ANTINUTRITIONAL FACTORS OF RAW SOYBEAN ON GROWTH AND HAEMATOLOGICAL RESPONSES OF NILE TILAPIA*

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ABSTRACT

This study was conducted to evaluate the effect of antinutritional factors of RSB (raw soybean) on hematology and growth performance of Nile tilapia (*Oreochromis niloticus*). The replacement was made the levels of 0.0, 15.0, 25.0 and 35.0% of the protein in soybean meal (SBM) by the crude soy protein. In this study, the effect of replacing SBM by RSB on haematological profile and growth performance of Nile tilapia were evaluated after 90 days of feeding. Urea activity was evaluated of RSB which indicated the presence of antinutritional factors. The hematological parameters (erythrogram and white blood cell) were evaluated. The parameters of growth performance were also evaluated. RSB affected the weight gain, feed intake and protein retention. Additionally, an increase of neuthrophils count and a decrease of albumin concentration were observed. RSB inclusion in the Nile tilapia diet changed the white blood cell number and negatively affected growth development. However, no effect on erythrogram was observed in this study. We can conclude that although the antinutritional factors present in RSB interfered the growth performance, it is recommended to use the replacement level of RSB below 35%, as it does not affect fish health.

Key words: urease activity; trypsin inhibitor; nutrition; *Oreochromis niloticus*; raw soybean.

FATORES ANTINUTRICIONAIS DA SOJA CRUA NO CRESCIMENTO E RESPOSTAS HEMATOLÓGICAS DA TILÁPIA DO NILO

RESUMO

O objetivo do estudo foi avaliar o efeito dietético dos fatores antinutricionais da SC (soja crua) na hematologia e no desempenho produtivo da tilápia-do-nilo (*Oreochromis niloticus*). Foi realizada a substituição de 0,0; 15,0; 25,0 e 35,0% da proteína do farelo de soja (FS) pela proteína da SC. Neste estudo, foram avaliados o efeito da substituição do FS por SC no perfil hematológico e desempenho produtivo da tilápia após 90 dias experimentais. Foi avaliado a atividade ureática da SC, indicando a presença de fatores antinutricionais. Os parâmetros hematológicos (eritrograma e serie branca do sangue) foram avaliados. Também foram avaliados os parâmetros de desempenho produtivo. A SC afetou o ganho de peso, o consumo de ração e a retenção proteica. Além disso, foram encontrados aumento do número de neutrófilos e diminuição da concentração de albumina. A inclusão de SC na dieta da tilápia alterou o número de células brancas no sangue e prejudicou o desenvolvimento produtivo. No entanto, nenhum efeito sobre eritrograma foi observado neste estudo. Assim podemos concluir que, embora os fatores antinutricionais presentes na SC interferiram no desempenho produtivo, recomenda-se utilizar o nível de substituição da SC abaixo de 35%, por não afetar a saúde dos peixes.

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INTRODUCTION

Antinutrients or antinutritional factors are defined as substances which by themselves, or through their metabolic products arising in living systems, interfere with food utilization and affect the health of animals (FRANCIS *et al*., 2001). They are most likely present in plant feedstuffs and can be divided into four broad groups: 1) factors affecting protein utilization and digestion; 2) factors affecting mineral utilization; 3) antivitamins; and 4) miscellaneous substances (FRANCIS *et al*., 2001). According to KROGDAHL *et al*. (2010), the most relevant antinutritional factors are soluble and insoluble fibers, phytic acid, enzyme inhibitors, hemagglutinins, lectins, saponins, phytoestrogens, phytosterols and oligosaccharides, which are found in great amounts in soybean products. The study of the antinutritional factors in plant feed ingredients have gained more attention recently due to the markedly increase on plant feedstuffs inclusion in aquafeed in the last two decades. However, the substances which depress fish growth and health are difficult to study since most of the plant feedstuffs presents more than one antinutrient and showns that they may interact to produce undesirable effects (FRANCIS *et al*., 2001; IWASHITA *et al*., 2008; KROGDAHL *et al*., 2010).

