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Addition of Yeast and/or Phytase to Diets with Soybean Meal as Main Protein Source: Effects on Growth, P Excretion and Lysozyme Activity in Juvenile Rainbow Trout (*Oncorhynchus mykiss* Walbaum)

Daniel Sánchez Ávila[1], Esleban Arvizu Sánchez[1], Luis Héctor Hernández Hernández[1,*], Mario Alfredo Fernández Araiza[1], Omar Angeles López[1]

[1] Laboratorio de Producción Acuícola, UNAM FES Iztacala, Av. de los Barrios 1, Los Reyes Iztacala, Tlalnepantla, Estado de México, C.P. 54090, México.

* Corresponding Author: Tel.: +52.55 56231197; Fax: +52.55 56231197;
E-mail: luish3@yahoo.com

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Abstract

Two feeding trials, one under laboratory conditions and the other under practical conditions in a farm, were performed to determine the effect of inclusion of yeast and/or phytase to diets with soybean meal as main protein source, on the growth, P and N excretion, oxygen consumption and serum lysozyme activity of juvenile rainbow trout (*Oncorhynchus mykiss*). A basal diet (soybean meal, 400 g/kg diet and fish meal, 200 g/kg diet as protein sources) was added either with yeast (Diet Y, 15 g/kg diet), phytase (Diet Ph, 0.4 g/kg diet) and yeast and phyatse (Diet Y+Ph, 15 and 0.4 g/kg diet of yeast and phytase, respectively). A commercial diet was used as a control. Triplicate groups were fed the diets during 70 days (laboratory trial, initial weight 1.2 ± 0.06 g, mean ± standard error) and 75 days (farm trial, initial weight 2.8 ± 0.1 g). At the end of the trials, growth performance, oxygen consumption, P and N excretion and serum lysozyme activity were determined. A trend of higher values of growth was observed in the juvenile fed the diets with soybean meal and yeast (Diets Y and Y+Ph) in both feeding trials. As well, lower values of excretion of P and N were observed in the fish fed Diet Y. Significantly higher value of serum lysozyme activity was observed in the fish fed diet Y under laboratory conditions. Use of diet with 75% of substitution of the fishmeal with soybean meal, added with yeast and/or phytase is reported for the first time to feed rainbow trout under laboratory and farm conditions. The results show the possibility to use such diets, particularly the one with yeast (Diet Y), to fed juveniles without affecting the growth and the immune responses.

Keywords: Growth performance, lysozyme activity, phytase, rainbow trout, soybean meal, yeast.

Introduction

The rapid growth of the aquaculture during the last 20 years has been accompanied by an increasing demand for aquafeeds (Gatlin *et al*., 2007). Fishmeal is the main source of protein in commercial diets and with no expectations to increase the production beyond the current levels (Hardy, 1996) the demand for fishmeal will exceed the supply in a few years (Gatlin *et al*., 2007). Plant origin meals are an alternative to fishmeal (Hardy, 2010), but substitutions are still low (Slawki *et al*., 2011) as such feedstuffs usually contain high levels of fiber, starch, non-soluble carbohydrates and anti-nutrients that affects digestibility, fish normal growth (Krogdahl *et al*., 2010) and immune responses (Burrells *et al*., 1999). Thus, the aquaculture requires find ways to use more effectively plant-origin feedstuffs for the future development of the industry (Gatlin *et al*., 2007; Kaushik and Seiliez, 2010).

High inclusions of plant-origin meals in fish diets might require the use of additives such enzymes or probiotics that allow a better digestibility and proper use of nutrients, which will be reflected not only in growth, but on an adequate immune response as well. Particularly, one of the major concerns in using high levels of soybean meal in fish diets is its content of phytic acid (*myo*-inositol 1,2,3,5,6-hexakis dihydrogen phosphate) that usually causes low availability of minerals and reduces digestibility (Cheryan, 1980). A way to reduce the effect of this compound is by using phytase, a phosphohydrolase enzyme that catalyse the release of P from the phytic acid (Jorquera *et al*., 2008). Several authors (Cheng and Hardy, 2003; Cheng *et al*., 2004; Wang *et al*., 2009) found that the apparent digestibility of protein from soybean meal was improved with the inclusion of phytase. Recently (Cruz *et al*., 2011) it was report that juveniles of rainbow trout fed a diet with soybean meal (400 g/kg diet) as main source of protein and phytase (0.8 g/kg diet) had a similar growth performance and showed a reduction in P and N excretion, compared to those fed a commercial diet and one with fishmeal as sole protein source. On the

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other hand, the inclusion of yeast of genus *Saccharomyces* on feed improved the growth of several species of fish (Lara-Flores *et al*., 2003; Adbel-Tawwab *et al*., 2008; Chiu *et al*., 2010; Barnes *et al*., 2006), and despite the fact that *Saccharomyces* yeast are able to produce phytase when the phytic acid is present (Nayini and Markakis, 1984) so far there are not reports of its use on diets with high contents of soybean protein sources. Therefore, the aim of the present research was to determine the effects of the inclusion of yeast and/or phytase to a basal diet with 75% of soybean meal (400 g/kg diet) and 25% of fishmeal (200 g/kg diet) in the growth, P and N excretion, oxygen consumption and serum lysozyme activity of juvenile rainbow trout (*Oncorhynchus mykiss*) reared under laboratory and farm conditions.

