BOLETIM DO INSTITUTO DE PESCA
ISSN 1678-2305 online version
Scientific Article

SOYBEAN MEAL IN DIETS FOR JUVENILES OF PIRARUCU*

Kaila de Assis CERDEIRA[1,2]
Karla Jaqueline Nogueira da Silva e SOUZA[3]
Jefferson Barros FERREIRA[2]
Aline ZAMPAR[4]
Eduardo Akifumi ONO[3]
Elizabeth Gusmão AFFONSO[3]

[1]Universidade Federal do Amazonas – UFAM, Instituto de Ciências Sociais, Educação e Zootecnia, Estrada Parintins/Macurany, 1805, Jacareacanga, CEP 69152-240, Parintins, AM, Brasil. E-mail: kaila_cerdeira@hotmail.com (corresponding author).

[2]Universidade Nilton Lins, Programa de Pós-graduação em Aquicultura, Avenida Professor Nilton Lins, 3259 Flores, CEP 69058-030, Manaus, AM, Brasil.

[3]Instituto Nacional de Pesquisas da Amazônia, Laboratório de Fisiologia aplicada à Piscicultura, Avenida André Araújo, 2936, Aleixo, CEP 69060-001, Manaus AM, Brasil.

[4]Universidade do Estado de Santa Catarina – UDESC, Departamento de Zootecnia, Rua Beloni Trombeta Zanin, 680, Santo Antônio, CEP 89815-630, Chapecó, SC, Brasil.

*Financial support by Darpa/Finep; INCT-Adapta II/Cnpq/Fapeam; PAPAC/Fapeam.

Received: November 10, 2017
Approved: April 04, 2018

ABSTRACT

In order to assess the effects of replacing fishmeal (FM) protein by soybean meal (SM) for juveniles of pirarucu (*Arapaima gigas*) feeds, five isonitrogenous (46% CP) and isocaloric (4,800 kcal kg^{-1} GE) diets containing 0, 15, 30, 45 and 60% replacement were tested. One hundred and five fish (233.5 ± 11.5 g) were distributed in fifteen 200-L tanks, fed three times daily to apparent satiation for 120 days. The highest growth rates were observed in diets with up to 30% replacement, but no significant difference was detected as regards to specific growth rate, feed conversion ratio, mean daily feed intake and mean daily protein intake in fish fed diets with up to 45% FM replacement. Survival rate, condition factor, hepatosomatic and viscerosomatic indexes were not influenced by the treatments, just as the biochemical analyses were not, except for cholesterol. Fish fed diets with increasing levels of SM presented lower hematological values, and higher mean corpuscular value, indicating probable anemia at 60% FM replacement and hypocholesterolemia. Diets with 15 and 30% FM replacement were the most cost effective suggesting that SM can replace up to 30% of FM in diets for pirarucu juveniles (200 to 1,200 g), without compromising growth performance, fish health, feed digestibility and feed cost effectiveness.

Key words: native fish; nutrition; physiology; protein replacement; carnivorous fish.

FARELO DE SOJA NA DIETA DE JUVENIS DE PIRARUCU

RESUMO

Para avaliar os efeitos da proteína do farelo de soja (FS), substituindo a proteína da farinha de peixe (FP) na alimentação de juvenis de pirarucu, foram testadas cinco dietas isoproteicas (46% PB) e isocalóricas (4800 kcal kg^{-1} EB), contendo 0, 15, 30, 45 e 60% de substituição. Cento e cinco exemplares (233,5 ± 11,5 g) foram distribuídos em 15 tanques (200 L), alimentados três vezes ao dia, até saciedade aparente, por 120 dias. Os maiores ganho de peso foram obtidos até 30% de substituição, sem diferenças significativas para taxa de crescimento específico, conversão alimentar aparente, consumo médio diário de ração e consumo médio diário de proteína com até 45% de FS. Não houve diferenças estatísticas para sobrevivência, fator de condição, índice hepatossomático e víscerossomático e nas análises bioquímicas, exceto colesterol. Os valores hematológicos diminuíram com os níveis de substituição, com elevação do volume corpuscular médio, indicando provável quadro de anemia em 60% de FS e de hipocolesterolemia. Dietas com 15 e 30% de FS foram mais viáveis, sugerindo a substituição até 30% da FP pelo FS (inclusão de 22%) em dietas para pirarucu (200 a 1.200 g), sem prejuízos aos parâmetros zootécnicos, saúde dos peixes e menor custo de produção.

Palavras-chave: peixe nativo; nutrição; fisiologia; substituição de proteína; peixe carnívoro.

INTRODUCTION

For carnivorous species, like pirarucu (*Arapaima gigas*), fishmeal is the main ingredient of the diet, representing from 30 to 50% of its composition, with high protein content, excellent amino acid profile, high nutrient digestibility and, in general, it has no antinutritional factors (HARDY, 1999; GATLIN *et al.*, 2007). However, with the rapid growth of fish farming, and consequently the increase in demand for this ingredient, there has been strong pressure on the stocks of forage species, raw material of this input, making its production reach the maximum sustainable limit (FAO, 2014). In this context, intensification of fish farming depends on the development of sustainable sources of protein for the replacement of fishmeal as the main protein nutrient in the diet, including plant sources (FAO, 2014).

From all sources of plant proteins, soybean meal is considered the most complete in its amino acid composition, in addition to its high availability and low cost (PEISKER, 2001). However, the nutritional value of soybean is limited by the presence of antinutritional factors such as protease inhibitors (trypsin and chymotrypsin), hemagglutinins (lectins), phenolic compounds (tannins), which affect the digestion and physiology of animals (STECH *et al*., 2010). According to the literature, the heating of soybean at 121 °C for 20 minutes destroys most of the trypsin inhibitors present, however, it presents some complications such as, large differences in heat stability of hemagglutinating and anti-tryptic activity (ARNDT *et al*., 1999; CSÁKY and FEKETE, 2004).

Despite this, soybean meal has been successfully used in the partial or total replacement of fishmeal for both freshwater and seawater carnivorous species (FREITAS *et al*., 2011; YE *et al*., 2011; LIM *et al*., 2011; YANG *et al*., 2011; KADER *et al*., 2012; SILVA-CARRILLO *et al*., 2012; KOKOU *et al*., 2012; SILVA, 2013; AZARM and LEE, 2014).

