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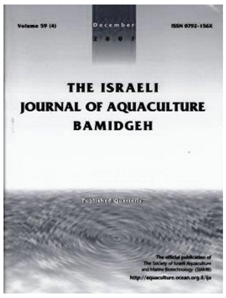

Effect of Enzyme Supplementation to Soybean Meal Based Diets on Growth, Feed Conversion Ratio, Nutrient Digestibility, and Body Composition of Rainbow Trout *Oncorhynchus mykiss,* (Walbaum) Fry

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Key words: soybean meal, Rainbow trout, growth, pectinase, xylanase, cellulase

Abstract

A 12 week feeding trial was conducted with rainbow trout *Oncorhynchus mykiss*, fry (3.98 ± 0.05 g, average initial weight) to determine the effects of supplemental exogenous enzymes on growth, digestibility, and body composition. Five diets were prepared adding pectinase, xylanase, cellulase enzymes, and a commercial enzyme complex, to diets containing 40% soybean meal. The experiment was conducted in triplicate in 15 tanks, each stocked with 45 juvenile fish. The results showed that addition of enzymes to diet containing 40% soybean meal had no significant effect on growth performance and feed conversion ratio. No significant differences were observed in the apparent lipid digestibility, apparent protein digestibility, and whole body composition among dietary treatments (P>0.05). In conclusion, the use of single or multi-components of commercial enzymes (tested in this case) are not sufficient to degrade the high levels of non-starch polysaccharides introduced into digestive tract by the diets containing soybean meal at the rate of 40%.

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Introduction

Soybean meal (SBM) is probably the most promising and most studied alternate protein source to fish meal. However, like other plant derived protein sources it contains 30.3% non-starch polysaccharides such as cellulose, xylose, pectins, hemicellulose, and galactonic acid (Huyghebaert and Groote 1995; Smits and Annison 1996) which constitute 70–90% of the plant cell wall (Bach Knudsen 2001). The anti-nutritive effect of non-starch polysaccharides (NSP) for monogastric animals is well defined in the literature (Choct et al., 1996; Bakker et al., 1998; Simon 1998). Most of the nutrients including protein cannot be fully utilized in nutrition due to the lack of NSP degrading enzymes in the digestive tracts of monogastric animals, therefore NSPs are anti-nutritive and reduce the performance of monogastric animals (Alloui et al., 1994). Pentosans hold significant amounts of water and, due to the resulting high viscosity, the absorption of nutrients is limited. In practical conditions this can be seen as reduced feed conversion ratio (FCR) and weight gain. These problems can be overcome by the addition of β-glucanases (Cellulase) and xylanases, resulting in improved animal performance (Paloheimo et al., 2011). Enzyme mixtures containing cellulase, xylanase, and pectinase, may assist in the digestion of soybean meal in vitro digestion (Malathi and Devegowda, 2001).

In addition, exogenous enzymes are widely used to reduce the anti-nutritional effects of non-starch polysaccharides in feeds of monogastric animals including pigs and poultry (Alloui et al., 1994; Ghazi et al., 2003; Esonu et al., 2005). However, there is very limited information regarding the effects of enzyme supplementation in fish feed, apart from phytase (Dalsgaard et al., 2012). Most of the studies in this area have used commercial mixed enzymes and there is limited information on the effects of the supplementation of pure xylanase to soybean meal based diets in fish (Dalsgaard et al., 2012).

The aim of this study was to examine effects of supplementation of pure cellulase, pectinase, xylanase, and commercial enzyme complex, to diets containing soybean meal on growth performance, feed conversion ratio, body composition, and nutrient digestibility, of rainbow trout fry.

Materials and methods

Experimental diets and analysis. Experimental diets were isonitrogenous and isoenergetic and formulated to fulfill the nutritional requirements for rainbow trout (NRC 1993). The diets were prepared by adding pectinase (10.000 unit/g), xylanase (100.000 unit/g), cellulase (5000 unit/g), and commercial enzyme complex (Farmazyme XP 2010) to trout diets. Cellulase was derived from *Trichoderma reseei*, pectinase from *Aspergillus niger,* and xylanase from *Bacillus subtilis*. Enzymes were obtained from FARMAVET(TR) Enzymes Company in Turkey. The inclusion level of the enzymes was 0.1% as recommended by the company. Formulation of the experimental diets is given in Table 1.

