Revista Brasileira de Zootecnia
© 2013 Sociedade Brasileira de Zootecnia
ISSN 1806-9290
www.sbz.org.br

R. Bras. Zootec., v.42, n.1, p.7-12, 2013

Digestible methionine + cystine requirement for Nile tilapia from 550 to 700 g

Mariana Michelato[1,2], Wilson Massamitu Furuya[3], Themis Sakaguti Graciano[2], Luiz Vítor Oliveira Vidal[2], Tadeu Orlandi Xavier[2], Lorena Batista de Moura[2], Valéria Rossetto Barriviera Furuya[3]

[1] *CNPq Scholar.*
[2] *Programa de Pós-graduação em Zootecnia/Universidade Estadual de Maringá.*
[3] *Universidade Estadual de Ponta Grossa, Departamento de Zootecnia.*

ABSTRACT - This trial was conducted to determine the dietary digestible methionine + cystine requirement of Nile tilapia (550 to 700 g) based on the ideal protein concept. Six hundred fish were distributed in a completely randomized design with five treatments and four replicates, with 30 fish per experimental unit. The fish were fed diets containing approximately 262 g of digestible protein/kg, 3,040 kcal of digestible energy/kg and 7.90, 9.40, 10.90, 12.40 or 13.90 g of methionine + cystine/kg. The fish were hand-fed three times a day until apparent satiation for 30 days. No effects of dietary methionine + cystine on feed conversion ratio, daily protein deposition, whole body moisture, fillet moisture, crude protein, ether extract and ash, plasmatic HDL and LDL cholesterol were observed. Dietary methionine resulted in a linear increase in whole body protein and linear reduction in lipid deposition rate, hepatosomatic index, whole body ether extract and ash, plasmatic total cholesterol, plasmatic total lipids and plasmatic triglycerides. According to the Linear Response Plateau, the daily weight gain and fillet yield increased up to a level of 9.00 and 9.90 g methionine + cystine/kg of diet, respectively. The digestible methionine + cystine requirement of Nile tilapia is 9.00 g/kg for weight gain and 9.90 g/kg for fillet yield, corresponding to methionine + cystine:lysine ratios of 0.60 and 0.66, respectively.

Key Words: amino acids, body composition, fillet yield, fish, performance, plasmatic lipid pattern

Introduction

Tilapia is one of the most important freshwater farm-raised fish and has had an increasing role in the global aquatic food trade. The Nile tilapia (*Oreochromis niloticus*) is an economically important freshwater fish in Brazil.

Methionine is an essential amino acid for fish (Lovell, 1989) and it is usually the first limiting amino acid in many fish diets, especially those containing higher levels of soybean meal protein source (Furuya et al., 2004). This amino acid acts as methyl donor (Bender, 2003) precursor of several substrates, including nucleic acids, proteins, phospholipids, biogenic amines (Mato et al., 1997), carnitine (Swenson & Reece, 1996) cystine (Zhou et al., 2006), choline (Swenson & Reece, 1996; Kasper et al., 2000), polyamines and other metabolic intermediates (Bender, 2003). Thus, methionine supplementation is associated with improvements of fish growth and health (Espe et al., 2008; Graciano et al., 2010).

The dietary methionine supplementation has been indicated mainly in diets based on soybean protein, which have methionine as the first limiting amino acid for carnivorous fish like rainbow trout, *Oncorhynchus mykiss* (Gaylord et al., 2007); Atlantic salmon, *Salmo salar* (Hansen et al., 2007); cobia, *Canadum rachycentron* (Chou et al., 2004); and cod, *Gadus morhus* (Hansen et al., 2007), and omnivorous fish like carp, *Carassius auratus* (Hu et al., 2008); Nile tilapia (Santiago & Lovell, 1988; Furuya et al., 2001; Nguyen & Davis, 2009); channel catfish, *Ictalurus punctatus* (Harding et al., 1977; Cai & Burtle, 1996); and pacu, *Piaractus mesopotamicus* (Abimorad et al., 2009).