Materials and Methods

Experimental Diets

A basal diet was formulated according to Cruz *et al.* (2011) with soybean meal (crude protein, 50.6±1%; Pronasoya, S.A. de C.V., Mexico) and fishmeal (crude protein, 55±1%; Vimifos S.A. de C.V., Mexico) as protein sources (Table 1). Three experimental diets were prepared by adding the Baker's yeast (Diet Y, 15 g/kg diet), phytase (Diet Ph, 0.4 g/kg diet) or a combination of both (Diet Y+Ph, 15 and 0.4 g, respectively). Phytase was added according to Cheng *et al.* (2004) and obtained from DSM Nutritonal Products of Mexico (Ronozyme P5000, 5,000 FYT/g). In the other hand, the yeast (*Saccharomyces* sp., approximately 8.5 x 10^9 cells/g) was added according with Lara-Flores *et al.* (2003) and obtained from a local baker shop. Cod liver oil (Drotasa S.A. de C.V., México) and soybean lecithin (Abastecedora de Productos Naturales, S.A. de C.V., Mérida, México) were use as lipid sources, and dextrin (Sigma Aldrich Co., St. Louis, MO, USA) was added as a source of carbohydrates. A vitamin-mineral mix (Micro Rovimix for carnivorous fish, DSM Nutritional Products of Mexico, Guadalajara, Mexico) and wheat gluten (Sigma Aldrich Co., St. Louis, MO, USA) as a binder were added. Finally, α-cellulose (Sigma Aldrich Co., St. Louis, MO, USA) was used to fill diet up to 100%. Diets were prepared according to Cruz *et al.* (2011) by mixing all the powdered ingredients with oils and water (40 %) to obtain wet dough, which was passed through a meat chopper and produce 5-mm diameter pellets. Diets were dried at 60°C for 2 hours and stored at -20°C until use. The experimental diets were compared with a commercial diet (Api-trucha 1, 45% protein, Malta-Cleyton de Mexico, Mexico).

Table 1. Basal formulation of the test diets fed to juvenile of rainbow trout. Proximate composition was of (% dry weight basis ± standard error): crude protein, 42.1±1.0; lipids, 11.2±0.7; ash, 12 ± 0.5

Ingredients	g/kg[1]
Fish meal[2]	200
Soybean meal[3]	400
Cod liver oil[4]	50
Soybean Lecithin[5]	50
Dextrin[6]	100
Vitamin and mineral mix[7]	40
Wheat gluten[6]	50

[1]α-cellulose was added to fill up the diets to 1 kg according to: Diet Y, 15 g yeast+95 g α–cellulose; Diet Ph, 0.4 g phytase+109.6 α-cellulose; Diet Y+Ph, 0.4 g phytase+15 g yeast+94.6 g α-cellulose.
[2]Vimifos S.A. de C.V., Mexico
[3]Pronasoya, S.A. de C.V., Mexico
[4]Drotasa S.A. de C.V., México
[5]Abastecedora de Productos Naturales, S.A. de C.V., México)
[6]Sigma Aldrich Co., St. Louis, MO, USA)
[7]DSM Nutritional Products of Mexico, Mexico

Experimental Fish

Rainbow trout of 60 days post-hatching were obtained at the rainbow trout reference centre of Mexico, "Centro de Producción Acuícola El Zarco", located in the State of Mexico. Fish came from two different spawning seasons, one in December 2011 and used for the trial under laboratory conditions at the Laboratorio de Producción Acuícola. The second batch was obtained on December, 2012 and use under practical conditions at the trout farm "Rancho Los Alevines", located in the municipality of Amanalco de Becerra, State Of México, Mexico.