The pirarucu has been farmed due to its biological, zootechnical and economic characteristics (LÜLING, 1964; BARD and IMBIRIBA, 1986; REBAZA *et al*., 2010), nevertheless, in order for commercial production to grow, alternatives are needed to make it viable for it to be farmed, such as the use of inputs that might reduce food costs.

In recent work with juveniles of pirarucu (25 to 200 g), SILVA (2013) replaced fishmeal with soybean meal, up to 30%, and did not observe significant changes in the zootechnical performance and physiology of fish. However, other results in the literature demonstrate that it is possible to increase the percentage of replacement of vegetal protein sources with the improvement in the quality of the diet for carnivorous fish, such as the inclusion of synthetic amino acids and feed extrusion, in an attempt to reduce the effects of antinutritional factors. In this regard, the objective of this paper was to evaluate the replacement of fishmeal protein with soybean meal protein in the diet of juveniles of pirarucu, determining the coefficient of apparent digestibility of the diets, productive performance, metabolic profile and economic viability of the feeds.

METHODS

Acquisition, acclimatization and fish farming system

A total of 200 fish (± 3.5 g) were obtained from Saint Antônio's Farm, located at Estrada de Balbina, municipality of Presidente Figueiredo, AM, Brazil. They were transported in plastic bags with water and oxygen to the Laboratory of Physiology Applied to Fish Farming - LAFAP in the National Institute for Research in the Amazon (INPA), Manaus - AM. They were conditioned in PVC tanks with capacity for 2000 L. These had aeration and a continuous flow system, with food conditioning based on artemia nauplii, gradually replacing the dry food (commercial feed with 55% CP) for 13 days. During the growth period, up until reaching a weight of approximately 200 g, the fish were fed with a feed containing 55% crude protein (CP) from four to six times a day until apparent satiation and, as a prophylactic against pathogens, they were often exposed to salt baths (5 g L^{-1} for one hour).

For the experiment, the fish were conditioned in 15 conical tanks of glass fiber of 200 L. The water supply came from an artesian well with constant renovation and aeration. During the experimental period, water quality presented the mean values of 6.0 ± 0.3 mg L^{-1} of dissolved oxygen; 28.3 ± 0.4 °C temperature; 4.6 ± 0.4 pH; 0.7 ± 0.3 mg L^{-1} of total ammonia; 0.003 ± 0.001 mg L^{-1} of nitrite. All of them remained constant and within acceptable limits for Amazonian fish (KUBITZA, 2003).

Experimental diets

The isonitrogenous and isoenergetic experimental diets were prepared in individual batches to contain five replacement levels of fishmeal protein with soybean meal protein (0, 15, 30, 45 and 60%), in relation to energy: protein ratio of 10 kcal g^{-1}, according to ONO *et al*. (2008) and 0.5% chromium oxide (Cr_2O_3) was used as an inert tracer for digestibility analysis, via indirect (NG and WILSON, 1997). The protein sources of animal and vegetable origin, selected to make up the feeds, were purchased in the local market, with the salmon fish meal imported by a national company. The other ingredients were obtained both locally and from other states. The bromatological composition of the ingredients is shown in Table 1 and the feed formulation described in Table 2.

For the manufacture of different feeds, the ingredients were finely milled, sieved, weighed, blended, extruded (10 mm) and oven dried at 55 °C for 24 hours. After that, the soybean oil was heated and sprinkled with a pressurized pump over the feeds, then packed and stored in a freezer.

Experimental design

The juveniles of pirarucu were fed diets containing different levels of fishmeal replacement with soybean meal (0, 15, 30, 45 and 60%) for a period of 120 days in a completely randomized design, with five treatments and three replicates. Seven fish were used in each experimental unit, with a mean initial weight of 233.5 ± 11.5 g, fed three times a day (08:00, 12:00 and 16:00), until apparent satiety.

Feed digestibility

In order to determine feed digestibility, feces were collected between the 30th and the 60th day of experiment following the method described by CHO (1990) and modified by

Table 1. Proximal composition (%) of dry matter (DM), crude protein (CP), ethereal extract (EE), nitrogen-free extract (NFE) and ashes (CZ) of the ingredients.

Ingredients	DM	CP	EE	NFE	CZ
Fishmeal	91.3	70.4	9.9	2.2	17.5
Soybean meal	88.8	52.8	1.8	38.3	7.1
Wheat flour	87.1	13.1	1.2	85.1	0.6
Corn gluten meal	91.7	71.8	1.6	24.7	1.9
Blood meal	88.7	97.7	0.5	0.1	1.7

Table 2. Ingredients and proximal composition of experimental feeds with different levels of replacement of fishmeal protein with soybean meal protein in the diet of juveniles of pirarucu.

Ingredients %	Protein Replacement Levels,%				
	0	15	30	45	60
Fishmeal	52.0	44.0	36.0	28.5	21.0
Soybean meal	0.0	11.0	22.0	33.0	44.0
Wheat flour	28.8	24.6	20.1	15.3	11.1
Corn gluten meal	4.0	4.5	4.9	5.2	5.2
Blood meal	5.5	5.5	5.5	5.5	5.5
Soybean oil	8.2	8.7	9.7	10.6	11.2
Vitamin-mineral premix[1]	1.0	1.0	1.0	1.0	1.0
DL-Methionine	0.0	0.1	0.2	0.2	0.3
L-Lysine	0.0	0.1	0.1	0.2	0.2
Chromium oxide	0.5	0.5	0.5	0.5	0.5
Proximal Composition					
Humidity (% HUM)	3.6	3.7	3.4	3.2	3.1
Crude protein (% CP)	46.9	47.2	46.6	46.4	46.8
Ethereal extract (% EE)	8.9	10.0	10.3	12.0	11.0
Crude fiber (% CF)	3.1	3.9	4.3	3.6	5.0
Ashes (% CZ)	10.6	10.0	9.3	8.9	8.2
Nitrogen free extract (% NFE)	30.0	29.1	30.4	29.5	30.9
Gross energy (kcal GE)	4,718.3	4,802.1	4,850.0	4,962.2	4,947.9
Lysine (%)[2]	3.5	3.6	3.6	3.6	3.6
Methionine (%)[2]	1.3	1.4	1.4	1.4	1.4
Starch (%)[2]	22.7	20.9	18.9	16.6	14.8
Calcium (% Ca)[2]	2.0	1.7	1.4	1.2	1.0
Phosphorus (% P)[2]	1.4	1.3	1.1	1.0	1.0
GE:CP (kcal g^{-1})	10.0	10.2	10.4	10.7	10.6
R$ kg^{-1} of feed[3]	4.32	3.96	3.61	3.28	2.94

