

## Effects of diets with soybean meal on the growth, digestibility, Phosphorus and Nitrogen excretion of juvenile rainbow trout *Oncorhynchus mykiss*

## Efectos de dietas con harina de soya en el crecimiento, digestibilidad, excreción de fósforo y nitrógeno de juveniles de trucha arco iris *Oncorhynchus mykiss*

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### ABSTRACT

A feeding trial was performed to evaluate the inclusion of high levels of soybean meal as a substitute for fishmeal in diets for juvenile rainbow trout and determine the phosphorus and nitrogen excretion. Fishmeal was replaced with soybean meal at levels of 50, 75 and 100 % with an inclusion of 0.8 g of phytase per kg of diet ( $\text{g kg}^{-1}$ ), whereas a diet with 100% substitution was used without phytase. A commercial diet and one with 100% of fishmeal were used as controls. Diets were fed to triplicate groups of juveniles ( $4.12 \pm 0.7$  g, mean  $\pm$  standard deviation) for a period of 50 days, and growth performance, P and N excretion and protein digestibility were determined at the end of this period. Data were analyzed with one-way ANOVA. The results showed that fish growth decreased when 100% of substitution was used, but diet with 75% of soybean meal did not affect growth. In addition, excretion values of P and N at this level of substitution were lower than those of the fish fed the control diets. The data suggest that diet with 75% soybean meal and 25% fishmeal might be used under practical conditions, as the growth performance observed on the juveniles fed such diet was similar to those of fish fed the commercial diet and the nutrient loading was reduced.

**Key words:** Rainbow trout, *Oncorhynchus mykiss*, soybean meal, fishmeal, substitution, phytase.

### RESUMEN

Se realizó una prueba de alimentación para evaluar la inclusión de altos niveles de harina de soya como sustituto de la harina de pescado en dietas para juveniles de trucha arco iris y determinar la excreción de fósforo y nitrógeno. La harina de pescado se sustituyó en niveles de 50, 75 y 100% con la inclusión de 0.8 g de fitasa por kg de dieta ( $\text{g kg}^{-1}$ ), así como una dieta con 100% de sustitución sin fitasa fue utilizada. Una dieta comercial y una con 100% de harina de pescado se utilizaron como controles. Las dietas se ofrecieron a grupos de juveniles por triplicado de juveniles ( $4.12 \pm 0.7$  g, promedio  $\pm$  desviación estándar) por un periodo de 50 días. Al finalizar este periodo, se determinó el crecimiento, la excreción de fósforo y nitrógeno y la digestibilidad de la proteína. Los datos se analizaron con un ANDEVA de una vía. Los resultados mostraron que el crecimiento de los peces disminuyó cuando se utilizó 100% de sustitución con o sin fitasa, pero el 75% de harina de soya en la dieta no afectó el crecimiento. A este nivel de sustitución también se observaron valores de excreción de fósforo y nitrógeno más bajos que los encontrados en los peces alimentados con las dietas control. Los datos sugieren que la dieta con 75% de harina de soya y 25% de harina de pescado puede ser uti-

lizada en condiciones prácticas de cultivo, pues el crecimiento observado en los juveniles alimentados con dicha dieta fue similar al de los peces alimentados con la dieta comercial y además, se redujo el aporte de nutrientes al agua.

