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Digestibilidade e composição química de *Oreochromis niloticus*, alimentados com dietas contendo diferentes níveis de fósforo

Digestibility and Chemical Composition of "Oreochromis niloticus", fed diets containing different phosphorus levels

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RESUMO

Com este estudo objetivou-se estimar o coeficiente de digestibilidade aparente e determinar a composição química da carcaça de alevinos de tilápia do Nilo (4,0 ± 0,4g), alimentados com rações que continham diferentes níveis de fósforo O delineamento utilizado foi o inteiramente casualisado, com quatro tratamentos e cinco repetições. Foram fornecidas dietas com 28% de proteína digestível e 3000kcal de ED/kg, que continham 6% de farinha de peixe e ultrapassada a relação lisina/metionina, suplementadas com fosfato bicálcico de forma a se obter níveis de 0,27; 0,32; 0,37 e 0,42% de fósforo disponível. Para determinação do coeficiente de digestibilidade, as dietas foram fornecidas quatro vezes ao dia com quantidade correspondente a 4% do peso corporal, durante sete dias. Para determinação da composição química da carcaça, a alimentação foi distribuída quatro vezes por dia, às 7h, 10h, 13h e 16h. Procedeu-se o arraçoamento manual, fornecido até saciedade aparente, quando não eram observadas captura, regurgitação dos grânulos e presença de ração no comedouro. Foi observada diferença significativa dos níveis de fósforo disponível sobre a digestibilidade da proteína bruta, matéria seca e extrato etéreo para alevinos de tilápia. Não foi observada diferença significativa dos níveis de fósforo disponível da dieta sobre proteína bruta, cinzas, cálcio e água na carcaça. Entretanto, houve redução da gordura corporal com os maiores níveis de fósforo na dieta. Diante dos resultados encontrados conclui-se que a dieta com 0,27% de fósforo disponível foi mais digestível em relação aos demais tratamentos e que dietas com o nível de 0,42% de fósforo proporcionam melhores índices de composição corporal.

Palavras-chave: alevinos, exigência, proteína digestível, tilápia do Nilo

SUMMARY

This study aimed to estimate the coefficient of aparent digestibility and determine the carcass chemical composition of Nile tilapia fingerlings (4.0 ± 0.4g) fed with diets containing different levels of phosphorus. A completely randomized design with four treatments and five replications was used. Diets with 28% digestible protein and 3000kcal DE / kg, containing 6% fish meal and surpassing the lysine / methionine ratio, supplemented with dicalcium phosphate in order to obtain levels of 0.27; 0.32; 0.37 and 0.42% available phosphorus. To determine the coefficient of digestibility of the diets, the feed was supplied four times a day which corresponds to 4% of body weight, for seven days. To determine the chemical composition of carcass, feed was supplied four times a day, at 7am, 10am, 1pm and 4pm. The feeding was manual and provided until apparent satiation, when capturing of food was not observed, nor regurgitation of food grains and there was presence of feed in the feeder. Significant

difference was observed in levels of phosphorus over the digestibility of crude protein, dry matter and ether extract for tilapia fingerlings. There was no significant difference observed in available phosphorus levels in the diet over: crude protein, ash, calcium and water for carcass. However, there was significant reduction of body fat with the highest levels of phosphorus in the diet. Considering the results it is concluded that the diet with 0.27% available phosphorus was more digestible than the other treatments and diets with the level of 0.42% phosphorus provided better indicators of body composition.

Keywords: digestible protein, fingerlings, Nile tilapia, requirement

INTRODUCTION

The digestibility is an important aspect in the evaluation of food, as for their biological efficiency. The nutritional value of food is based not only on the chemical composition but also in the amount of nutrients and energy that fish can absorb and use (NRC, 1993). The efficiency in food digestion can be influenced, among other factors, by the food surface exposure to digestive secretions as well as by the time of passage through the gastrointestinal tract. In nutrition studies, the coefficients of apparent digestibility are generally used in order to determine the nutritional value of a given food, in which the digestibility of a food depends primarily on the chemical composition and also the animal's capacity to digest food. The digestibility has fundamental importance to meet the nutritional requirements of a species, since knowing eating habits and providing a balanced diet are not sufficient to ensure a positive response in animal performance. (GONÇALVES & CARNEIRO, 2003).