The dietary methionine requirements have been estimated for several species of fish, ranging from 1.80 to 4.00 g/kg of dietary protein (Wilson, 2002). Because of the interrelation between methionine and cystine, it is more appropriate to show their requirements as methionine plus cystine. The fingerlings of tilapia mossambica (*Oreochromis mossambicus*) require a diet with 10.10 g of methionine + cystine/kg (Jackson & Capper, 1982), whereas Nile tilapia require 9.00 g/kg and 10.00 g/kg according to the recommendations of the NRC (1993) and Furuya et al. (2004), respectively. Based on the ideal protein concept, the methionine + cystine:lysine ratio for Nile tilapia varies from 0.57 to 0.6 (Nguyen & Davis, 2009; Furuya et al., 2001).

Received November 8, 2011 and accepted September 19, 2012.
Corresponding author: marianamichelato@hotmail.com

There is no information about the dietary methionine + cystine requirement for Nile tilapia weighing more than 500 g considering growth, feed efficiency, body composition, economical analysis and fillet yield. Due to the high cost of the feed during the final phase, it is important to determine the methionine + cystine requirements to develop cost-effective diets considering not only the fish growth, but the fillet yield and the economic analysis. The present study was conducted to determine the dietary digestible methionine + cystine requirement of Nile tilapia from 550 to 700 g.

Material and Methods

The experiment was conducted in Rio do Corvo, Diamante do Norte, Paraná State, Brazil, from December 2009 to January 2010, during 30 days. Six hundred (563.40 ±10.50 g) of all-male population of Nile tilapia obtained by hormonal induction during the larval phase were distributed into 20 cages with a unit volume of 1 m^3 (1.00 × 1.00 × 1.20 m high) in a completely randomized design with five treatments and four replicates.

Five diets were formulated to contain approximately 3,040 kcal of digestible energy/kg and 262 g of crude protein/kg and crystalline amino acids were added to keep the amino acid requirement for the Nile tilapia, except for methionine + cystine (NRC, 1993), and 7.90, 9.40, 10.90, 12.40 or 13.90 g of methionine + cystine/kg (Table 1). The values of digestible protein and amino acids, digestible energy and available phosphorus were obtained from Furuya et al. (2001b), Pezzato et al. (2002) and Guimarães et al. (2008a, b), in studies conducted with the Nile tilapia.

The diets were extruded at Universidade do Oeste do Paraná - UNIOESTE, Paraná, Brazil, using a single screw extruder which obtained granules of approximately 5.50 mm of diameter. All ingredients were previously ground in a hammer mill with a sieve of 0.80 mm of diameter.

The fish were hand-fed until apparent satiation three times a day (9h00, 13h00 and 17h00), seven days per week. Each diet was randomly assigned to triplicate cages. Fish were slowly hand-fed little by little until apparent satiation on the basis of visual observation of the fish feeding behavior. During the experiment, the water quality was checked every week. The average values of temperature, dissolved oxygen and pH of the water cages were 27.50±1.20 ºC, 5.70±0.12 mg L^{-1} and 7.37±0.57, respectively. The data obtained are within the range recommended by Sipauba-Tavares (1994) for tropical freshwater fish.

At the beginning of the experimental period, 30 fish were homogenized, ground in an electric meat grinder and