Feeding Trials

The first trial was performed under laboratory conditions on a recirculation system with 100-L polypropylene tanks. Each diet was fed to triplicate groups of 15 juveniles (1.2±0.06 g) at 7% of their body weight daily, divided into two equal feeding at 09:^{00} and 16:^{00}. Juveniles were weighed every 10 days and the ration size was adjusted accordingly. In order to reduce the leaching of P from fecal matter, it was collected after 30 min of feeding (Windell *et al*., 1978) by siphoning the bottom of each tank and kept to determine the P contents. After that, unconsumed feed was removed and quantified for measurement of the diet intake. This trial lasted 70 days. Through the feeding trial, the water quality parameters were: dissolved oxygen 6.5±1.0 mg/L (mean±SD), pH 7.9±0.5 and water temperature of 16±1°C. Water flow on each tank was of 1.5 L/min during the entire experiment. All tanks were maintained under a natural photoperiod. The second trial was under farm conditions in flow-through system with 12 glass-fibre tanks of 1000-L. Each diet was fed to triplicate groups of 65 juveniles (2.8±0.1 g) at 7% of their body weight daily, as before, divided into two equal rations size. The juveniles were weight every 15 days and ration size was adjusted accordingly. The fecal matter was removed as previously described. This trail was performed for 75 days. Water parameters (mean±SD)

were: dissolved oxygen 5.5±1.0 mg/L, pH 7.0±0.5 and water temperature of 12±1°C. Water flow on each tank was of 5 L/min during the entire experiment.

At the end of both trials, the fish were starved for 24 h and weighed to determine the growth performance. In first feeding trial a sample of 5 fish for each replication were selected randomly to evaluate the oxygen consumption, and nitrogen and phosphorus excretion. Fish, finally, were sacrificed with an over-dose of MS-222 (ethyl 3-aminobenzoate, methanesulfonic acid, Sigma Aldrich Co., St. Louis, MO, USA) at 200 mg/L and blood samples were taken to determine the serum lysozyme activity. Blood was allowed to clot at 4°C and three h later, the serum was separated by centrifugation at 3,000 rpm for 10 min, pooled and frozen at -20°C until used.

Oxygen Consumption, Nitrogen and Phosphorus Excretion

Fish were fed their respective diets 24 h before perform the tests were used. A closed recirculated system of twenty 1-L flask connected in a series by plastic tubes, was slowly filled with water and one fish was place in each flask. The initial concentrations of oxygen, nitrogen and phosphorus were obtained and the flasks were closed hermetically. Fish were maintained in such conditions for a period of 30 min and then, flask were open and dissolve oxygen was measured again and samples of water were taken for the determination of nitrogen and phosphorus concentrations. Dissolved oxygen was measured with an oxymeter (model 556MPS, YSI Inc., OH, USA) and oxygen consumption was calculated according with Hernández *et al.* (2012). The P excretion (as PO_4^{3-}) was determined by method of molibdovanadate and nitrogen (as NH_3-N) by the Nessler method (Clescerl *et al.*, 1995) respectively. Dry feces were digested with persulfate acid at 400°C and then, total phosphorus was determined in by the molybdovanate method (Clescerl *et al.*, 1995).

Lysozyme Activity

It was determined according with Caruso *et al.* (2002) and briefly, 100 µl of the blood serum were added to an aqueous solution of *Micrococcus lysodeikticus* (lyophilized cells, Sigma Aldrich Chemical, St. Louis, MO, USA) to obtain a mix of 1000 µl, which was incubated at 25°C and absorbance was read at 530 nm exactly 0.5 and 4.5 min after adding the sample. One unit of lysozyme activity (U) was defined as the amount of enzyme that caused a decrease in absorbance of 0.001 min/L.

Statistical Analysis

Data were tested with the Shapiro and Wilk W test and Barlett´s test for normality and homoscedasticity, respectively (Zar, 1999). Data expressed as percentage were arcsine transformed and then tested. Since all data showed normality and homoscedasticity, they were compared by one-way ANOVA (package Prism 6.0 for Mac, GraphPad Sofware, Inc.). When found the statistical differences between treatments were evaluated by a Tukey multiple comparison test, considering an error of 5% ($P<0.05$).

Results

The growth performance of the fish feed on the trial under laboratory conditions is show in Table 2. The weight gain (WG) of the juveniles was significantly higher in the fish fed the diets with soybean meal than the observed for those fed with the commercial diet. Among the diets with soybean meal, the one added with yeast showed the highest WG, but no significant differences were observed when compared with the other two treatments. Higher values was observed on the specific growth rate (SGR), feed conversion ratio (FCR) and protein conversion ratio (PCR) on the groups fed the experimental diets than those observed in the commercial diet, but no significant differences were found. Survival was higher of 96% all the groups and mortality was not related to the treatment with the diets.

