Effect of dietary phytase supplementation on Rainbow trout *Oncorhynchus mykiss (Walbaum)* productivity and nutritional properties of feed

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Abstract

Effects of dietary supplementation of phytase "Ronozyme P5000 (CT)"-DSM in concentration of 1000 FYT/kg in salmonid aquaculture feeds has been investigated in relation to production performance of rainbow trout Oncorhynchus mykiss (Walbaum), nutritional properties of feed, Ca and P content in bone tissue, feces and blood.

Obtained results demonstrated a strong beneficial effect of used enzyme on analyzed morphometric parameters in analyzed fish. Phytase supplemented feeds for rainbow trout have been shown to have statistically significant effects on: biochemical parameters in blood serum and whole blood, mineral content of bones and nutritional properties of absorbed nutrients.

Keywords: rainbow trout, phytase, phosphorus, weight gain, blood

Introduction

Adequate environmental conditions and good quality feedstuffs are key factors for achieving efficient and profitable aquaculture production. Modern nutritional formulations in intensive fish production systems are based on the application of different feed supplements for maximizing productivity.

Phosphorus (P) is an essential trace element for maintaining normal physiological- nutritional process in fish body, and for that reason the sources of phosphorus supplementation, organic vs. inorganic, as well as its increased availability from less available forms are of paramount importance [20,34]. The most essential functions of P in fish body are: constituent of phospholipids, takes part in glycide metabolism, component of DNA and RNA, bone component in the form of hydroxypatite (about 80%) etc. [4,12].

A great number of aquaculture feeds of vegetable origin contains significant quantities of P, however, about 60-80% of vegetable P is either unavailable or in the form of less available phytate. As the result of poor P digestibility (4-6%), dietary supplementation of inorganic forms of phosphorus in fish feed has become a routine practice. Dietary P supplementation in salmonid aquaculture nutrition has been shown to have many disadvantages, the main of which are excretion of substantial quantities of unabsorbed phosphorus in the form of primary and secondary phosphates from the fish digestive tract, thus contributing to eutrophication and degradation of qualitative-quantitative ambient parameters [17,37,23] and consequently, impacting cost-effectiveness of trout production and increasing price of fish meat.

Having in mind all these facts, modern nutritional practices involve dietary supplementation of phytase enzymes in trout rations for improving phosphorus availability in the fish digestive tract by 60-80% [14]. Phytase addition in fish feed positively affects other minerals, amino acids and digestive ferments, increases nitrogen retention for several times and reduces P excretion in the environment [7, 38].

Numerous positive effects have been obtained with phytase inclusion in the diets for monogastric animals. On the other hand, however, literature data on the effects of dietary phytase supplementation in fish nutrition are scarce and insufficient. The objective of this paper is to examine phytase application in trout nutrition, and its effects on increased phosphorus availability, reduced excretion in water and improved production and performance efficiency.

Materials and Methods

Conducted 40-day trial involved 300 trout divided in two ponds, with volume of 0,48 m^3 each, having equal incoming water supply of 24 l/min, that is, water flow of 72 flow recycles during 24 hours.

Broodstock of 1+ year of age rainbow trout was used in trial, as follows, initial mean body weight 77,49 g, average body length 18,90 cm, in total 300 trout assigned in two groups of 150 fish per pond. Stocking density was 24,22 kg/ m^3 with a density index of 0.128 , flow index 1.54 and body condition coefficient 0.011.

Experimental groups (C-control and T-trial) received complete feed for grower trout of equal composition, whereas trial diets were supplemented with phytase in concentration of 1000 FYT/kg. Raw material and chemical composition of used feeds is shown in Table 1. Phytase additive “Ronozyme P5000 (CT)” manufactured by company DSM, containing 5000 FYT/g feed was used.

Table 1. Raw material and chemical composition of used feed (%).