[1]Premix Nutron, Enrichment with microminerals and vitamins in mg kg^{-1}; manganese (26); zinc (140); iron (100); copper (14); cobalt (0,2); iodine (0,6); selenium (0,6), Vitamin A (10,000 IU); Vitamin D3 (4,000 IU); Vitamin E (100); Vitamin K (5); Vitamin B1 (25); Vitamin B2 (25); Vitamin B6 (25); Vitamin B12 (30); niacin (100); folic acid (5); pantothenic acid (50); biotin (0.8); choline (2,000); inositol (50); Vitamin C (350); [2]Calculated according to NRC (1993) and ROSTAGNO *et al.* (2011); [3]Fishmeal: R$ 7,000.00 t^{-1}; soybean meal: R$ 1,590.00 t^{-1}; wheat flour: R$ 490.00 t^{-1}; corn gluten meal: R$ 2,400.00 t^{-1}; blood meal: R$ 1,760.00 t^{-1}; soybean oil: R$ 2,000.00 t^{-1}; premix: R$ 18,150.00 t^{-1}; DL-methionine: R$ 24,720.00 t^{-1}; L-lysine: R$ 7,430.00 t^{-1}; (Prices obtained in the local market: March 31, 2016).

CANTELMO *et al*. (1999). Feces were freeze dried and stored for proximal composition analysis.

Analyses methods

Samples of the ingredients and experimental feeds were analyzed for proximal composition, following the AOAC (2000) guidelines (Table 2). The gross energy of the ingredients was estimated based on energy values obtained from NRC (1993), where proteins, ethereal extract and carbohydrates presented values of 5.6; 9.4, and 4.1 kcal g^{-1}, respectively.

The concentration of Chromium Oxide was determined by digestion with nitric and perchloric acids and read using a spectrophotometer (FURUKAWA and TSUKAHARA, 1966). The apparent digestibility coefficient (ADC) was calculated according to HOULIHAN *et al*. (2001): ADC = 100 –100 x ((%Cr_2O_3 in feed/%Cr_2O_3 in feces) x (%nutrient in the feces/%nutrient in feed)).

To evaluate the growth in weight and length of juveniles of pirarucu, an analysis was performed at the beginning and another one at the end of the experiment. The following zootechnical indexes were analyzed: weight gain (WG = final mean weight (g) - initial mean weight (g) of the fish), apparent feed conversion (AFC = amount of feed offered (g)/weight gain (g)), specific growth rate (SGR = 100 x (natural logarithm of final weight (g) - natural logarithm of initial weight (g))/experiment length (days)), mean daily feed intake (MDFI = feed amount (g)/number of fish/time (days)), mean daily protein intake (MDPI = 100 x (MDFI/gross protein content), condition factor (CF = final weight (g)/final length^3 (cm) x 100), survival (S = 100 x (final number of fish/initial number of fish)).

At the end of the experiment, three fish per experimental unit were anesthetized with eugenol (20 mg L^{-1}) (INOUE *et al*., 2011), euthanized by concussion according to the recommendations of AVMA (2007) and subsequently submitted to collection and weighing of the liver and visceral fat for calculations, regarding the hepato-somatic indexes (HSI,%=liver weight/fish body weight x 100) and visceral-somatic fat index (VSFI,%= visceral fat weight/ fish body weight x 100).

At the end of the experiment, blood samples from five fish per experimental unit were collected. Blood was obtained by caudal vein puncture using 3 ml syringes and 18 Gx 11/2" needles with EDTA (10%), stored in a 2ml microtube, and kept under refrigeration (17 °C). The hemoglobin concentration ([Hb]) was determined by the cyanometahemoglobin method; hematocrit (Ht), by the microhematocrit method; number of erythrocytes (RBC) in a Neubauer chamber, after dilution in citrate formaldehyde modified by OLIVEIRA-JUNIOR *et al*. (2009). With the results of these parameters, the hematimetric indexes of Wintrobe were calculated: Mean Corpuscular Volume (MCV=Ht x 10/RBC); Mean Corpuscular Hemoglobin (MCH= [Hb] x 10/RBC) and Mean Corpuscular Hemoglobin Concentration (MCHC = [Hb] x 100 / Ht). After blood centrifugation, the plasma was used to determine the glucose, cholesterol and triglycerides by means of the colorimetric enzymatic method with a lipid clearing factor (C=LCF); total proteins by the biuret reaction method; albumin by the bromocresol green reaction (BCG) and; bilirubin by the Sims-Horn method, all of them using commercial kits.

Economic viability among feeds tested

To evaluate the economic viability of experimental feeds, the average cost of diets per kilogram of live weight gain was determined according to BELLAVER *et al*. (1985), from Yi= Qi x Pi/Gi, where: Yi=average cost of feed per kg gained in the ith treatment; Qi=the average amount of feed consumed in the ith treatment; Pi=average price per kilogram of the feed used in the ith treatment; Gi=mean weight gain of the ith treatment.

Statistical analysis

Before the beginning of the experiments, the biometric results of the fish groups were submitted to the Cochran test to guarantee the homoscedasticity of the experiment. The results of the variables analyzed were expressed by mean and standard deviation and submitted to the simple variance analysis ($p < 0.05$). When there were significant differences among the treatments, the averages were compared by means of the Tukey test at the 5% significance level. In order to determine the optimum replacement level, Principal Component Analysis (PCA) was used, followed by the Tukey test and linear regression of the two main components. The SAS program, version 9.2 was used.

RESULTS

Feed digestibility

The values of the apparent digestibility coefficients (ADC) of the nutrients and the energy of the experimental diets, containing increasing levels of replacement of fishmeal protein with soybean meal protein in the diet of juveniles of pirarucu, presented significant differences ($p<0.05$) among treatments (Table 3).

The ADCs of dry matter (DM) and gross energy (GE) were not statistically different for the 0, 15 and 30% replacement treatments, whereas ethereal extract (EE) and crude protein (CP) values remained constant with replacements that varied from 0 to 45%. There was a significant decrease in the ADC of the nitrogen-free extract (NFE) with the increase of soybean meal levels in the diet. The mean values of the ADCs of the nutrients and the energy of the fish fed with the highest level of replacement (60%) were lower when compared to the other treatments.

