Revista Brasileira de Zootecnia

ISSN 1806-9290
www.sbz.org.br

R. Bras. Zootec., v.41, n.6, p.1346-1352, 2012

Mathematical modeling for digestible protein in animal feeds for tilapia

Luiz Vítor Oliveira Vidal[1], Wilson Massamitu Furuya[2], Elias Nunes Martins[3], Tadeu Orlandi Xavier[1], Mariana Michelato[1], Themis Sakaguti Graciano[1]

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

ABSTRACT - The objective of this study was to formulate mathematical models to estimate digestible protein in some animal feeds for tilapia. Literature results of the proximate composition of crude protein, ether extract, and mineral matter, as well as digestible protein obtained in biological assays, were used. The data were subjected to multiple linear stepwise backward regression. Path analysis was performed to measure the direct and indirect effects of each independent variable on the dependent one. To validate the model, the experience used data from independent studies and values obtained from a digestibility trial with juvenile Nile tilapia testing five meat and bone meals, using the Guelph feces collecting system and chromium oxide (III) as an indicator. The obtained model used to estimate digestible protein values (DP) of animal origin is: $DP\,(g\,kg^{-1}) = -204.15 + 1.203 \times CP; R^2 = 0.953$. The path coefficients showed a high direct positive effect (0.900) of crude protein on the digestible protein content. The mineral matter content has an indirect negative effect on protein digestibility (-0.710), reducing the crude protein content and quality.

Key Words: chemical composition, estimate, feed, linear models

Introduction

Tilapia is one of the most promising species for aquaculture, due to its rapid growth in intensive farming. Feed is the most expensive component in tilapia farming, representing over 50% of the operating costs (El Sayed, 2006).

The way fish use protein varies by species, and is influenced by feeding habits (Dabrowski & Portella, 2006). Thus, the values of digestible protein in each ingredient are important to develop well-balanced and sustainable diets (Pond et al., 2005). Reliable data on nutrient digestibility are critical to evaluate the inclusion potential of a dietary ingredient, to elaborate low-cost diets, and to minimize the environmental impact of livestock production (Vandenberg & De La Noüe, 2001).

Digestibility values are obtained based on *in vivo* fecal collection, a methodology routinely used in animal nutrition studies in digestibility trials. In terms of practical conditions, it is costly and difficult to subject every raw material batch to digestibility trials.

Due to the possibility of obtaining the values of crude protein, ether extract, and mineral matter contents by low-cost chemical analysis, and their use in regression equations, the estimation of digestible protein values can be of great practical application (Sakomura & Rostagno, 2007). They may also be an important tool in complementing the biological assays, which depend on a more complex, expensive and prolonged methodology. Mathematical modeling has been widely used to estimate digestible lipids (Sales, 2009; Hua & Bureau, 2009a), available phosphorus (Hua & Bureau, 2006), carbohydrates (Hua & Bureau, 2009b), and protein (Sales, 2008). The development of individual models, both for feed and species will make it possible to obtain data applicable to new situations and physiological features of fish.

The objective of this study was to develop mathematical models to estimate the digestible protein for animal feedstuff for tilapia and to validate them with data from a biological digestibility trial, using meat and bone meal as standard feed and independent studies from the literature.

Material and Methods

Chemical composition and digestible protein, data from some ingredients of animal origin were collected from scientific papers published between 2002 and 2008, mostly obtained for Nile tilapia. The search was conducted on the Scopus and ISI Web of Science databases.

Articles that contained values of dry matter (DM), crude protein (CP), ether extract (EE), mineral matter (MM), and digestible protein (DP) of fish meal, shrimp meal, meat and bone meal, and poultry by products meal were used. At

Received August 18, 2011 and accepted December 21, 2011.
Corresponding author: luizvitor.vidal@gmail.com

the end of the selection, eight articles were obtained, which resulted in the database described below (Figure 1). For standardization, the data on chemical composition and protein digestibility were expressed in dry matter values.