The main source of residues in aquaculture is feeding, because the nutrients that are not retained by fish are excreted into the water and discharged as effluent into the system, with nitrogen and phosphorus as the primary causes for eutrophication in the aquaculture environment. Phosphorus is considered an essential nutrient for the bone structure formation and body metabolism, being indispensible that is in adequate levels to meet the nutritional requirement of the animal. Furthermore, excessive concentrations of this mineral in the aquatic environment may lead to eutrophication of the environment, compromising water quality and tolerance capacity of aquaculture systems (VAN Der PLOEG & BOYD, 1991) and in the case of cyanobacteria predominance affect the organoleptic characteristics of the fish carcass.

Thus, researches that maximize production and, on the other hand, minimize the load of pollutants in the aquatic environment, especially phosphorus, constitute basic need to the development of this activity (OLIVEIRA et al., 2008).

The present study was conducted to determine the coefficient of apparent digestibility for the feed and body composition of Nile tilapia fingerlings fed diets containing different levels of phosphorus.

MATERIAL E METHODS

The experiment was conducted at the Laboratório de Digestibilidade de Peixes da Estação de Piscicultura (Laboratory of Fish Digestibility in the Fish Culture Station), UFLA, Lavras, Minas Gerais, for 90 days.

Eight hundred (800) tilapia fingerlings (Oreochromis niloticus), sexually reverted from the same spawn, with an average initial weight of 4.0 ± 0.4g, average initial length of 5.0 ± 0.5cm were used. The fish were randomly

distributed into 20 incubators adjusted to digestibility trials, featuring feeders at 30cm from the top of water with a volume of 250L at a density of 40 fish / hatchery.

The incubators are part of a closed system with re-circulating water, with aeration and a flow rate of 90L/hora, controlled heating by electric heaters connected to thermostat (27.0 ± 1.0°C) and physical and biological filtration of water through a biofilter. Four feeds containing 0.27; 0.32; 0.37 and 0.42%42% of available phosphorus were evaluated (Table 1).

Table 1. Percentages and calculated composition of experimental feeds

Ingredients	Feeds			
	Basal feed 0.42% available P	0.37% available P	0.32% available P	0.27% available P
Corn	30,00	30,00	29,00	29,30
Soybean meal	57,73	57,75	59,24	60,51
Fish meal	6.00	6,00	5,00	4,01
Limestone	0,00	0,19	0,40	0,47
Dicalcium phosphate	0,50	0,25	0,0	0,00
Soybean oil	4,24	4,24	4,44	4,35
L- Lysine	0,06	0,09	0,10	0,12
DL-methionine	0,24	0,24	0,24	0,24
Supremais	0,50	0,50	0,50	0,50
Vitamin C	0,10	0,10	0,10	0,10
Butylated hydroxytoluene	0,02	0,02	0,02	0,02
Alginate	0,10	0,10	0,10	0,10
Salt	0,10	0,10	0,10	0,10
Kaolin	0,31	0,32	0,56	0,08
Chromium Oxide	0,10	0,10	0,10	0,10
Total	100,00	100,00	100,00	100,00
Calculated composition of the nutrients[3]				
DP (%)	28,00	28,00	28,00	28,00
DE (kcal/kg)	3000,36	3000,36	3000,15	3000,04
Ca (%)	0,63	0,63	0,63	0,63
Avail. P. (%)	0,42	0,37	0,32	0,27
FB (%)	4,95	4,95	5,03	5,12
Lysine (%)	1,77	1,77	1,78	1,78
Methionine (%)	0,65	0,65	0,65	0,64

[1]Mineral and vitaminic Premix: Composition/kg of product: vit. A – 900,000 UI; vit. D3 – 50,000 UI; vit. E – 6,000 mg; vit. K3 – 1,200mg; vit. B1 – 2,400mg; vit. B2 – 2,400mg; vit. B6 – 2,000mg; vit. B12 – 4,800mg; folic acid – 1,200mg; calcium pantothenate – 12,000mg; vit. C – 24,000mg; biotin- 6.0mg; choline - 65.000mg; nicotinic acid – 24,000mg; Fe – 10,000mg; Cu - 600mg; Mn -4,000mg; Zn – 6,000mg; I - 20mg; Co – 2.0mg; and Se - 25mg.
[2]Butylated hydroxytoluene (antioxidant).
[3]According to Furuya et al. (2001).
[4]Analyses conducted at the Laboratório de Pesquisa Animal (Laboratory Of Animal Research) at DZO/UFLA.
DP = digestible protein, DE = digestible energy, P. Avail. = phosphorus available, CF = crude fiber.