No.	Component ,%	Control group	Trial group
1	Corn	**11.25**	**11.25**
2	Fishmeal	**48**	**48**
3	Soybean meal	**19**	**19**
4	Sunflower meal 33%	**3**	**3**
5	Limestone	**1.9**	**1.9**
6	Mono-Ca-phosphate	**0.5**	**0.5**
7	Iodized salt	**0.3**	**0.3**
8	Premix	**1**	**1**
9	Soya oil	**15**	**15**
10	Chromium trioxide (Cr_2O_3)	**0.05**	**0.05**
11	TOTAL:	**100**	**100**
Chemical composition of feed, (%)			
12	Water	**9.35**	**9.31**
13	Ash	**11.02**	**11.01**
14	Protein	**40.39**	**40.37**
15	Fiber	**2.67**	**2.65**
16	Dry matter (DM)	**90.65**	**90.69**
17	Calcium, Ca	**2.99**	**2.97**
18	Total phosphorus, P	**1.58**	**1.58**
19	Available phosphorus, P	**0.97**	**0.97**
20	Metabolizable energy ME MJ/kg	**15.10**	**15.10**

Quantity and number of daily rations were determined based on pre-defined nutritional tables, adjusted to water temperature and fish body mass throughout the trial. Used feed and physico-chemical parameters of water were sampled and analyzed at the beginning of trial with standard examination methods [2,*27,39*]**.**

Feed conversion ratio of feed was determined in ten-day intervals based on weight gain measurements (representative sample of 30 fish per group) and feed consumption. Feces collected from the ponds bottom was analyzed for total Ca and P content. Chromium trioxide (Cr_2O_3) was dosed in the amount of 0.05% as an indicator method for determination of feed and nutrient digestibility. Chromium content was established with atomic absorption spectrophotometry [28]**.** Nutrient utilization was determined with indirect method of digestibility determination [24,29].

ADCs (%) = 100 x [1 - (% marker in feed / % marker in feces) x (% nutrient in feces / % nutrient in feed)]

At the end of trial, 10 fish from each group were sacrificed and subjected to biochemical blood analyses (glucose-GLU, total protein-TP, aspartate amino-transferase -AST, alanine amino-transferase-ALT, lactate dehydrogenase-LDH, Ca, P, erythrocyte, leukocyte and hemoglobin concentrations), ash content and Ca and P content in bone tissue. Ca content in feed, bones and feces was determined with flame photometric method, and in blood with atomic absorption. Phosphorus (P) was determined by spectrophotometric method [2,33], i.e. by colorimetric analyzing of blood [40]. Hematological examinations were carried out with standard methods (erythrocyte and leukocyte count- Thoma-Zeiss counting chamber; hemoglobin estimation by Sahli’s method). Trout blood serum was analyzed on automatic analyzer Hitachi-705 (Boehringer Mannhein Deutschland-tests). Blood samples were obtained by caudal vessel puncture, and all blood analyses were conducted on random samples taken from 10 fish per treatment at the end of trial.

Fish health status and mortality were recorded on daily basis, for each group separately. Condition coefficient and total growth coefficient were calculated according to Fulton formula [35].

$$P.I. = \frac{\text{Body mass (kg) x \% survived fish}}{\text{Rearing period (days) x feed conversion}} \text{ x } 100$$

$$Fk = \frac{g\overline{X} \cdot 100}{(L\overline{X})^3} \qquad KUR = \frac{g\overline{X}}{L\overline{X}}$$

Fk – condition coefficient, KUR- total growth coefficient, PI-production coefficient

g $\overline{X}$ –mean body mass value, gL $\overline{X}$ –mean body length value, cm

Tests for statistical significance were performed to determine differences between mean values with the application of standard mathematical statistic procedures, that is, analysis of variance, F-tests and Lsd tests.

Results and Discussion

Trial results related to physico-chemical quality of fish pond water are given in Table 2. Obtained results indicate that essential water quality parameters were within optimum limits for salmonid waters [1,25] and meeting all criteria necessary for successful trout fattening.

Table 2. Physico-chemical analyses of water

ra- eter	Water flow	Water temperature	pH	CO_2	$^o dH$	O_2	BOD_5	$KMnO_4$	NO_2	NO_3	NH_3	PO_4
nit	l/sec	C^o	pH	mg/l	degree	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
alue	33	13.5	7.45	1.30	11.23	11.90	0.41	2.65	0.00	0.04	0.0011	0.00

Data from Table 1 are indicative of raw material and chemical composition of used trout feed. Based on shown chemical composition of feed, it may be concluded that complete feeds were of balanced chemical composition and contained all necessary ingredients for meeting nutritional requirements of rainbow trout [29,34].