All data were analyzed by multiple linear regression, testing models with and without intercept, respectively:

$$Y_i = \beta_0 + \beta_1 X_{i1} + \beta_2 X_{i2} + \beta_3 X_{i3} + e_i$$

$$Y_i = \beta_1 X_{i1} + \beta_2 X_{i2} + \beta_3 X_{i3} + e_i$$

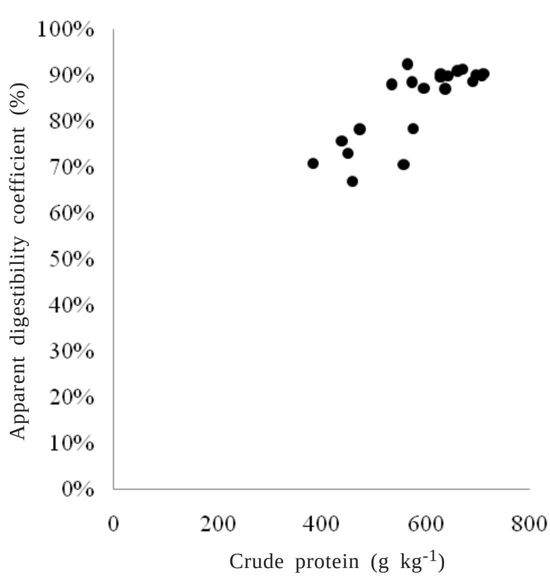
β_0 value was obtained according to the follow equation:

$$\beta_0 = (\overline{Y}_1 - \beta_1 \overline{X}_{i1}) + (\overline{Y}_2 - \beta_2 \overline{X}_{i2}) + (\overline{Y}_3 - \beta_3 \overline{X}_{i3})$$

where: Yi = apparent digestible protein (ADP) of the ingredients obtained in a digestibility test; β_0 = β_0 = intercept; X_{i1}, X_{i2} X_{i3} = feed chemical composition variables, respectively, crude protein, ether extract, and mineral matter.

The backward stepwise method was used to remove insignificant independent variables ($P<0.05$). To determinate which equation best estimates digestible protein, linear equation with intercept and without intercept, the root mean squared error (RMSE) of each one was used. Path analysis was performed to measure the direct and indirect effects of each independent variable on the dependent.

To validate the models, a digestibility assay was conducted at the Universidade Estadual de Maringá - Fish Culture "Estação de Piscicultura de Floriano" - UEM/CODAPAR, from January to February 2010



Source: Boscolo et al. (2008), Goddard et al. (2008), Guimarães et al. (2008), Köprücü & Özdemir, Boscolo et al. (2004), Sklan et al. (2004),; Maina et al. (2002) and Pezzato et al. (2002).

Figure 1 - Modeling dataset consisting of data from 8 studies.

A practical reference diet was formulated to contain approximately 320 g kg^{-1} of crude protein, 3120 kcal of digestible energy, 34.0 g kg^{-1} of crude fiber and 5.0 g kg^{-1} of phosphorus (Table 1).

Meat and bone meal with different protein levels (Table 2) were used as standard feed to validate the equations and replaced 30% of the reference diet.

In the preparation of test diets, after grinding, weighing, and mixing of ingredients, water was added at 60 °C at a ratio of 25% of the total weight of the diet. The mixture was pelleted in a meat mill and dried in a forced ventilation oven (55 °C) for 48 h.

The apparent digestibility coefficients (ADC) of crude protein were determined by the indirect method using chromic oxide III (5 g kg^{-1}) as an inert indicator. Conical fiberglass tanks were used for fecal collection.

Table 1 - Percentage composition of reference diet

Feed	(g kg^{-1})
Corn	326.2
Soybean	437.0
Poultry by-product meal	149.5
Corn starch	29.9
Dicalcium phosphate	19.9
Soybean oil	14.9
L-lysine HCl	1.0
DL-methionine	1.0
L-threonine	1.0
L-tryptophan	0.5
L-arginine	1.0
Ascorbic acid[1]	1.0
NaCl	5.0
Choline chloride	1.0
Mineral and vitamin premix[2]	5.0
BHT[3]	0.2
Calcium propionate[4]	1.0
Chromic oxide III	5.0
Total	1000.0

