

Partial substitution of bean (*Phaseolus vulgaris*) flour for fishmeal in extruded diets for rainbow trout (*Oncorhynchus mykiss*): Effects on yield parameters

Rodríguez-Miranda J.¹; Reyes-Jáquez D.²; Delgado E.^{3*}; Ramirez-Wong B.⁴; Esparza-Rivera J.R.⁵; Solís-Soto A.²; Vivar-Vera M.A.¹; Medrano-Roldán H.²

Received: November 2014

Accepted: September 2015

Abstract

The objective of this research was to evaluate yield parameters (gained weight, weight percentage, survival, feed conversion factor (FCR), feed conversion efficiency (FCE), condition factor (K), specific growth rate (SGR) and hepatosomatic index (HSI) of trouts fed with experimental diets elaborated with bean (*Phaseolus vulgaris* L.) flour instead of fishmeal with 15, 30 and 45% (BF15, BF30 and BF45, respectively) for 32 days, as well as a control diet (CD). The greatest weight gain was presented by fish fed with BF15 and BF30 (14.48 and 14.14 g, respectively) with no significant differences ($p>0.05$) and an approximate increase of 50% of their initial weight. FCR did not show significant differences ($p>0.05$) among CD, BF15 and BF30 diets with an average value of 2.05. FCE did not show significant differences ($p>0.05$) between diets BF15 and BF30 with an average value of 46.70%. SGR did not show significant differences ($p>0.05$) between BF15 and BF30 diets with an average value of 1.25. It is concluded that 30% is the maximum substitution without causing a decrease in yield and nutritional parameters in rainbow trout under the experiment conditions, although further research is suggested.

Keywords: Extruded diets, Fishmeal, *Oncorhynchus mykiss*, Specific growth rate.

1-Instituto Tecnológico de Tuxtepec, Av. Dr. Víctor Bravo Ahuja s/n Col. 5 de Mayo. C.P. 68360, Tuxtepec, Oaxaca, México.

2-Instituto Tecnológico de Durango. Blvd. Felipe Pescador 1830 Ote., Col. Nueva Vizcaya, C.P. 34080, Durango, Durango, México.

3-New Mexico State University, Department of Family and Consumer Sciences, NMSU Gerald Thomas Hall Room 308 P.O. Box 30003 MSC 3470 Las Cruces, NM 88003 USA.

4-Universidad de Sonora, Blvd.Luis Encinas, Apto. Postal 1658, C.P. 83000. Hermosillo, Sonora México.

5-Universidad Juárezdel Estado de Durango. Artículo 123 Avenue S/N, C. P. 35010, Gómez Palacio, Durango, México.

* Corresponding autor email:edelgad@ad.nmsu.edu, edelgad 2000@yahoo.com

Introduction

Rainbow trout (*Oncorhynchus mykiss*) is a carnivorous fresh-water salmonid fish (Kaushik and Médale, 1994) important to the national and international aquaculture industry (Dabrowski, 1984). Trout requires diets with protein content of 29-50% and low levels of starch (1-2%) (Uys and Hecht, 1985; Charlton and Bergot, 1986). Generally, carnivorous fresh-water species have higher protein requirements than herbivorous fish (Cowey *et al.*, 1995). Hence, protein is considered one of the most important factors in carnivorous fish nutrition (Glencross *et al.*, 2006). Salmonid diets are mainly elaborated with fishmeal, increasing production cost by 60% (Thompson *et al.*, 2005). Protein is the most important component of the diet for fish because it contributes to growth and if present in excess, can be a source of backup power (Bureau *et al.*, 2006). The results of studies of salmon indicate that optimal growth is achieved when about half of the food energy is supplied by protein (Jobling, 1993). Protein is required in the diet to provide the essential amino acids and nitrogen for the synthesis of non-essential amino acids. The protein in the muscles of the body incorporates about 23 amino acids including, 10 (essential) amino acids supplied in the diet because the fish cannot synthesize or cannot synthesize sufficient amounts for maximum growth. It is for this reason that the composition of dietary protein containing vegetable raw materials, can