Soybean is the most commonly used plant protein source to replace fish meal in aquaculture diets. The recommended upper limit for including soy products in herbivorous/omnivorous fish is 20% in grow-out phase, while for carnivorous species half of this amount is recommended (HERTRAMPF and PIEDAD-PASCUAL, 2003). However, previous studies have reported the suitability of total replacement of fish meal by standard soybean meal in Nile tilapia diets, however, it is important to have adequate supplementation with synthetic amino acids and the incorporation of the enzyme phytase to improve the availability of phosphorus (FURUYA *et al*., 2004a; DANWITZ *et al*., 2016). Additionally, other soy products, such as full-fat soybean meal (heat processed) may be limited up to 17% in Nile tilapia diets due to the several antinutrient contents that still are detected in this product after processing (FURUYA *et al*., 2004b).

The effects of the incorporation of raw soybean (whole unprocessed beans) has been reported in an early study with Nile tilapia (WEE and SHU, 1989). These authors observed a reduced growth performance and feed utilization in all inclusion levels of raw soybean compared to the diets with soybean meal or boiled full-fat soybean. Additionally, they concluded that Nile tilapia grew better when trypsin inhibitor levels were lower than 0.09%. EL-SAYED *et al.* (2000) reported a modification on secreted proteases of Nile tilapia fed with diets, which contained raw and processed soybeans. These authors also observed an inhibitory effect of wheat bran on protease activity of fish.

DANWITZ *et al.* (2016) observed a decrease in feed intake and in growth performance when they included the rapeseed protein in the diets for turbot (*Psetta maxima* L.). This seems to be a result of antinutritional factors present in used rapeseed, because ANFs like glucosinolates, their breakdown products and also phytic acid adversely affected feed palatability and fish growth.

Haematological and serum protein profile has been used as a tool to evaluate fish welfare and disturbance on fish metabolism (BARROS *et al*., 2009; BARBIERI and BONDIOLI 2015). It can also be influenced by antinutritional factors, such as lectins presented in greater quantities in grain legumes, such as soybeans, peas and grasses. Lectins or hemagglutinins can be detected and characterized for their ability to agglutinate erythrocytes, in some cases with high specificity. They can also enhance mitogenic stimulation of lymphocytes and agglutination of cancer cells (LIS and SHARON, 1973). However, few studies on the effects of the antinutritional factors of soybean on Nile tilapia hematology are available.

Thus, based on the lack of available data about effect of raw soybean on fish haematology and the hypothesis that antinutritional factors may influence haematological profile of Nile tilapia, we designed a feeding trial with Nile tilapia fingerlings to test our hypothesis. Additionally, we evaluated the effect of different levels of raw soybean inclusion on growth performance and feed utilization.

METHODS

This study was approved by the ethics committe of animal experimentation, protocol nº 208/2008.

Raw soybean grains (RSB) were ground for chemical composition analysis. Dry matter [DM], gross energy [GE], crude protein [CP], ether extract [EE], ASH, crude fiber [CF] of raw soybean and soybean meal were analyzed according to AOAC (2000) protocols. For trypsin inhibitor activity analysis, raw soybean was finely ground and passed in a sieve with 60 mesh, and the crude soybean meal was cold defatted, successively washed in petroleum ether and analyzed according to KAKADE *et al.* (1969). The chemical composition, trypsin inhibitor (TI) and urease activity values, protein solubility in KOH (g 100^{-1}), and other antinutrients in soybean meal (SBM) and RSB are shown in Table 1. Urease activity (pH) was determined according to AOCS (1980) and protein solubility in KOH (g 100^{-1}) were determined according to ARABA and DALE (1990) (LABTEC, Campinas, SP, Brazil).

Table1. Chemical composition and antinutrients in standard soybean meal and raw soybean

Chemical composition	Raw soybean	Soybean meal
Dry matter (%)	86.83[a]	87.71[f]
Crude protein (%)	36.04[a]	51.21[g]
Gross energy (kcal kg^{-1})	5,390[a]	3,990[g]
Lipid (%)	24.6[a]	1.48[g]
Crude fiber (%)	9.65[a]	7.12[g]
Ash (%)	4.88[a]	7.44[g]
Lysine (%)	0.6[b]	3.97[g]
Met + cyst (%)	0.27[b]	1.25[g]
Antinutrients		
Trypsin inhibitor (UTI mg^{-1})	257[a]	58[h]
Urease activity (pH)	2.21[a]	0.00[i]
Protein solubility in KOH (%)	86.57[a]	69.16[i]
Non-starch polysaccharides (%)	10.9[b]	20.00[j]
Phytic acid (%)	1.27[c]	1.00[k]
Tannin (mg g^{-1})	1.52[c]	8.22[i]
Lectins (mg g^{-1})	6.5[d]	0.05 – 0.18[l]
Saponins (%)	0.1 – 0.5[e]	0.43 – 0.67[m]