**Palabras clave:** Trucha arcoiris, *Oncorhynchus mykiss*, harina de soya, harina de pescado, sustitución, fitasa.

## INTRODUCTION

The rapidly expansion of aquaculture during the last 20 years has been accompanied by an increasing demand for aquafeeds (Gatlin *et al.*, 2007). Fishmeal is the major source of protein in most feeds (Drew *et al.*, 2007), but it is becoming an expensive resource because of its rising demand (Dalsgaard *et al.*, 2009). Moreover, the use of fishmeal for aquafeeds has negative effects on the environment, since its use increases the level of nutrients as phosphorus (P) and nitrogen (N) in wastewaters from the farms (Hardy, 2002). The use of plant meals has been suggested as an alternative to fishmeal: they are cheaper, readily available and their phosphorus content is lower than that of fishmeal (Dalsgaard *et al.*, 2009). Among plant meals, soybean meal has been pointed out as the most promising alternative sources of protein source for aquafeeds (Hardy, 1996) because of its protein level (44-48%), reasonable well-balanced amino acid profile, availability and cost (Cheng *et al.*, 2004; Gatlin *et al.*, 2007). The use of soybean meal as partial substitute of fishmeal has been reported for several species of fish (Fowler, 1980; Olli *et al.*, 1989; Arnesen *et al.*, 1989; Krogdahl, 1994; Arndt *et al.*, 1999; Refstie *et al.*, 2000; Refstie *et al.*, 2001). However, its use is still limited (up to 25% of substitution), in part because of the large quantity of indigestible carbohydrates, although the presence of phytic acid or *myo*-inositol hexaphosphate is the major concern (Laining *et al.*, 2010). Phytic acid is the main form of storing P in seeds (Jorquera *et al.*, 2008), and usually causes low availability of minerals and reduces the apparent digestibility of protein (Gatlin *et al.*, 2007). The most efficient way to reduce the effect of this acid is by using phytase, a phosphohydrolase enzyme that catalyzes the sequential release of inorganic orthophosphate from phytic acid (Jorquera *et al.*, 2008) and has the potential to improve digestibility of nutrients for aquatic organisms (Cheng & Hardy, 2003).

The use of soybean and phytase in rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) has been reported by Cheng and Hardy (2003), who found that inclusion of phytase increased the apparent digestibility coefficients of crude protein and minerals of diet prepared with full-fat soybean meal. Cheng *et al.* (2004) reported that the apparent digestibility of crude protein of diets prepared with soybean meal in rainbow trout juveniles increased using microbial phytase. The same authors suggested 500 FTU of phytase  $\text{kg}^{-1}$  diet as optimal for the rainbow trout. Wang *et al.* (2009) reported that use of soybean meal pretreated with phytase or sprayed phytase allowed improvement of the apparent digestibility coefficient of diets, and that pretreatment at a level of 1,000 U of phytase/kg diet was the best option. In spite of those results,

there is no report of how high levels of inclusion of soybean meal might affect the levels of dissolved nitrogen (N) and phosphorus (P) excretion, which is a major concern for many fish culture operations (Bureau & Cho, 1999). Thus, the aim of the present research was to evaluate the inclusion of high levels of soybean meal as substitute of fishmeal in diets for juvenile rainbow trout, and to determine the P and N excretion.

## MATERIAL AND METHODS

**Test fish and test diets.** Rainbow trout, *Oncorhynchus mykiss*, of an age of 60 days post-hatching, were obtained from a private farm in the State of Mexico, Mexico and transported to the Laboratorio de Producción Acuícola, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México. Fish were maintained in 500-L tanks and fed a commercial pelleted diet for trout (Api-trucha 1, 50% protein, Malta-Cleyton de México, México) until 7 d before the feeding trial.

Four test diets were formulated with different levels of substitution of soybean meal (Protein,  $50.6 \pm 1\%$ ; total phosphorus,  $0.25 \pm 0.002\%$ ; Pronasoya, S.A. de C.V., México, México) for fishmeal. The formulation of these diets are shown in Table 1 and are based on the species requirements reported by NRC (1993), with a minimum of 40% of protein content. Cod liver oil (Drotasa, S.A. de C.V., México, México) and soybean lecithin (Abastecedora de Productos Naturales, S.A. de C.V., Mérida, Mexico) were used as additional lipid sources, whereas dextrin (Droguería Cosmopolita S.A. de C.V., Mexico, Mexico) was used as carbohydrate source. Moreover, a vitamin and mineral mix (Micro Rovimix for carnivorous fish, DSM Nutritional Products de Mexico, S.A. de C.V., Guadalajara, Mexico) and wheat gluten as binder (Sigma Aldrich Co., St. Louis, MO, USA), were used.  $\alpha$ -cellulose (Sigma Aldrich Co., St. Louis, MO, USA) was added as filler up to 100%. Three of the four experimental diets (substitution of 50, 75 and 100%, Soy1, Soy2 and Soy3) included 0.8 g of phytase (Phytase Ronozyme P5000, DSM Nutritional Products de México, S.A. de C.V., Guadalajara, México) per kg of diet ( $\text{g kg}^{-1}$ ). The fourth diet (Soy4) was with 100% substitution, and no enzyme added. A diet with 100% of fishmeal (Table 1) and a commercial diet (Api-trucha 1, 50% protein, Malta-Cleyton de Mexico, Mexico) were used as control diets.