affect fish physiologically manifesting immediate effects on growth or mortality (Storebakken *et al.*, 2000). Therefore, the correct formulations of the food together with a suitable power level are critical aspects, which should not be underestimated. Failures in diets, usually attributed to improper formulation or deficiencies in its manufacturing, have often been the result of poor farm management due to inadequate particle size, poor feeding frequency and/or excessive or poor power supply (Uys and Hetcht, 1985; Charlton and Bergot, 1986). Extensive research has been performed using different vegetable protein sources as substitution for fishmeal. Legumes have been studied for their high protein content. Most of the researches were made on the most known legume: soy. However, this legume is becoming inaccessible to rural inhabitants (Eneche, 2005). Hence it is necessary to potentiate the use of other locally available legumes, aiming to encourage a greater planting. Bean (*P. vulgaris* L.) is a rich source of proteins, minerals (phosphorus and iron), vitamins and energy for humans and monogastric animals (Gatel and Grosjean, 1990; Olvera-Novoa and Olivera-Castillo, 2000). Fishmeal replacement with bean (*P. vulgaris* L.) flour in diets elaboration for salmonids species has not been reported. This research objective was to evaluate yield parameters (growth, feed conversion efficiency, condition factor, specific growth rate and hepatosomatic index)

in trout fed experimental diets with bean (*P. vulgaris* L.) flour substitution.

Materials and methods

Diets

Three diets and a control (CD) were elaborated (Table 1). Fishmeal was replaced in the three diets with bean flour in 15, 30 and 45% (BF15, BF30 and BF45, respectively). The diets were extruded at 120°C with 20% of moisture and were later dried (60°C/5h) and kept in refrigeration until feeding. Further details of feed processing can be found in a previous article (Rodríguez-Miranda *et al.*, 2012).

Diets chemical analyses

Proximal chemical analyses were performed according to AOAC (2005) methods for each one of the diets: moisture content by drying (AOAC 925.45), ashes by dry incineration (AOAC 938.08), crude fat by petroleum ether extraction (AOAC 989.05), crude protein by Kjeldahl method using N x 6.25 factor (AOAC 991.20) and nitrogen free extracts were determined using 100% difference minus protein, ash and fat contents percentages. Gross energy was calculated according to Ekanayake *et al.* (1999) and metabolic energy according to Fenerci and Şener (2005).

Experimental conditions and feeding

Juvenile rainbow trout were used (*Oncorhynchus mykiss*) with a specific weight of 29.1±3.7g, with 20 trout per tank. All treatments were evaluated in

duplicate. Eight polyester-fiber rectangular tanks with a 0.88m³ (2.44×0.6×0.7m) volume were used in a recirculated-closed system (Ras), with filtered and thermo regulated water (16±1°C) and a water flow rate of 3-6L/min. Fish underwent a 48 h previous adaptation period. Feeding was manual at 2.2% rate of total biomass performed three times a day and seven days a week for 32 days.

Evaluation of growth parameters

Samples were taken of six fish at the beginning of the experiment and were sacrificed before fish distribution in the tanks. In the same way, three fish per tank were retrieved at the end of the feeding period. Survival (%) = 100 × (final amount of fish / initial amount of fish), percentage weight gain = [(final weight - initial weight) / initial weight × 100], condition factor (K) = Weight of fish (g) / (length (cm)³ × 100), feed conversion ratio (FCR) = (feed intake / wet weight gain), feed conversion efficiency (FCE) = [(final weight - initial weight (g)) / (feed consumed (g))] × 100, specific growth rate (SGR) = [(ln final weight - ln initial weight) / time (days)] × 100 and hepatosomatic index (HSI) = (liver weight / body weight) × 100, were determined.

Statistical analyses

Obtained results were compared using a one-way variance analyses (ANOVA) and differences among media were determined with a least significant

difference test at a confidence level of 95% using statistical software Statistica Version 8 (StatSoft, Inc. 1984-2008, USA).

Results

Diets chemical composition

Results of chemical analyses of the diets are shown in Table 2. Bean flour substitution percentage affects significantly ($p < 0.05$) protein content compared with the control diet (CD). Fat content increased ($p < 0.05$) in extrudates with 15% of bean flour compared with CD. Regarding ash content and nitrogen free fraction no significant effect ($p < 0.05$) was found in the extrudates compared with the CD. Results of protein content are within the range reported in literature (26-50%) for rainbow trout (*O. mykiss*) diets (Lovell, 1989). Significant differences ($p < 0.05$) were found in total energy and metabolic energy.