[a] Analyzed values; [b] values reported by MORRIS *et al.* (2005); [c] values reported by EGOUNLETY and AWORH (2003); [d] values reported by VASCONCELOS *et al.* (2001); [e] values reported by KROGDAHL *et al.* (2010); [f] values reported by GONÇALVES *et al.* (2009); [g] values reported by GUIMARÃES *et al.* (2008); [h] values reported by CARVALHO *et al.* (1997); [i] values reported by STECH *et al.* (2010); [j] values reported by VIANA *et al.* (2011); [k] values reported by BERGAMIN *et al.* (2013); [l] values reported by BARROWS *et al.* (2008); [m] values reported by BUREAU *et al.* (1998).

Experimental Diets

The practical basal diet was formulated to contain approximately 30.0% crude protein and 4,000 kcal kg^{-1} of crude energy based on the feedstuff values reported in NRC (1993). Diets were mechanically mixed with water (25% of dry weight) in a Kitchen Aid multi-function mixer (Ação Científica®, Piracicaba, SP, Brazil) and pelleted a meat grounder aiming to obtain a 4.0 mm pellet diameter (Ação Científica®, Piracicaba, SP, Brazil) (Table 2). The experimental diets consisted of a basal control diet (without RSB, diet RSB0) and three test diets containing 10.62% (RSB15); 17.70% (RSB25) and 24.78% (RSB35) of RSB due to the replacement of 0.0, 15.0, 25.0 and 35.0% of protein in soybean meal SBM for protein of RSB (Table 2).

Feeding and Sampling

One hundred and sixty all-male Nile tilapia with an initial body weight of 17.0 ± 1.55 g (mean ± SD) were randomly assigned into 32, 200 L-aquaria, at a stocking density of five fish/aquarium, four treatments with eight replicates. The tanks were inserted in a recirculation system with a biofilter and controlled heating system, that maintained the water quality and temperature at 26.0 ± 1.0 ºC. Each tank was considered a replicate for the growth performance trial.

Hematology

After 90 days, the haematological parameters were evaluated on eight fish per treatment (one fish from each replicate). The fish were anesthetized (1.5 g benzocaine: 15L water), and blood samples were drawn from the caudal vein using a tuberculin syringe, rinsed on 3% EDTA. For Red blood cell [RBC] and leukocyte counts [WBC] were determined by dilution and counting using a hemocytometer in Neubauer's chamber, using 0.01% Merck® Toluidine Blue diluted in 0.9% physiological solution in a 1: 200 ratio Thoma pipette. It was prepared of blood-staining blades stained with May-Grünwald-Giemsa-Wright for total thrombocyte count and for leukocytes differentiation. Differential counting was performed on a light microscope under immersion (100 x). Two hundred cells were counted to establish percentages for each target cellular, targeted component. The haemoglobin [Hb] was determined by the cyanometahaemoglobin colorimetric method using a commercial kit (Gold Analisa Diagnóstica, Belo Horizonte, MG, Brazil). The haematocrit [Ht] was determined using the microhematocrit method. The total plasma protein [TPP] was measured using a manual Goldberg refractometer by breaking the microhaematocrit capillary just above the leukocyte layer after the haematocrit reading. The mean corpuscular volume [MCV = (Ht x 10)/RBC] and the mean corpuscular haemoglobin concentration [MCHC = (Hb x Ht) x 100], were calculated. The albumin concentration [ALB] was determined by the bromocresol method using a commercial kit (Analisa Diagnóstica^{Ò}) for colorimetric determination. The albumin:globulin ratio [A/G] was calculated as following Globulin = TPP – ALB; A/G = ALB/Globulin.