A similar trend was observed in the growth performance during the trial under practical

Table 2. Growth performance and survival of juvenile rainbow trout fed diets with high content of soybean meal as protein source and the addition of yeast and/or phytase under laboratory conditions for a period of 70 days

	Diets			
	Diet Y	Diet Ph	Diet Y+Ph	Commercial
Wt gain (%)[1]	1,043±16a	992±31a	984±23a	893±8b
SGR (%/day)[2]	3.4±0.07	3.4±0.01	3.4±0.02	3.3±0.06
FCE[3]	0.70±0.01	0.65±0.02	0.64±0.02	0.67±0.1
PCE[4]	2.3±0.2	2.1±0.1	2.3±0.1	2.7±0.8
Survival	98	100	98	96

Data are the means of triplicate groups±standard error. Means with different letters in the same line differ significantly ($P<0.05$).
[1]Weigth gain = ((Final weight – initial weight) / Initial weight) x 100
[2]Specific growth rate = ((ln final weight – ln initial weight)/70) x 100
[3]Feed conversion efficiency = Weight gain (g) / total feed intake (g dry weight basis)
[4]Protein conversion efficiency = Weight gain (g) / total protein intake (g dry weight basis)

conditions (Table 3), with higher values of the diets with the soybean meal diets than the commercial diet. However, no significant differences were observed between the groups. Again, highest values were observed on the group fed the diet with yeast and followed by the Diet Y+PH. Survival rates were higher the 78% and the mortality observed was not related to the treatments.

Regarding the physiological responses during the trial under laboratory conditions, the oxygen consumptions during the first trial was not significantly different among the treatments, higher values were observed on the fish fed the Diets Y and the commercial (Figure 1a). Nitrogen excretion (expressed as NH_3-N, Figure 1b) showed higher values in the group fed the commercial diet, but not significant differences were observed among the treatments. Organisms fed the Diet Y+Ph showed the highest N excretion between the groups fed the experimental diets. Regarding the P excretion (as PO_4^{3-}, Figure 1c) the fish fed the commercial diet showed significantly higher values when compared the juveniles fed Diets Y and Y+Ph. Regarding the P content in feces, no significant differences among the groups were observed in both feeding trials, but a tendency of higher values were observed on the commercial diet (Figures 1d and 1e).

Serum lysozyme activities of the juvenile rainbow trout reared under laboratory conditions are show in Figure 2a and significant higher values were observed in the group fed diet Y than the others. In Figure 2b is show the lysozyme activity of the juveniles fed under practical conditions and not significant differences were found among the groups.

Discussion

With a limited supply of fishmeal for aquafeeds on the next decades (Hardy, 1996), aquaculture requires to find ways to use more effectively plant-origin feedstuffs for the future development of the industry (Gatlin *et al*., 2007; Kaushik and Seiliez, 2010). In the present research we report for the first time, the use of yeast and/or phytase in diets with 75% of substitution of the fishmeal with soybean meal to feed rainbow trout under laboratory and farm conditions. The results show the possibility to use such diets, particularly the one with yeast (Diet Y), to fed juveniles without affecting the growth and the lysozyme activity, an important non-specific immune response in fish.

Both, under laboratory and farm conditions, data of growth performance were higher than the previously reported by Cruz *et al.* (2011), in which they fed rainbow trout juveniles with a similar formulation of 75% soybean and 25% fishmeal as protein sources and the addition of 0.8 g phytase per kg of diet. The same was found when SGR was compared with those obtained by Gomes *et al.* (1995) and Wang *et al.* (2009) when fed rainbow trout juveniles with soybean meal and the inclusion of phytase. The addition of phytase has been related to an improvement of growth in salmonids, mainly because phytic acid is hydrolysed and allows a better digestion process (Cheng *et al*., 2004). Phytase was added to the experimental diets at a level of 0.4 g/kg, about 2,000 FYT/kg, and it seems to be an adequate level to be included when 400 g/kg of soybean is used. Regarding the use of yeast, it has been reported previously as growth promoter in several species of fish such as Nile tilapia *Oreochromis niloticus* (Lara-Flores *et al*., 2003; Adbel-Tawwab *et al*., 2008), grouper *Epinephelus coioides* (Chiu *et al*., 2010) and rainbow trout (Barnes *et al*., 2006). However, for the first time is reported its use in diets with high contents of soybean meal. The improved growth of juveniles fed the Diets Y and Y+Ph might be related to a better nutrient digestibility, particularly the protein fraction (Lara-Flores *et al*., 2003; Waché *et al*., 2006) and the results of the present research indicates that yeast might contribute to this. During the feeding trials we were not able to determine the protein digestibility, but Bureau *et al.* (2002) reported that N excretion and oxygen consumption might be useful to estimate the nutrient utilization of diet, as usually, low values indicates a better use of the protein (less N excretion) and less energy spent in its oxidation (less oxygen consumption). We observed lower values of NH_3-N excretion and similar values of oxygen consumption on the fish fed the experimental diets. Cruz *et al.*

Table 3. Growth performance and survival of juvenile rainbow trout fed diets with high content of soybean meal as protein source and the addition of yeast and/or phytase under practical conditions for a period of 75 days