[1] Vitamin C: calcite salt, active principle ascorbic 2 acid-42%-monophosphate.
[2] Mineral and vitamin mix (per kg): vitamin A - 1.2 million IU; vitamin D3 - 200,000 IU; vitamin E - 12,000 mg; vitamin K3 - 2,400 mg; vitamin B1 - 4,800 mg; vitamin B2 - 4,800 mg; vitamin B6 - 4,000 mg; vitamin B12 - 4,800 mg; folic acid - 1,200 mg; calcium D-pantothenate - 12,000 mg; ascorbic acid - 48,000 mg; biotin - 48 mg; choline - 65,000 mg; nicotinic acid - 24,000 mg; iron - 10,000 mg; copper sulfate - 600 mg; manganese sulfate - 4,000 mg; zinc sulfate - 6,000 mg; potassium iodine - 20 mg; cobalt - 2 mg; selenium - 20 mg.
[3] Butylated Hydroxytoluene.
[4] Calcium propionate.

Table 2 - Chemical composition of meat and bone meal with different levels of crude protein

Variable	Crude protein (g kg^{-1})				
	337.0	374.9	401.7	434.8	463.8
DM, g kg^{-1}	936.4	940.5	947.6	951.5	956.4
CP, g kg^{-1}	337.0	374.9	401.7	434.8	463.8
EE, g kg^{-1}	89.9	106.0	115.7	131.6	144.6
MM, g kg^{-1}	454.5	420.9	387.6	355.2	323.0
GE, kcal kg^{-1}	3031.40	3249.37	3462.88	3767.10	4011.39

DM - dry matter; CP - crude protein; EE - ether extract; MM - mineral matter; GE - gross energy.

Fish (180 juveniles of Nile tilapia GIFT strain with an average weight of 32.65±4.52 g) were kept in the fecal collection tanks during the entire trial and fed to satiation, every 2 h from 8:30 to 17:00 by hand feeding. Collector tubes were installed and the feces were collected in the morning and kept frozen at -21 °C until the end of the collection period, when the tanks were cleaned and all the water was replaced.

Each test diet was assessed in triplicate for five days, each tank was considered a collection repetition. Before feces collection, fish were adapted to the conical tanks, handling, and fed pellet diets for seven days. For each new ingredient, the feces were discarded in the first three days to avoid contamination with the previous diet. At the end of each sampling period, the feces were dried in a forced ventilation oven at 55 °C (48 h) and milled and analyzed according to the methodology described by the AOAC (1990). The gross energy was determined by an adiabatic bomb calorimeter (Parr Instrument Company, Moline, IL, USA).

The chromic oxide content of diets and feces were determined according to Bremer Neto et al. (2005).

The apparent digestibility coefficients for crude protein were calculated according to the equations described by Pezzato et al. (2002).

$$ADC = 100 - \left[100 \times \left(\frac{g\ kg^{-1} I_d}{g\ kg^{-1} I_f}\right) \times \left(\frac{g\ kg^{-1} N_f}{g\ kg^{-1} N_d}\right)\right]$$

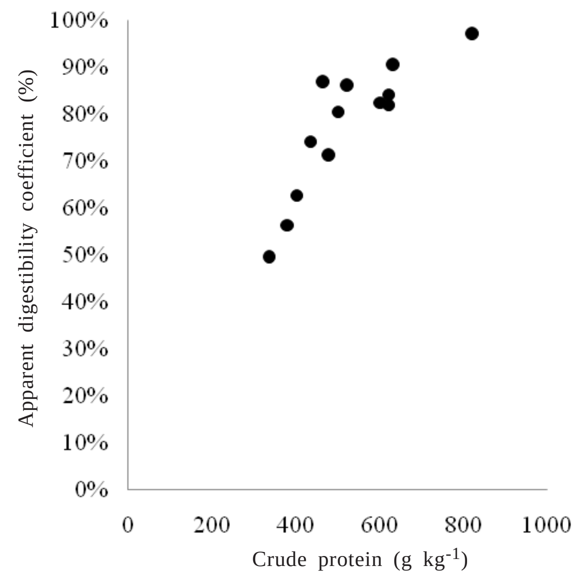
where: ADC (n) = apparent digestibility coefficient; I_d = % of chromic oxide in the diet; I_f = % of chromic oxide in feces, N_d = nutrients in the diet, N_f = nutrients in feces.

$$ADC_{ing} = \frac{ADC_{(td)} - b \times ADC_{rd}}{a}$$

where: ADC (ing) = apparent digestibility coefficient of the ingredients, ADC (td) = apparent digestibility coefficient of the test diet, ADC (rd) = apparent digestibility coefficient of the reference diet, b = percentage of the reference diet, a = percentage of test ingredient.