Diets were prepared according to Hernández *et al.* (2004): briefly, the powdered ingredients were mixed with the oils and distilled water (40%). The wet dough was passed through a meat chopper to obtain 5-mm diameter pellets, which were dried at 60

Table 1. Formulation of the test diet fed to juveniles of rainbow trout.

Ingredient (g/kg)	Diets (% FM-% SM)				
	Soy 1 (50%-50%)	Soy 2 (25%-75%)	Soy 3 (0%-100%)	Soy 4 (0%-100%)	Control (100%-0%)
Fish meal (FM)	300	200	—	—	600
Soybean meal (SM)	300	400	600	600	—
Cod liver oil	50	50	50	50	50
Soybean lecithin	50	50	50	50	50
Dextrin	100	100	100	100	100
Vitamin and mineral mix <sup>1</sup>	40	40	40	40	40
Gluten	50	50	50	50	50
$\alpha$ Cellulose	109.2	109.2	109.2	110	110
Phytase	0.8	0.8	0.8	—	—
Proximate composition (%)					
Protein	42.9	45.2	42.5	42.5	43.9
Lipid	9.3	8.9	8.7	8.8	9.0
Ash	10.1	9.8	9.8	9.3	10.1
Moisture	9.2	9.4	9.6	10.2	9.1

<sup>1</sup>Vitamin and mineral mix (g kg<sup>-1</sup>): *p*-aminobenzoic acid, 1.45; biotin, 0.02; myo-inositol, 14.5; nicotinic acid, 2.9; Ca-pantothenate, 1.0; pyridoxine-HCl, 0.17; riboflavin, 0.73; thiamine-HCl, 0.22; menadione, 0.17;  $\alpha$ -tocopherol, 1.45; cyanocobalamin, 0.0003; calciferol, 0.03; L-ascorbyl-2-phosphate-Mg, 0.25; folic acid, 0.05; choline chloride, 29.65; retinol, 0.015; NaCl, 1.838; MgSO<sub>4</sub>·7H<sub>2</sub>O, 6.85; NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O, 4.36; KH<sub>2</sub>PO<sub>4</sub>, 11.99; Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>·2H<sub>2</sub>O, 6.79; Fe-citrate, 1.48; Ca-lactate, 16.35; AlCl<sub>3</sub>·6H<sub>2</sub>O, 0.009; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.17; CuCl<sub>2</sub>, 0.0005; MnSO<sub>4</sub>·4H<sub>2</sub>O, 0.04; KI, 0.008 and CoCl<sub>2</sub>, 0.05.

°C in a constant temperature oven for 60 min and stored at -20 °C until used.

**Feeding trial.** For the feeding experiment, a recirculation system with 18 plastic tanks (100-L capacity, filled with 80-L water) was used. Fifteen juveniles with an initial weight of 4.12 ± 0.5 g (mean ± standard deviation) were randomly stocked in each tank. Each test diet was fed to triplicate groups of juveniles. Fish were fed each day the respective diet at 7% of the body weight and the daily ration was divided into two equal feedings at 800 and 1500 h. The juveniles were weighed every 10 days and the ration size was adjusted accordingly. Dead fish were not replaced during the experiment. Thirty minutes after feeding, the remaining feed was removed and quantified for measurement of the diet intake and then, the fecal matter was taken out by siphoning the bottom of each tank. Throughout the feeding trial, mean (± SD) water temperature was 14 ± 1 °C, and dissolved oxygen and pH were 6.8 ± 1.0 mg L<sup>-1</sup> and 7.9 ± 0.5, respectively. The water flow through each tank was 1.5 L min<sup>-1</sup> during the entire experiment. All tanks were maintained under a natural photoperiod (11 hours light, 13 hours dark). The feeding trial lasted 50 d, from December 2007 to January 2008.

