Enzyme Supplementation to Soybean Based Diet in Rainbow trout (Oncorhynchus mykiss) Effects on Growth Parameters and Nitrogen and Phosphorus Digestibility

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Abstract

The aim of the this study was to examine the effects of the diets based on Soybean Meal (40%) supplemented with exogenous enzymes on growth performance, feed utilization, apparent digestibility and reduce environment pollution waste output of nitrogen and phosphorus in rainbow trout (Oncorhynchus mykiss) culture. Trout groups (initial weight 87.00±1,5) method of random plots with 3 replications of 50 fish/pond with the ratio of 1050 fish in the concrete ponds. Diets consisted of 25% fish meal (FM) and 40% dehulled hexane extracted soybean meal (SBM) in control group (C0) and diet supplemented with protease enzyme (PRT; 2g/kg-1), diet supplemented with enzyme cocktail (MIX; cellulose, xylanase, endo-ß-1,3:1,4-glucanase; 2g/kg-1) and diet supplemented with pyhtase enzyme (PHY; 2g/kg-1). About of growth performance were found while condition factor (1,21-1,23) were statistically similar ($p>0.05$), however specific growth rate, SGR, (1,118-1,340) and feed conversion ratio, FCR, (1,26-1,30) were obtained significantly different among groups ($p<0,05$). PRT and PHY groups significantly improved SGR and FCR better than control group. In this study, showed the highest nitrogen apperent digestibility coefficient, ADC (85,49±1,98) in PRT group while, the poorest value obtained (72,82±0.01) C0 group

respectively ($p<0,05$). Also the best (58.57±0.49) and lowest (42.85±1.98) ADC was obtained PHT and C0 groups for phosphorus respectively ($p<0,05$).

Keywords: rainbow trout, enzyme, growth performance, nitrogen, phosphorus, digestibility

1.INTRODUCTION

Natural feed additives as referred to the use of enzymes; made in the areas of biotechnology and animal feed baits, intensive research has become common parallel developments in nowadays (Nir and Senköylü 2000). Developments in biotechnology, with a significant effect in feeding the fish is quite expensive but can be substituted for fish meal with plant-based raw materials to be used in a more effective and beneficial, increasing digestibility, reduction factors of antinutritional have opened new horizons in the use of these feeds as effective. The most appropriate herbal product.is soybean meal feed raw materials of vegetable origin that can be used instead of fish meal in fish feeds affordability, availability and nutritional value in the markets. The addition of enzyme increases of nutrition value, because of non-starch polysaccharide in soybean meal feed value limited (Deguara et al. 1999, Hardy 2000, Hardy and Gatlin 2002, Cho and Bureau 2003). Enzyme with the use of lower-priced raw materials from more expensive raw materials used in equal and sometimes better performance (Deguara et al. 1999, Nir and Senköylü, 2000). Feed manufacturers are prefers dry and granular enzymes, because of its importance in terms of homogeneous distribution of enzyme activity instead of liquid enzyme preparations (Collier and Hardy 1986, Inbor 1990). Usually the most limiting factor in plant development is phosphorus in freshwater and is nitrogen in the seas. The majority of phosphorus in vegetable feed ingredients commonly used (50-80%), phytic acid or phytate-bound form is found and undigestibility by the monogastric animals. Such animals hasn't got any enzymes to break down phytate and phosphorus reveals, adding has been sufficient amount of the phytase enzyme feed stuffs in the structure of phytate phosphorus digestibility remain free (Lantzsch 1989, Sugiura et al. 2000, Sugiura et al. 2001, Saçaklı 2002). Microbial phytase enzyme is effective in reducing environmental pollution by fecal excretion of phosphorus caused, depending on the form of phytic phosphorus in feedstuffs monogastric animal diets vegetarian diet significantly increases of evaluation. (Gibson and Ulah 1990, Graham and Inborr 1993, Gordon and Roland 1997). Discarded and retained in reducing the level of nitrogen and phosphorus levels are the key strategies in the ration protein level needed to keep the fish, the energy: protein and amino acid digestibility of high raw materials (Vergara et al. 1996, Sugiura and Hardy 2000, Cho and Bureau 2003). In fish fed is observed and improvement in performance additon of enzymes addition increased the digestibility of the nutrients, specific growth rate and protein efficiency ratio and a marked improvement (Tandler and Kolkovski 1992, Deguara 1998, Deguara et al. 1999, Ayhan et al. 2008).