	Diets			
	Diet Y	Diet Ph	Diet Y+Ph	Commercial
Wt gain (%)[1]	513±35	446±84	480±28	396±38
SGR (%/day)[2]	2.4±0.07	2.2±0.2	2.3±0.06	2.1±01
FCR[3]	1.07±0.02	1.06±0.02	1.05±0.02	1.26±0.04
PCE[4]	1.5±0.03	1.7±0.008	2.1±0.007	1.8±0.8
Survival	79	86	80	78

Data are the means of triplicate groups±standard error. Not significant differences were observed at this level (P<0.05)
[1]Weigth gain = ((Final weight – initial weight) / Initial weight) x 100
[2]Specific growth rate = ((ln final weight – ln initial weight)/75) x 100
[3]Feed conversion efficiency = Weight gain (g) / total feed intake (g dry weight basis)
[4]Protein conversion efficiency = Weight gain (g) / total protein intake (g dry weight basis)

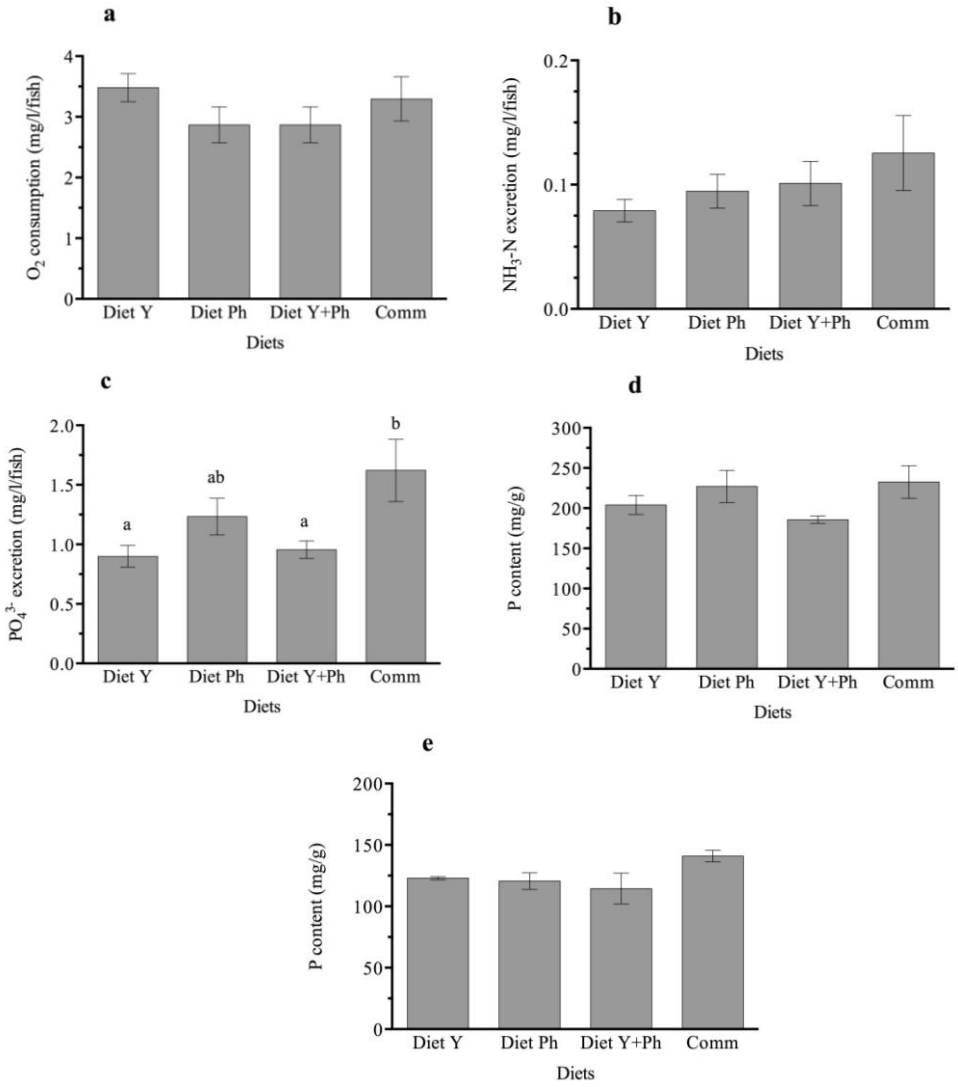

Figure 1. Physiological responses of juvenile rainbow trout fed diets with high content of soybean meal as protein source and the addition of yeast and/or phytase. Data are the means of three replicate groups ± standard error. Bars with different letters differ significantly (P<0.05). (a) Oxygen consumption, (b) nitrogen excretion, (c) phosphorus excretion and (d) total phosphorus in feces of the fish fed under laboratory conditions. (e) Shows the total phosphorus in feces of fish fed under practical conditions.

(2011) reported that feeding juveniles of rainbow trout with a diet based on soybean meal, the oxygen consumption was as high as double of the found during the feeding trial under laboratory conditions.