At the end of the 50 d, fish were starved for 24 h, and weighed to obtain growth performance. Five fish, collected randomly, were

returned to the tanks and were fed their respective diets added with 1% of chromium oxide during 10 d, for apparent digestibility determination. The remaining fish were used to evaluate oxygen consumption, and P and N excretion.

**Digestibility determination.** The apparent digestibility coefficients (ADC) of protein were determined with the NRC (1993) technique, using chromic oxide at a concentration of 1% (Cr<sub>2</sub>O<sub>3</sub>, powder, J.T. Baker, Phillipsburg, N.J., USA) as an inert marker. As fish rapidly accepted the diets with chromic oxide, no conditioning period was deemed necessary and the juvenile were fed with these diets for 10 days, during which feces were collected on a daily basis. Feces were dried in an oven and digested in a mixture of perchloric acid and nitric acid at 250 °C. Chromic oxide was quantified with the equation:

$$Cr_2O_3 = \frac{Y - 0.032}{0.2089} \cdot 4$$

Where: Y = absorbance at 350 nm (Furukawa & Tsukahara, 1966).

The ADC were calculate with the equation:

$$ADC(\%) = 100 - \left[ \left( \frac{\%MF}{\%MH} \right) * \left( \frac{\%PF}{\%PH} \right) * 100 \right]$$

Where: MF and MH = marker in feed and in feces; PF and PH = nutrient (protein) in feed and in feces.

**Oxygen consumption, P and N excretion.** Fish were fed their respective diets 24 hours before the test. A closed recirculation system of twenty 1-L flasks connected in series, by plastic tubes was slowly filled with water and one fish was placed per flask. Water samples were taken (initial P and N concentrations = 0 mg L<sup>-1</sup>) and the dissolved oxygen was measured, then flasks were hermetically closed. After 30 min, the flasks were opened, dissolved oxygen was measured with an oxygen meter (model 85, YSI Incorporated, OH, USA), and water samples were taken to determine P and ammonia N concentrations with the molybdo vanate and Nessler methods (Clesceri *et al.*, 1998). Oxygen consumption was calculated as the difference between the initial and final concentration.

**Statistical analysis.** Data on final body weight (FBW), weight gain (WG), specific growth rate (SGR), feed conversion efficiency (FCE), feed intake (FI), survival, oxygen consumption, P and N excretion and ADC were tested for normality and homoscedasticity with the Shapiro and Wilk W test and Barlett's test, respectively (Zar, 1999), after arcsine transformation in the case of survival, expressed as percentage. Since all data were normal and homoscedastic, the data were compared with one-way ANOVA tests. Significant differences among treatments were determined by Tukey multiple comparison tests (Zar, 1999), with a significance level of 5% ( $p < 0.05$ ). All statistical analysis were performed with the package Minitab version 15 (Minitab Inc., State College PA, USA).

## RESULTS

The means of final body weight (FBW), weight gain (WG), specific growth rate (SGR), feed conversion efficiency (FCE), protein conversion efficiency (PCE) and feed intake (FI) of the juvenile rainbow trout are given in Table 2. The fish fed with diet Soy2 showed the highest values of FBW, WG, FCE and PCE among the diets with soybean meal, and did not show significant differences when compared with those observed with the control and commercial diets.

The growth performance of the fish fed with diet Soy4, which had 100% soybean meal without phytase, was lower than those of the juveniles fed with phytase-added diets. Feed intake was similar with diets Soy1, Soy2, control and commercial, and these were significantly higher than with Soy3 and Soy4.

Survival ranged from 66 ± 11% with diet Soy3 to 83-91 % with the other diets, and there were no significant differences among treatments (Table 2). Oxygen consumption was significantly lower in the juveniles fed diets Soy3 and Soy4 than those determined for the rest of the experimental groups. The significantly highest consumption was observed in the fish fed the control diet with 100% of fishmeal as protein source (Fig. 1a).