The aim of this study, with the addition to fish feed as protease enzyme, enzyme cocktails and phytase enzyme, more efficient use of raw materials of vegetable origin, fresh water fish feaces and discharge of nitrogen and phosphorus excretion, thus reducing the environmental pollution, increase growth performance, feed conversion to raise the utilization efficiency.

2.MATERIALS AND METHODS

Fish and texperimental design: Rainbow trout (Oncorhynchus mykiss, initial weigh 87,0±1,5g), used in the experiment were obtained from the Ministry of Food Agriculture and Livestock, Institute of Mediterranean Fisheries Research, Production & Training, Kepez Unit, Antalya. As a triplicates the design with 4 groups of 50 fish per group for a total of 600 pieces of fish are grouped according to the random subdivision method. Trial; a 6,3 m3 capacity were 4 pieces of concrete ponds. Each concrete pond was seperated equal to 2,1 m3 in capacity for three section.

Feed materials: Feeds in the experiment were prepared at feed prepare units in the institution. Utilization rate of feedstuffs used in the experiment and prepared feeds and nutrient contents in the feed are given in Table 1.

As a dietary supplement protease enzyme, the enzyme cocktail and phytase enzyme were used. Trial feed grinding-mixing-ratios given in Table 1 was passed through a pelletizing operations. The ration was obtained and pelletted, 50 kg.hour-1 capacity in dry press-pellet machinery. Feeds were stored at +4°C the cooling cabinet. During the experiment, the total feed intake and feed digestibility with biometric measurements were analyzed.

In feed and feaces has been analysed dry matter, crude protein, crude fat and crude ash by Weende Analysis and phosphorus analysis according to the method of Vanadat (AOAC, 1995). The digestible energy values calculated in MJ.kg-1 (New, 1987). Chromic oxide, and digestibility of feed and feaces for analysis and the growth performance and value were determined by Steffens 1989; Goddard 1996).

Table 1. Ingerdients, nutrient composition, growth performance and digestibility of experiment trial [1]

INGREDIENTS, (g.kg $^{-1}$)	**DIETS**			
	C0 [1]	**PRT**[1]	**MIX**[1]	**PHY**[1]
Fish meal	25	25	25	25
Soybean meal	40	40	40	40
Full fat soybean meal	9,,22	9,12	9,12	9,12
Blood meal	5	5	5	5
Wheat middlings	9,23	9,13	9,13	9,13
Menhaden oil	7	7	7	7
Vitamin premix [2]	2	2	2	2
Mineral premix [3]	1	1	1	1
Vitamin C [4]	0,3	0,3	0,3	0,3
Choline [5]	0,15	0,,15	0,,15	0,,15
Pellet binder [6]	0,4	0,4	0,4	0,4
Chromic oxide [7]	0,4	0,4	0,4	0,4
Antioxidant [8]	0,3	0,3	0,3	0,3
Allzyme Vegpro [9]		0,2		
Roxazyme-G [10]			0,2	
Ronozyme-P [11]				0,2
TOTAL	100,00	100,00	100,00	100,00
Nutrition composition				
Dry matter, %	92,33	92,92	91,94	93,54
Crude protein, %	44,85	44,72	42,81	43,14
Crude oil, %	13,36	12,45	13,52	11,89
Crude cellulose, %	3,25	3,25	3,25	3,25

Crude ash, %	13,74	15,15	11,38	12,29
Metabolizable energy, (MJ. kg^{-1})	14..39	14..39	14.39	14.39
Calcium, %	1.09	1.09	1.09	1.09
Phosphorus, %	1.45	1.45	1.45	1.45
Lysine, %	3,17	3,17	3,17	3,17
Methionine+cystine, %	1,50	1,50	1,50	1,50
Growth Performance				
Initial Weight (g) [3]	76,0±11,5	76,1±0,5	75,0±2,5	75,0±1,5
Final Weight (g) [4]	208,0±5,0b	211,0±1,5a	206,0±5,5ab	206,0±5,5ab
SGR [6]	1,118±0,02b	1,340±0,02a	1,122±0,02ab	1,290±0,02ab
FCR [7]	1,29±0,01a	1,26±0,01b	1,30±0,01a	1,27±0,01b
CF [8]	1,21±0,01	1,23±0,01	1,22±0,04	1,23±0,04
Digestibility				
ADC Dry matter % [12]	63,79±0,49bc	68,73±1.52c	61,58±0,03b	66,92±0,52a
ADC Protein % [12]	85,03±0,75ab	87,42±l,17c	86,96±0,55b	87,21±0,46a
ADC Nitrogen % [12]	72,82±0.15b	85,491.98c	69,711.19b	78,950.68a
ADC Phosphorus % [12]	42,85±0.58a	48,77±2.01a	45,69±0.48a	58,57±0.49b

[1] Data (mean±SD) with different letters within a row are significantly different ($p<0.05$).