Growth performance

The results did not show statistical differences for GP up to 30% of replacement; for specific growth rate (SGR), apparent feed conversion (AFC), mean daily feed intake (MDFI) and mean daily protein intake (MDPI) up to 45%, however, the 60% diet presented the worst performance results, although the survival rate was not significantly different in relation to the other treatments, according to Table 4. There were no changes in the in ther condition factor (CF), hepatosomatic index (HSI) and visceral-somatic fat index (VSFI), according to Table 5.

Blood parameters

Different levels of soybean meal decreased the hematocrit (Ht), hemoglobin concentration (Hb) and red cell count (RCC) values whereas they increased the mean corpuscular volume (MCV), indicating anemia when there was a 60% replacement (Table 6). Among the biochemical parameters evaluated (glucose, triglycerides,

Table 3. Mean ± standard deviation of apparent digestibility coefficient (%) of dry matter (DM), ethereal extract (EE), crude protein (CP), nitrogen-free extract (NFE) and gross energy (GE) of pirarucu juveniles (*Arapaima gigas*) fed with different levels of replacement of fishmeal with soybean meal (0, 15, 30, 45 and 60%). N=3.

Diet (%)	DM	EE	CP	NFE	GE
0	91.8±0.1^{a}	98.5±0.3^{a}	94.3±0.6^{ab}	90.5±1.2^{a}	94.1±0.6^{a}
15	90.3±0.3^{a}	98.0±0.3^{ab}	94.4±0.4^{ab}	82.6±3.0^{b}	92.2±0.7^{ab}
30	89.2±0.4^{ab}	98.0±0.4^{ab}	94.9±0.2^{a}	80.7±0.9^{b}	91.9±0.4^{ab}
45	87.0±1.3^{b}	98.6±0.1^{a}	94.3±0.6^{ab}	73.6±2.6^{c}	90.3±1.0^{b}
60	84.2±1.7^{c}	97.2±0.7^{b}	92.3±1.5^{b}	69.5±2.5^{c}	87.5±1.5^{c}
P value	0.0001	0.0113	0.0215	0.0001	0.0001

Means followed by different letters, in the same column, differ significantly by the Tukey test ($p<0.05$).

Table 4. Mean ± standard deviation of initial weight (IW), final weight (FW), weight gain (WG), specific growth rate (SGR), apparent feed conversion (AFC), mean daily feed intake (MDFI), mean daily protein intake (MDPI), and survival (S) of pirarucu juveniles (*Arapaima gigas*) fed with different levels of fishmeal partially replaced with soybean meal (0, 15, 30, 45and 60%). N=21.

Indexes	Replacement Levels (%)					P value
	0	15	30	45	60	
IW (g)	220.7± 4.9	241.6±9.9	233.3±12.6	233.6±15.2	238.3±5.5	0.2161
FW (g)	1,204.7±52.5[a]	1,117.1±113.4[ab]	985.3±46.5[abc]	904.8± 139.3[bc]	703.7±141.6[c]	0.0017
WG (g)	984.0±54.2[a]	875.5±122.6[ab]	751.9±37.5[ab]	671.2±125.7[bc]	465.5±138.0[c]	0.0011
SGR (%)	5.87±0.0[a]	5.8±0.1[a]	5.7±0.0[a]	5.6±0.1[ab]	5.4±0.2[b]	0.0024
AFC	1.0±0.0[a]	1.0±0.1[a]	1.1±0.1[ab]	1.1±0.2[a]	1.5±0.3[b]	0.0115
MDFI (g)	7.9±0.5[a]	6.5±1.2[ab]	7.00±0.6[ab]	5.9± 0.6[ab]	4.8±1.4[b]	0.0222
MDPI (g)	3.7±0.3[a]	3.0±0.6[ab]	3.2±0.3[ab]	2.8±0.3[ab]	2.2±0.7[b]	0.0242
S (%)	95.3±8.2	90.5±8.2	100.0±0.0	100±0.0	85.7±14.3	0.2263

Means followed by different letters on the same line differ significantly by the Tukey test (p<0.05).

Table 5. Mean ± standard deviation of the condition factor (CF), hepatosomatic index (HSI) and visceral-somatic fat index (VSFI) of pirarucu juveniles (*Arapaima gigas*) fed with different levels of replacement of fishmeal with soybean meal (0, 15, 30, 45 and 60%). N=9.

Indexes	Replacement Levels (%)					P value
	0	15	30	45	60	
CF	0.6±0.0	0.6±0.1	0.6±0.0	0.6±0.0	0.7±0.0	0.1077
HSI (%)	1.2±0.4	1.5±0.4	1.4±0.4	1.2±0.3	1.2±0.3	0.1989
VSFI (%)	1.3±0.4	1.3±0.6	1.3±0.3	1.4±0.4	1.0±0.5	0.2796

Means followed by different letters, on the same line, differ significantly by the Tukey test (p<0.05).

Table 6. Mean ± standard deviation of hemoglobin concentration (Hb), hematocrit (Ht), number of erythrocytes (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of pirarucu juveniles (*Arapaima gigas*) fed with different levels of replacement of fishmeal with soybean meal (0, 15, 30, 45 and 60%). N=15.

Diets (%)	Hematologic parameters					
	Ht (%)	[Hb] (g dL^{-1})	RBC (x10^6cl μL^{-1})	MCV (fL)	MCH (pg)	MCHC (g dL^{-1})
0	35.2 ± 1.7	7.9 ± 0.3[a]	1.7 ± 0.2[ab]	203.0 ± 23.9[ab]	44.7 ± 4.7[ab]	22.6 ± 0.7
15	33.2 ± 1.9	7.4 ± 0.4[ab]	2.1 ± 0.1[a]	158.7 ± 11.4[b]	35.6 ± 3.9[b]	22.3 ± 0.1
30	32.9 ±0.2	7.8 ± 0.2[ab]	1.8 ± 0.1[ab]	185.5 ± 14.9[ab]	42.9 ± 2.8[ab]	23.8 ± 0.9
45	31.4 ± 1.8	7.0 ± 0.2[ab]	1.7 ± 0.1[ab]	194.9 ± 14.0[ab]	43.2 ± 3.9[ab]	22.4 ± 0.8
60	31.3 ± 1.6	6.8 ± 0.5[b]	1.5 ± 0.3[b]	222.0 ± 28.6[a]	51.3 ± 7.9[a]	21.9 ± 0.5
P value	0.0641	0.0135	0.0054	0.0297	0.0399	0.0625

Means followed by different letters, in the same column, differ significantly by the Tukey test (p<0.05).