There was a tendency of lower values of P excretion (as mg of PO<sub>4</sub><sup>-3</sup> L<sup>-1</sup>) with increasing soybean meal content in the experimental diets with phytase. The excretion of the fish fed diet Soy4 (100% soybean meal without phytase) was significantly higher than that observed in the diet Soy3 (100% soybean with phytase), and the highest value was observed in the fish fed the diet control with 100% of fishmeal (Fig. 1b).

Table 2. Final body weight (BW), weight gain (WG), specific growth rate (SGR), feed conversion efficiency (FCE) and feed intake (FI) of juveniles of rainbow trout fed diets with high levels of soybean meal. Values are means of the three replicate groups ± standard deviation. Means with different letters in the same line differ significantly ( $p < 0.05$ ).

Treatments	Soy1	Soy2	Soy3	Soy4	Control	Comm
FBW (g)	10.6 ± 0.9ab	12.5 ± 1.3a	9.4 ± 0.8b	8.9 ± 1.5b	13.9 ± 1.8a	12.9 ± 1.9a
WG <sup>1</sup> (%)	157 ± 21ab	203 ± 24a	131 ± 8b	116 ± 35b	236 ± 49a	216 ± 49a
SGR <sup>2</sup> (% day <sup>-1</sup> )	1.7 ± 0.2a	2.2 ± 0.3b	1.7 ± 0.1a	1.5 ± 0.2a	2.4 ± 0.2b	2.3 ± 0.2b
FCE <sup>3</sup>	1.7 ± 0.1a	1.5 ± 0.1b	1.7 ± 0.07a	1.7 ± 0.06a	1.2 ± 0.07c	1.2 ± 0.05c
PCE <sup>4</sup>	1.5 ± 0.06a	1.7 ± 0.04b	1.5 ± 0.1a	1.5 ± 0.04a	1.9 ± 0.1 c	1.8 ± 0.1 c
FI (g fish <sup>-1</sup> day <sup>-1</sup> )	2.2 ± 0.1a	2.5 ± 0.1a	1.8 ± 0.1b	1.6 ± 0.1b	2.4 ± 0.1a	2.3 ± 0.1a
Survival (%)	91 ± 4	89 ± 8	66 ± 11	87 ± 23	83 ± 4	89 ± 8

$${}^1\text{Weight Gain} = \frac{(\text{Final BW} - \text{Initial BW})}{\text{Initial BW}} * 100$$

$${}^2\text{Specific growth rate} = \frac{(\ln \text{Final BW} - \ln \text{Initial BW})}{50} * 100$$

$${}^3\text{Feed conversion efficiency} = \frac{\text{Weight gain (g)}}{\text{Total feed intake in dry weight basis}}$$

$${}^4\text{Protein conversion efficiency} = \frac{\text{Weight gain (g)}}{\text{Total protein intake in dry weight basis}}$$