[2] C0: Control group, no added enzyme, PRT: Protease group: 2%o protease; MIX: Mix group : 2%o enzyme cookteyl; PHT: Phytase group : 2%o phytase

[3] Vitamin mixture; Included of per kg; 18.000 IU A, 2000 IU D, 200 mg E, 12 mg K, 150 mg B2, 20 mg B1, 0,05 mg B12, 20 mg pyridoxine, 10 mg panthotenic acid, 220 mg niacine, 120 mg inositol, 5 mg folic acid, 0,5 mg biotine, 2000 mg choline.

[4] Mineral mixture: Included of per kg: 70 mg zinc, 60 mg mangenese, 60 mg magnesium, 4 mg ferro, 2 mg copper, 1.5 mg iode, 0.5 mg cobalt, 0.05 mg selenium.

[5] Vitamin C, Hoffman La-Roche Inc.

[6] Choline, Ufuk Kimya İlaç San. ve Tic. Ltd. Sti Istanbul.

[7] Lignosulphanate,

[8] Cr_2O_4, Merck

[9] Buthylhidroxitoluoen, (powder form)

[10] Allzyme Vegpro, All-Tech Inc.

[11] Roxazyme G, Hoffman La-Roche Inc.

[12] Ronozyme-P, Hoffman La-Roche Inc.

[13] Body weight of initial (WI)

[14] Body weight of final (WF)

[15] Specific growth rate, SGR (%/day) = ((Ln final body weight – Ln initial body weight)/days) x 100

[16] Feed conversion ratio, FCR = dry feed intake (g) / weight gain (g).

[17] Condition factor = (W/L^3) x 100

[18] Apparent Digesitbility Coefficient (ADC) = 100 – (100 x ((Cr_2O_3 in diet, % / Cr_2O_3 in feaces, %) x (nutrient in feaces, % / nutrient in diets, %))

Experimental process: Total duration of the experiment, was carried out in 14 weeks including 2 weeks of adaptation time. During the experiment, a biometric measurements were made every two weeks. During the trial, daily feed intake is calculated depending on the temperature of the water and total live weight from 1.5 to 3%. Feeding was 2 times per day (09:00am, 16:00pm). Each fish in the tank every two weeks weighed in bulk. Trial groups from 14.5 to 18.5°C change in water temperature, dissolved oxygen 7.84 ± 0.46 mg.L-1, 7.78 ± 0.06 pH, ammonia and nitrite 0001 0:01 to 0:10 mg.L-1 was determined to be. The study of natural fotoperyot (15 daylight: 9 nightlight) were applied.

Digestive work: 15 days trial period at the end of the experiment the fish groups fed in the milking stools on a daily basis via the -20°C until analyzed were collected and incubated freezer. After analysis of nutrient digestibility in feed and feces rates calculated Apparent Digestibility Coefficient, ADC (Steffens 1989; Goddard 1996).

Statistical analysis: The results of the trial groups were analysed by one-way analyses of variance (ANOVA) with Duncan's multiple comparison tests used to determine the groups which are responsible for the difference, made with SAS 5.0 statistical package. Significance was tested at the p=0,05 level and homogeneity of variance was performed by Levene test and variance was found to be homogenous (Orhan et al., 2004).

3.RESULTS AND DISCUSSION

3.1.Growth performance in trial groups

Data on body weight gain and performance criteria of the trial groups given in Table 1. Table 1 According to the Tablo 1 in terms of initial live weight there was no difference statistically significant ($p>0.05$) but at the end of the experiment statistically significant difference between groups ($p<0.05$). Thus, specific growth, SGR, in data, the best value protease (PRT, 1.340±0,02) and phytase (PHY, 1.290±0,02) group was obtained. The lowest value by SGR phytase (C0 1.1150,02) group was obtained (Table 2, $p<0,05$). In terms of feed conversion ratio the best (lowest) value was obtained protease (PRT, 1.26±0,01) group and the worst (highest) of the mix group (MIX 1,30±0,01) (Table 1, $p<0,05$). Condition factor (CF)were obtained the best value PRT (1,23±0,01) and PHY, 1,23±0,04) groups, while the lowest in C0 group (1,20±0,01) (Table 1, $p<0.05$).