The differences between the digestible protein of the meat and bone meals were determined by analysis of variance (ANOVA; $P<0.05$), significant values were submitted to linear regression.

The T test of Student was applied to investigate the differences between the mean obtained values from the digestibility trial and the estimated values. The performance of determined prediction equations were evaluated by linear regression analysis between predicted (y) and obtained (x) values, adapted from Sales (2008). The values used in the validation procedure were obtained in the digestibility assay and from four independent studies (Figure 2). All calculations were performed in the statistical package SAS 9.1.3.



Source: Present digestibility trial; Vásquez-Torres et al., 2010; Gonçalves et al., 2009; Meurer et al., 2003; Sampaio et al., 2001.

Figure 2 - Validating dataset consisting of data from 5 studies.

Results and Discussion

The standard model for estimating digestible protein, obtained by multiple linear regression, was highly significant ($P<0.0001$) and had a coefficient of determination $R^2 = 0.953$ (Figure 3) and accurately described the observations of the dataset (Figure 4). Sales (2008a) used data from animal ingredients measured in 35 species and obtained the equation: $DP\,(g\ kg^{-1}) = -1039.33 + 0.997 \times CP; R^2 = 0.761$. According to Dabrowiski & Portella (2006), different species of fish have different digestive metabolisms, which depend on the feeding habits; therefore, this biological factor should be taken into consideration in the development of mathematical models.

Analyzing the equations, one can notice that the root mean squared error of the equation with intercept was lower than the equation without intercept; therefore, the equation with intercept is the best option to estimate digestible protein (Table 3).

The value of an equation intercept creates a trend in the estimated values, since it is obtained from the average values of the dependent and independent variables (Bhujel, 2008). Thus, the use of prediction models with a constant is not indicated when the values of the chemical composition of the feed used to obtain the model are in a short range. On the other hand, the equations without an intercept can

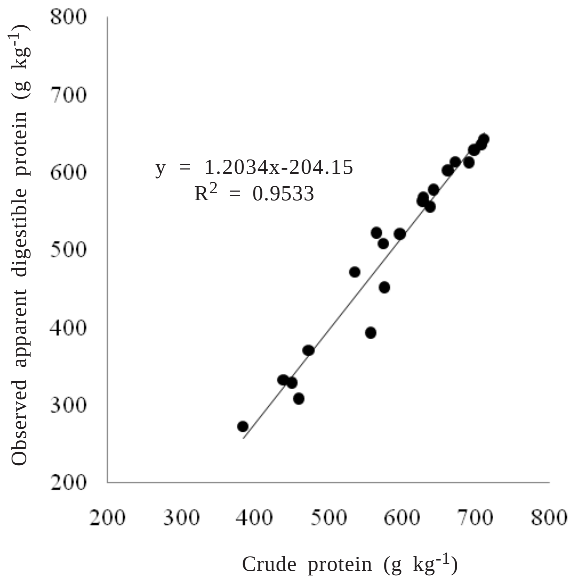


Figure 3 - Mathematical model to estimate apparent digestible protein values in animal feeds.