Weight gain and survival

Weight gain results reflect fish growth in grams using the different diets (CD, BF15, BF30 and BF45) for 32 days (Table 3). The greatest average weight gain at the end of the experiment was shown by the fish fed with CD diet (18.10 g) which represents a 62% weight gain from their initial weight. Significant differences ($p < 0.05$) were found between CD and all the other diets (BF15, BF30 and BF45). However, no significant differences ($p > 0.05$) were found between diets BF15 and BF30 (14.48 and 14.14 g,

respectively) with an increase of approximately 50% of their initial weight. The minimum weight gain was obtained in fish fed with BF45 diet (1.59g = 5.46%).

Condition factor (K)

No significant differences were found ($p > 0.05$) between fish fed CD, BF15 and BF30 diets (Table 4), with a K average value of 1.73. The maximum value was found in fish fed BF45 diet (2.00).

Feed conversion ratio and food conversion efficiency

Table 4 shows FCR and FCE results. No significant differences ($p > 0.05$) were found in FCR between CD, BF15 and BF30 diets with an average value of 2.05. The maximum value was found in diet BF45 (13.71). FCE did not show significant differences ($p > 0.05$) between BF15 and BF30 diets (average value of 46.79%). Significant differences ($p < 0.05$) were found with CD and BF45 diets. The minimum value was found in BF45 diet with 7.35% and the maximum value was found in CD diet with 54.39%.

Specific growth rate

Significant differences ($p < 0.05$) were found among treatments fed CD and BF15, BF30 and BF45 diets (Table 4). However, no significant differences ($p > 0.05$) were found between fish fed BF15 and BF30 diets, with an average SGR of 1.25.

Table 1: Formulation of diets.

Ingredients(%)	Diets			
	CD	BF15	BF30	BF45
Fishmeal ^a	62	52.7	43.4	34.1
Bean flour	--	9.3	18.6	27.9
Wheat flour	20	20	20	20
Fish oil ^b	12	12	12	12
Dried whey ^c	3.4	3.4	3.4	3.4
Choline chloride ^d	0.5	0.5	0.5	0.5
Vitamin and mineral premix ^e	2	2	2	2
Chromium oxide ^f	0.1	0.1	0.1	0.1
Total	100	100	100	100

^aCaliforniaPlant'sChoice, Ensenada Baja California, México. ^bProteínas de Calidad, México D.F., México. ^cF&ADairyproducts, Inc. Las Cruces NM 88007 Product of The U.S.A. ^dCholine chloride Sigma-Aldrich, Co., 3050 spruce street, St. Louis, MO 63103 USA. Reagent Grade \geq 98%, ^eComposition of the vitamin and mineral premix: Ca,196 g/kg; P,46 g/kg; Na, 57 g/kg; NaCl, 111 g/kg; Mg,12 g/kg; Fe,2.4 g/kg; Cu, 14 mg/kg; Mn, 1698 mg/kg; Se, 150 mg/kg; vitamin A, 4000,000,000 UI; vitamin D3,40,000,000 UI; vitamin E, 400,000 UI, vitamin K, 160g/kg; vitamin B1, 61g/kg; vitamin B2, 160 g/kg; vitamin B6, 84g/kg; vitamin B12, 0.4 g/kg, folic acid, 4g/kg; calcium pantothenate, 540 g/kg, ^fInert marker, chromic oxide, Sigma Chemical Co., St Louis, Mo, USA.

Table 2: Chemical composition of the diets evaluated.

Component (g 100g ⁻¹)	Diets			
	CD	BF15	BF30	BF45
Dry matter	91.7±0.23 ^a	91.6±0.17 ^a	91.9±0.00 ^a	92.5±0.13 ^b
Protein (N x 6.25)	48.7±2.00 ^a	41.9±1.10 ^b	40.9±1.02 ^c	38.7±1.17 ^c
Lipid	16.9±1.03 ^a	18.8±0.15 ^b	16.7±0.19 ^a	16.4±0.01 ^a
Ash	12.9±0.15 ^c	11.4±0.10 ^b	10.4±0.46 ^a	9.7±0.31 ^a
Free extract nitrogen	29.2±0.95 ^a	27.8±1.00 ^a	24.1±0.99 ^b	35.0±0.91 ^c
Gross energy (kJ 100 g ⁻¹)	1923.1±21.21 ^c	1862.8±14.14 ^a	1729.1±19.80 ^b	1833.1±22.63 ^a
Metabolizable energy(Kcal kg ⁻¹)	364.4±10.61 ^c	353.3±7.07 ^b	325.7±8.49 ^a	330.1±11.31 ^a

Values are mean±standard deviation. Different superscripts within the row denote significant differences ($p<0.05$).