Table 7. Mean ± standard deviation of biochemical parameters of pirarucu juveniles (*Arapaima gigas*) fed with different levels of fishmeal replaced with soybean meal (0, 15, 30, 45 and 60%). N=15.

Diet (%)	Biochemical Parameters					
	Glucose (mg dL^{-1})	Cholesterol (mg dL^{-1})	Triglycerides (mg dL^{-1})	Proteins totais (g dL^{-1})	Albumin (g dL^{-1})	Bilirubin (mg dL^{-1})
0	78.4±14.7	114.7± 0.6[a]	56.0±7.7	2.8±0.1	0.9±0.0	0.7±0.4
15	79.3±4.3	81.8±4.1[bc]	59.4± 6.2	2.8±0.5	0.9±0.1	0.6±0.2
30	86.3±23.1	105.2±12.0[ab]	64.0±9.9	2.6±0.1	0.8±0.0	0.4±0.2
45	75.0±7.8	85.3±10.0[bc]	62.2±4.4	2.8±0.3	0.9±0.1	0.5±0.2
60	58.1±4.5	68.6±7.4[c]	60.5±1.6	2.4±0.5	0.8±0.0	0.6±0.1
P valor	0.1780	0.0007	0.6500	0.6480	0.1648	0.0962

Means followed by different letters, in the same column, differ significantly by the Tukey test (p<0.05).

total proteins, cholesterol, bilirubin and albumin), Table 7, only cholesterol showed a significant decrease in all diets with soybean meal, with a higher hypocholesterolemia in the 60% replacement.

Economic viability among feeds tested

Considering the expenses with the experimental diets in comparison to weight gain (Figure 1), the treatment with 15 and 30% of soybean meal protein reduced the feed cost by 14,4 and 10%, respectively, in relation to the one without any replacement (0%). The results found as regards to the feed price per grams gain (R$ g gain^{-1}) did not differ statistically from each other (p> 0.05).

Optimum replacement level

The Principal Component Analysis (PCA) of the treatments identified in a single principal component (PCA1), called body performance, in which 99.7% of the covariance was explained,

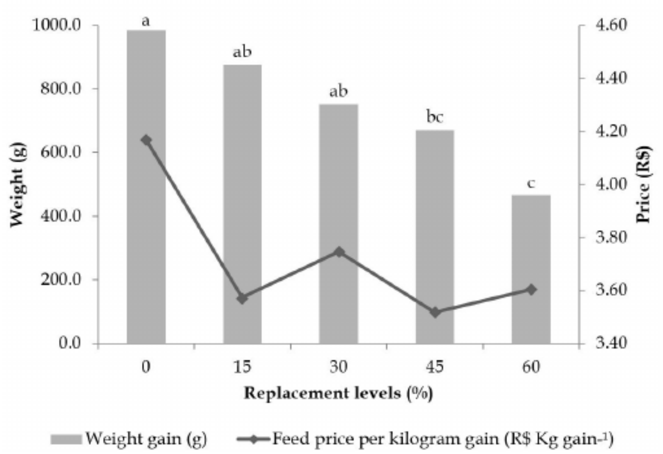

Figure 1. Average feed cost per kg of weight gain of pirarucu juveniles (*Arapaima gigas*) fed with different levels of replacement of fishmeal with soybean meal (0, 15, 30, 45 and 60%). Means followed by different lowercase letters, in the bars, differ significantly by the Tukey test (p <0.05).

Table 8. Mean ± standard deviation of the single principal component variable (PCA1) of pirarucu juveniles (*Arapaima gigas*) fed with different levels of fishmeal replacement with soybean meal (0, 15, 30, 45 and 60%). N=21.

Diet (%)	PCA1
0	1,546.6±75.4^{a}
15	1,407.8±166.9^{ab}
30	1,227.2±59.0^{ab}
45	11,113.2±187.3^{bc}
60	825.6±197.6^{c}
P value	0.001

Means followed by different letters, in the same column, differ significantly by the Tukey test (p<0.05).

represented by the final weight and weight gain variables. The PCA1 decreases with increasing levels of replacement (Table 8).

The best replacement level determined by the analysis of variance (p <0.001) and the orthogonal regression model considered as a variable response was the body performance and, as an explanatory variable, the diet. The only significant adjustment was the model (Y = 1571 - 11.58x) of degree 1. Through the model, it is observed that with each 15 percent increase in the percentage of soybean meal in the diet, there was a reduction of 11.58 g in body performance during the 120-day experiment.

The Tukey test did not differ statistically among the treatments 0, 15 and 30% of replacement (Table 8), indicating that it is possible to replace up until 30% of fishmeal with soybean meal, without compromising the development and the health of pirarucu, when they weigh between 200 and 1.200 g.

DISCUSSION

Due to the large share of feed in the cost of production, having as its more expensive ingredients the ones from animal origin, the replacement of fishmeal with alternative raw materials will considerably reduce the costs for the production system. Consequently, the pirarucu price will be more competitive, generating more income.

SILVA (2013) replaced up to 30% of the fishmeal with soybean meal in pirarucu (25 to 200 g) diets with no significant changes in fish performance and physiology. However, results of other studies have shown the possibility of higher replacement with vegetable protein sources with the inclusion of synthetic amino acids and feed extrusion, reducing the effects of antinutritional factors.

The present work suggests that soybean meal protein can replace up to 30% of fishmeal protein in the diet of pirarucu juveniles, weighing from 200 to 1,200 g, without compromising nutrient and energy digestibility, zootechnical performance and the health of fish. The relation between feed cost and fish weight gain corroborate these results. Comparing those with results obtained by PHAM *et al*. (2007), who evaluated the mix of cotton and soybean meal supplemented with methionine, we see that the authors obtained good results by replacing up to 40% of fishmeal protein in diets for the Japanese flatfish (*P. olivaceus*).