Analysis of obtained length and weight growth rates is shown in Table 3. Length and weight growth morphometric properties ranged within standards for trout of 1^+ year of age. At the end of experiment, mean values of analyzed parameters in T-group were higher than values established in C-group, as follows: 4.27% (body mass); 21.42% (weight gain); 3.06% (overall body length); 20.90% (overall body length growth). Significance test of analyzed body properties indicated significant differences (p<0.05) between experimental groups, for all four tested morphometric parameters. Obtained results are in accord with the results of other authors, showing that supplementary phytase in fish diets positively affects growth and weight gain [22,36,41].

Table 3. Review of morphometric parameters (40 days)

Period days	Parameters	EXPERIMENTAL GROUPS	
		Control C	**Trial T**
Average body mass (g)			
	n = 30	**94.08**	**98.10**
40	Difference (%)		**+4.27**
statistically different			*p<0,05
Average total body length (cm)			
	n = 30	**20.27**	**20.89**
40	Difference (%)		**+3.06**
statistically different			*p<0,05
Average weight gain (g)			
	n = 30	**5.32**	**6.46**
	Difference (%)		**+21.42**
1 – 40	statistically different		*p<0,05
Average body length gain (cm)			
1 – 40	**n = 30**	**0.51**	**0.79**
	Difference (%)		**+20.90**

	statistically different	*p<0,05

Based on the obtained field results (Table 4) it can be concluded that phytase supplemented feed improved feed intake and feed conversion ratio in T-group. Found differences in feed conversion were statistically significant ((p<0.05), as opposed to numerical values for feed intake (p>0.05).

Mortality rate in T-group was lower by 1.33%. T-group has also showed better performance results at the end of the trial and obtained higher numerical values for condition coefficient (CC) and total growth coefficient (TGC), though achieved differences were not statistically significant (p>0.05).

Table 4. Production indexes at the end of experiment, 40 days

Indexes	**Experimental groups**	
	C groups	**T- groups**
Mortality,%	**4**	**2.67**
Difference, %		**-33.25**
statistically different		p>0,05
CC - Condition coefficient	**0.54**	**0.63**
Difference, %		**16.67**
statistically different		p>0,05
TGC- Total growth coefficient	**3.60**	**3.62**
Difference, %		**0.56**
Feed consumption (kg)	**13.547**	**14.322**
Difference, %		**5.72**
Total for all days by 1 fish, g	**43.759**	**44.852**
Daily by 1 fish, g	**1.09**	**1.12**
statistically different		p>0,05
Feed conversion ratio	**2.63**	**2.17**
Difference, %		**-17.49**
statistically different		*p<0,05

Considering data given in Table 5, it can be concluded that phytase supplementation in T-group improved values of P (by 16.79%), inorganic P (by 11.22%) and Ca concentrations (by 7.90%) in blood serum of examined trout.

Table 5. Biochemical parameters of blood serum and whole blood (40 days)

Parameters	**Experimental groups**		
	C groups, n =10	**T- groups, n =10**	**statistically different**
GLU, mmol/l^{-1}	**5.49**	**5.52**	
TP, g/l^{-1}	**35.08**	**35.12**	
AST, U/l^{-1}	**3.23**	**3.18**	
ALT, U/l	**0.17**	**0.17**	
LDH, U/l	**17.60**	**17.40**	
Ca^{2+}, mmol/l^{-1}	**1.90**	**2.05**	***p<0,05**
P, mmol/l^{-1}	**2.86**	**3.34**	****p<0,01**
P, mmol/l^{-1} (inorganic)	**0.98**	**1.09**	****p<0,01**
Blood elements			
Er, x 10^{12}	**1.26**	**1.29**	
Leu x 10^9/l	**20.09**	**20.23**	
Hb, g/l^{-1}	**47.20**	**54.61**	***p<0,05**

Obtained mean differences between analyzed groups of rainbow trout were statistically significant ($p<0.05$) for Ca content and highly significant ($p<0.01$) for total and inorganic phosphorus content. Achieved values for biochemical parameters are in compliance with normal physiological values for rainbow trout [*5,11,26*].

P concentration in trout complete feed is in direct correlation with total and inorganic phosphorus content in fish blood serum and bones [14,15]. Inadequate digestibility of dietary P from feeds for monogastric and aquaculture animals is automatically reflected in blood parameters. To that end, Ca and P contents in blood serum and bones are reliable indicator of availability of these two minerals in fish feeds [31].