Table 1 - Composition of the experimental diets

Ingredient	Levels of methionine + cystine (g/kg)				
	7.90	9.40	10.90	12.40	13.90
Soybean meal	420.00	420.00	420.00	420.00	420.00
Corn	416.80	417.00	417.30	417.00	416.80
Rice	80.00	80.00	80.00	80.00	80.00
Poultry viscera meal	40.00	40.00	40.00	40.00	40.00
Dicalcium phosphate	20.00	20.00	20.00	20.00	20.00
L-glutamic acid	7.00	5.50	4.00	2.50	1.00
Salt	5.00	5.00	5.00	5.00	5.00
Soybean oil	3.00	2.80	2.50	2.30	2.00
L-lysine	1.00	1.00	1.00	1.00	1.00
L-threonine	1.00	1.00	1.00	1.00	1.00
L-tryptophan	0.50	0.50	0.50	0.50	0.50
DL-methionine	0.00	1.50	3.00	4.50	6.00
Mineral and vitamin mix[1]	5.00	5.00	5.00	5.00	5.00
Vitamin C[2]	0. 50	0. 50	0. 50	0.50	0.50
Antioxidant[3]	0.20	0.20	0.20	0.20	0.20
Antifungic[4]	1.00	1.00	1.00	1.00	1.00
Calculated composition (g/kg, as fed basis)[5]					
Dry matter	877.70	877.80	877.80	877.90	877.90
Digestible energy (kcal/kg)	3041.20	3041.20	3041.20	3041.20	3041.20
Crude protein	262.00	262.00	262.00	262.00	262.00
Ether extract	27.50	27.50	27.50	27.50	27.50
Crude fiber	33.90	33.90	33.90	33.90	33.90
Calcium	8.20	8.20	8.20	8.20	8.20
Available phosphorus	4.50	4.50	4.50	4.50	4.50
Methionine	4.00	5.50	7.00	8.50	10.00
Methionine + cystine	7.90	9.40	10.90	12.40	13.90
Lysine	15.00	15.00	15.00	15.00	15.00
Threonine	11.10	11.10	11.10	11.10	11.10
Tryptophan	3.80	3.80	3.80	3.80	3.80
Arginine	17.80	17.80	17.80	17.80	17.80
Phenylalanine + tyrosine	21.00	21.00	21.00	21.00	21.00
Histidine	6.50	6.50	6.50	6.50	6.50
Isoleucine	11.30	11.30	11.30	11.30	11.30
Leucine	21.60	21.60	21.60	21.60	21.60
Valine	12.20	12.20	12.20	12.20	12.20

[1] Mineral and vitamin supplement (Supre Mais®): composition per kg: vit. A - 1,200.000 IU; vit. D3 - 200,000 IU; vit. E - 12,000 mg; vit. K3 - 2,400 mg; vit. B1 - 4,800 mg; vit. B2 - 4,800 mg; vit. B6 - 4,000 mg; vit. B12 - 4,800 mg; folic acid - 1,200 mg; Capantothenate - 12,000 mg; vit. C - 48,000 mg; biotin - 48 mg; choline - 65;000 mg; niacin - 24,000 mg; Fe - 10,000 mg; Cu - 600 mg; Mg - 4,000 mg; Zn - 6,000 mg; I - 20 mg; Co - 2 mg; Se - 20 mg.
[2] Vitamin C: 3;500 mg/kg.
[3] Banox®. Composition: BHA, BHT, propyl gallate and calcium carbonate (Alltech Agroindustrial Ltda, Brazil).
[4] Mold Zap Aquatica®. Composition: ammonium dipropionate, acetic acid, sorbic acid and benzoic acid (Alltech Agroindustrial Ltda, Brazil).
[5] According to Furuya et al. (2001b), Pezzato et al. (2002) and Guimarães et al. (2008a,b), based on digestible values of amino acids.

store frozen (-20 ºC) of initial whole body composition. At the end of the experimental period, fish were feed-deprived for 24 hours prior to sampling, then anesthetized with clove oil at 300 mg/L (Vidal et al., 2008), counted and weighed to determine survival rate, final body weight, daily weight gain and feed conversion ratio. Eight fish from each cage were randomly collected for determination of the hepatosomatic index and fillet yield. Five fish were collected for determination of the whole body composition and three fish were collected for plasmatic lipid pattern.

Samples of whole fish body and fillets were ground in an electric meat grinder to obtain homogeneous samples.

All samples were dried at 55 °C for 62 hours. The protein deposition ratio and lipid deposition ratio were determined using the methodology described by Fraga (2002).

Blood samples were taken from the caudal vein of three fish per cage. The plasma was obtained by centrifugation at 1200 g for 8 minutes at room temperature. After centrifugation, the plasma was used for analysis of total cholesterol, total lipids, triglycerides, LDL and HDL cholesterol. The levels of plasma triglycerides and cholesterol were determined using colorimetric enzymatic method and photometric enzymatic method, respectively. The HDL and LDL were determined according to Friedewald et al. (1972).