These results corroborate those obtained by KOKOU *et al*. (2012) in which WG and food efficiency were not influenced when diets had a 40% replacement with bioprocessed soybean meal for gilt-head seabream (*S. aurata*), although protein efficiency was affected because of this inclusion, indicating a possible deficiency in certain amino acids.

Similar results in AFC values from (1,0 to 1,5) were found for spotted rose snapper (*Lutjanus guttatus*) (SILVA-CARRILLO *et al*., 2012) and the rainbow trout (*O. mykiss*) (SLAWSKI *et al*., 2012). On the contrary, SILVA (2013) and MEDEIROS (2014) found higher values of AFC for pirarucu under laboratory and farming conditions, ranging from 2.8-3.5 and 1.5-1.7, respectively. These high AFC values can probably be attributed to the kind of processing used for experimental diets (pelleted rations) or to amino acid deficiency, which could have favored greater waste of nutrients present in foods. During the experiment, it was possible to observe, in some moments, the rejection of feed intake on the part of animals fed with a 60% replacement diet. That may have influenced the lower MDPI and lower WG. Therefore, it is suggested that, in the future, an assessment on the palatability of these diets with soybean meal replacement be made.

Synthetic amino acids are used in the formulation of diets with the aim of improving the qualitative and quantitative profile of amino acids, since those are deficient mainly in plant protein sources. In this sense, it is assumed that the similar nutritional values among diets did not promote changes in HSI and VSFI and that the extrusion process efficiently reduced the antinutritional factors found in soybean meal without causing any damage to the fish organism. ZHOU *et al*. (2011), KADER *et al*. (2012) and SILVA (2013) also did not observe changes for black snapper (*A. schlegelii*), red snapper (*P. major*) and pirarucu (*A. gigas*), respectively.

The tools used to evaluate fish stress due to endogenous and exogenous factors are the haematological parameters. The apparent anemia, when there are high levels of soybean in the diet, could characterize a macrocytic and normochromic anemia, as it was demonstrated by the increase in the mean corpuscular volume (MCV) and normal levels in mean corpuscular hemoglobin concentration (MCHC), respectively. Thus, the results suggest that the negative effects of soybean meal can be evidenced in the diet with the highest level of this ingredient, that means 60% replacement, in juveniles of pirarucu, indicating alteration in its homeostasis. That may restrict the ability of blood to transport oxygen to tissues (HEATH, 1995; TAVARES-DIAS *et al*., 2008).

However, hyperglycemia found under stress conditions was not observed in this study, as well as in the one by SILVA-CARRILLO *et al*. (2012), using the same protein sources in spotted rose snapper juveniles (*L. guttatus*) and for the carnivorous species red snapper (*P. major*) fed with dehydrated soybean meal supplemented with squid by-products (KADER *et al*., 2012).

According to the literature, plant proteins are known to affect cholesterol metabolism in fish, reducing plasma concentrations. Also, the hypocholesterolemia observed in all levels of soybean meal in the present study agree with LIM and LEE (2009), LIM *et al*. (2011) and YE *et al*. (2011).

Regarding protein metabolism, such as total proteins, albumin and bilirubin, it can be stated that they did not change among treatments, reflecting the quality of this nutrient in the diet. Therefore, it is suggested that the increasing replacement with soybean meal in the diets did not influence the hepatic activity of the fish, in which the liver maintained its capacity to assimilate and store nutrients (CLARKE and WISEMAN, 1999; CABALLERO *et al*., 1999; KADER *et al*., 2010). Similar results were found for red snapper (*P. major*) (KADER *et al*., 2012), spotted rose snapper (*L. guttatus*) (SILVA-CARRILLO *et al*., 2012) and white sturgeon (*H. huso*) (JAHANBAKHSHI *et al*., 2013).

Regarding the economic feasibility, the price of feeds in the present study, R$ 3.52 to 4,17, is below those tested for pirarucu, described by MEDEIROS (2014) with a cost of R$ 5.51 to 7.69, and SILVA (2013) from R$ 5.66 to 7.37, as well as for the commercial feeds used for carnivorous fish, with 45% CP, purchased in the Manaus market, with an average value of R$ 108,00 (bag with 25 kg), allowing for the production of experimental feeds on a commercial scale. The price fluctuations of ingredients vary according to availability in the market.

CONCLUSION

The hematological results and the cost of feed compared to the weight gain suggest that there is an influence on fish performance due to different levels of soybean meal and, in association with the results of zootechnical parameters and feed digestibility, the soybean meal protein can replace up to 30% of fishmeal protein in the diet of pirarucu juveniles. In addition, diets with high levels of soybean meal, as observed in 60% replacement, are not recommended for pirarucu farming, causing hemolytic anemia.

REFERENCES

AOAC – Association of Official Agriculture Chemists. 2000 *Official methods of analyses of the Association of Agriculture Chemists*. Washington: AOAC. 937p.

ARNDT, R.E.; HARDY, R.W.; SUGIURA, S.H.; DONG, F.M. 1999 Effects of heat treatment and substitution level on palatability and nutritional value of soy defatted flour in feeds for Coho salmon, *Oncorhynchus kisutch*. *Aquaculture*, *180*(1-2): 129-145. **http://dx.doi.org/10.1016/S0044-8486(99)00186-6**.

AVMA – American Veterinary Medical Associations. 2007 *Guidelines on euthanasia*. Washington: AVMA. 36p.

AZARM, H.M.; LEE, S. 2014 Effects of partial substitution of dietary fish meal by fermented soybean meal on growth performance, amino acid and biochemical parameters of juvenile black sea bream *Acanthopagrus schlegeli*. *Aquaculture Research*, *45*(6): 994-1003. **http://dx.doi.org/10.1111/are.12040**.

BARD, J.; IMBIRIBA, E.P. 1986 *Piscicultura de pirarucu, Arapaima gigas*. Belém: EMBRAPA-CPATU. v. 52. 52p.

BELLAVER, C.; FIALHO, E.T.; PROTAS, J.F.S. 1985 Radícula de malte na alimentação de suínos em crescimento e terminação. *Pesquisa Agropecuária Brasileira*, *20*(8): 969-974.

CABALLERO, M.J.; LÓPEZ-CALERO, G.; SOCORRO, J.; ROO, F.J.; IZQUIERDO, M.S.; FÉRNANDEZ, A.J. 1999 Combined effect of lipid level and fish meal quality on liver histology of gilthead seabream (*Sparus aurata*). *Aquaculture*, *179*(1-4): 277-290. **http://dx.doi.org/10.1016/S0044-8486(99)00165-9**.

