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# PERSPECTIVES OF PHYTASE APPLICATION IN FEED MIXTURES FOR REDUCTION OF PHOSPHORUS AMOUNTS IN FISH FARM EFFLUENTS

# PERSPEKTIVE PRIMJENE FITAZE U KRMNIM SMJESAMA ZA SMANJENJE KOLIČINE FOSFORA U IZLAZNOJ VODI RIBNJAKA

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## SUMMARY

Six-month-old tilapia (Oreochromis niloticus) were fed with experimental diet containing 0.01 $g.kg^{-1}$ enzime phytase (P2 mixture) and compared with fish fed with the same diet however with reduced inorganic phosphorus content (P1 - negative control) and untreated feed mixture (K - positive control). The outflow water from variants with phytase supplementation (P1 and P2) showed lower loading with phosphorus by 33.2 and 36.7% respectively in comparison with control feed mixture, namely in mean and absolute values. In recounting per 1 kg of weight gain, the phosphorus loading was lower by 27.7 and 25.5% in variants P1 and P2 respectively. Fish retained by 6.1 and 8.8% phosphorus more in groups P1 and P2 respectively in comparison with positive control.

## INTRODUCTION

Recent technologies in fish farming are characterised by two apparently antagonistic trends - the intensification of production on one side, and the effort for minimization of adverse impacts of farming upon the water ecosystem on the other side. Continuously increasing perspective importance of fish production in closed and through-flowing systems provokes an urgent need for the application of farming technologies appropriate and usable under these conditions. The solution of problems connected with fish feeding and nutrition is an important prerequisite for the minimization of energy demand during the process of water treatment and for reduction of effluents loading with nutrients and organic matter.

Solid and dissolved fish metabolites, and non-digested and unconsumed feed particles are the main source of pollution originating in fish farming. Their discharging into surface waters results in increased organic pollution and euthropication processes. Following undesirable phenomena are characteristic for water ecosystems affected by fish farm effluents: the decrease of dissolved oxygen concentration, rapid development of algae and macrophytes, sedimentation of suspended solids, changes of hygienic and organoleptic conditions etc. The application of traditional procedures of water treatment in fish farming is extremely difficult

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due to large amounts of water discharged with low concentration of pollution in comparison with e.g. municipal effluents.

Phosphorus is generally considered to be an important macronutrient in fish diets since its availability form water and some feed sources (e.g. plant protein) is limited (Phillips et al., 1958). On the other hand, phosphorus is considered to be a nutrient deciding about the intensity of eutrophication processes in surface water bodies (Ryden et al. 1973) where it is discharged as the waste component of fish farm effluents (e.g. Phillips et al., 1993).

At present, some possibilites how to solve this problem are available - however extremely high requests for energy and expenses are their disadvantage which should result in such a high product (fish) price which cannot be accepted by the market. Tendencies to ecological approaches in animale husbandry in countries with high fish production and comsumption resulted in the development and manufacture of such feed mixtures which composition and processing guarantee minimum mechanical and metabolic losses and low costs for energy. There was confirmed that despite high price of these feeds, the economical result of their application in farming process is the same or even more convenient in comparison with the traditional feed mixtures. the reason is above all in lower feed consumption and reduced amount of feed waste and fish excreta due to more efficient feed conversion into fish flesh (lower conversion coefficient) resulting also in lower costs of the operation of culture system and recycling water treatment.

As already mentioned above, this objective can be achieved particularly by supplementation of feed mixtures with high quality (and that is why also costly) feed components. Our approach was based on the application of phytase as the biological active substance in feeds with the aim to enhance the efficiency of digestive processes and consequently also the aplicability of traditional (or prospectively non-traditional cheapper local by-products) with contemporary reduction of pollution produced by intensive farming units.

Phosphorus retained in plant components of feed mixtures is available for fish just from 30% in average with no respect to stomach presence (e.g. salmonids) or absence (e.g. cyprinids). about 50-80% of phosphorus in vegetable components occurs in the form of calcium of magnesium salt of phytic acid which is a hexaphosphate ester of inositol. This organic phosphorus compound must be first hydrolyzed in the digestive tract by enzyme phytase to inositol and phosphoric acid, and only after that it can be absorbed and utilized by fish organism. The absorption is easier in acidic environment. (Tacon 1990).

So, the enzyme phytase is able to unblock from organic compounds phytate bound phosporus which is not available for fish digestive processes. The aplicability of phytase for these purposes in monogastric farm animals nutrition was proved by Zobač et al. (1995) and Šimeček et al. (1995). Meyer-Burgdorf (1992) and Cain and Garling (1995) tested the application of phytase in rainbow trout (Oncorhynchus mykiss) nutrition.

The aim of the study was to evaluate the possibilities of phytase application for the enhancement of digestive processes efficiency and the reduction of phosporus loading in Nile tilapia (Oreochromis niloticus) culture. Tilapia was selected for these experiments since it is characteristic by extremely low pH value in its stomach, and acidic environment is an important prerequisite for optimal phytase funciton.

## MATERIAL AND METHODS

The experiments were performend with six-month-old tilapia in flowthrough rectangular oblong tanks of volume 1,420 $m^3$ and flow rate 0.3 $l.s^{-1}$. The tanks were areated with compressed air through ceramic diffusers in the way to reach at least 50% exygen saturation. The temperature of inflow water amounted to 21.8±0.7°C. The identical initial biomass was stocked into individual tanks. The evaluated production parameters included biomass increment, conversion coefficient and specific growth rate (SGR) in individual tanks.

The experimental feed mixtures were pelleted using 3 mm matrix and were given to fish in seven doses daily in the total weight of 3% of initial fish biomass. Three feed mixtures (Table 1) were compared: K - control feed mixture witl full inorganic phosphorus content (positive control), P1

- feed mixture without inorganic phosphorus supplement (negative control) and P2 - feed mixture without inorganic phosphorus supplement however with the addition of 0.1 g.kg-1 enzyme phytase (Natuphos, product of Gist-Brocades Co., Delft, NL). Feed composition is presented in table 2.

**Table 1. Experimental feeds formulation (in percentage)**
**Tablica 1. Sastavi pokusnih krmnih smjesa (%)**

| Component | Positive control Pozitivna kontrola | Negative control Negativna kontrola | Enzyme phytase Enzim fitaza |
|---|---|---|---|
| | K | P1 | P2 |
| Fish meal - Riblje brašno | 10.0 | 10.0 | 10.0 |
| Meat-bone meal - mesno-koštano brašno | 15.0 | 15.0 | 15.0 |
| Soybean meal - Sojina sačma | 25.0 | 25.0 | 25.0 |
| Wheat meal - Pšenično brašno | 44.0 | 44.0 | 44.0 |
| Alfalfa meal - Lucerna dehidrirana | 4.5 | 4.5 | 4.5 |
| Biovitamin K | 0.5 | 0.5 | 0.5 |
| Mineral supplement - Mineralni dodatak | 1.0 | - | - |
| Natuphos | - | - | 0.01 |
| Binder (g/12 kg) - Vezivo | 200.0 | 200.0 | 200.0 |

**Table 2. The composition of tested feed mixtures (percentage in dry matter)**
**Tablica 2. Hranjva vrijednost pokusnih krmnih smjesa (u % suhe tvari)**

| Feed - Hrana | Positive control Pozitivna kontrola | Negative control Negativna kontrola | Enzyme phytase Enzim fitaza |
|---|---|---|---|
| | K | P1 | P2 |
| Dry matter - Suha tvar | 89.93 | 90.04 | 89.76 |
| Crude protein - Sirove bjelančevine | 31.5 | 31.4 | 31.9 |
| Fat - Mast | 3.97 | 3.78 | 3.87 |
| Fibre - Vlaknina | 3.44 | 3.32 | 3.46 |
| Ash - Pepeo | 8.87 | 8.87 | 9.05 |
| Calcium - Kalcij | 2.26 | 2.23 | 2.24 |
| Phosphorus - Fosfor | 1.030 | 0.963 | 0.970 |

The experimental feeding lasted for 11 days. 24-hour pooled water samples of inflow and outflow water served for the analysed. Twelve 100 ml samples were collected regularly in two-hour intervals from individual tanks. Total phosphorus content was analysed in the laboratory. Water temperature, oxygen concentration and pH values were registered in situ using transportable measurement devices. The flow rate in individual tanks was registred in two-hour intervals as well. Phosphorus loading of discharged water ($g.day^{-1}$) was consequently calculated from the values of its concentrations in inflow and outflow water, and flow rates.

At the end of the experiment, the representative pattern of 5 fish from each tank was taken for analysis. Sacrificed fish were ground, homogenized and analysed for dry matter, crude protein, fat, ash, calcium and phosphorus contents.

Calculated values of water loading with phosphorus in individual tanks were tested for ANOVA analysis. The homogeneity of dispersion was confirmed by Bartlett's test, and Newmann-Keuls Multiple Range Test was utilised for further evaluation at the 0.05 level of significance.

## RESULTS

The groups of fish, which were supplied with reduced prosphorus (P1, P2), feed showed higher conversion coefficient and lower specific growth rate in comparison with the control group (Table 3).

**Table 3. Production results of tilapia culture during the experimental period**
**Tablica 3. Proizvodni rezultati tilapija u tijeku pokusnog razdoblja**

| | Positive control Pozitivna kontrola | Negative control Negativna kontrola | Enzyme phytase Enzim fitaza |
|---|---|---|---|
| | K | P1 | P2 |
| Duration - Trajanje (days - dana) | 11 | 11 | 11 |
| Initial stock - Početni broj | 860 | 860 | 860 |
| Mean weight - Prosječna težina ($g.ind^{-1}$) | 33 | 33 | 33 |
| Total weight - Ukupna težina (g) | 28540 | 28520 | 28480 |
| ($g.l^{-1}$) | 20 | 20 | 20 |
| Final Stock - Završni broj (ks) | 860 | 860 | 860 |
| Mean weight - Prosječna težina ($g.ind^{-1}$) | 42 | 41 | 41 |
| Total weight - Ukupna težina (g) | 36020 | 35420 | 34840 |
| ($g.l^{-1}$) | 25 | 25 | 25 |
| Survival rate - Stupanj preživljavanja | 100 | 100 | 100 |
| Weight gain - Prirast težine (g) | 7480 | 6900 | 6360 |
| Individual gain - Pojedinačni prirast (g) | 9 | 8 | 7 |
| Feed consumption - Uzimanje hrane (g) | 9460 | 9460 | 9460 |
| Conversion coefficient - Konverzija (kg/kg) | 1.26 | 1.37 | 1.49 |
| Weight gain - Prirast težine (%) | 26 | 24 | 22 |
| SGR ($\%.d^{-1}$) - Specifični stupanj rasta | 2.12 | 1.97 | 1.83 |

The outflow water from variants with phytase supplementation (P1 and P2) showed lower loading with phosphorus by 33.2 and 36.7% respectively in comparison with control feed mixture, namely in mean and absolute values (Table 4). In recounting per 1 kg of weight gain, the phosphorus loading was lower by 27.7 and 25.5% in variants P1 and P2 respectively. Fish retained by 6.1 and 8.8% phosphorus more in groups P1 and P2 respectively in comparison with positive control.

Statistical evaluation of effluents loading with phosphorus in individual days of the experiment did not prove statistically significant difference ($P>0.05$) between individual variants.

## DISCUSSION

Phosphorus is considered as one of the most important inorganic elements in fish nutrition. It is the main component of bone tissues and metabolic processes including transformation of energy, cell membranes permeability and genetic formations. Its content in fish depends above all on taxonomic position and related number of intermuscular bones and scales development (Rothschein et al. 1983).

**Table 4. Daily values (24-hour pooled samples) of total phosphorus concentration (mg.l$^{-1}$) during the experimental period**

**Tablica 4. Dnevne vrijednosti (24-sata uzoraka) ukupne koncentracije fosfora (mg.l$^{-1}$) tijekom pokusnog razdoblja**

| Days - Dani | P (mg.l$^{-1}$) | | | | P - Pinilow (g.day$^{-1}$) | | |
|---|---|---|---|---|---|---|---|
| | Inflow | Positive control | Negative control | Enzyme phytase | Positive control | Negative control | Enzyme phytase |
| | | K | P1 | P2 | K | P1 | P2 |
| 1 | 0.29 | 0.37 | 0.30 | 0.30 | 2.06 | 0.26 | 0.25 |
| 1 | 0.28 | 0.31 | 0.33 | 0.31 | 0.77 | 1.29 | 0.76 |
| 3 | 0.23 | 0.33 | 0.34 | 0.32 | 2.69 | 2.96 | 2.36 |
| 4 | 0.34 | 0.56 | 0.46 | 0.47 | 5.97 | 3.27 | 3.49 |
| 5 | 0.58 | 0.77 | 0.79 | 0.65 | 5.22 | 5.73 | 1.89 |
| 6 | 0.88 | 1.00 | 0.89 | 1.06 | 3.39 | 0.28 | 5.01 |
| 7 | 0.80 | 0.90 | 0.80 | 0.83 | 2.88 | 0.00 | 0.83 |
| 8 | 0.23 | 0.49 | 0.42 | 0.41 | 6.60 | 4.78 | 4.46 |
| 9 | 0.25 | 0.34 | 0.37 | 0.29 | 1.77 | 2.37 | 0.77 |
| Mean | | | | | 3.48 | 2.20 | 2.33 |
| median | | | | | 2.88 | 1.89 | 2.37 |
| S.D. | | | | | 2.00 | 1.75 | 2.06 |

The amount of phosphorus dischargted form fish farming units is in a close correlation with its content in feeds (Ketola, Richmond 1994, Summerfelt et al. 1994). Maximum demand in the diet of rainbow trout, the farming of which is considered to be the most important source of “fischeries” pollution in countries of temperate climate, amounts to 0.8% (Philips, Beveridge 1986). The demand for carp and channel catfish amounts to 0.6-0.7% (Ogino, Takeda 1976) and 0.45% respectively. However, these data concern the available (utilizable) phosphorus, which percentual proportion on total phosphorus content differs according the origin of particular feed component and fish species (New, 1987). So, there is obvious that the interventions into feed mixtures composition (Alster, 1988) and their optimization are besides treamtnet facilities, feeding regime and water recirculation an essential processes deciding about the discharged water quality (Cripps, Kelly 1994). The essential role of feeds for loading of surface waters with nutrients was proved also by data of Philips and Beveridge (1986), according whom 85% of phosphorus and 80% of nitrogen from feeds enter the environment as pollutant.

Two approaches are aplicable for reduction of loading with phosphorus:

- enhanced utilization of phosphorus from feed.

- reduced ingestion of phosphorus by reduction of its content in feed (Dekker, 1986).

Dietary phosphorus requirements for Nile tilapia (Oreochromis niloticus) amount to 0.46% (Haylor et al. 1988). This could probably be the reason for fluctuating of results of analyses in our experiments where fish were supplied with more than twice higher amount of phosphorus in feeds namely also in negative control (0.96%). However, phosphorus requirements are very probably affected by qualitative composition of the diet because according to the data of Watanabe et al. (1980), the demand for phosphorus amounted to 0.9% in juvenile Nile tilapia as well as according to Viola et al. (1986) for hybrids Oreochromis aureus x 0. niloticus (0.7-1.0%).

**Table 5. Phosphorus balance in tilapia culture using feeds with different phosphorus content and enzyme phytase**

**Tablica 5. Balans fosfora u uzgoju tilapije upotrebom hrane s različitim sadržajem fosfora i enzima fitaze**

| | Positive control K | Negative control P1 | Enzyme phytase P2 |
|---|---|---|---|
| P ingested - Unesen (gl | 97.44 | 91.10 | 91.76 |
| P non - retained - Ne zadržan (g) | 31.34 | 20.94 | 19.83 |
| (g/kg feed) - g/kg hrane | 3.31 | 2.21 | 2.10 |
| (g/kg gain) - g/kg prirasta | 4.19 | 3.03 | 3.12 |
| P retained - Zadržan (g) | 66.10 | 70.16 | 71.93 |

On the other hand, the feed we have used was not extremely rich in phosphorus in comparison with the most of commercial feed mixtures which usually contain about 1% of phosphorus in dry matter (DOP 1.0 ECOlife 0.9-1.1% - Alsted 1991. Alter Mole 0.9-1.3%), and some of them even up to 2.3% (Philips and Beveridge 1986).

The raito between calcium and phosphorus is very important for the utilization of both elements by fish organism (New 1987). Sakamoto and Yone (1973) present the optimal Ca/P ratio 1:2, whilst it was opposite (2.05-2.32:1) in our feed mixtures.

The idea of phytase supplementation into fish diets simultaneously with the replacement of organic phosphorus source by its inorganic form was verified first time in rainbow trout farming by Meyer-Burgdorf (1992). The application of phytase in diets based on soya and fish meal resulted in the enhancement of phosphorus digestibility from 39 to 79%. Similar success with the applicatoin of phytase in the rainbow trout diet reported Cain and Garling (1995). Using feeds with phytase addition, they reached both identical or even better conversion rate, and 65-88% reduction of phosphorus amount in effluents.

In our experiments with tilapia, the reductin of phosphorus concentration in the diet resulted in higher conversion coefficient and lower specific growth rate. However on the other hand, expected reduction appeared in effluent water loading with phosphorus in both average and absolute values in variants with reduced content of dietary phosphorus. As concerns the mixture with phytase supplementation, the difference in comparison with positive control was slightly higher (_36.7%) than between negative and positive contol (-33.2%). However, due to a big dispersion of registered values, even so marked differences were not statistically significant (P>0.05).

The data about the balance of dietary phosphorus (in percentage) as presented by various authors in comparison with our results are presented in Table 6.

**Table 6. Literature data about the percentage of excreted and waste phosphorus**

**Tablica 6. Podaci iz literature o (%) izlučenog i neiskorištenog fosfora**

| Author | Renained in fish Zadržan u ribi | Dissolved (water) Otopljen u vodi | Deposited (solids) Deponiran u čvrstom obliku | Fish - Riba |
|---|---|---|---|---|
| Wallin, Hakanson, 1991 | 15-30 | 16-26 | 51-59 | Marine - Morska |
| Ketola, Richmond, 1994 | 32-76 | 19-64 | 4-32 | Rainbow - Pastrva |
| Adámek (unpubl.) with phytase - s fitazom | 6-37 | 23-25 | 33-58 | Tilapija |
| Without phytase - Bez fitaze | 12-47 | 22-32 | 47-55 | Tilapija |

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## SAŽETAK

Šestmjesečne tilapie bile su hranjene pokusnim obrocima koji su sadržavali 0.01 $g*kg^{-1}$ enzima fitaze (P2 smjesa) i uspoređena s ribom hranjenom s istom krmnom smjesom, ali sa smanjenim sadržajem anorganskog fosfora (P1 - negativna kontrola) te netretiranim krmnim smjesama (K - pozitivna kontrola). Izlazna voda od varijanata s dodatkom fitaze (P1 i P2) pokazale su niže opterećenje fosforom za 33.2 i 36.7% prema pojedinoj supini u usporedbi s kontrolnom krmnom smjesom u prosječnim i apsolutnim vrijednostima. U preračunu po 1 kg prirasta, opterećenje fosforom bilo je manje za 27.7 i 25.5% u varijantama P1 i P2. