{Final body N(\%)} \times \text{final weight (g)}] - [\text{Initial body N(\%)} \times \text{initial weight (g)}] \times 100\}}{[(\text{Feed consumption, g}) \times (\text{\% crude protein of the ration} / 6.25)]}$$

Statistical analyses were performed using SAEG 9.1 (SAEG, 2007). The data were interpreted through analysis of variance. The effects of the methionine plus cystine:lysine ratios were analyzed using linear or quadratic regression models, based on the decomposition of the treatments into orthogonal polynomials ($P < 0.05$). It was also verified whether there was adjustment for the discontinuous model "Linear Response Plateau" (LRP). The model of best fit was chosen considering the P value (significance) and the coefficient of determination (R^2) (model sum of squares/sum of treatment squares).

RESULTS AND DISCUSSION

The water quality parameters were kept within the recommended standards for the species, according to known values (GOMES et al., 2013; MENDONÇA et al., 2012). The maximum and minimum water temperatures remained at 28.0 ± 0.38 ° C and 26.2 ± 0.57 °C, respectively. The concentration of dissolved oxygen in the water was 8.96 ± 0.84 mg L^{-1}, pH was 7.1 ± 0.13, and total ammonia was ≤1.00 mg L^{-1}.

For the performance and food efficiency variables, it was verified that only the weight gain, feed consumption, and protein efficiency for the weight gain of the fish did not vary ($P > 0.05$) as a function of the elevation of the ratio of digestible methionine plus cystine to lysine. Considering that the rations contained different levels of digestible methionine plus cystine, the consumption of digestible sulfur amino acids increased linearly ($P < 0.05$) with elevated dietary levels (Tables 3 and 4).

Table 3. Zootechnical performance variables of tambaqui juveniles: feed consumption (FC), consumption of digestible methionine plus cystine (CDMC), weight gain (WG), specific growth rate (SGR), feed conversion (FC), and protein efficiency for weight gain (PEG). Summary of variance analysis, as a function of the digestible methionine plus cystine:lysine ratio of the rations.

Methionine ratio plus cystine to lysine (%)	Variable					
	FC (g)	CDMC (mg)	WG (g)	SGR (% day^{-1})	FC (g g^{-1})	PEG (g g^{-1})
50.0	9.19	66.65	6.91	4.57	3.59	1.98
55.0	8.25	65.77	6.86	4.70	2.59	2.20
60.0	10.13	88.16	8.99	5.59	1.97	2.58
65.0	8.62	81.15	9.06	5.92	1.30	2.30
70.0	9.86	100.11	8.02	5.11	2.70	2.25
75.0	8.92	97.01	8.05	5.03	2.35	2.38
P>F linear	1.000	0.001	0.184	0.121	0.075	0.150
P>F quadratic	1.000	1.000	0.110	0.006	0.007	0.118
CV (%)	13.24	17.00	22.64	12.40	38.64	14.21

CV - Coefficient of variation; *P>F* - Significance of the "F" test for the analysis of variance.

The feed consumption, as well as the nutrient and energy utilization, did not vary among treatments, except for the digestible methionine plus cystine consumption, which increased linearly with the elevation of the ratio of methionine plus cystine to lysine in the rations. The results indicate that the significant effects observed in the other variables were only caused by the differences in the consumption of digestible methionine plus cystine, since this is the only nutritional variation in all the experimental rations. The non-variation in the feed intake may be associated with the fact that diets are isoenergetic, since the energetic level of the diet may limit the amount the fish consumed (NRC, 2011).

The specific growth rate and feed conversion of the fish increased in a quadratic manner ($P < 0.01$ and $P < 0.01$, respectively) with the elevation of methionine plus digestible cystine levels. This improved the feed efficiency until the estimated digestible methionine plus cystine:lysine ratio of 64.4% and 64.8%, corresponding to dietary levels of 0.934% and 0.939% of digestible methionine plus cystine, respectively (Tables 3 and 4, Figure 1).