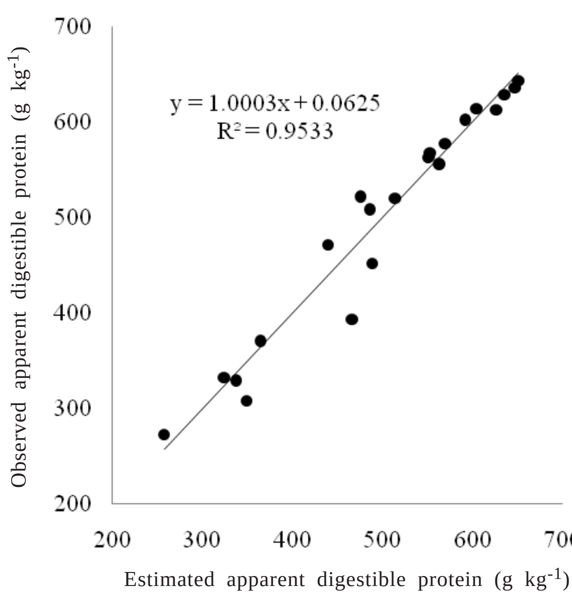


Figure 4 - Comparison of observed and estimated digestible protein content (g/kg) models of the diets from dataset.

reduce the accuracy of the estimated data when the linear coefficient is distant from zero (Rencher & Schaalje, 2008).

The path analysis showed determination coefficients of 0.957 for digestible protein (Table 4).The path coefficients showed a high direct positive effect (0.8996) of crude protein on the digestible protein content (Figure 5). The mineral matter negatively affected the digestibility of protein by reducing the crude protein content and/or increasing the collagen content, an indigestible protein from the bone matrix (Butolo, 2010), shown by the indirect effect (-0.7101). Protein is the most costly nutrient in diets for domestic animals (Wilson, 2002), and the use of low-quality animal feeds, such as toasted blood meal and feather meal results in high nitrogen excretion into the aquatic environment (Pezzato et al., 2002; Sampaio et al., 2001). This process can reduce water quality, with possible excessive proliferation of algae or microorganisms, both of which are either directly or indirectly harmful to the integrity of the fish. The formulation of diets closely adjusted to the nutritional needs of the species, made from high-quality and properly processed ingredients are the main tools for reducing the negative impacts of aquaculture waste (Cyrino et al., 2010).

Differences ($P<0.05$) were observed for the ADC of crude protein of feeds (Table 5). Thus, as the composition of the reference diet may influence the results, the feed processing, fecal collecting method, and nutrient levels used for determining the feed ADC are important factors in determining the biological value of each feed, which may

Table 3 - Linear models used to estimate crude protein and digestible energy of ingredients of animal origin for tilapia

Equation				R^2	RMSE
Intercept	CP	EE	MM		
-204.15	1.203	-	-	0.953	2.609
-	0.970	-	-0.290	0.998	2.651

CP - crude protein; EE - ether extract; MM - mineral matter; R^2 - determination coefficient; RMSE - root mean squared error.

Table 4 - Path coefficients between the variables of chemical composition and contents of digestible protein and energy

Variable	Effect	Digestible protein
CP	Direct	0.900
CP	EE	0.000
CP	MM	0.077
Total		0.976
EE	Direct	-0.001
EE	CP	-0.156
EE	MM	0.003
Total		-0.153
MM	Direct	-0.097
MM	CP	-0.710
MM	EE	0.000
Total		-0.807
R^2		0.957

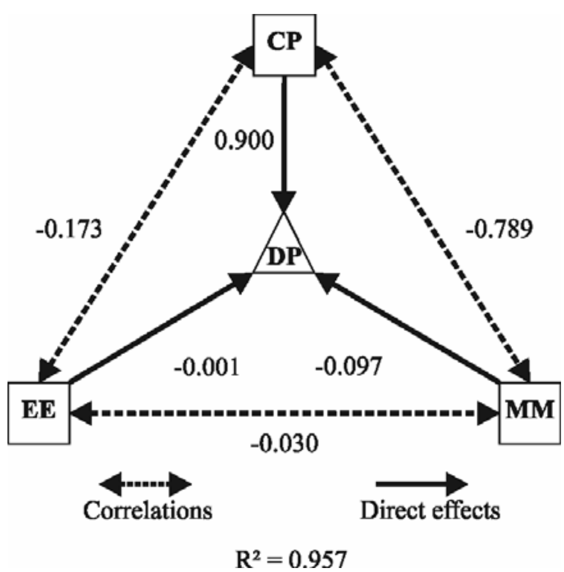
CP - crude protein; EE - ether extract; MM - mineral matter; R^2 - determination coefficient.

present differences with regard to each methodology (Gonçalves et al., 2009).