Table 1. Formulation and chemical composition of the experimental diets (%)

Ingredients	Groups				
	Control	***Commercial enzyme***	***Cellulase***	***Pectinase***	***Xylanase***
Fish meal[1]	34.00	34.00	34.00	34.00	34.00
Soybean meal[2]	40.00	40.00	40.00	40.00	40.00
Wheat flour[3]	6.00	6.00	6.00	6.00	6.00
Corn flour[4]	4.00	4.00	4.00	4.00	4.00
Menhaden oil	14.00	14.00	14.00	14.00	14.00
Vitamin[5]	0.80	0.80	0.80	0.80	0.80
Mineral[6]	0.40	0.40	0.40	0.40	0.40
Cr_2O_3	0.50	0.50	0.50	0.50	0.50
Binder[7]	0.30	0.30	0.30	0.30	0.30
Mix Enzyme	-	0.1	-	-	-
Cellulase	-	-	0.1	-	-
Pectinase	-	-	-	0.1	-
Xylanase	-	-	-	-	0.1
Chemical composition					
Dry matter	10.75±0.12	10.82±0.39	12.11±0.14	11.47±1.47	11.92±0.21
Crude protein	41.05±1.76	40.01±1.65	40.66±1.90	40.83±1.71	40.52±1.32
Crude fat	13.78±0.29	12.51±0.78	12.36±0.61	12.82±0.33	13.15±0.21
Crude ash	10.97±0.03	10.93±0.12	10.60±0.12	10.90±0.35	9.77±0.12
Digestible energy (kcal/kg)[8]	4200	4200	4200	4200	4200

[1,2]Purchased from USA, [3,4]Purchased from Turkey, [5]Vitamin premix contained the following per kilogram; 4 000 000 IU vitamin A. vitamin D3 480 000 IU. 2400 mg vitamin E. 2400 mg vitamin K3. 4000 mg vitamin B1. 6 000 mg vitamin B2. 4 000 mg Niacin. 10 000 mg Cal.D. Pantothenate. 4 000 vitamin B6. 10 mg vitamin B_{12}. 100 mg D-Biotin. 1200 mg folic acit. 40 000 mg vitamin C. 60 000 mg inositol. [6] Mineral premix contained the following per kilogram; 23750 mg manganese. 75 000 mg zinco. copper 5 000 mg. cobalt 2 000 mg. iodine 2750 mg. selenium 100 mg. magnesium 200 000 mg. [7]Lignosülfanat, [8] Digestible energy value was calculated from published values for the diet ingredients (NRC. 1993).

All ingredients and enzymes were thoroughly mixed in a mixer and 20% distilled water was added. Diets were minced with a 0.4 mm sieve and the spaghetti-like strands were dried (20°C) for 24 hour in a convection oven. After drying, the feed was broken up into 2 mm pellets and stored at 4°C until use.

The moisture, crude protein, crude fiber, and ash contents of feed ingredients, experimental diets, feces samples, and body composition, were determined according to standard AOAC methods (AOAC 1990), whereas lipid percentage was determined by the chloroform methanol extraction method (Bligh and Dyer, 1959).

Experimental Design. Rainbow trout fry were obtained from Egirdir Fisheries Faculty, Turkey. This experiment, carried out in 15 tanks, was performed in triplicate. At the start of the experiment, 45 rainbow trout fry (mean weight of 3.98 ± 0.05 g) were stocked into each tank. During the 90 day feeding trial the rainbow trout were hand fed twice a day (09:00 and 16:00 hours). All fish were fed to satiation. At the end of the feeding trial, 5 fish per tank were given a lethal dose of anesthesia (500 mg/l MS-222), homogenized in a blender, and stored at -20°C until analysis of protein, lipid, ash, and moisture was performed.

During the experimental period, water quality parameters - dissolved oxygen level, temperature, and pH, were maintained at 7.0±0.09 mg/l, 11±0.9°C, and 7.05±0.47, respectively.

Digestibility study. Apparent digestibility coefficients were measured by the indicator method using 5 g/kg chromic oxide as a marker (Hardy and Barrows, 2002). During the feeding trial, fish feces was collected and prepared for chemical analysis (Lim et al., 2001).

Apparent digestibility coefficients (ADC) were calculated using the following equations;

ADC=100-[100x (Cr_2O_3 in diet (%)/Cr_2O_3 in feces (%) x (nutrient in feces (%) / nutrient in diet (%)].

Statistical analysis. A one-way analysis of variance (ANOVA) was used to compare growth, feed conversion ratio and nutrient digestibility among treatments. All data were analyzed using SPSS computer program (SPSS 2000). Duncan test was used to determine significant difference between treatment means ($P<0.05$)

Results

Growth performance of rainbow trout fry fed with the different enzyme diets is shown in Table 2.