Deguara's (1998) study was obtained avg. 50g. seabream fish were added fish meal 26% +soybean meal 32% of the study, 26% basic diets trial groups by 0,01% protease+alpha-galactosidase enzyme with low pH and high pH protease+alpha-galactosidase enzymes. Study in terms of live weight than the control group with low pH protease+alpha-galactosidase enzyme supplemented group of about 18 g. while providing a increasing, a high pH protease+alpha-galactosidase enzyme according to the control group was obtained from 10 g good growth. 25%+40% soybean meal based on fish meal similar to the results of the trial group with Deguara's study protease (PRT) and phytase (PHY) enzyme-supplemented groups, the growth has been between 4-6 g live weight than the other groups (Table 1). Deguara's (1998) study, the control group in terms of specific growth rate of 0.53% protease+alpha-galactosidase enzyme to grow, but with low pH and high pH group, 0.77% protease+alpha-galactosidase enzyme group,% 0.71 a value of was obtained. In this study about of SGR, the control group (C0) 1.12% although, the best growth of the protease (PRT, 1.34%) and phytase (PHY, 1.29%) groups, a similar growth trend was observed (Table 1, $p<0,05$). Deguara's (1998) study were obtained control group in terms of feed conversion coefficient of 2.62, but with low pH value, enzyme protease+alpha-galactosidase group of high pH protease+alpha-galactosidase enzyme group, 2.18 and 2.46 value. Yan et al., (2001) in their study, 12.4g The channel catfish (Ictalurus punctatus), addition of phytase (0, 500, 1000, 2000, 4000, 8000 unit.kg-1) fed, 0.57% 0,16% usefull of total phosphorus between, there was no effect in weight gain, but feed consumption was higher than in the control group reported. In our study by feed conversion coefficient, PRT (1,26) and PHY (1,27) groups enzyme-supplemented groups, particularly the control (1,29) group to a more positive level that is lower than for other groups were obtained (Table 1, $p<0,05$). Similarly Jahan et al (2001), carp and tilapia fingerlings diets in combination karbonhidrase and protease enzyme has an additional 19% increase in weight and mirror carp, more increased 5% in FCR, more value 16% in SGR and had improved, while 9% tilapiada stated that the weight gain. Our study is based on the values

of the FCR and protease enzymes, especially phytase supplemented groups provided a positive contribution to the utilization of the feed can be said. This is because the fish with vegetable protein sources by means of the enzyme phytase phytic phosphat considered to be useful in making the result can be assumed. Terms of condition factor Deguara (1998)'s study in gilthead seabream fish was obtained, in control group, 1.42 value, in alpha-galactosidase enzyme with low pH protease group, 1.49 value and a high pH protease+alpha-galactosidase, group 1, 44 value. In our study, trout condition factor ranged from 1.21 to 1,23 value (Table 1, $p<0,05$).

3.2.Nitrogen-phosphorus digestibility (ADC) in trial goups

In terms of the value of nitrogen digestibility in the trial groups were obtained the high digestibility of 85.49% protease (PRT)and the lowest digestibility 69.71% enzyme cocktail (MIX) group. The control group (C0) with 72.82% in the second last. In the same way by phosphorus digestibility among groups with the highest digestibility value of 86.58% phytase (PHY) 74.99% and lowest value in the control group (C0)74,99% (Tablo 1, $p<0.05$).

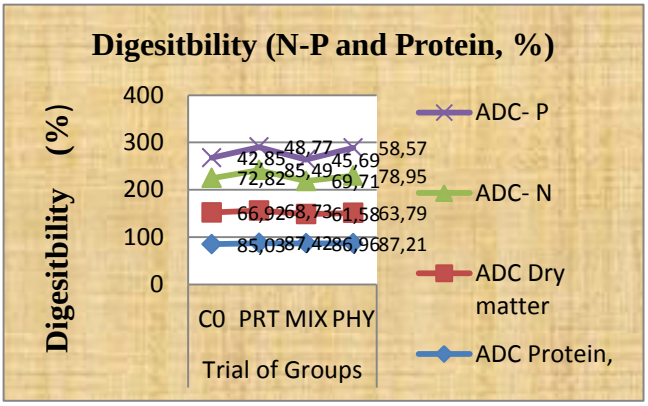

Fig 1. Digestibility nitrogen-phosphorus and dry matter-protein in experimental diets (%)