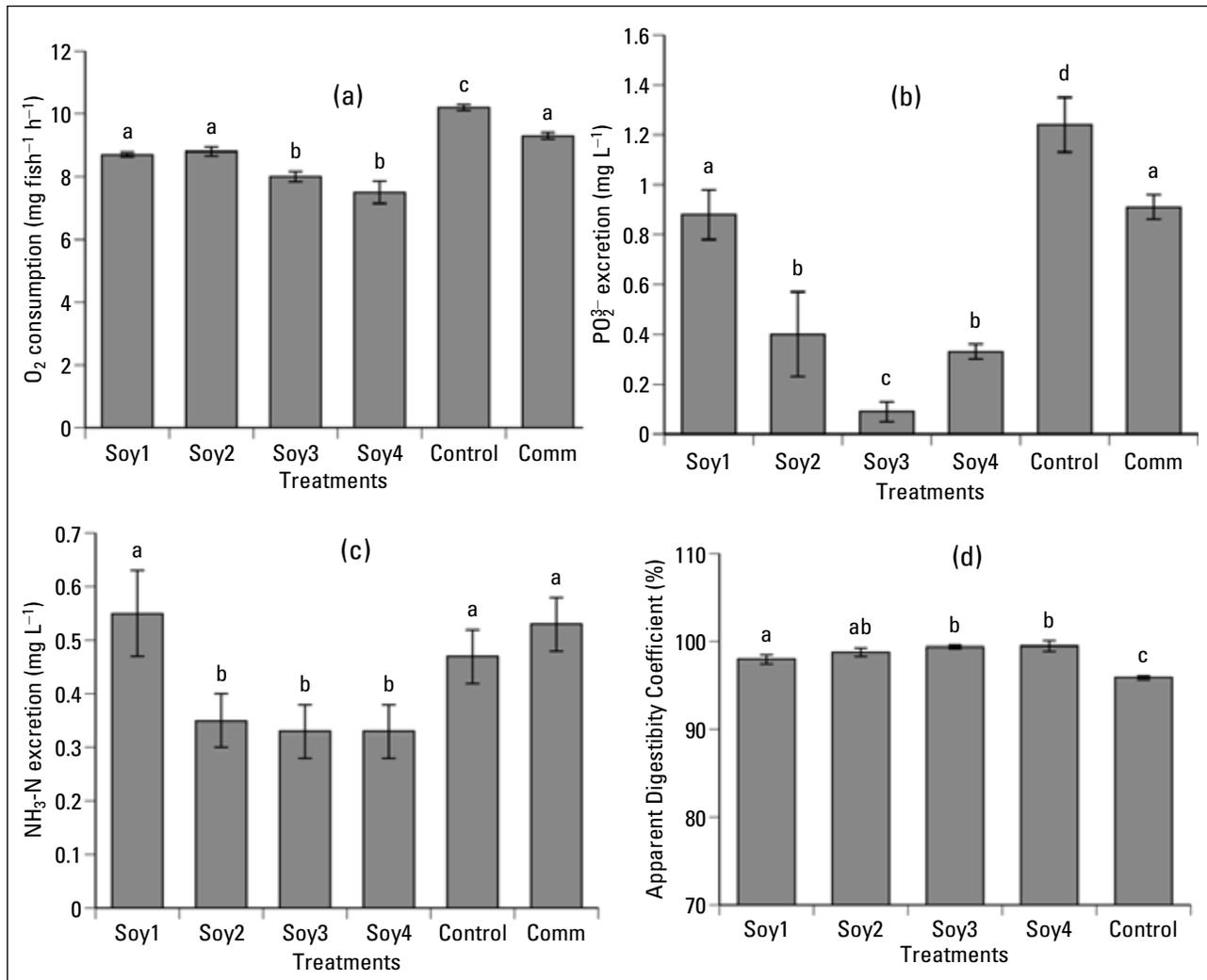


Figure 1a-d. Different parameters evaluated on juveniles of rainbow trout fed diets with high levels of soybean meal. (a) Oxygen consumption. (b) Phosphorus excretion. (c) Nitrogen excretion. (d) Apparent digestibility coefficient. Values are means of the three replicate groups  $\pm$  standard deviation. Means with different letters in the same column differ significantly ( $p < 0.05$ ). Comm refers to the commercial diet.

The N excretion of the fish fed with diets Soy2, Soy3 and Soy4 showed significantly lower values than those observed in the commercial and the control diet and in the diet with a substitution of 50% of soybean meal (Fig. 1c).

Higher values of ADC were observed in the diets with soybean meal substitution, particularly with total fishmeal replacement. The control diet with 100% fishmeal showed the lowest values of ADC (Fig. 1d).

## DISCUSSION

One of the main challenges of aquaculture industry for the years to come is to find alternative ingredients, which are cheaper and

environmental friendly (Gatlin *et al.*, 2007). Soybean meal has been pointed out as an alternative source of protein for aquafeeds (Hardy, 1996), because of its protein content and reasonable well-balanced amino acid profile. However, its use at high levels of supplementation (greater than 50%) has been discouraged due to the presence of some anti-nutritional compounds, particularly the content of phytic acid. This compound has been reported to be indigestible and to cause negative effects on growth, feed conversion and macronutrient digestibility (Laining *et al.*, 2010). Previous reports have suggested that the use of the enzyme phytase (Cheng *et al.*, 2004; Wang *et al.*, 2009) allows to improve the digestibility of diets and thus, growth. The data obtained in the present research show that soybean meal might be used up to

75% without affecting significantly the growth performance of juvenile rainbow trout when  $0.8 \text{ g kg}^{-1}$  of phytase is added to the diet. The fish fed this diet (Soy2), showed a growth performance similar to that reported by Wang *et al.* (2009) in rainbow trout of an initial weight of 1.7 g and fed a diet with 60% of soybean meal as source of protein.