The chemical analyses of diets, whole body and fish fillet samples were determined following the methodology described by Silva & Queiroz (2002). All amino acid analyses of the experimental diets were performed at Ajinomoto Animal Nutrition Laboratory (São Paulo, Brazil).

The statistical analysis was performed through program SAEG (Sistema para Análises Estatísticas e Genéticas, version 8.0). The quantitative dietary methionine + cystine requirement was determined with linear regression, quadratic or discontinuous Linear Response Plateau (LRP) analysis according to the best fit obtained for each variable, based on the significance of regression coefficients for the *F* test, the coefficient of determination, the sum of squared deviation and the phenomenon under study.

Results and Discussion

The levels of digestible methionine + cystine did not affect (P>0.05) the feed conversion ratio and protein deposition rate of fish, but they affected the daily lipid deposition and hepatosomatic index, which decreased linearly, and fillet yield, which increased linearly. The regression analysis of daily weight gain and fillet yield data using the Linear Response Plateau model indicated that daily weight gain and fillet yield increased up to 9.00 and 9.90 g of methionine + cystine/kg, respectively (Table 2).

The digestible methionine + cystine influenced (P>0.05) daily weight gain in the fish of the present study, close to the values obtained by Santiago & Lovell (1988) and Furuya et al. (2001a) for Nile tilapia, of 9.00 and 10.50 g methionine + cystine/kg, respectively. On the other hand, Jackson & Capper (1982) determined the requirement of 12.70 g of methionine + cystine/kg for tilapia mossambica (*Oreochromis mossambicus*) for maximum weight gain. Likewise, values higher than those found in this study were obtained by Burtle & Cai (1995) and Schwarz et al. (1998) for channel catfish (13.40 g/kg) and common carp (12.40 g/kg).

The proportion of methionine:cystine can also affect the weight gain of fish, as described by Nguyen & Davies (2009), who observed the best weight gain of Nile tilapia fed diets containing 0.50:0.50, 0.60:0.40 or 0.70:0.30 of methionine:cystine ratios. In the present study, the proportion of methionine to cystine estimated for weight gain and fillet yield production were approximately 0.60:0.40 and 0.70:0.30, respectively.

The result obtained for the feed conversion ratio differs from those found by several authors, who determined the effects of dietary methionine + cystine on feed conversion for juvenile rainbow trout (Kim et al., 1992), channel catfish (Burtle & Cai, 1995), yellow perch (Twibell et al., 2000) and Nile tilapia (Furuya et al., 2004). Discrepancies among values also may be due to differences in basal diet composition, size or age, genetic differences, feeding regime and culture conditions of the fish.

There was no effect (P>0.05) of the dietary digestible methionine + cystine levels on the protein deposition

Table 2 - Performance of Nile tilapia fed diets with increasing levels of digestible methionine + cystine

Variable	Methionine + cystine (g/kg)					CV	Effect
	7.90	9.40	10.90	12.40	13.90		
Daily weight gain (g/fish)	4.41	5.21	4.95	4.67	5.19	15.41	LRP
Feed conversion ratio (g/g)	1.65	1.64	1.49	1.44	1.43	13.35	NS
Protein deposition ratio (mg/day)	625.25	808.56	808.56	611.65	936.48	14.54	NS
Lipid deposition ratio (mg/day)	725.25	714.62	628.09	649.29	440.48	19.80	Linear
Fillet yield (g/100 g of BW)	34.97	37.58	38.01	39.45	39.38	7.83	LRP
Hepatosomatic index	1.89	1.90	1.77	1.78	1.70	8.37	Linear

	Equation	P value
Daily weight gain	$Y = 1.630 + 0.363X$, $R^2 = 0.78$; met + cys = 9 g/kg; daily weight gain = 4.90 g	0.023
Fillet yield	$Y = 11.835 + 2.739X$, $R^2 = 0.89$, met + cys = 9.9 g/kg; fillet yield = 38.95 g/100 g of body weight	0.018
Lipid deposition rate	$Y = 1092.000 – 42.320X$, $R^2 = 0.77$	0.024
Hepatosomatic index	$Y = 2.171 – 0.0337 X$, $R^2 = 0.87$	0.020