CANTELMO, O.A.; PEZZATO, L.E.; BARROS, M.M.; RIBEIRO, M.A.R. 1999 Influência de diferentes aglutinantes na digestibilidade aparente da matéria seca e da proteína, no pacu (*Piaractus mesopotamicus*) arraçoado com rações elaboradas com ou sem vapor arraçoado com rações elaboradas com ou sem vapor. *Acta Scientiarum*, *21*(2): 277-282.

CHO, C.Y. 1990 Fish nutrition, feeds and feeding: with special emphasis on salmonid aquaculture. *Food Reviews International*, *6*(3): 333-357. **http://dx.doi.org/10.1080/87559129009540876**.

CLARKE, E.; WISEMAN, J. 1999 Extrusion temperature impairs trypsin inhibitor activity in soybean meal. *Feed Technology*, *3*(8): 29-31.

CSÁKY, I.; FEKETE, S. 2004 Soybean: feed quality and safety. Part 2: pathology of soybean feeding in animals: a review. *Acta Veterinaria Hungarica*, *52*(3): 315-326. **http://dx.doi.org/10.1556/AVet.52.2004.3.7**. PMid:15379446.

FAO – Food and Agriculture Organizations of the United Nations. 2014 *The state of world fisheries and aquaculture*. Rome: FAO. 244p.

FREITAS, L.E.L.; NUNES, A.J.P.; SÁ, M.V.C. 2011 Growth and feeding responses of the mutton snapper, *Lutjanus analis* (Cuvier, 1828), fed on diets with soy protein concentrate in replacement of Anchovy fish meal. *Aquaculture Research*, *42*(6): 866-877. **http://dx.doi.org/10.1111/j.1365-2109.2010.02793.x**.

FURUKAWA, A.; TSUKAHARA, H. 1966 On the acid digestion method for the determination of chromic oxide as an index in the study of digestibility of fish feed. *Nippon Suisan Gakkaishi*, *32*(6): 502-506. **http://dx.doi.org/10.2331/suisan.32.502**.

GATLIN, D.M.; BARROWS, F.T.; BROWN, P.; DABROWSKI, K.; GAYLORD, T.G.; HARDY, R.W.; HERMAN, E.; HU, G.; KROGDAHL, Å.; NELSON, R.; OVERTURF, K.; RUST, M.; SEALEY, W.; SKONBERG, D.; J SOUZA, E.; STONE, D.; WILSON, R.; WURTELE, E. 2007 Expanding the utilization of sustainable plant products in aquafeeeds: a review. *Aquaculture Research*, *38*(6): 551-579. **http://dx.doi.org/10.1111/j.1365-2109.2007.01704.x**.

HARDY, R.W. 1999 Aquaculture's rapid growth requeriments for alternate protein sources. *Feed Management*, *50*(1): 25-28.

HEATH, A.G. 1995 *Water pollution and fish physiology*. 2nd ed. Boca Raton: CRC.

HOULIHAN, D.; BOUJARD, T.; JOBLING, M. 2001 *Food intake in fish*. Oxford: Blackwell Science. 418p. **http://dx.doi.org/10.1002/9780470999516**.

INOUE, L.A.K.A.; BOIJINK, C.L.; RIBEIRO, P.T.; SILVA, A.M.D.; AFFONSO, E.G. 2011 Avaliação de respostas metabólicas do tambaqui exposto ao eugenol em banhos anestésicos. *Acta Amazonica*, *41*(2): 327-332. **http://dx.doi.org/10.1590/S0044-59672011000200020**.

JAHANBAKHSHI, A.; IMANPOOR, M.R.; TAGHIZADEH, V.; SHABANI, A. 2013 Hematological and serum biochemical indices changes induced by replacing fish meal with plant protein (sesame oil cake and corn gluten) in the Great sturgeon (*Huso huso*). *Comparative Clinical Pathology*, *22*(6): 1087-1092. **http://dx.doi.org/10.1007/s00580-012-1532-4**.

KADER, M.A.; BULBUL, M.; KOSHIO, S.; ISHIKAWA, M.; YOKOYAMA, S.; NGUYEN, B.T.; KOMILUS, C.F. 2012 Effect of complete replacement of fishmeal by dehulled soybean meal with crude attractants supplementation in diets for red sea bream, *Pagrus major*. *Aquaculture*, *350-353*: 109-116. **http://dx.doi.org/10.1016/j.aquaculture.2012.04.009**.

KADER, M.A.; KOSHIO, S.; ISHIKAWA, M.; YOKOYAMA, S.; BULBUL, M. 2010 Supplemental effects of some crude ingredients in improving nutritive values of low fishmeal diets for red sea bream, *Pagrus major*. *Aquaculture*, *308*(3-4): 136-144. **http://dx.doi.org/10.1016/j.aquaculture.2010.07.037**.

KOKOU, F.; RIGOS, G.; HENRY, M.; KENTOURI, M.; ALEXIS, M. 2012 Growth performance, feed utilization and non-specific immune response of gilthead sea bream (*Sparus aurata* L.) fed graded levels of a bioprocessed soybean meal. *Aquaculture*, *364-365*: 74-81. **http://dx.doi.org/10.1016/j.aquaculture.2012.08.009**.

KUBITZA, F. 2003 *Qualidade de água no cultivo de peixes e camarões*. Jundiaí: Edição do Autor. 229p.

LIM, S.J.; KIM, S.S.; KO, G.Y.; SONG, J.W.; OH, D.H.; KIM, J.D.; KIM, J.U.; LEE, K.J. 2011 Fish meal replacement by soymeal in diets for *Tiger puffer, Takifugu rubripes*. *Aquaculture*, *313*(1-4): 165-170. **http://dx.doi.org/10.1016/j.aquaculture.2011.01.007**.

LIM, S.J.; LEE, K.J. 2009 Partial replacement of fish meal by cottonseed meal and soybean meal with iron and phytase supplementation for parrot fish *Oplegnathus fasciatus*. *Aquaculture*, *290*(3-4): 283-289. **http://dx.doi.org/10.1016/j.aquaculture.2009.02.018**.