Digestibility of nitrogen were mesured at feed and feaces in experimental groups, which is one of the media in order to determine what percentage of the amount of nitrogen and fecal nitrogen values. If you need to compare the groups in terms of enzyme-supplemented groups less than the control group to be noted that levels of nitrogen excretion (Table 1). Ramseyer and Garling (1998) reported without any additives in fish feeds were assessed 30% of the nitrogen by the body and discarded of 70% in feaces and urine, and feeding was assessed 32% of the phosphorus by the body and discarded of 68% with the urine and feaces. In our study, nitrogen values of between 6,41% - to 6,63% in feed. Based on measurements of nitrogen derived from nitrogen digestibility were obtained minimum percent of 69.72±0.01 in control group (C0) while, the addition of percent of 83,2±1,98 PRT and 81,25±0,68 in PHY supplemented group.

Generrally in rainbow trout need to 0.5 to 0.8% of digestible phosphorus in feed, but should be find approximately 1,5 times more phosphorus in that value (Ketola and Harland 1993;Ketola and Richmond 1994; Garcia-Ruiz and Hail 1996). Phytate in the same way, the

total phosphorus in plant protein sources 70% of the phytate phosphorus monogastric animals (fish) are considered very low (Lall, 1991). Phytate phosphorus; channel fish, trout and salmon (Ketola and Richmond 1994), redseabream (NRC,1993) and carp phytase enzyme can not be assessed adequately. This is why the commercial phytase enzyme by the addition of vegetable protein sources in fish feed for fish, the phosphorus becomes evaluated (Riche and Brown, 1996). In feeding studies have found by Lanari et al. (1998) 2% rate of phosphorus excretion in the control group, 1,8g.kg-1 of phytase supplemented group and 0,89g.kg-1 was found. In the same study, 115,.3 g. trout fish of 33% soy trout feed 1000 IU/kg of added phytase enzyme was added at the end of the study group, 9.52% phosphorus digestibility (control group: 58.01%; phytase enzyme group: 68.1% ($p<0.01$) an increase. Schafer et al. (1994) investigeted by the juvenile carp fish 25%, suggests that the excretion of phosphorus phytase supplemented group. However Deguara (1998) has founded 45.66% of the phosphorus digestibility in the control group, but with low pH value, protease+alpha-galactosidase enzyme group, 59.52% and 53.68% as a group of high pH protease+alpha-galactosidase. In our study, phosphorus obtained 1.44 to 1.53% in feed while the experimental group (Table 1) by measurements digestibility of phosphorus were identified in the control group was percent of 42.85 while, percent of 48.77 in the protease and percent of 58.57 as phytase group ($p<0,05$).

In plant-based feed find the phosphorus, while which is available at a limited level for the fish to be in the form of phytic acid (Sugiura and Hardy, 2000; Cheng and Hardy, 2002; Lall 1991), but Riche and Brown (1999) and Satoh et al (2003) according to by the studies, the plant protein sources rations based on the availability of more high phosphorus showed, this feed is shown as a control group to include more low phophorus and calcium. While many studies of enzyme supplementation of diets are usually small, such as fry or fingerlings while achieving positive results in individuals, Degura (1998) and this study shows that the big fish in a similar positive results are obtained. Fixing only the performance of these positive results, but also allows the use of fish meal diet, less level. However, similar studies are needed anyway.

As a result of, in the raw materials of herbal origin digesitbility of phythin phosphat better to increase of fish feed nitrogen and phosphorus to take advantage of an additional increase of the protease and phytase enzymes, enzyme-supplemented groups had higher rates of nitrogen and phosphorus retention (ie, a higher rate digestibility by the fish's body) in this study were determined. In the same way in aqauculture, environmental pollution is responsible for the largest food nitrogen and phosphorus into the water excretion rate of feaces from the addition of protease, and phytase enzyme were found to be lower in groups. This would mean reducing the parameters of the results of pollution from aquaculture feed. Water left in an improvement of nitrogen-phosphorus ratio of unity, even one in a thousand of waters and protection of the environment adds value to a very positive sense. These studies are possible by increasing the feed rates of reducing pollution from water.

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