Met + cys = methionine + cystine; CV = coefficient of variation; BW - body weight; NS = not significant; LRP = Linear Response Plateau.
Linear effect (P<0.05).

rate. On the contrary, Burtle & Cai (1995) estimated the requirement of 9.90 g/kg of methionine + cystine (5.80 g/kg of methionine) in the diet for maximal utilization of the dietary protein for channel catfish, while Kim et al. (1992), determined the requirement of 7.80 g/kg for maximum utilization of the dietary protein by rainbow trout. The differences in the results compared with this study may be related to the levels of protein, energy and amino acids balance, since the previous studies did not consider the balance of energy:protein and essential amino acids:lysine ratios.

There was no effect (P>0.05) of the dietary digestible methionine + cystine levels on fillet composition. The levels of digestible methionine + cystine affected (P<0.05) fillet yield, which increased up to 9.90 g of methionine + cystine /kg.

The higher fillet yield may be related to a lower hepatosomatic index value with increasing levels of dietary methionine + cystine (Table 2), in addition to a higher protein and lower ether extract and ash depositions in the whole body (Table 3).

It is important to consider that the requirements of methionine + cystine also have to be assessed separately in fish diets, as well as other non-ruminants that require a minimum of dietary methionine, considering that methionine can not be obtained from cystine (trans-sulfidation). Thus, based on the results of this study, a minimum of 5.30 and 5.70 g/kg of methionine is required for weight gain and fillet yield, respectively, in diets for the Nile tilapia. In this study, the highest levels of protein in the whole body are in accordance with the results obtained by Yan et al. (2007). However, the authors did not observe reduction in the whole body fat.

The highest concentration of protein in the whole body, in addition to lower levels of fat, are probably related to the functions of methionine on the synthesis of body protein and lipid metabolism. Methionine is an essential amino acid for normal growth of fish, because it participates in the protein synthesis and other metabolic functions (Teshima et al., 2002; Alam et al., 2001).

The increasing levels of methionine + cystine did not affect (P>0.05) the serum HDL and LDL cholesterol levels, but resulted in a linear reduction (P<0.05) in the values of total cholesterol, total lipids and serum triglycerides (Table 4).

The reduction of the total cholesterol, total lipids and plasma triglycerides may be related to the different functions of methionine. Methionine affects the profile of plasma amino acids in many species of fish (Twibell et al., 2000; Mai et al., 2006) as well as the metabolism of sulfur amino acids (Espe et al., 2008). The S-adenosylmethionine, synthesized from methionine, originates compounds such as carnitine (Swenson & Reece, 1996) and choline (Swenson & Reece, 1996; Kasper et al., 2000). Choline also acts as a lipotropic factor, enhances the synthesis of lipoproteins and prevents fatty liver (Swenson & Reece, 1996), which may explain the hepatosomatic index reduction of fish fed adequate levels of methionine + cystine (Table 2).

The choline molecule has three methyl groups (-CH3), and reacts with acetyl coenzyme A, acting as a precursor of acetylcholine, a neurotransmitter (Zeisel, 2000) and phosphatidylcholine, which is a structural component of cell membranes acting also in the metabolism and transport of lipid-cholesterol (Bender, 2003). It also acts as a lipotropic factor, and improves the synthesis of lipoproteins

Table 3 - Whole body and fillet compositions of the Nile tilapia fed diets containing increasing levels of digestible methionine + cystine

Variable	Methionine + cystine (g/kg)					CV	Effect
	7.90	9.40	10.90	12.40	13.90		
	Whole body (g/100 g of wet weight)						
Moisture	65.28	64.87	65.37	63.80	65.33	1.08	NS
Crude protein	14.60	14.89	15.06	15.06	15.11	3.98	Linear
Ether extract	12.11	12.21	11.98	11.85	11.08	5.67	Linear
Ash	3.76	3.73	3.64	3.53	3.50	8.12	Linear
	Fillets (g/100 g of wet weight)						
Moisture	76.56	75.25	75.83	75.56	75.81	0.59	NS
Crude protein	17.16	17.80	17.73	17.88	17.76	2.06	NS
Ether extract	2.58	2.97	2.69	2.52	2.50	9.19	NS
Ash	1.12	1.19	1.50	1.20	1.40	22.22	NS
	Equation						P value
Whole body crude protein	$Y = 14.070 + 0.0789X, R^2 = 0.80$						0.021
Whole body ether extract	$Y = 13.61 - 0.161X, R^2 = 0.73$						0.033
Whole body ash	$Y = 4.155 - 0.048X, R^2 = 0.96$						0.001

CV - coefficient of variation; NS - not significant.
Linear effect (P<0.05).