All feeds were formulated with 28% digestible protein (NRC, 1993) and supplemented with amino acids, methionine and lysine in order to present a lysine:methionine ratio cited by Furuya et al., (2004) for tilapia.

The diets were isocaloric (3,000kcal of digestible energy) and balanced according to the values of proteins and amino acids by Furuya et al. (2001).

The ingredients were finely ground until they reach a diameter equal to or smaller than 0.5mm. In the preparation of the test feeds, after weigthing and mixing of ingredients, 1000FTU/Kg commercial phytase was added to the mixture to improve phosphorus availability of plant origin.

Then water was added (45°C) at a ratio of 18% of the total weight of the feed. The mixture was pelleted in meat grinder and dried in forced ventilation oven (45^{o}C) for 18 hours. Later, it was disintegrated in diameters from 2 to 5mm and stored in refrigerator.

The experiment design used was completely randomized, with four levels of available phosphorus and five replications. The experiment had a seven-day period for adaptation to the diet and environment, when the dead fingerlings were replaced. The fish were fed four times a day (8am; 11am; 2pm and 5pm) with an amount corresponding to 4% of their body weight. To determine the coefficients of apparent digestibility, the experimental diets were grounded and then 0.1% chromium oxide was added (Cr_2O_3) as indicator. Then, they were pelleted again using the same procedures previously described for this process.

Feces collection was performed daily at 7am for seven consecutive days after the pre-experimental period and stored in a freezer for further analysis. Samples collected from each tank were dried in a forced air oven (60°C) for 36 hours and, after drying, the material was grounded, identified and stored in a refrigerator for analysis. At the end of this period of seven days for feces collection, the fish in each experimental unit were weighted and returned to their respective treatments.

The water temperature and oxygen content dissolved into water were checked daily, at 8am and 4pm, and the pH checked every two days.

The coefficients of apparent digestibility for energy, dry matter, ether extract and crude protein in the feed were determined according to the expression proposed by Nose (1960) $CAD = 100 - [100 \times (\%DI/\%FI) \times (\%FN/\%DN)]$

in which: CAD = coefficient of apparent digestibility (%);

%DI and %FI = % of indicators in the diet and feces, respectively;

%FN and %DN = % of dry matter, ether extract or crude protein, in the feces and diet, respectively.

After collecting the digestibility data, the experiment was continued for another 60 days to evaluate the chemical composition of the carcasses.

For the chemical composition test, the feed was supplied four times a day, at 7am; 10am; 1pm and 4pm. The feeding was manual and provided until apparent satiation, when capturing of food was not observed, nor regurgitation of food grains and there was presence of feed in the feeder.

After the last feeding of the day, the incubators were cleaned, in this process 40% of its water volume was emptied in order to remove any residue.

The parameters of dissolved oxygen (mg / L) and water temperature were checked daily and the water pH of each experimental unit was tested every two days using the kit “Laboratório de Medição Portátil F-1003” (Laboratory Notebook Measurement F-1003), Bernauer.

The fish fasted for 24 hours and, after weighting, blood was collected from all fish in the experiment for blood tests.
For hematological analysis, all fish were anesthetized (benzocaine, 1g / 15 L of water) and, after complete desensitization, blood was collected by jugular vein puncture with 3 mL syringe washed with anticoagulant EDTA, at 4%. Hemoglobin concentration was determined by the cianometahemoglobin method, using a commercial kit Hemoglobin Diagnostic Scans for colorimetric determination in atomic absorption spectrophotometer. The hematocrit was obtained by microhematocrit method. The variables mentioned before were evaluated using the techniques described by Jain (1986). Blood samples were centrifuged at 3,000rpm for five minutes for reading in hematocrit percentages table.
Then the fish were stored in an isothermal box with ice, slaughtered and eviscerated for evaluation of carcass traits.
The treatment averages were compared by nonparametric test Kruskal-Wallis.
The chemical analysis to determine the water content of crude protein (CP), ether extract (EE), ash (A), calcium and phosphorus in the carcass were conducted at the Laboratório de Análises de Alimentos do Departamento de Ciências dos Alimentos (Laboratory of Food Analysis in the Department of Food Sciences) at UFLA, following the methodology mentioned by AOAC (2002).
The experiment design used was completely randomized, with four levels of available phosphorous and five replications. Analysis of variaence (ANOVA) to assess the significance between treatments was used. Once significance between treatments was detected, the decomposition of the degrees of freedom by means of orthogonal polynomials to fit the rules were used. In situations where there was a polynomial fit of order three, was opted for the multiple comparison test, Scott-Knott, since when, the present study has no biological meaning to the cubic spline. In cases where was detected the ANOVA assumptions violation was opted for the type transformation Box-Cox:

$$y' = \frac{y^{\lambda}-1}{\lambda}$$

where is:
y = original variable;
y' = transformed variable;
λ = parameter to be estimated from data.