Table 4. Adjusted regression equations, determination coefficients, and requirement values for the variables of the specific growth rate (SGR), consumption of digestible methionine plus cystine (CDMC), and feed conversion (FC) of tambaqui juveniles as a function of the ratio of digestible methionine plus cystine:lysine (R) of the rations.

Variable	Model	Equation	R^2	Requeriment
SGR (% day^{-1})	Quadratic	$SGR = – 17.797 + 0.7256R – 0.0056R^2$	0.71	64.4
CDMC (mg)	Linear	$MCDC = – 5.3675 + 1.4161R$	0.81	-----
FC ($g\ g^{-1}$)	Quadratic	$FC = 35.758 – 1.0486R + 0.0081R^2$	0.73	64.8

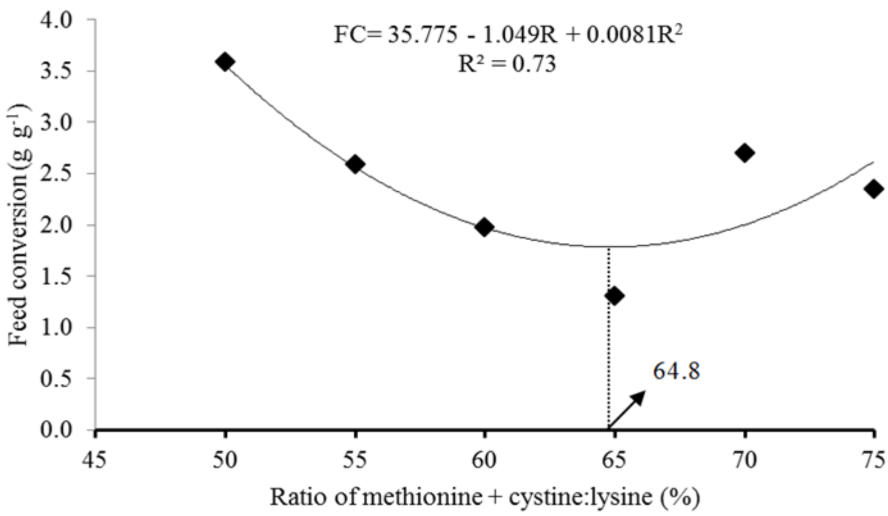

Figure 1. Graphical representation of the feed conversion of tambaqui juveniles, as a function of the methionine plus digestible cystine:lysine ratio (R) of the rations.

Although the weight gain did not change ($P > 0.05$), a 31% increase was observed in the diet containing the ratio of methionine plus cystine to digestible lysine of 65.0% (0.942% of sulfur amino acids), in relation to the basal diet. This value is similar to the estimated level that provided the best specific growth rate (0.934%), indicating that nutrient levels below this value would limit the deposition of body protein. The fact that no statistical variation was detected is probably due to the coefficient of variation (22.64%) obtained for this variable.

Similar results were observed by Gonçalves Junior (2015), when evaluating the effects of five digestible isolisinic rations (1.45%) containing different levels of digestible methionine plus cystine (0.66% to 0.94%), in tambaquis in the fingerling stage, who found that there was no effect of treatments on the consumption of feed, but the consumption of methionine plus cystine, weight gain, and specific growth rate all increased linearly. In this sense, they concluded that the highest level assessed (0.94%), similar to that estimated to optimize the specific growth rate in this study (0.934%), would be appropriate to meet the nutritional needs of the species.

The increase in the fish growth rate associated with a similar consumption of feed, resulted in improved feed conversion, indicating that there was a greater efficiency of protein utilization for the formation of lean tissue (deposition of corporal protein). This hypothesis is based on the fact that the deposition of lean tissue is more energy efficient than the deposition of fat, because it uses a higher percentage of water in its composition (BOMFIM et al., 2010).