Table 4 - Plasmatic lipid profile of tilapia fed diets containing increasing levels of digestible methionine + cystine

Variable	Methionine + cystine (g/kg)					CV	Effect
	7.90	9.40	10.90	12.40	13.90		
Total cholesterol (mg/dL)	231.34	217.29	194.13	199.68	188.07	20.84	Linear
HDL cholesterol (mg/dL)	76.31	76.64	70.98	78.88	78.73	19.68	NS
LDL cholesterol (mg/dL)	61.68	52.76	43.40	63.73	54.08	30.96	NS
Total lipids (mg/dL)	861.87	870.95	806.52	771.97	767.47	20.43	Linear
Triglycerides (mg/dL)	363.68	385.70	337.63	312.04	325.28	44.81	Linear
	Equation						P value
Total cholesterol (mg/dL)	$Y = 281.80 – 6.944X, R^2 = 0.85$						0.023
Total lipids (mg/dL)	$Y = 1024.00 – 19.180X, R^2 = 0.87$						0.020
Triglycerides (mg/dL)	$Y = 454.20 – 10.030X, R^2 = 0.64$						0.044

HDL - high-density lipoprotein; LDL - low-density lipoprotein; CV - coefficient of variation; NS - not significant.
Linear effect (P<0.05).

and prevents fatty liver (Swenson & Reece, 1996). This can resulted lower levels of whole body fat.

Conclusions

The dietary digestible methionine + cystine requirements for weight gain and fillet yield of Nile tilapia from 550 to 700 g are 9.00 and 9.90 g/kg, respectively.

Acknowledgements

The authors would like to thank Ajinomoto Animal Nutrition, São Paulo, Brazil, for all support for providing the amino acids analysis, and CNPq, for the financial support for the project and the grant of a research productivity fellowship.

References

ABIMORAD, E.G.; FAVERO, G.C.; CASTELLANI, D. et al. Dietary supplementation of lysine and/or methionine on performance, nitrogen retention and excretion in pacu *Piaractus mesopotamicus* reared in cages. **Aquaculture**, v.295, p.266-270, 2009.

ALAM, M.S.; TESHIMA, S.; ISHIKAWA, M. et al. Methionine requirement of juvenile Japanese flounder *Paralichthys olivaceus* estimated by the oxidation of radioactive methionine. **Aquaculture Nutrition**, v.7, p.201-209, 2001.

BENDER, D.A. **Nutritional biochemistry of the vitamins**. 2.ed. Cambridge, USA: Cambridge University Press, 2003. 488p.

BURTLE, G.J.; CAI, Y.J. Additional methionine supplementation required in soybean meal/corn-based diets for channel catfish. **UGA Animal & Dairy Science**, p.2-10, 1995.

CAI, Y.; BURTLE, G.J. Methionine requirement of channel catfish fed soybean meal-corn-based diets. **Journal of Animal Science**, v.74, p.514-521, 1996.

CHOU, R.L.; HER, B.Y.; SU, M.S. et al. Substituting fish meal with soybean meal in diets of juvenile cobia *Rachycentron canadum*. **Aquaculture**, v.229, p.325-333, 2004.

ESPE, M.; HEVROY, E.M.; LIASET, B. et al. Methionine intake affects hepatic sulfhur metabolism in Atlantic salmon, *Salmo salar*. **Aquaculture**, v.274, p.132-141, 2008.