Enzyme in trout diet

Table 2. Growth parameters of trout fed diets with various supplemental enzymes (mean±SE)

	Control	*Commercial enzyme*	*Cellulase*	*Pectinase*	*Xylanase*
Initial weight (g)	3.93±0.07	3.94±0.08	4.10±0.22	3.75±0.13	3.93±0.02
Final weight (g)	15.45±0.63	15.97±0.32	14.81±0.99	13.78±0.44	15.21±1.11
WG (g)	11.52±0.55	12.03±0.32	10.71±0.80	10.03±0.48	11.29±1.14
SGR (% day^{-1})	1.52±0.02	1.56±0.03	1.42±0.04	1.45±0.06	1.50±0.09
FCR	1.36±0.20	1.22±0.15	1.39±0.13	1.74±0.19	1.28±0.35
PER	1.17±0.14	1.37±0.10	1.20±0.06	1.09±0.06	1.25±0.14

Weight gain (WG) = (final body weight. g - initial body weight. g)
Specific growth rate (SGR) = [(ln final body weight - ln initial body weight)/days] x 100
Feed conversion ratio (FCR) = (total feed intake. g)/ (final body weight. g - initial body weight. g)
Protein efficiency ratio (PER) = weight gain (g)/protein intake (g)

Final weight, weight gain (WG), specific growth rate (SGR) and protein efficiency ratio (PER) of rainbow trout fry fed with enzyme addition to diet containing 40% soybean meal showed no significant differences with fish fed control diet ($P>0.05$). The supplement of enzymes to diet had no significant effect on feed conversion ratio of rainbow trout. In addition, protein digestibility and lipid digestibility were not significantly affected by the enzyme supplementation (Table 4). There were no differences among the groups in the body composition (Table 3). The survival rate was 100% in all groups.

Table 3. Body composition of trout fed diets with various supplemental enzymes (mean±SE)

%	*Moisture*	*Crude protein*	*Crude lipid*	*Crude ash*
Control	7.95±0.99	14.46±0.24	4.18±0.13	2.45±0.35
Commercial enzyme	76.26±1.25	14.75±0.09	4.04±0.38	2.15±0.15
Cellulase	78.72±1.56	14.22±0.04	4.25±0.21	2.35±0.05
Pectinase	75.65±2.68	14.02±0.59	4.05±0.55	2.20±0.01
Xylanase	79.49±1.27	14.55±0.37	4.42±0.42	2.25±0.05

Table 4. Mean percent protein and lipid digestibility of trout fed diets with various supplemental enzymes (mean±SE)

%	*Crude protein*	*Crude lipid*
Control	83.97±0.51	91.56±0.48
Commercial enzyme	84.60±0.29	90.72±1.75
Cellulase	83.52±0.32	90.53±1.27
Pectinase	83.17±0.33	91.52±0.78
Xylanase	84.12±0.40	93.20±2.48

Discussion

In this study, the addition of three single enzyme preparations (pectinase, xylanase, cellulase) or a mixture of multi-component enzymes (pectinase, xylanase, cellulase, β glucanase, ɑ-amylase) to a soybean meal based diet had no significant effects on growth parameters and nutrient digestibility in trout fry. They appeared to be unable to degrade the high levels of NSPs in the soybean based feeds, and growth parameters were not affected by enzyme addition. These results are in agreement with results reported regarding trout fingerlings (Ogunkoya et al., 2006; Dalsgaard et al., 2012). The reported improvements in nutrient digestibility by enzyme supplementation of the diets containing 34.4% soybean meals did not improve SGR and FCR (Dalsgaard et al., 2012).

Performance has been significantly improved by enzyme supplementation in catfish (Yildirim and Turan (a) 2010; Lin et al., 2007) and tilapias (Yildirim and Turan (b), 2010).

Water temperature could regulate the activation of enzymes in fish diets. High water temperatures may account for the increased high efficiency of exogenous feed enzymes in rainbow trout and other fish (Vandenberg et al., 2011; Ghomi et al., 2012; Yıldırım and Turan 2010, Lin et al., 2007; Jackson et al., 1996).

In the current study, no significant differences were detected in whole body moisture, lipid, and ash among the dietary treatments ($p>0.05$). Similarly, no significant differences were detected in whole body moisture, protein, lipid and ash in tilapia fed diets with exogenous enzyme (Ng and Chong, 2002; Lin et al., 2007). There were insignificant changes in body crude protein, lipid and ash of rainbow trout due to dietary enzyme supplementation (Ogunkoya et al., 2006).

This study evaluated the effect of pectinase and cellulase on growth and the apparent digestibility of nutrients in a 40% soybean meal based diet. We found that pectinase, xylanase, cellulase, and commercial enzyme complex supplementations to trout fry diet containing 40% soybean meal at 11^{o}C do not have a significant effect on the growth parameters, feed conversion ratio, body composition, and nutrition digestibility values at the dose evaluated in this trial. Further studies on the effect of supplemented enzymes, the optimal temperatures, and the rate of soybean meal in combination with supplemented enzymes, are needed.

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