Phosphorus excretion (in urine and feces) was similar to that reported previously by Cruz *et al.* (2011) and Hernández *et al.* (2012). Soybean storages most of its P as phytic acid (*myo*-inositol 1,2,3,5,6-hexakis dihydrogen phosphate), an organic compound that once in the digestive tract, usually forms complexes phytate-protein or phytate-mineral-protein (Cheryan, 1980) or binds to trypsin (Singh and Krikorian, 1982) that reduces the digestibility (Gatlin *et al*., 2007) and P is usually excreted in the urine and/or feces (Coloso *et al*., 2003), which is a major concern of aquaculture operations, as it can cause eutrophication of water bodies surrounding the farms (Bureau and Cho, 1999). Phytase is a phosphohydrolase enzyme that catalyses the sequential release of inorganic orthophosphate from the phytic acid (Jorquera *et al*., 2008) and has been suggested as supplement in diets with soybean meal (Wang *et al*., 2009) to improve P retention and thus, decreased its excretion. After the feeding trials and with the addition of phyatse to diets with soybean meal, as expected, the P excretion was lower than the commercial diet. However, P excretion in feces and urine tended to be lower on the juveniles fed with Diet Y. Phytase has been reported to be present in yeasts of genus *Saccharomyces* (Nayini and Markakis, 1984) and the activity of the enzyme has been found to increase when the phytic acid is in the growth medium (Gontia-Mishra and Tiwari, 2013). Our results suggest that the yeast included in the experimental diets helped to catalyze the phytic acid present in the soybean meal in a better way than the phytase used in Diet Ph.

According to Burrells *et al.* (1999), the

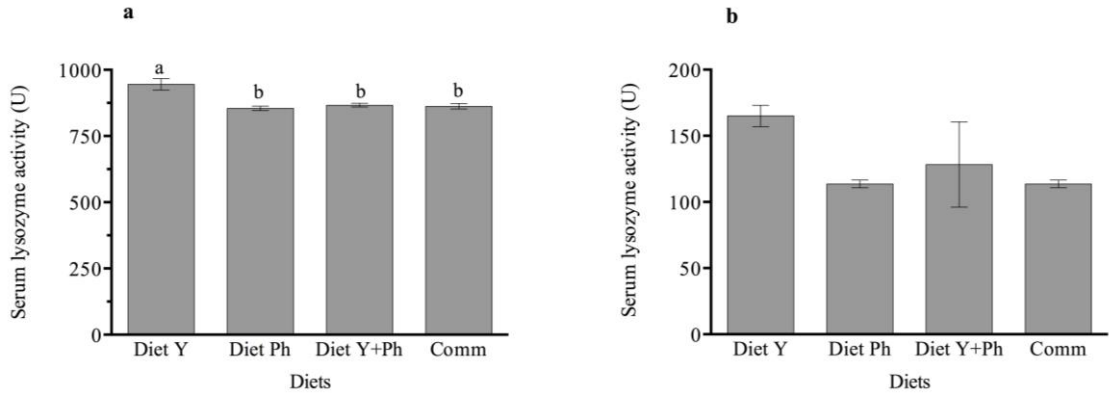

Figure 2. Non-specific immunological responses of rainbow trout juveniles fed diets with high contents of soybean meal as protein source and the addition of yeast and/or phytase. Data are the means of three replicate groups ± standard error. Bars with different letters differ significantly (P<0.05). (a) Serum lysozyme activity under laboratory conditions and (b) serum lysozyme activity under practical conditions.

incorporation of high levels of soybean protein in fish feed affects the immune capacity of salmonids, as nutrition of the organisms play an important role in maintain the normal response and particularly, the lysozyme activity is the non-specific response that often is use to evaluate the fish condition. The non-specific immune responses are the first line of defence in fish and usually can avert many microbial until the specific responses have been developed (Saurabh and Sahoo, 2008). The data obtained under laboratory conditions of serum lysozyme activity are between the normal ranges reported previously for rainbow trout fed a diet based on fish meal (Verlhac *et al*., 1986) and those fed on a diet with plant-origin proteins (Hernández *et al*., 2012; Jalili *et al*., 2013). However, data under the practical conditions are lower that those reported. Several environmental factors have been reported to affect lysozyme activity and are well establish that temperature has an effect in it (Saurabh and Sahoo, 2008). During the trial under farm conditions, temperature was lower by 3 to 4 °C than the performed under laboratory conditions and this might influencing the lower lysozyme activities as reported previously for carp (Studnicka *et al*., 1986), Atlantic halibut (Langston *et al*., 2002) and southern bluefin tuna (Watts *et al*., 2002). Regardless of the two feeding trials, we were able to identify a trend in which the yeast and the phytase added to the experimental diets caused higher lysozyme activities. Besides, the yeast has been reported to improve the lysozyme activity in several species of fish mainly by contributing with nucleic acids and β-1,3-glucans (Abdel-Tawwab *et al*., 2008; Chiu *et al*., 2010), but the mechanisms are still not fully understood (Nayak, 2010).