For the statistical analysis of the water variable, the nonparametric test Kruskal-Wallis was used, as for the other variables the Scott-Knott test was used to compare averages. All tests were performed using the statistical package R version 2.8.1 (2008).

RESULTS E DISCUSSION

The values obtained for water temperature, pH and dissolved oxygen (26.0 ± 0.55° C, 6.5 ± 0.12 and 6.9 ± 0.1mg / L, respectively) were similar to those cited by Popma & Green (1990) Mercante *et al*. (2006) as suitable for breeding of this species.
The average rate of hematocrit was 26.7 (± 10.3%) and hemoglobin values were (6.76 ± 0.12g / dL) in the tilapia fingerlings, which was similar to the averages reported in the literature.
No significant differences ($p>0,05$) between the levels of available phosphorus as the hematocrit variable were detected. For hemoglobin variable significant ($p<0,01$) between the levels of available phosphorus adjusted polynomial regression of order two (Figura 1) was detected. The maximum amount of

hemoglobin in the blood (3.17g/dL) was observed when using 0.32% available phosphorus.

According Hrubec et al. (1996) Tavares-Dias & Moraes (2003), the hemoglobin values for *hybrid bass Tilapia rendalli* are higher than in mammals, usually between 3,3 and 14g / dL. However, more active fish may have higher values.

The average values for the coefficients of apparent digestibility of dry matter, crude protein and ether extract from the present study are presented on Table 2.

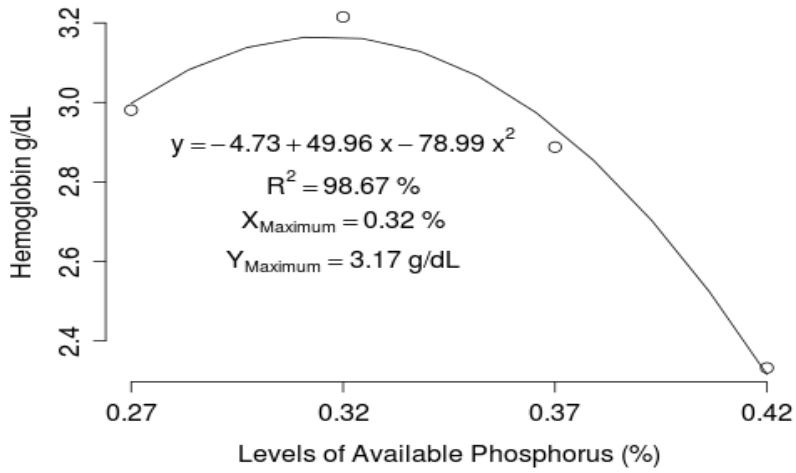

Figure 1. Quadratic regression of Hemoglobin variable (g/dL) depending of available phosphorus levels (%)

Table 2. Coefficient of apparent digestibility for Nile tilapia fingerlings, fed diets containing different available phosphorus levels

Variables	CV (%)	Phosphorus levels (%)			
		0.27	0.32	0.37	0.42
Dry Matter^{L}	1,73	99,81	97,76	95,77	94,72
Crupe proteinP^{L}	1,25	99,92	98,46	96,85	96,42
Ether extract^{Q}	1,76	99,85	98,28	95,55	96,76

^{L}Linear regression significant, P<0.01; ^{Q}Quadratic regression significant, P<0.01.

The increase 0.1% of available phosphorus in the diet resulted in a reduction of 3.45% of dry matter digestibility (Figure 2).

Available phosphorus levels in the diet also resulted in a decrease on apparent digestibility of crude protein (CP). The increase at 0.1% of phosphorus causes a reduction of 2.42% on the digestibility coefficient (Figure 3).

Decrease of digestibility coefficient of ether extract (EE) with the increased available phosphorus level, provided the 0.39% available phosphorus level in the diet cause worse digestibility (96,41%) - (Figure 4).