As mentioned before, growth of fish is related to the digestibility of the diet and particularly to that of the protein fraction. The ADC of protein of the diets with soybean meal was higher than the reported by Wang *et al.* (2009) and Dalsgaard *et al.* (2009), but coincides with those reported by Cheng *et al.* (2004). It seems that phytase incorporation into diet is related to the digestibility of protein portion. When added after diet preparation (Dalsgaard *et al.*, 2009) or is sprayed (Wang *et al.*, 2009) on the diet, ADC tends to be lower than when the phytase is added before preparation, as in this study.

Interestingly, fish fed with diets Soy3 and Soy4 (100% of substitution of fishmeal) showed the highest values of ADC of protein. In addition, those diets showed less excretion of  $\text{NH}_3\text{-N}$  and oxygen consumption. According to Bureau *et al.* (2002), N excretion and oxygen consumption may be used to estimate the oxidation of nutrients from the diet and, usually, low values indicate a better use of the protein source (less excretion) and less energy spent in its oxidation (less consumption). However, fish fed those diets showed significantly lower growth than that observed with Soy1, Soy2, control and commercial diets. Soybean is deficient in lysine, methionine and threonine (Gatlin *et al.*, 2007), three essential amino acids. This probably caused the poor growth performance in 100% soybean meal based diets, even if these had the highest digestibility of all diets. The fishmeal inclusion in the other diets could allow an increase in the levels of such amino acids and better growth performance.

Phosphorus plays an important role in fish, because it is an important constituent of the fish endoskeleton, phospholipids, nucleic acids, cell membranes and energy-rich compounds (Kaushik, 2001). Although fish can absorb minerals from natural water (NRC, 1993), food is the main source of phosphorus because of the low concentration both in freshwater and seawater (Lall, 2002). The phytic acid usually binds to trypsin (Singh & Krikorian, 1982) or forms complexes of phytate-protein or phytate-mineral-protein that are resistant to proteolytic digestion (Cheryan, 1980). P bioavailability is one of the most relevant aspects affected by phytic acid and P usually is excreted in the urine and/or feces (Coloso *et al.*, 2003). According to Bureau & Cho (1999), dissolved phosphorus form is excreted via urine, which is a major concern for aquaculture operations, as it can cause eutrophication of water bodies surrounding the farms. The addition of phytase (a phosphohydrolase enzyme that catalyzes the sequential release of inorganic orthophosphate from the phytic acid (Jorquera *et al.*, 2008)) was suggested (Wang *et al.*, 2009) to improve P reten-

tion, and thus, decrease the excretion of dissolved phosphorus to water, as it was observed when fish were fed diets with high replacement of fishmeal by soybean meal. Decreasing levels of P excretion were observed as soybean meal increased in diet. Values were significantly lower than those observed in the control diets and such values represent almost a third of the P excreted with 100% fishmeal and a commercial diet. This might help in reducing the waste output from cultures, as reported by Cain & Garling (1995). Phytase inclusion, as expected, allowed to reduce P excretion and indicates that enzyme is working on the phytic acid and that it is active, even after feed processing. Similar results were observed in the Atlantic salmon (Storebakken *et al.*, 1998) and other salmonid species (Sugiura *et al.*, 2001), but this is the first time that a study on rainbow trout fed with high levels of substitution of soybean meal for fishmeal is reported.

The inclusion of phytase in the diet with a 75% of replacement of fishmeal with soybean meal allows improvement of the digestibility and this diet is capable to support the normal growth and development of juvenile rainbow trout. Moreover the use of this diet would allow the reduction of excretion of N and P to water. Even that this diet was used under laboratory conditions, the data suggests that might be used under practical conditions and will help reduce the nutrient pollution of water bodies surrounding the farms and reduce the feeding costs, as soybean is cheaper than fishmeal.

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