There was an increase ($P<0.05$) in crude protein (CP) digestibility coefficients of meat and bone meal. Comparing these values with others from the literature, proximity is observed between the ADC of 434.80 g kg^{-1} CP in meat and bone meal and that studied by Pezzato et al. (2002), who obtained an ADC of 731.90 g kg^{-1}, using a meat and bone meal composition similar to the one used in the present study. The 463.80 g kg^{-1} CP meat and bone meal had a higher ADC than that obtained by Guimarães et al. (2008), who determined a 78.4% ADC, despite the proximity to the crude protein value. The current study was conducted with a meat and bone meal containing less mineral matter (323.00 g kg^{-1}) than that used by Guimarães et al (2008), 411.3 g kg^{-1}. When evaluating alternative feeds for Australian silver perch (*Bidyanus bidyanus*) using two meat and bone meals with 492.00 g kg^{-1} and 543.00 g kg^{-1} crude protein, Allan et al. (2000) obtained ADC values of crude protein of 715.00 and 739.00 g kg^{-1} respectively, which were lower than the values obtained with tilapia, in the present study, when considering the chemical composition of the tested ingredients.

The T test did not determine differences between the values obtained and estimated for the meat and bone meals. Furthermore, the linear regression between the observed and estimated values demonstrates highly significant ($P<0.0001$) linear relationship, an intercept close to zero, a slope close to one, and a high determination coefficient (Figure 6).

In practical terms, the use of mathematical models to estimate the digestible protein values is an important tool, since it is common to buy feed of different chemical compositions, and it would be difficult to carry out digestibility trials for all of them.



DP - digestible protein, CP - crude protein, EE - ether extract; MM - mineral matter.

Figure 5 - Path analysis between chemical composition and digestible protein content of animal ingredients for tilapia.

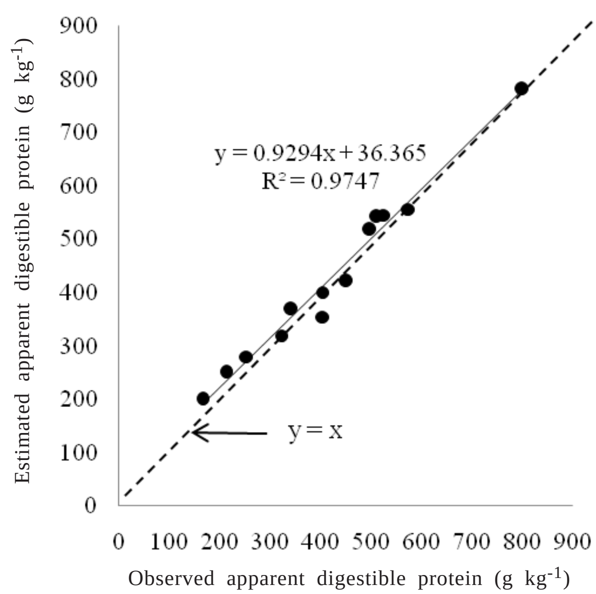


Figure 6 - Comparison of observed and estimated apparent digestible models of protein values from independent studies and digestibility trial.

Table 5 - Apparent digestibility coefficients of meat and bone meal with different levels of crude protein for Nile tilapia

Variable	Crude protein (g kg^{-1})					
	337.0	374.9	401.7	434.8	463.8	Mean
ADC (%)[1]	49.67	57.01	62.77	74.28	87.03	66.15
DP (g kg^{-1})[2]	167.4	213.7	252.1	323.0	403.6	272.0NS
Estimated without intercept (%)	195.1	241.6	277.2	318.7	355.7	277.7NS
Estimated with intercept (%)	201.3	246.9	279.1	318.9	353.1	279.9NS

ADC - apparent digestibility coefficient; NS - non-significant by the T test; DP - digestible protein
[1] linear effect: $y = -51.275 + 2.918x$, $R^2 = 0.967$.
[2] linear effect: $y = -470.52 + 1.845x$, $R^2 = 0.969$.