Table 3: Juvenile rainbow trouts' (*Oncorhynchus mykiss*) (initial weight 29.1g) results after 32 days of feeding.

Diet	Final weight (g)	Gained weight (g)	% Gained weight	Survival (%)
CD	47.14±2.29 ^c	18.10±2.29 ^a	62.31±7.89 ^a	100±0.00 ^a
BF15	43.52±2.18 ^a	14.48±2.18 ^b	49.86±7.50 ^b	100±0.00 ^a
BF30	43.18±0.64 ^a	14.14±0.64 ^b	48.67±2.22 ^b	100±0.00 ^a
BF45	30.63±0.19 ^c	1.59±0.19 ^c	5.46±0.64 ^c	100±0.00 ^a

Values are mean±standard deviation. Different superscripts within the row denote significant differences ($p<0.05$).

Table 4: Condition factor (K), feed conversion ratio (FCR), feed conversion efficiency (FCE), specific growth rate (SGR) and hepatosomatic index (HSI) of the experimental diets evaluated at 32 days of feeding.

Diet	K (--)	FCR (--)	FCE (%)	SGR (--)	HSI (%)
CD	1.76±0.12 ^a	1.85±0.15 ^a	54.39±4.26 ^a	1.51±0.15 ^a	1.18±0.44 ^b
BF15	1.71±0.30 ^a	2.14±0.20 ^a	47.10±4.64 ^b	1.26±0.15 ^b	0.82±0.24 ^{ab}
BF30	1.72±0.26 ^a	2.15±0.07 ^a	46.49±1.44 ^b	1.24±0.05 ^b	0.64±0.08 ^a
BF45	2.01±0.26 ^b	13.71±1.58 ^b	7.35±0.82 ^c	0.17±0.02 ^c	0.51±0.05 ^a

Values are mean±standard deviation. Different superscripts within the row denote significant differences ($p<0.05$).

The highest SGR (1.51) was found in the rainbow trout group fed with CD, while the lowest SGR (0.17) was found in the fish fed with BF45 diet.

Hepatosomatic index

No significant differences ($p>0.05$) were found among the treatments fed substitution diets (Table 4). However, there are significant differences ($p<0.05$) when compared against CD diet, except BF15 diet, which did not show significant differences ($p>0.05$). The highest value was found in fish fed CD and BF15 diets with an average of 1%, while all the other treatments presented an average value of 0.65%.

Discussion

The protein content is within those reported in the literature (26-50g 100g⁻¹) for diets of rainbow trout (*O. mykiss*) (Lovell, 1989). The differences are due to the increased bean flour content, and decrease in the concentration of fishmeal, which is the raw material in the diet that contributes to the formulation of higher protein content. The protein content found in BF45 is within the range of existing protein diets in the market (28-42g 100g⁻¹). The protein content found in this research is higher than those reported by Fallahi *et al.* (2013) in fish feed using distillers dried grains with solubles (DDGS) (38.25g 100g⁻¹) and Ayadi *et al.* (2011) in diets for yellow perch (*Perca flavescens*) using DDGS (28.5-30.4 g 100g⁻¹) and within the range reported

by Mjoun and Rosentrater (2011) in aquaculture diets containing DDGS (27.9g 100g⁻¹), soybean meal (51g 100g⁻¹) and fishmeal (64.2g 100g⁻¹) and below that reported by Kader *et al.* (2012), in diets for sea bream (*Pagrus major*) with replacement of fishmeal with dehulled soybean meal (48.8-50.4g 100g⁻¹), An-Hen *et al.* (2014) extruded food for Atlantic salmon made with partial or total replacement of wheat flour with oatmeal and tapioca starch (58.4-60.6 g 100g⁻¹). Soy protein is considered the most nutritious vegetable component and is widely used in animal feed and aquaculture. However, bean protein by its amino acid composition, also represents an alternative to aquaculture feed. Vegetable protein also has advantages compared to fishmeal, because it has biogenic amines (Swick *et al.*, 1995). In this paper up to 45% of bean flour substitution for fish meal was obtained in the extruded diets; the literature shows that it can be replaced with up to 50% of soy protein without affecting the growth of rainbow trout (Stickney *et al.*, 1996; Médale *et al.*, 1998; Mambrini *et al.*, 1999).

The amount of feed required by a fish in order to achieve a certain weight gain mainly depends on the feed quantity and quality. Besides, harvest conditions (water temperature) affect the developing animal physiology and nutrition at the same time that feed properties affect environment conditions (due detritus accumulation,

excretal products, etc.) (Bailey and Alanärä, 2006; Bureau *et al.*, 2006).