Table 2. Diet composition and proximate analysis of the experimental diets (dry matter)

	Replacement level (%)			
	0	15	25	35
Ingredient				
Wheat middlings	13.50	13.18	8.50	3.00
Meat and bone meal 45	10.50	10.50	10.50	10.50
Soybean meal	61.10	53.00	48.00	42.00
Raw soybean	0.00	10.62	17.70	24.78
Corn	2.00	6.00	11.40	18.92
Corn starch	2.00	0.00	0.00	0.00
Soybean oil	10.23	6.00	3.15	0.00
NaCl	0.10	0.10	0.10	0.10
Methionine	0.22	0.25	0.30	0.35
Ascorbic acid	0.08	0.08	0.08	0.08
Vitamin premix[1]	0.15	0.15	0.15	0.15
Mineral premix[2]	0.10	0.10	0.10	0.10
BHT[3]	0.02	0.02	0.02	0.02
Total	100	100	100	100
Proximate Composition				
Crude energy (kcal kg^{-1})	4,053	4,090	4,114	4,120
Crude protein (%)	30.87	31.68	31.98	31.91
Ether extract (%)	12.86	11.21	10.09	8.69
Crude fiber (%)	4.10	4.77	4.90	4.94

[1]Vitamin premix supplied the following (IU or mg kg^{-1} diet): vitamin A, 16060; vitamin D3, 4510; vitamin E (DL-α tocopherol), 250; vitamin K (menadione sodium bisulphite), 30; thiamine, 32; riboflavin, 32; Ca-D-pantothenate, 80; niacin, 170; biotin, 10; folic acid, 10; cyanocobalamin, 0.032; pyridoxine, 32. [2]Mineral premix supplied the following (mg kg^{-1} diet): Na2SeO3, 0.7; MnO, 50; ZnO, 150; FeSO4, 150; CuSO4, 20; CoSO4, 0.5; I2Ca, 1.0. [3]Antioxidant: butylated hydroxytoluene.

Growth performance

At the end of the feeding trial (90 days), growth performance was evaluated by calculation of weight gain [WG = final weight – initial weight], feed intake [FI = total food offered during the experimental period], feed conversion ratio [FCR = feed fed/weight gain], protein efficiency ratio [PER = weight gain/total protein intake], protein retention [PR = (((final body weight x whole-body protein) - (initial body weight x initial whole-body protein)) / (dietary protein)) x 100] and relative percentage survival [RPS% = (number of surviving fish/number of initial fish)/100].

Chemical analyzes of diets and Whole-body

At the beginning of the feeding trial 25 fish from the initial stock were anesthetized with an alcoholic solution of benzocaine diluted in water (1g 10L^{-1}) and euthanized by anesthetic overdoses (1g L^{-1}) for the determination of initial whole-body composition. The same euthanasia procedure was performed at the end of feeding trial with eight fish from each treatment for the determination of final whole-body compositions. The analyzes performed for the initial and final whole-body composition, and for the diets were: Dry matter [DM], crude protein [CP], ether extract [EE], crude fiber [CF] and ash were determined according to AOAC (2000); energy contents were determined in adiabatic, calorimetric bomb (IKA® Werke – JK C2000 basic).

Statistical analysis

Design in this study was completely randomized. Data on growth performance were analyzed by one-way ANOVA using the general linear model [GLM] procedure, complemented by Tukey test (SAS, 2004). Data on survival rate were submitted to the Kruskal-Wallis non-parametric statistical test complemented with multiples comparisons of Dunn (ZAR, 1999). Data on whole-body composition were submitted to ANOVA and Tukey test to the pair of averages (ZAR, 1999). Haematology data were submitted to ANOVA for repeated measures models in independent groups (JOHNSON and WICHERN, 2002).

RESULTS

The water quality parameters, such as pH 7.0 ± 0.5, dissolved oxygen 7.05 ± 0.15 mg L^{-1}, total ammonia 0.11 ± 0.07 mg L^{-1}, and alkalinity 100.00 ± 10.00 eq mg $CaCO_3$ L^{-1} are within the optimal range for this species (BOYD, 1996). Raw soybean levels did not affect erythrogram (Figure 1). The presence of raw soybean significantly increased the absolute number of neutrophils (P < 0.05) (Figure 2) and affected the albumin content (P < 0.05) (Table 3).