Because of the lower cost of meat and bone meal in some countries, as compared with fish meal, it has been widely used as a source of energy, protein (amino acids), minerals, and vitamins. However, its protein, fat, and mineral composition is highly variable, which even affects the nutritional value of other feeds in the diet.

Multiple linear models are useful to estimate the values of digestible protein, using the chemical composition values of ingredients of animal origin. The values estimated by mathematical models are very close to those obtained by independent experiments.

Digestibility trial data are consistent with those in the literature and confirm the effectiveness of the models.

Conclusions

It is possible to estimate the digestible protein in animal ingredients of tilapia feed through the prediction models. The developed model is $DP\,(g\,kg^{-1}) = -204.15 + 1.203 \times CP; R^2 = 0.953$.

Acknowledgements

This research is part of the Masters dissertation of the first author and was supported by the Universidade Estadual de Maringá and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

ALLAN, G.L.; ROWLAND, S.J.; MIFSUD, C. et al. Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus*: V. Least-cost formulation of practical diets. **Aquaculture,** v.186, n.3-4, p.327-340, 2000.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTRY – AOAC. **Official methods of analysis of Association of Official Analytical Chemists**. 15.ed. Arlington: AOAC, 1990. 1298p.

BHUJEL, R.C. **Statistics for aquaculture**. Ames: Wiley-Blackwell, 2008. 240p.

BOSCOLO, W.R.; HAYASHI, C.; FEIDEN, A. et al. Composição química e digestibilidade aparente da energia e nutrientes da farinha de resíduos da indústria de filetagem de tilápias, para a tilápia do Nilo (*Oreochromis niloticus*). **Ciência Rural,** v.38, n.9, p.2579-2586, 2008.

BOSCOLO, W.R.; HAYASHI, C.; MEURER, F. et al. Digestibilidade aparente da energia e proteína das farinhas de resíduo da filetagem da tilápia do Nilo (*Oreochromis niloticus*) e da corvina (*Plagioscion squamosissimus*) e farinha integral do camarão canela (*Macrobrachium amazonicum*) para a tilápia do Nilo. **Revista Brasileira de Zootecnia**, v.33, n.1, p.8-13, 2004.

BREMER NETO, H.; GRANER, C.A.F.; PEZZATO, L.E. et al. Determinação de rotina do crômio em fezes, como marcador biológico, pelo método espectrofotométrico ajustado da 1,5-difenilcarbazida. **Ciência Rural**, v.35, n.3, p.691-697, 2005.

BUTOLO, J.E. **Qualidade de ingredientes na alimentação animal**. 2.ed. Campinas: Colégio Brasileiro de Nutrição Animal, 2010. 430p.

CYRINO, J.E.P.; BICUDO, Á.J.D.A.; SADO, R.Y. et al. A piscicultura e o ambiente: o uso de alimentos ambientalmente corretos em piscicultura. **Revista Brasileira de Zootecnia,** v.39, p.68-87, 2010.

DABROWSKI, K.; PORTELLA, M.C. Feeding plasticity and nutritional physiology in tropical fishes. In: VAL, A.L.; ALMEIDA-VAL, V.M.F.D.; RANDALL, D.J. et al. (Eds.). **The physiology of tropical fishes**. Amsterdam: Elsevier, 2006. v.21, p.155-224. (Fish Physiology).

EL-SAYED, A.F.M. **Tilapia culture**. Wallingford: CABI Publishing, 2006. 277p.

GODDARD, S.; AL-SHAGAA, G.; ALI, A. Fisheries by-catch and processing waste meals as ingredients in diets for Nile tilapia, *Oreochromis niloticus*. **Aquaculture Research**, v.39, n.5, p.518-525, 2008.

GONÇALVES, G.S.; PEZZATO, L.E.; BARROS, M.M. et al. Energia e nutrientes digestíveis de alimentos para a tilápia do nilo. **Boletim do Instituto de Pesca,** v.35, n.2, p.201-213, 2009.

GUIMARÃES, I.G.; PEZZATO, L.E.; BARROS, M.M. Amino acid availability and protein digestibility of several protein sources for Nile tilapia, *Oreochromis niloticus*. **Aquaculture Nutrition**, v.14, n.5, p.396-404, 2008.