LÜLING, K.H. 1964 Zur biologte und ökologte von *Arapaima gigas* (Pisces, Osteoglossidae). *Zoomorphology*, *54*(4): 436-530.

MEDEIROS, P.A. 2014 *Dietas práticas com diferentes níveis de proteína e energia na alimentação de juvenis de pirarucu Arapaima gigas (Schinz, 1822) durante a engorda em tanque-rede*. Manaus. 61f. (Dissertação de Mestrado. Instituto Nacional de Pesquisas da Amazônia). Available from: <http://bdtd.inpa.gov.br/handle/tede/1518>. Access on: 6 mar. 2015.

NG, W.K.; WILSON, R.P. 1997 Chromic oxide inclusion in the diet does not affect glucose utilization or chromium retention by channel catfish, *Ictalurus punctatus*. *The Journal of Nutrition*, *127*(12): 2357-2362. **http://dx.doi.org/10.1093/jn/127.12.2357**. PMid:9405586.

NRC – National Research Council. 1993 *Nutrient requirements of fish*. Washington: National Academy Press. 114p.

OLIVEIRA-JUNIOR, A.A.; TAVARES-DIAS, M.; MARCON, J.L. 2009 Biochemical and hematological reference ranges for Amazon freshwater turtle, *Podocnemis expansa* (Reptilia: Pelomedusidae), with morphologic assessment of blood cells. *Research in Veterinary Science*, *86*(1): 146-151. **http://dx.doi.org/10.1016/j.rvsc.2008.05.015**. PMid:18617203.

ONO, E.A.; NUNES, E.S.S.; CEDANO, J.C.C.; PEREIRA-FILHO, M.; ROUBACH, R. 2008 Digestibilidade aparente de dietas práticas com diferentes relações energia:proteína em juvenis de pirarucu. *Pesquisa Agropecuária Brasileira*, *43*(2): 249-254. **http://dx.doi.org/10.1590/S0100-204X2008000200014**.

PEISKER, M. 2001 Amino acids in aquafeeds: a fresh approach to amino acid nutrition. *Aquafeed International*, *4*: 22-26.

PHAM, M.A.; LEE, K.J.; LIM, S.J.; PARK, K.H. 2007 Evaluation of cottonseed and soybean meal as partial replacement for fishmeal in diets for juvenile Japanese flounder *Paralichthys olivaceus*. *Fisheries Science*, *73*(4): 760-769. **http://dx.doi.org/10.1111/j.1444-2906.2007.01394.x**.

REBAZA, M.; REBAZA, C.; DEZA, S. 2010 Densidad de siembra para cultivos de Paiche en jaulas flotantes. *Aquavisión*, 6: 26-27.

ROSTAGNO, H.S.; ALBINO, L.F.T.; DONZELE, J.L.; GOMES, P.C.; OLIVEIRA, R.F.; LOPES, D.C.; FERREIRA, A.S.; BARRETO, S.L.T.; EUCLIDES, R.F. 2011 *Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais de aves e suínos*. 3ª ed. Viçosa: Universidade Federal de Viçosa. 252p.

SILVA, R.M. 2013 *Substituição parcial da farinha de peixe pelo farelo de soja na dieta de juvenis de pirarucu Arapaima gigas (Schinz, 1822)*. Manaus. 47f. (Dissertação de Mestrado. Instituto Nacional de Pesquisas da Amazônia). Available from: <http://pgaquicultura.inpa.gov.br/index.php/2-uncategorised/83-2013>. Access on: 19 dec. 2014.

SILVA-CARRILLO, Y.; HERNÁNDEZ, C.; HARDY, R.W.; GONZÁLEZ-RODRÍGUEZ, B.; CASTILLO-VARGASMACHUCA, S. 2012 The effect of substituting fish meal with soybean meal on growth, feed efficiency, body composition and blood chemistry in juvenile spotted rose snapper *Lutjanus guttatus* (Steindachner, 1869). *Aquaculture*, *364-365*: 180-185. **http://dx.doi.org/10.1016/j.aquaculture.2012.08.007**.

SLAWSKI, H.; ADEM, H.; TRESSEL, R.P.; WYSUJACK, K.; KOOPS, U.; KOTZAMANIS, Y.; WUERTZ, S.; SCHULZ, C. 2012 Total fish meal replacement with rapeseed protein concentrate in diets fed to rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquaculture International*, *20*(3): 443-453. **http://dx.doi.org/10.1007/s10499-011-9476-2**.

STECH, M.R.; CARNEIRO, D.J.; CARVALHO, M.R.B. 2010 Fatores antinutricionais e coeficientes de digestibilidade aparente da proteína de produtos de soja para o pacu (*Piaractus mesopotamicus*). *Acta Scientiarum: Animal Sciences*, *32*(3): 255-262.

TAVARES-DIAS, M.; MARCON, J.L.; LEMOS, J.R.G.; FIM, J.D.I.; AFFONSO, E.G.; ONO, E.A. 2008 Índices de condição corporal em juvenis de *Brycon amazonicus* (Spix&Agassiz, 1829) e *Colossoma macropomum* (Cuvier,1818) na Amazônia. *Boletim do Instituto de Pesca*, *34*(2): 197-204.

YANG, Y.; WANG, Y.; LU, Y.; LI, Q. 2011 Effect of replacing fish meal with soybean meal on growth, feed utilization and nitrogen and phosphorus excretion on rainbow trout (*Oncorhynchus mykiss*). *Aquaculture International*, *19*(3): 405-419. **http://dx.doi.org/10.1007/s10499-010-9359-y**.

YE, J.; LIU, X.; WANG, Z.; WANG, K. 2011 Effect of partial fish meal replacement by soybean meal on the growth performance and biochemical indices of juvenile Japanese flounder *Paralichthys olivaceus*. *Aquaculture International*, *19*(1): 143-153. **http://dx.doi.org/10.1007/s10499-010-9348-1**.

ZHOU, F.; SONG, W.; SHAO, Q.; PENG, X.; XIAO, J.; HUA, Y.; OWARI, B.N.; ZHANG, T.; NG, W.-K. 2011 Partial replacement of fish meal by fermented soybean meal in diets for black sea bream, *Acanthopagrus schlegelii*, juveniles. *Journal of the World Aquaculture Society*, *42*(2): 184-197. **http://dx.doi.org/10.1111/j.1749-7345.2011.00455.x**.