FRAGA, A.L. **Exigência de lisina para suínos em fase inicial (15-30 kg), de dois grupos genéticos, em rações formuladas de acordo com o conceito de proteína ideal.** 2002. 46f. Dissertação (Mestrado em Zootecnia) – Universidade Estadual de Maringá, Maringá.

FRIEDEWALD, W.T.; LEVI, R.I.; FREDRICKSON, D.S. Estimation of the concentration of low density lipoproteins cholesterol in plasma without use of the ultracentrifuge. **Clinical Chemistry**, v.18, p.499-502, 1972.

FURUYA, W.M.; HAYASHI, C.; FURUYA, V.R.B. et al. Exigências de metionina + cistina total e digestível para alevinos revertidos de tilápia do Nilo, *Oreochromis niloticus* (L.), baseadas no conceito de proteína ideal. **Acta Scientiarum**, v.23, n.4, p.885-889, 2001.

FURUYA, W.M.; PEZZATO, L.E.; BARROS, M.M. et al. Use of ideal protein concept for precise formulation of amino acid levels in fish-meal-free diets for juvenile Nile tilapia (*Oreochromis niloticus* L.). **Aquaculture Research**, v.35, p.1110-1116, 2004.

FURUYA, W.M.; PEZZATO, L.E.; PEZZATO, A.C. et al. Coeficientes de digestibilidade e valores de aminoácidos digestíveis de alguns ingredientes pela tilápia do Nilo (*Oreochromis niloticus*). **Revista Brasileira de Zootecnia**, v.30, p.1143-1149, 2001b.

FURUYA, W.M.; SILVA, L.C.R.; NEVES, P.R. et al. Exigências de metionina + cistina para alevinos revertidos de tilápia do Nilo (*Oreochromis niloticus*), baseadas no conceito de proteína ideal. In: REUNIÃO DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, 2001, Piracicaba. **Anais...** Piracicaba: Sociedade Brasileira de Zootecnia, 2001a. p.1418-1419.

GAYLORD, T.G.; BARROWS, F.T.; TEAGUE, A.M. et al. Supplementation of taurine and methionine to all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*). **Aquaculture,** v.269, p.514-524, 2007.

GRACIANO, T.S.; FURUYA, W.M.; NATALI, M.R.M. et al. Metionina e colina em rações para juvenis de Tilápia do Nilo, desempenho produtivo, morfologia hepática e muscular. **Pesquisa Agropecuária Brasileira**, v.45, p.737-743, 2010.

GUIMARÃES, I.G.; BARROS, M.M.; TACHIBANA, L. et al. Nutrient digestibility of cereal grain products and by-products in extruded diets for Nile tilapia. **Journal of the World Aquaculture Society**, v.39, p.781-789, 2008b.

GUIMARÃES, I.G; PEZZATO, L.E; BARROS, M.M. et al.Amino acid availability and protein digestibility of several protein sources for the Nile tilapia, *Oreochromis niloticus*. **Aquaculture Nutrition**, v.14, p.396-404, 2008a.

HANSEN, A.; ROSENLUND, G.; KARLSEN, O. et al. Total replacement of fish meal with plant proteins in diets for the Atlantic cod (*Gadus morhua* L.) I — Effects on growth and protein retention. **Aquaculture**, v.272, p.599-611, 2007.

HARDING, D.E.; ALLEN, O.W.; WILSON, R.P. Sulfur amino acid requirement of channel catfish: L-methionine and L-cystine. **The Journal of Nutrition**, v.107, p.2031-2035, 1977.

HU, M.; WANG. Y.; WANG. Q. et al. Replacement of fish meal by rendered animal protein ingredients with lysine and methionine supplementation to practical diets for the gibel carp, *Carassius auratus gibelio*. **Aquaculture**, v.275, p.260-265, 2008.

JACKSON, A.J.; CAPPER, B.S. Investigations into requirements of the tilapia (*Sarotherodon mossambicus*) for dietary methionine, lysine and arginine in semi-synthetic diets. **Aquaculture**, v.29, p.289-297, 1982.

KASPER, C.S.; WHITE, M.R.; BROWN, P.B. Choline is required by Tilapia when metionine is not in excess. **Journal of Nutrition,** v.130, p.238-242, 2000.