HUA, K.; BUREAU, D.P. Modelling digestible phosphorus content of salmonid fish feeds. **Aquaculture,** v.254, n.1-4, p.455-465, 2006.

HUA, K.; BUREAU, D.P. Development of a model to estimate digestible lipid content of salmonid fish feeds. **Aquaculture**, v.286, n.3-4, p.271-276, 2009a.

HUA, K.; BUREAU, D.P. A mathematical model to explain variations in estimates of starch digestibility and predict digestible starch content of salmonid fish feeds. **Aquaculture,** v.294, n.3-4, p.282-287, 2009b.

KÖPRÜCÜ, K.; ÖZDEMIR, Y. Apparent digestibility of selected feed ingredients for Nile tilapia (*Oreochromis niloticus*). **Aquaculture,** v.250, n.1-2, p.308-316, 2005.

MAINA, J.G.; BEAMES, R.M.; HIGGS, D. et al. Digestibility and feeding value of some feed ingredients fed to tilapia *Oreochromis niloticus* (L.). **Aquaculture Research,** v.33, n.11, p.853-862, 2002.

MEURER, F.; HAYASHI, C.; BOSCOLO, W.R. Digestibilidade aparente de alguns alimentos protéicos pela tilápia do Nilo (*Oreochromis niloticus*). **Revista Brasileira de Zootecnia,** v.32, n.6, suppl.2, p.1801-1809, 2003.

PEZZATO, L.E.; MIRANDA, E.C.D.; BARROS, M.M. et al. Digestibilidade Aparente de Ingredientes pela Tilápia do Nilo (Oreochromis niloticus). **Revista Brasileira de Zootecnia,** v.31, n.4, p.1595-1604, 2002.

POND, W.G.; CHURCH, D.C.; POND, K.R. **Basic animal nutrition and feeding**. Hoboken: Wiley, 2005. 608p.

RENCHER, A.C.; SCHAALJE, G.B. **Linear models in statistics**. 2.ed. New Jersey: John Wiley & Sons, 2008. 688p.

SAKOMURA, N.K.; ROSTAGNO, H.S. **Métodos de pesquisa em nutrição de monogástricos**. Jaboticabal: FUNEP, 2007. 283p.

SALES, J. The use of linear regression to predict digestible protein and available amino acid contents of feed ingredients and diets for fish. **Aquaculture**, v.278, n.1-4, p.128-142, 2008.

SALES, J. Linear models to predict the digestible lipid content of fish diets. **Aquaculture Nutrition**, v.15, n.5, p.537-549, 2009.

SAMPAIO, F.G.; HISANO, H.; YAMAKI, R.A. et al. Digestibilidade aparente das farinhas de peixe nacional e importada e das farinhas de sangue tostada e spray-dried, pela tilápia do Nilo, *Oreochromis niloticus* (L.). **Acta Scientiarum, Animal Science**, v.23, n.4, p.891-896, 2001.

SKLAN, D.; PRAG, T.; LUPATSCH, I. Apparent digestibility coefficients of feed ingredients and their prediction in diets for tilapia *Oreochromis niloticus* x *Oreochromis aureus* (Teleostei, Cichlidae). **Aquaculture Research**, v.35, n.4, p.358-364, 2004.

VANDENBERG, G.W.; DE LA NOÜE, J. Apparent digestibility comparison in rainbow trout (*Oncorhynchus mykiss*) assessed using three methods of faeces collection and three digestibility markers. **Aquaculture Nutrition**, v.7, p.237-245, 2001.

VÁSQUEZ-TORRES, W.; YOSSA PERDOMO, M.I.; HERNÁNDEZ ARÉVALO, G. et al. Digestibilidad aparente de ingredientes de uso común en la fabricación de raciones balanceadas para tilapia roja híbrida (*Oreochromis* sp.). **Revista Colombiana de Ciencias Pecuarias**, v.23, p.207-216, 2010.

WILSON, R.P. Amino acids and proteins. In: HALVER, J.E.; HARDY, R.W. (Eds.). **Fish nutrition**. Amterdam: Elsevier, 2002. p.144-181.