According to these results, the treatment with 0.27% available phosphorus showed digestibility superior than the others. Furuya et al. (2006), also working with phosphorus levels in diets for tilapia, found lower values than those found in this study. Studies carried out to determine P requirement of tilapia are discrepant (WATANABE et al. 1980; HAYLOR et al. 1988; SIGNOR et al., 2004). These differences may be related to several factors such as the phosphorus source used, stage of development of the fish, since the requirements can vary in different stages of the animals' life, feed management, environment, minerals dissolved in the aquatic environment and the statistical model used.

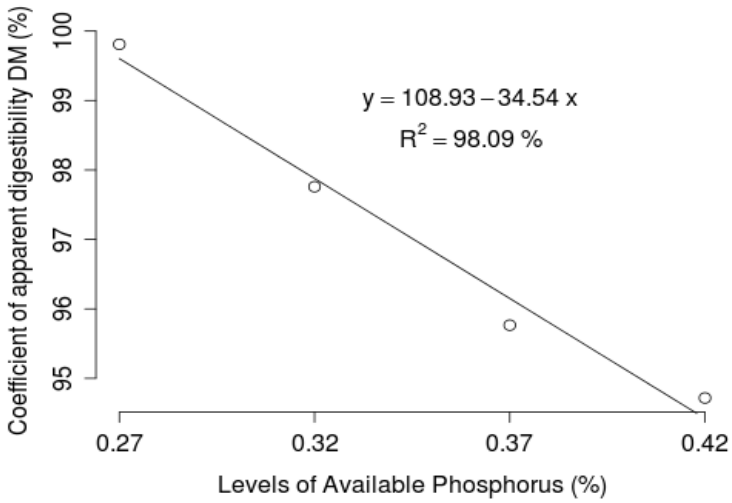

Figure 2. Linear regression of dry matter (DM) digestibility apparent accordin to available phosphorus levels

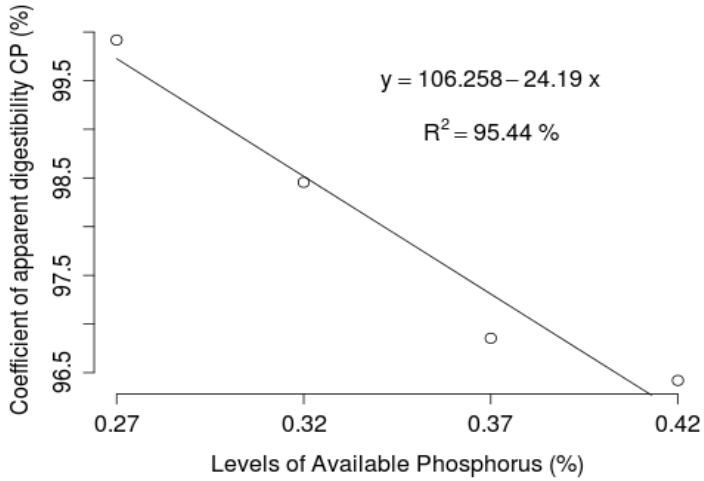

Figure 3. Linear regression of apparent digestibility coefficient of crude protein (CP) depending of available phosphorus levels

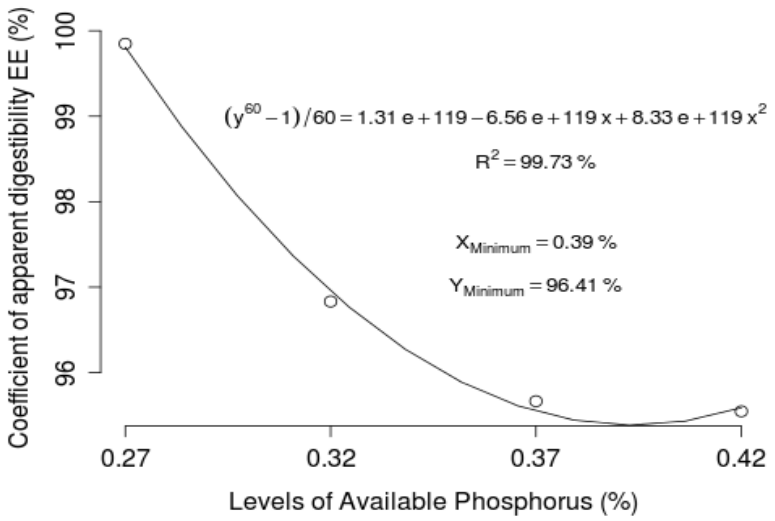

Figure 4. Linear regression of apparent digestibility coefficient of ether extract (EE) according to the level of available phosphorus

The average values of body composition for the fish carcasses are presented on Table 3. Regarding the water percentage, the level of 0.37% was higher than the 0.32% and 42% phosphorus and similar to the level of 27%, this being similar at all studied levels.