The treatments did not influence ($P > 0.05$) the levels of body protein or moisture. However, the body fat content of the fish decreased linearly ($P < 0.05$) with the increase of the digestible methionine plus cystine:lysine ratio. In relation to the body fat deposition, body protein deposition, and nitrogen retention efficiency, no effects ($P > 0.05$) of the treatments were observed (Tables 5 and 6).

The linear reduction in the body fat contents with increasing the digestible methionine plus cystine to lysine ratio, can be explained by the amino acid balance in the diets that favored the deposition of lean tissue, which consequently reduced the energy available for the deposition of body fat (BOMFIM et al., 2008a; QUADROS et al., 2009; BOMFIM et al., 2010).

Table 5. Composition of the carcasses of tambaqui juveniles: body moisture content (BM), fat content (BF), protein content (BP), deposition of body protein (DBP), deposition of fat (DBF), and nitrogen retention efficiency (NRE). A summary of the analysis of variance, as a function of the digestible methionine plus cystine:lysine ratio of the rations.

Methionine ratio plus cystine to lysine (%)	Variable					
	BM (%)	BF (%)	BP (%)	DBF (mg day^{-1})	DBP (mg day^{-1})	NRE (%)
50.0	79.42	1.32	15.84	3.69	24.20	30.91
55.0	78.56	1.24	16.65	3.35	24.24	35.07
60.0	79.01	1.28	16.47	4.10	31.40	40.76
65.0	82.72	1.09	16.52	3.47	29.57	37.60
70.0	79.78	1.05	15.40	2.98	27.41	34.04
75.0	79.55	1.12	15.70	3.58	28.43	36.75
P>F linear	0.219	0.028	0.225	1.000	0.198	0.272
P>F quadratic	0.200	1.000	0.112	1.000	0.187	0.060
CV (%)	1.13	16.95	5.77	37.60	21.08	15.21

CV - Coefficient of variation; *P>F* - Significance of the "F" test for the analysis of variance.

Table 6. Adjusted regression equations, coefficients of determination, and requirement values for the body fat content (BF) of tambaqui juveniles as a function of the digestible methionine plus cystine:lysine ratio (R).

Variable	Model	Equation	R^2	Requeriment
BF (%)	Linear	BF = 1.8187 – 0.0101R	0.73	-----

In relation to the tissue deposition, it was expected that there would be an improvement in at least one of the variables (fat or body protein) and the nitrogen retention efficiency, due to the statistical variation for the specific growth rate as a function of the amino acid relations evaluated in this study. This may have occurred due to the corresponding coefficient of variation (CV%) obtained for these variables. There was an improvement of 26.5% in the fish fed with rations containing 0.870% and 0.942% of sulfur amino acids (digestible methionine plus cystine:lysine ratios of 60% and 65%, respectively) in relation to that obtained by fish fed with the basal diet (digestible methionine plus cystine:lysine ratio of 50%).

Considering the minimum levels of total and digestible lysine recommended for tambaqui juveniles of 1.95% and 1.73% (SILVA, 2016), the total and digestible sulfur amino acid levels of 1.18% and 1.12%, estimated based on the digestible methionine plus cystine:lysine ratios of 60.5% and 64.8%, can be recommended in the rations for this life stage, respectively.

In this study, the recommended ratio of methionine plus cystine:digestible lysine of 64.8% is slightly higher than those recommended for the Nile tilapia by Furuya et al. (2001), Bomfim et al. (2008), and Quadros et al. (2009), which were 61%, 59.5%, and 60%, respectively.

CONCLUSION

The ratio of digestible methionine plus cystine:lysine in rations for tambaqui juveniles in the weight range of 0.28 g to 9.60 g is 64.8%.

ACKNOWLEDGMENTS

To the Fundação de Amparo à Pesquisa a Desenvolvimento Científico e Tecnológico do Maranhão – FAPEMA, for the financial support, and the Brazilian Agricultural Research Corporation - EMBRAPA, for their collaboration with the analysis.

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