The present research shows the possibility of using high contents of soybean meal by adding yeast or yeast and phytase in diets for juvenile rainbow trout reared under different conditions. Diets Y and Y+Ph did not affected the growth and the serum lysozyme activity, besides they might be contributing with reducing the P excretion. As well represents an option to use more plant-origin ingredients in the commercial diets for trout, even that is necessary to establish a way to deliver the live yeast during the industrial process of diet manufacturing, which might include the use of encapsulated cells to ensure the viability of yeast.

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References

Abdel-Tawwab, M., Abdel-Rahman, A.M. and Ismael N.E.M. 2008. Evaluation of commercial live bakers´yeast, *Saccharomyces cerevisiae* as a growth and immunity promoter for fry Nile tilpia, *Oreochromis niloticus* (L.) challenged *in situ* with *Aeromonas hydrophila*. Aquaculture, 280: 185-189.

Barnes, M.E., Durben, D.J., Reeves, S.G. and Sanders, R. 2006. Dietary yeast culture supplementation improves initial rearing of McConaughy strain rainbow trout. Aquaculture Nutrition, 12: 388-394.

Bureau, D.P. and Cho, C.Y. 1999. Phosphorus utilization by rainbow trout (*Oncorhynchus mykiss*): estimation of dissolved phosphorus waste output. Aquaculture, 179: 127-140.

Bureau, D.P., Kaushik, S.J. and Cho, C.Y. 2002. Bioenergetics. In: J.E. Halver and R.W. Hardy (Eds.). Fish Nutrition, 3rd edition, Academic Press, San Diego, CA, USA: 1-59.

Burrells, C., Williams, P.D., Southgate, P.J. and Crampton, V.O. 1999. Immunological, physiological and pathological responses of rainbow trout (*Oncorhynchus mykiss*) to increasing dietary concentrations of soybean proteins. Veterinary Immunology and Immunopathology, 72: 277-288.

Caruso, D., Schlumberger, O., Dahm, C. and Proteau, J.P. 2002. Plasma lysozyme levels in sheatfish *Silurus glanis* (L.) Subjected to stress and experimental infection with *Edwardsiella tarda*. Aquaculture

Research, 33: 999-1008.

Cheng, Z.J. and Hardy, R.W. 2003. Effects of extrusion and expelling processing, and microbial phytase supplementation on apparent digestibility coefficients of nutrients in full-fat soybeans for rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 218: 501–514.

Cheng, Z.J., Hardy, R.W., Verlhac, V. and Gabaudan, J. 2004. Effects of microbial phytase supplementation and dosage on apparent digestibility coefficients of nutrients and dry matter in soybean product-based diets for rainbow trout *Oncorhynchus mykiss*. Journal of the World Aquaculture Society, 35: 1-15.

Cheryan, M. 1980. Phytic acid interactions in food systems. Critical Reviews on Food Science and Nutrition, 13: 114-119.

Chiu, C.H., Cheng, C.H., Gua, W.R., Guu, Y.K. and Cheng, W. 2010. Dietary administration of the probiotic, *Saccharomyces cerevisiae* P13, enhanced the growth, innate immune responses, and disease resistance of the grouper, *Epinephelus coioides*. Fish Shellfish Immunology, 29: 1053-1059,

Clescerl, L.S., Greenberg A.E. and Eaton, A.E. 1995. Standard Methods for the Examination of Water and Wastewater, 17th Ed., Am. Public Health Assoc., Washington, DC.

Coloso, R.M., King, K., Fletcher, J.W., Hendrix, M.A., Subramanyam, M., Weis, P. and Ferraris, R.P. 2003. Phosphorus utilization in rainbow trout (*Oncorhynchus mykiss)* fed practical diets and its consequences on effluent phosphorus levels. Aquaculture, 220: 801-820.

Cruz, C.A.C., Hernández, H.L.H., Fernández, A.M.A., Ramírez, P.T. and Angeles, O.L. 2011. Effects of diets with soybean meal on the growth, digestibility phosphorus and nitrogen excretion of juvenile rainbow trout *Oncorhynchus mykiss*. Hidrobiológica, 21: 118.125.

Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G.S., Krogdahl, Å., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R. and Wurtele, E. 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. Aquaculture Research, 38: 551-579.

Gomes, E., Rema, P. and Kaushik, S. 1995. Replacement of fishmeal by plant proteins in the diet of rainbow trout (*Oncorhynchus mykiss*): digestibility and growth performance. Aquaculture, 130: 177-186.