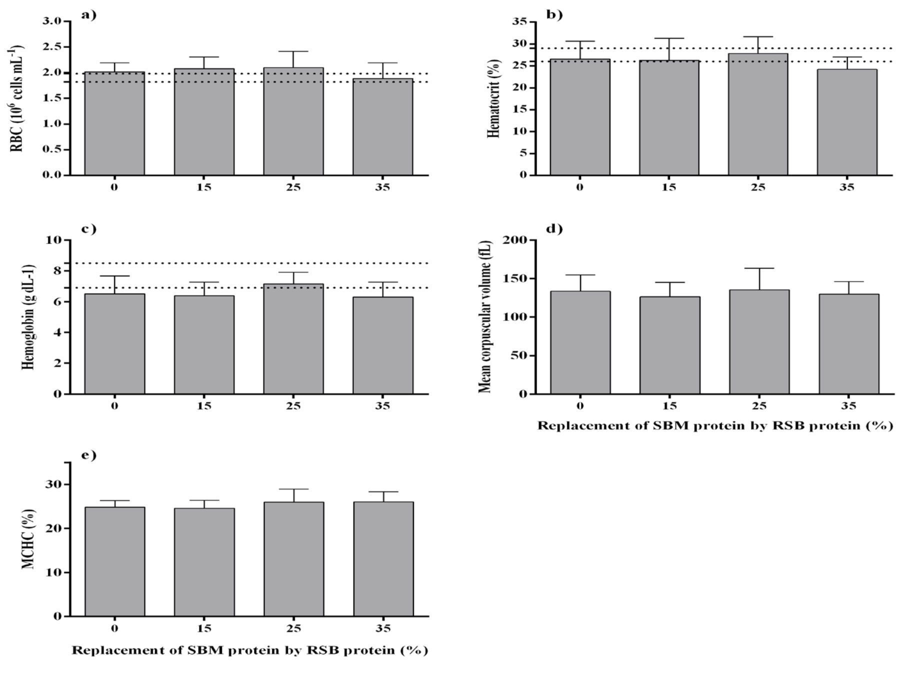

Figure 1. Red blood cell [RBC] count (a), hematocrit (b), hemoglobin concentration (c), mean corpuscular volume (d) and mean corpuscular hemoglobin concentration [MCHC] (e) of Nile tilapia fed diets containing different levels of RSB; the area between the two dotted lines (Fig. 1a, 1b and 1c) represents reference interval for Nile tilapia (BARROS *et al.*, 2009); values are mean ± SD of eight fish per diet, where (n=32).