KIM, K.; KAYES, T.B.; AMUNDSON, C.H. et al. Requirements for sulfur amino acids and utilization of D-methionine by the rainbow trout *(Oncorhynchus mykiss)*. **Aquaculture**, v.101, p.95-103, 1992.

LOVELL, R.T. **Nutrition and feeding of fish**. New York: Van Nostrand Reinhold, 1989. 267p.

MAI, K.; WAN, J.; AI, Q. et al. Dietary methionine requirement of large yellow croaker, *Pseudosciaena crocea* R. **Aquaculture**, v.253, p.564-572, 2006.

MATO, J.M.; ALVAREZ, L.; ORTIZ, P. et al. S-Adenosylmethionine synthesis: Molecular mechanisms and clinical implications. **Pharmacology & Therapeutics - Elsevier**, v.73, p.265-280, 1997.

NATIONAL RESEARCH COUNCIL - NRC. **Nutrient requirements of warm water fish and shellfish**. Washington: National Academy Press, 1993. 102p.

NGUYEN, T.N.; DAVIS, D.A. Re-evaluation of total sulphur amino acid requeriment and determination of replacement value of cystine for methionine in semi-purified diets of juvenile Nile tilapia, *Oreochromis niloticus*. **Aquaculture nutrition**, v.15, p.247-253, 2009.

PEZZATO, L.E.; MIRANDA, E.C.; PEZZATO A.C. et al. Digestibilidade aparente de ingredientes pela tilápia do Nilo (*Oreochromis niloticus*). **Revista Brasileira de Zootecnia**, v.31, p.1595-1604, 2002.

SANTIAGO, C.B.; LOVELL, R.T. Amino acid requirements for the growth of the Nile tilapia. **Journal of Nutrition**, v.118, p.1540-1546, 1988.

SCHWARZ, F.J.; KIRCHGESSNER, M.; DEURINGER, U. Studies on the methionine requirement of carp **(***Cyprinus carpio* L.). **Aquaculture**, v.161, p.121-129, 1998.

SILVA, D.J.; QUEIROZ, A.C. **Análise de alimentos**: métodos químicos e biológicos. 3.ed. Viçosa, MG: Universidade Federal de Viçosa, 2002. 235p.

SIPAÚBA-TAVARES, L.H. **Limnologia aplicada à aqüicultura**. Jaboticabal: Funep, 1994. 70p.

SWENSON, M.J.; REECE, W.O. **DUKES**: Fisiologia dos animais domésticos. 11.ed. Rio de Janeiro: Guanabara Koogan, 1996. 856p.

TESHIMA, S.; ALAM, M.S.; KOSHIO, S. et al. Assessment of requirement values for essential amino acids in the prawn, *Marsupenaeus japonicas*. **Aquaculture Research**, v.33, p.395-402, 2002.

TWIBELL, R.G.; WILSON, K.A.; BROWN, P.B. Dietary sulfur amino acid requirement of juvenile yellow perch fed the maximum cystine replacement value for methionine. **Journal of Nutrition**, v.130, p.612-616, 2000.

VIDAL, L.V.O.; ALBINATI, R.C.B.; ALBINATI, A.C.L. et al. Eugenol como anestésico para a tilápia-do-nilo. **Pesquisa Agropecuária Brasileira**, v.43, p.1069-1074, 2008.

WILSON, R.P. Amino acids and proteins, In: HALVER, J.E.; HARDY, R.W. (Eds.) **Fish nutrition**. 3.ed. New York: Academic Press, 2002. p.143-179.

YAN, Q.; XIE, S.; ZHU, X. et al Dietary methionine requirement for juvenile rockfish, *Sebastes schlegeli*. **Aquaculture nutrition**, v.13, p.163-169, 2007.

ZEISEL, S.H. Choline: an essential nutrient for humans. **Nutrition**, v.16, p.669-671, 2000.

ZHOU, Q.C.; WU, Z.H.; TAN, B.P. et al. Optimal dietary methionine requirement for juvenile cobia (*Rachycentron canadum*). **Aquaculture**, v.258, p.551-557, 2006.