This weight gain decrease happens when bean flour concentration is increased in feeds due to reduction in protein content and increase in carbohydrate content (Table 2), although no significant differences ($p>0.05$) were found in protein content, a significant effect was found ($p>0.05$) in weight gain decrease in feeds with lower protein content and higher carbohydrate content. Obtaining a 100% survival during the experiment period may be an indicator of good quality fish feed because preservation of good health during the feeding period was observed.

K indicates organisms' nutritional state and, in harvesting, it is useful to numerically compare and quantify fish condition or state associated with a valuation (physical shape) and from a nutritional point of view, it is not fat accumulation or gonadal development (Le Cren, 1951; Abowei *et al.*, 2009). K also provides information on feed activity of a species and to verify if good handling of its source of nutriment is being followed (Bagenal and Tesch, 1978). Interspecific variations within K, and for a specific species may widely vary, since such factors as temperature, reproductive state, feed quantity and quality influence them (Wootton 1990; García-García, *et al.*, 2002).

The results observed for K were due to protein and lipid content in the diets (Table 2). However, these results are

within the reported ranges. Bilgüven and Barış (2011) reported values of 1.15 and 1.14 in rainbow trout diets (*O. mykiss*) using sunflower and cottonseed meal flour, respectively. Fenerci1 and Şener (2005) reported 1.2 for rainbow trout (*O. mykiss*) pelletized and extruded diets. Bastardo and Sofia (2003) reported 1-1.2 for rainbow trout (*O. mykiss*) diets. Henceforth, the results found from this research indicate that fish fed with bean flour diets were in good physical state, up until the highest bean flour concentration (BF45). Mendo and Samame (1988) confirmed that fish state is an important factor in the development of sexual organs. This is corroborated with some species that have been studied, in which condition factor depends on reproduction, such as *Mugil incilis* (Blanco, 1983), *M. curema* (Flórez, 1986) and *Oligoplites saurus* (Duque *et al.*, 1996).

Feed conversion rate is closely related with digestibility and metabolic use in diets (Morales *et al.*, 1994), while FCE determines feed quantity assimilated by an animal related to what it has consumed; feed is transformed into body weight. Caballero *et al.* (2002) points out that a high FCR could be product of a low feed digestibility as well as a diet's poor nutritional value, hence a lower FCE. This occurs due to the diets' proteins and lipids content (Table 2). The BF45 diet had the highest nitrogen free extract in which higher starch content could be found and so required a greater feed amount

to become a gram of meat; when FCR is high, FCE is low. Improving feed efficiency leads to reaching biological optimal, which is maintained until maximum growth values are achieved with an *ad libitum* feed (maximal voluntary feeding). FCR and FCE improvement with the increase of animal-originated protein is related to an improvement in essential amino-acids profile, energetic value increase and ration consumption increase due to better palatability. Carneiro *et al.* (2006) states that animal-originating ingredients have a good amino-acid profiles (essentials, specifically) according to fish requirements, besides, show n-3 series essential fatty acids, low carbohydrates levels, high digestibility and low anti-nutritional factor levels. Hence FCR close to 1.0 would reflect good digestibility, and protein and lipid content; which are within the minimal 16% requirement for rainbow trout, and were sufficient to maintain a low FCR. However, these FCR results are below those reported for rainbow trout (*O. mykiss*) fed *ad libitum* with values of 0.80–1.60 (Okumuş and Mazlun, 2002; Morales and Quirós, 2007; Bhat *et al.*, 2011; Haghghi *et al.*, 2011), hence FCE are below the reported values of 69.4–6.89% (Ojeda-Ojeda, 2005; Yamamoto *et al.*, 2000). Results obtained from this research are not discouraging since feeding was at 2.3% of total biomass.