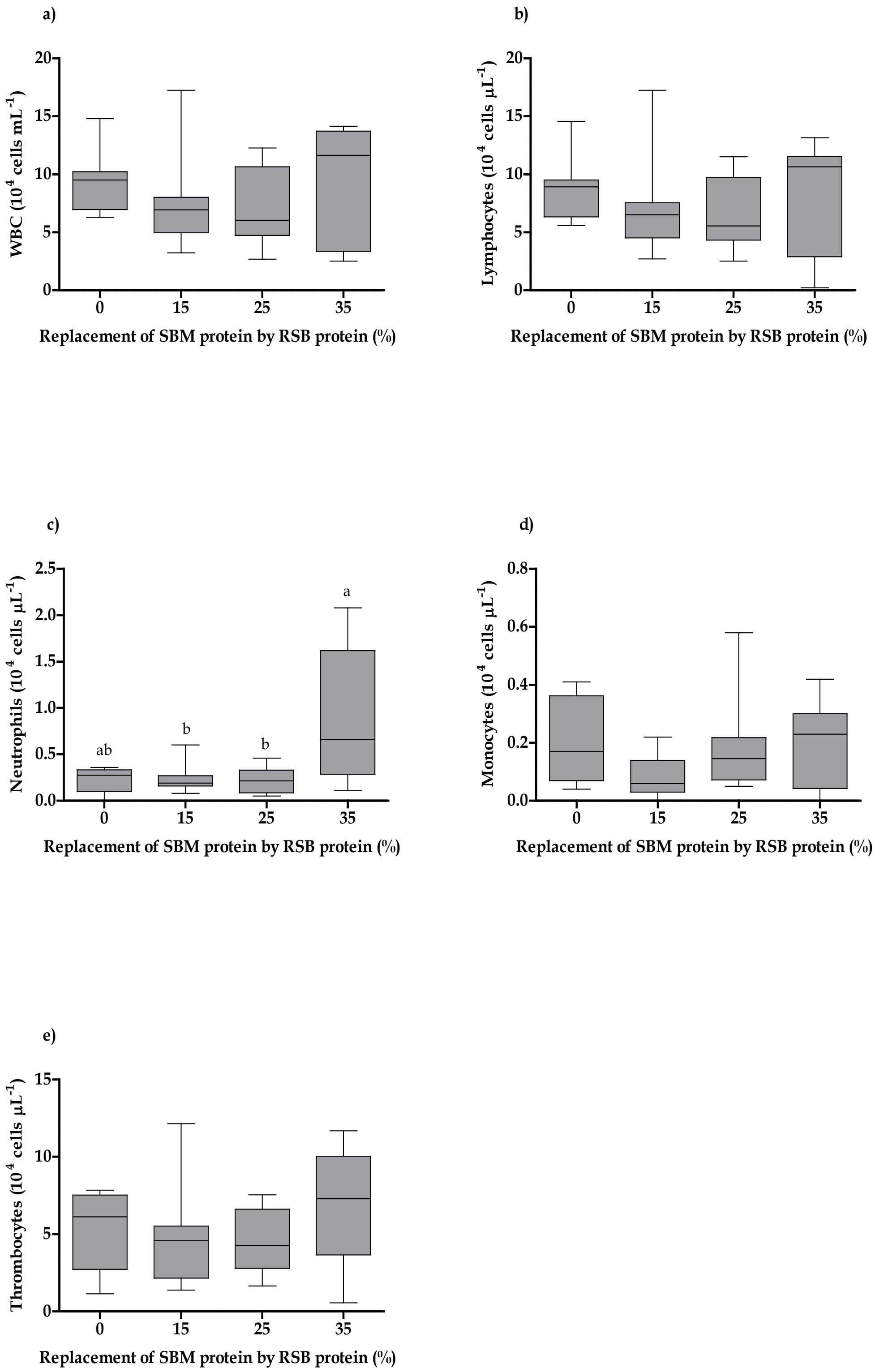

Figure 2. Total leukocyte count [WBC] (a), lymphocytes (b), neutrophils (c), monocytes (d) and thrombocytes (e) count of Nile tilapia fed diets containing different levels of raw RSB; values are means ± 95% confidence interval of eight fish per diet; different letters indicate significant differences at $P < 0.05$ by Dunn test, where (n=32).

Table 3. Means and standard deviations of total plasma protein [TPP], albumin [ALB], globulin [GLOB] and albumin/globulin ratio [A/G] of Nile tilapia fed experimental diets

	Replacement of SBM protein by RSB protein (%)[1]				
Parameter	0	15	25	35	P-value
TPP (mg dL^{-1})	3.06 ± 0.50	3.09 ± 0.60	3.07 ± 0.53	2.69 ± 0.47	P > 0.05
ALB (mg dL^{-1})	1.16 ± 0.30^{ab}	1.40 ± 0.20^{a}	1.03 ± 0.20^{b}	1.00 ± 0.26^{b}	P < 0.05
GLOB (mg dL^{-1})	2.05 ± 0.70	2.05 ± 0.92	2.43 ± 0.65	1.81 ± 0.49	P > 0.05
A/G	0.77 (0.37 – 0.88)	0.79 (0.58 – 1.91)	0.51 (0.36 – 0.71)	0.59 (0.35 – 0.96)	P > 0.05

[1]SBM: soybean meal; RSB: raw soybean; Different superscript letters indicate significant differences at P < 0.05.

Replacement of SBM protein by RSB protein negatively affected weight gain, feed intake and protein retention, but did not significantly influence on feed conversion, protein efficiency rate and survival rate (Table 4).

Raw soybean did not influence protein content. Fish fed diets with the highest inclusion level of RSB (35%) showed the highest moisture content, which it was not different from fish fed the 25% inclusion level. The fat content was inversely correlated with the increase on RSB inclusion in the diets (Table 5).

Table 4. Means and standard deviations of weight gain [WG], feed intake [FI], feed conversion ratio [FCR], protein efficiency ratio [PER], protein retention [PR] and relative percentage survival [RPS] of Nile tilapia fed experimental diets.