Regarding the dry matter percentage, the level of 0.37% was higher than the 0.32%, 0.27% and 0.42%.

There were no significant differences (P> 0.05) observed for protein, ash and calcium as the level of phosphorus increased in the diet. Corroborating this result, Ribeiro et. al. (2006) and Furuya et. al. (2008a) found no significant difference for moisture, protein and ash in the body composition of tilapia fingerlings (Table 3).

Table 3. Chemical composition of the carcass of Nile tilapia fingerlings fed diets containing different phosphorus levels

Variables	CV (%)	Phosphorus level (%)			
		0.27	0.32	0.37	0,42
Dry matter *	0,91	89,34[b]	89,12[b]	89,99[a]	88,63[b]
Crude protein[NS]	4,54	72,59	73,06	71,25	71,41
Ether extract[L]	8,53	19,64	18,40	18,19	17,37
Ashes[NS]	8,42	12,86	12,97	13,82	13,00
Calcium[NS]	10,12	4,83	4,61	4,96	4,81
Phosphorus*	7,50	3,42[b]	3,31[b]	3.75[a]	3,56[a]

*Averages with the same letter, in the line, do not differ by the Scott-Knott test at 5% probability level; [NS]Regressão não significativa; [L]Regressão linear significativa (P<0.01).

Significant differences were observed for fat and phosphorus (P< 0.05) when using different levels of phosphorus, and the highest amount of ether extract was observed in the use of 0.27% phosphorus in the diet.

Significant differences were observed for fat and phosphorus (P<0.05) when using different levels of phosphorus. The increase of 0.1% in available phosphorus level decreases by 1.4% carcass ether extract (Figure 5). Concerning the phosphorus percentage in the carcass, the levels of 0.37 and 0.42% available phosphorus that provided a higher percentage of phosphorus in the carcass compared to the levels 0.27 and 0.32%, the latter two are those who have provided similar percentage of carcass phosphorus. According to Ribeiro et al.(2011), increasing age leads to a decrease in body content water and increase fat content, with small changes in protein and minerals.

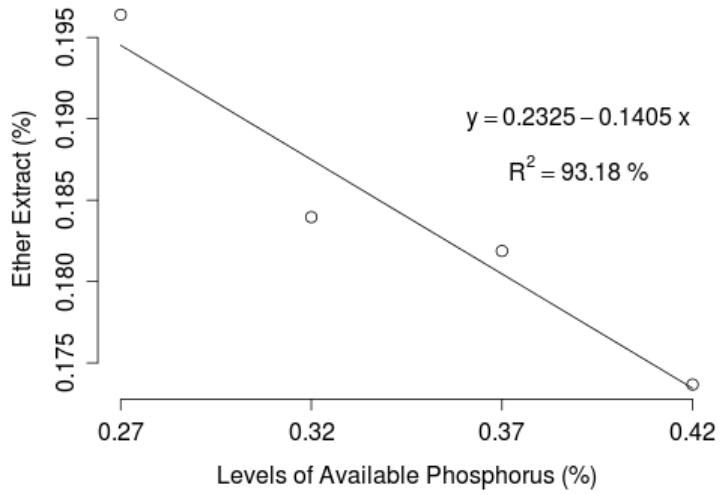

Figure 5. Linear regression of ether extract in function of available phosphorus levels

The results for fat were different from those found by Furuya et al. (2008a), who did not obtain significant differences for this parameter. Similar results found in this study were also described in the experiments of Zhang et al. (2006) with silver perch and Yang et al. (2006) with Japanese seabass.

Roy & Lall (2003), working with juvenile haddock, observed that the increase in phosphorus content of diet resulted in decrease of carcass fat content.

The reduction of fat content is related to inhibition of the citric acid cycle and the accumulation of acetil-CoA (FURUYA et al (2008b).

Phosphorus deposition was higher (P< 0.05) in higher phosphorus levels in the diet. The requirement results obtained in this work is close to the levels reported by NRC (1993) for the main species used in fish farming. The level of 0.42% phosphorus in the diet showed better indicators of body composition in Nile tilapia fingerlings, showing smaller amount of ether extract and higher phosphorus retention on the animal's body.

Considering the results it is concluded that the diet with 0.27% available phosphorus was more digestible than the other treatments and diets with the level of 0.42% provided a better body fat yield and higher deposal of this mineral for Nile tilapia fingerlings.

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