Numerous authors [17,42] reported that dietary supplemented phytase beneficially affects Ca and P levels in fish blood serum. Upon review of literature data and their comparison with the results of present study, it may be suggested that through dietary supplementation of phytase in 1000 FYT/kg complete feed, Ca and P concentrations in fish blood serum are significantly improved.

Other biochemical parameters in blood serum of analyzed trout (GLU, TP, AST,ALT, LDH) are close to values obtained by other authors [*3*]**,** with non-statistically significant numerical differences.

Trial results related to whole blood analyses (erythrocyte, leukocyte and hemoglobin) are given in Table 5. Statistically significant difference has been determined only for average Hb concentrations ($p<0.05$), as oppose to erythrocyte and leukocyte concentrations ($p>0.05$). According to [*11,30*] increase of transferrin iron, as an ingredient of respiratory pigment Hb effects, to certain extent, increase of P in animals and fish. Phytase supplemented feeds positively affects fish blood picture, in particular, content of hemoglobin and erythrocyte.

Table 6. Effect of phytase on phosphorus digestibility

Treatment	**Experimental groups**		
	C groups	**T- groups**	**statistically different**
Content of Ca in feces, (%)	**1.85**	**1.76**	p>0,05
Difference, %		**5.12**	
Content of P in feces, (%)	**0.94**	**0.68**	**p<0,01
Difference, %		**38.24**	
Digestibility of P (%)	**40.50**	**56.96**	**p<0,01
Difference, %		**40.65**	

Data shown in table 6 indicate that P content in feces of T-group was significant ($p<0.01$) in relation to C-group, whereas differences in Ca content were not so significant ($p>0.05$). Additionally, obtained results indicate that phytase supplementation significantly ($p<0.01$) improved the digestibility of P in T-group.

Many authors [*7,18*] suggest that substantial amount of feces is excreted during trout fattening phase in the environment, i.e. 44.6 tons/annually/100 tons of feces, containing about 1.1 tons – 1.8 tons of phosphorus (P). Through dietary supplementation with phytase, P digestibility is significantly improved P (56-80%), and hence P excretion reduced by 22.30-35.60% [17,37,23].

Table 7. Content of ash, Ca and P in trout bones

Treatment	**Experimental groups**		
	C groups n = 10	**T- groups n = 10**	**statistically different**
Dry bones mass, g	**13.20**	**14.92**	*p<0,05
Difference, %		**10.62**	
Ash content, %	**56.21**	**59.13**	p>0,05
Difference, %		**5.20**	
Ca content, %	**26.77**	**27.84**	p>0,05
Difference, %		**4.00**	
P content , %	**9.29**	**10.02**	*p<0,05
Difference, %		**7.86**	
Ca : P ratio	**2.88 : 1**	**2.77 : 1**	

Content of ash, Ca and P in fish bones is an important indicator of P availability in feed, and is also used for calculating bioavailability of P in some feedstuffs. Data given in Table 7 show that T-group had higher content of ash, Ca and P in bones than C-group, namely, ash content by 5.20%; Ca by 4.00%; P by 7.86%; dry bone mass by 14.92%, respectively. Furthermore, the Ca : P ratio in bones was higher in T-group (2.88), than in C-group (2.77).

Mean value differences between analyzed groups were not statistically significant ($p>0.05$) for ash and Ca content, while statistical significance was found for P content in fish bones and dry bone mass. Similar beneficial effects of phytase supplementation are reported by other authors, too [20,21,43].

At the end, it can be concluded that dietary phytase supplementation in trout feeds improved P availability from phytate, as suggested by the results obtained for ash concentration and Ca and P content in bones.

Conclusion

Phytase supplemented feeds for rainbow trout have been shown to have positive effects on: performance (weight gain, feed conversion, mortality, condition coefficient (CC) and total growth coefficient (TGC), biochemical parameters in blood serum and blood, P content in feces and bones, and enhanced P digestibility in treated fish. Determined numerical values for majority of examined parameters were statistically significant ($p<0.05$ i $p<0.01$).

Through dietary supplementation with phytase a strong positive effect is demonstrated on: improved phosphorus availability and digestibility, better performance, reduced environmental pollution, enhanced product quality, lower costs of aquaculture production, decreased share of inorganic ingredients in complete feeds.

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