Gontia-Mishra I. and Tiwari, S. 2013. Molecular characterization and comparative phylogenetic analysis of phytases from Fungi with their prospective applications. Food Technology and Biotechnology, 51: 313-326.

Hardy, R.W.1996. Alternate protein sources for salmon and trout diets. Animal Feed Science and Technology, 59:71-80.

Hardy, R.W. 2010. Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aquaculture Research, 41: 770–776.

Hernández, F.G., Hernández, H.L.H., Fernández, A.M.A. and Angeles, L.O. 2012. Effects of total replacement of fishmeal with *Spirulina* powder and soybean meal on juvenile rainbow trout (*Oncorhynchus mykiss* Walbaum). Israeli Journal of Aquaculture- Bamidgeh, IIC: 64.2012.790.

Jalili, R., Tukmechi, A., Agh, N., Noorin, F. and Ghasemi, A. 2013. Replacement of dietary fish meal with plant sources in rainbow trout (*Oncorhynchus mykiss*); effect on growth performance, immune responses, blood indices and disease resistance. Iranian Journal of Fisheries Science, 12: 577-591

Jorquera, M., Martínez, O., Maruyama, F., Marschner, P. and Mora, M.L. 2008. Current and future biotechnological applications of bacterial phytases and phytase-producing bacteria. Microbes and Environments, 23: 182-191.

Kaushik, S.J. and Seiliez, I. 2010. Protein and amino acid nutrition and metabolism in fish: current knowledge and future needs. Aquaculture Research, 41: 322–332.

Krogdahl, Å., Penn, M., Thorsen, J., Refstie, S. and Bakke, A.M. 2010. Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding response in salmonids. Aquaculture Research, 41: 333-344.

Langston, A.L., Hoare, R., Stefansson, M., Fitzgerald, R., Wergeland, H. and Mulcahy, M. 2002. The effect of temperature on non-specific defences parameters of three strains of juvenile Atlantic halibut (*Hippoglossus hippoglossus* L.). Fish and Shellfish Immunology, 12: 61-76.

Lara-Flores, M., Olvera-Novoa, M.A., Guzman-Mendez, B.E. and Lopez-Madrid, W. 2003. Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). Aquaculture, 216: 193-201.

Nayak, S.K. 2010. Probiotics and immunity: a fish perspective. Fish and Shellfish Immunology, 29: 2-14.

Nayini N.R. and Markakis, P. 1984. The phytase of yeast. Lebensmittel-Wissenschaft und Technologie, 17: 24–26.

Saurabh, S. and Sahoo, P.K. 2008. Lysozyme: an important defence molecule of fish immune system. Aquaculture Research, 39: 223-239.

Singh, M. and Krikorian, A.D. 1982. Inhibition of trypsin activity *in vitro* by phytate. Journal of Agriculture and Food Chemistry, 193: 799-800.

Slawski, H., Adem, H., Tressel, R.P., Wysujack, K., Koops, U. and Schulz, C. 2011. Replacement of fishmeal by rapeseed protein concentrate in diets for common carp (*Cyprinus carpio* L.). Israeli Journal of Aquaculture-Bamidgeh, IIC: 63.2011.605.

Studnicka, M., Siwicki, A. and Ryka, B. 1986. Lysozyme level in carp (*Cyprinus carpio* L.). Israeli Journal of Aquaculture-Bamidgeh, 38: 22-25.

Verlhac, V., Gabaudan, J., Obach, A., Schuep, W. and Hole, R. 1996. Influence of dietary glucan and vitamin C on nonspecific and specific immune response of rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 143: 123-133.

Waché, Y., Auffray, F., Gatesoupe, F.J., Zambonino, J., Gayet, V., Labbé, L. and Quentel, C. 2006. Cross effects of the strain of dietary *Saccharomyces cerevisiae* and rearing conditions on the onset of intestinal microbiota and digestive enzymes in rainbow trout *Oncorhynchus mykiss* fry. Aquaculture, 258: 470-478.

Wang, F., Yang, Y.H., Han, Z.Z., Dong, H.W., Yang, C.H. and Zou, Z.Y. 2009. Effects of phytase pretreatment of soybean meal and phytase-sprayed in diets on growth, apparent digestibility coefficient and nutrient excretion of rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquaculture International, 17: 143-157.

Watts, M., Munday, B.L. and Burke, C.M. 2002.

Investigation of humoral immune factors from selected groups of southern bluefin tuna, *Thunnus maccoyii* (Castelnau): implications for aquaculture. Journal of Fish Diseases, 25: 191-200.

Windell, J.T., Foltz, J.W. and Sarokan, J.A. 1978. Methods of fecal collection and nutrient leaching in digestibility studies. Progressive Fish Culturist, 49: 51–55.

Zar, J.H. 1999. Biostatistical Analysis. 4th Edition. Prentice Hall. New Jersey, USA, 663 pp.