SGR in harvesting and fish feed studies is important because it is

affected by the kind of feed given to the organisms (Papoustdoglou and Papaparaskeza-Papoutsoglou, 1978; Soriano-Salazar and Hernández-Ocampo, 2002) and it is an indicator of diets' protein quality, and also, under controlled conditions fish weight gain is in proportion to the essential amino-acids fed (Tacon, 1987; Soriano-Salazar and Hernández-Ocampo, 2002); it is a growth index that indicates if a given feed is efficient or not. Lowest SGR was found in BF45 diet, because it contains the lowest fishmeal content. Celis (1996) indicates that feeding with low essential amino-acids levels does not allow animals to fulfill all their genetic potential, because it is not being produced efficiently. One of the protein sources that present the best amino-acids profile for monogastric and aqueous species feeding is fishmeal. These results are below those reported by some authors. Okumuş and Mazlum (2002), reported 1.99-2.00, evaluating three commercial diets with different protein (45-48 g/100 g) and lipid (9-14 g/100 g) content fed *ad libitum*. Morales and Quirós (2007), in a research performed with three different feeding regimes: *ad libitum*, growth ration and maintenance ration, diets with protein contents of 46g/100g and lipids 17g/100g, reported values of 3.35, 1.94 and 0.07 respectively. Thiessen *et al.*, (2003) researched diets with fishmeal substituted by soy flour (180 g kg⁻¹, and proteic content of 52.3g/100g), shell-less pea flour (250g kg⁻¹, and proteincontent of

52.5g/100g) and canola flour (200g kg⁻¹, and protein content of 51.7 g/100 g), fed *ad libitum*, reported values of 1.87, 1.93 and 1.87, respectively. Yamamoto *et al.* (2001) reported SGR values of 1.68, 1.83, 1.74 and 1.65 in fish fed *ad libitum* on diets with different levels of pregelatinized potato starch (90, 180, 270 and 360 g kg⁻¹ and protein contents of 41.0, 41.7, 41.7 and 42.6g/100g, respectively). However, results from this research are within the reported range by Türker and Yıldırım (2011), who fed commercial diets (47g/100g of proteins and 20g/100g of lipids) until satiety, and reported SGR of 0.98-1.2 within the first 30 feeding days. Fenerci and Şener (2005), in four commercial diets (two extruded and two pelletized) with protein content of 42.3, 45.3, 45.2 and 39.1g/100g and lipid content of 12.1, 18.1, 13.2 and 10.9g/100g respectively, reported SGR values of 1.3 for extruded diets and 1.1-1.2 for pelletized diets. SGR values close to 1.6 indicate a complete feed (Johansson *et al.*, 1995; Okumuş and Mazlun, 2002). These differences found among reports may be related to fishmeal quality because its efficiency depends on the type of process used for its elaboration, fish species, if it is whole or in parts, apart from protein contents used in the research as well as the feeding regime and water temperature during feeding.

HSI is a liver weight to body weight ratio, allowing inferring on the possible organisms' nutritional state. Hence the higher HSI, indicates a better fish

nutritional condition. Legume and cereals use in fish nutrition, can have adverse effects on the liver, after long periods of feeding, due a tendency to increase glycogen deposits (Russell *et al.*, 2001; Serrano-Gutiérrez, 2004). Serrano-Gutiérrez (2004), points a greater liver gluconeogenesis activity when lupine inclusive levels are increased. On the other hand, Russell *et al.* (2001) reported that an increase in pea flour in the diet decreases HSI progressively. However, HSI close to 1.0% in rainbow trout suggests good health, by being fed with diets formulated with vegetable diets (Watanabe *et al.*, 1993; Serrano-Gutiérrez, 2004). These results are given below. Thiessen *et al.* (2003) reported values of 0.98-1.08% in diets with fishmeal substituted by soy flour. McCallun *et al.* (2000) reported values of 1.1% in fish fed *ad libitum* on diets with pea flour (250 and 200 g kg⁻¹), and protein contents of 52.4 and 59.9g/100g, respectively. Serrano-Gutiérrez (2004), with partial fishmeal substitution by white lupine flour (*Lupinus albus*) at 100, 150 and 200 g kg⁻¹ and protein contents of 46.96, 46.96, 45.34 and 45.07 g/100 g, fed *ad libitum*, reported values of 1.1%. Gümüş and İkiz (2009) reported values of 1.1-1.5% in diets with 40g/100g of proteins with different inclusion levels of pregelatinized corn starch (30, 120 and 180 g Kg⁻¹). HSI decrease could be associated to a change in the initial feeding regime. Despite the fact that each organ tends to represent a fixed

fish total weight percentage independent of its size, weight and liver size correspond to nutritional characteristics of the feed. High carbohydrate levels in diet are responsible for increase in size (Shearer, 1994; Serrano-Gutiérrez, 2004). Nevertheless, it is not possible to disregard the participation of other factors associated with an increase in available phosphorus for fish in the feed, which is responsible for generating a suppression of liver size due its effect on lipid metabolism (Robaina 1998; Serrano-Gutiérrez, 2004).

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