	Replacement of SBM protein by RSB protein (%)[1]				
Variable	0	15	25	35	P-value
WG (g)**	522.30 ± 41.25^{a}	381.17 ± 62.51^{b}	360.94 ± 65.46^{b}	358.62 ± 31.62^{b}	P < 0.05
FI (g)*	829.59 ± 51.28^{a}	739.53 ± 20.14^{b}	730.99 ± 29.60^{b}	704.99 ± 30.75^{b}	P < 0.05
FCR	1.60 ± 0.15	2.00 ± 0.40	2.10 ± 0.46	1.98 ± 0.16	P > 0.05
PER (%)	1.75 ± 0.17	1.45 ± 0.25	1.35 ± 0.28	1.41 ± 0.12	P > 0.05
PR (%)**	24.79 ± 0.57^{a}	20.92 ± 0.65^{b}	18.16 ± 0.71^{c}	19.12 ± 0.66^{c}	P < 0.05
RPS (%)	100.00 ± 0.00	92.00 ± 16.00	100.00 ± 00.00	100.00 ± 0.00	P > 0.05

[1]SBM: soybean meal; RSB: raw soybean; Different superscript letters indicate significant differences at P < 0.05; *Linear effect FI = 815.853-3.444x, R^2 = 0.56; **Quadratic effect: WG = 520.792-12.106x+0.216x^2, R^2 = 0.58; PR = 24.935-0.410x+0.007x^2, R^2 = 0.90.

Table 5. Means and standard deviations of whole-body composition of Nile tilapia fed experimental diets, wet weight basis.

	Whole-body	Replacement of SBM protein by RSB protein (%)[1]				
Variable (%)	(initial)	0	15	25	35	P-value
Moisture	74.02	70.35 ± 1.00^{b}	71.27 ± 2.29^{b}	72.12 ± 1.13^{ab}	73.62 ± 0.54^{a}	P < 0.05
Protein	15.74	15.77 ± 0.40	17.50 ± 2.55	15.85 ± 0.53	15.64 ± 0.62	P > 0.05
Lipid	4.78	10.49 ± 1.14^{a}	8.61 ± 0.72^{b}	8.56 ± 0.99^{b}	7.06 ± 0.29^{c}	P < 0.05
Ash	3.99	3.56 ± 0.27^{a}	2.81 ± 0.69^{b}	3.81 ± 0.31^{a}	4.03 ± 0.26^{a}	P < 0.05

[1]SBM: soybean meal; RSB: raw soybean; Different superscript letters indicate significant differences at P < 0.05 by Tukey test, where (n=32).

DISCUSSION

Haematological profile can be used as an indicator of fish health. However, reference intervals for normal fish must be available, mainly due to the extent of physiological variation and the characteristics of each species and environment (RANZANI-PAIVA and SILVA-SOUZA, 2004; WEISS and WARDROP, 2010; BARBIERI and FERREIRA, 2011). In this study, haematological parameters were considered normal for the species, as described by FELDMAN *et al.* (2000), and by BARROS *et al.* (2009), in the same conditions and for the same species. Similar results were observed in Atlantic cod when fish meal was replaced by a mixture of plant protein (OLSEN *et al.*, 2007); however, the authors used high quality plant protein sources, such as soy protein concentrate and wheat gluten which contain reduced amounts of antinutrients. AJALA *et al.* (2015) also observed general healthy status for fish fed with *Vernonia amygdalina* Del; it could also be due to the method of processing causing a reduction in saponin content of the leaf.

The results of this study were surprisingly since we assumed to observe an effect of the antinutrients on haematology. Although there were no significant differences in erythropoiesis, the highest percentage of raw soybean addition in the diet resulted in a 6.4% decrease on erythrocytes, 8.95% on haematocrit, and 3.22% for haemoglobin rate, which can also be related to agonistic behavior of the fish. NCHA *et al.* (2015) observed decreased haematocrit, erythrocyte and hemoglobin as the moringa leaf meal level increased in the diet for the *Clarias gariepinus*. The authors suggested that the reduction in the concentration of the haematocrit in the blood usually occurs due to the presence of toxic factor, which has adverse effect on blood formation. The decrease in erythrocytes may be ascribed to the higher concentration of anti-metabolites especially phytates in the diets containing more moringa leaf meal. The reduction in the hemoglobin concentration could imply that diets having higher substitutions contained low quality protein, and this may have resulted in poor transportation of oxygen from the respiratory organs to the peripheral tissue.

The antinutrients in RSB may have induced an immune response in Nile tilapia since a significant increase on neutrophils was observed when the highest levels of RSB was included in the diets. Previous studies on salmonids and carp have demonstrated a soybean-induced enteritis characterized by infiltration of lymphocytes, macrophages, eosinophils and neutrophils in the lamina propria which may induce functional alterations such as reduction in brushborder enzyme activities and reduced uptake of macromolecules like proteins (KROGDAHL *et al.*, 2010; URÁN *et al.*, 2008). Although we did not evaluate the effect of RSB on intestinal morphology, the increased number of neutrophils in Nile tilapia blood may be a secondary effect of an inflammation in the gut of fish once a recently study have reported the adverse effect of SBM on Nile tilapia intestinal and hepatic enzyme activities (LIN and LUO, 2011). However, further studies are needed to prove this hypothesis. The lack of difference compared to the control diet may be related to the high data variation, which is common for this type of parameter (WEISS and WARDROP, 2010).

Plasmatic protein concentration has been used to indicate the resistance of fish to stress since it refers to albumin and globulin fractions in plasma or serum. Albumin is the most abundant fraction and it is responsible for nutrient transportation and blood osmotic balance, while globulin fraction is involved in immune defense mechanisms (THOMAS, 2000). Although there were no statistical differences, we observed a decrease in the A:G ratio in fish fed diets with the highest RSB inclusion. Significant changes in ALB levels can reflect a possible stress condition of the fish.

Antinutritional factors found in RSB, such as lectins, phytic acids, saponins, protease inhibitors and allergens, can influence feed intake (FRANCIS *et al.*, 2001). Feed intake was affected by the replacement of RSB in the diets, as the 35% replacement level caused an 18% decrease on feed intake compared to the control group. In salmonids, which causes enteritis in fish fed with soy products have been attributed to soy saponins and/or their interaction with other antinutrients, such as tannins (KROGDAHL *et al.*, 2010). For instance, saponins induced growth depression due to reduced feed intake in Chinook salmon (*Oncorhynchus tshawytscha*) fed both *Quillaja saponaria* and a purified soybean extract containing saponins (BUREAU *et al.*, 1998), while rainbow trout have significant decrease on growth performance. Serious damage to the intestinal mucosa has been reported for both species (BUREAU *et al.*, 1998); however, Atlantic salmon seems to be more affected than other salmonid species (BUREAU *et al.*, 1998) showing reduced microvilli height and absorptive vacuole (BOONYARATPALIN *et al*., 1998).

Chemical indicators (trypsin inhibitor, protein solubility and urea activity) and biological indicators (weight gain, feed conversion and subclinical signs) have been used to determine the presence of antinutritional factors in feeds and its effects on animals (FRANCIS *et al.,* 2001). RSB has urea activity, which indicated the presence of antinutritional factors; protein solubility and high levels of trypsin inhibitors indicates the inadequate soybean processing which contributed to impaired growth performance and explains the low weight gain and protein retention results. Similar responses were described for tilapia (WEE and SHU, 1989), salmonids (BUREAU *et al.,* 1998; CHENG and HARDY, 2003), pacu *Piaractus mesopotamicus* (STECH et al., 2010), and channel catfish *Ictalurus punctatus* (PERES *et al.,* 2003).

Survival rate was not affected by RSB inclusion, and the mortality recorded was likely due to agonistic confrontation among animals (MEDEIROS *et al.,* 2005; GONÇALVES-DE-FREITAS *et al.,* 2008).

Inverse correlation between moisture and lipid content in carcass are expected, and reflected the influence of antinutritional substances in the non-processed raw soybean grains (FRANCIS *et al*., 2001). Similar results were previously reported with territorial species (CASTELL and CLIPLEF, 1988) and channel catfish (PERES *et al*., 2003), whereas high content of RSB decreased visceral and hepatic somatic indices.

The 90-day feeding period may have been decisive to the outcome of the study. Common carp seems to recover from soybean-induced enteritis after 45 days showing an adaptive response to soybean inclusion, could be attributed to the omnivorous nature of species which have a higher ability to modulate digestive functions to digest plant protein (URÁN *et al*., 2008). Although we did not evaluate specific inflammation sites in this study, an adaptive response of Nile tilapia could have occurred after 90 days of feeding, where it was not possible to observe statistical differences for the erythrogram only for the neutrophils with the diet containing the highest level of raw soybeans.

CONCLUSION

In this study we can conclude that although the antinutritional factors present in raw soybean interfered the growth performance, it is recommended to use values below 35% of replacement as it does not affect fish health.

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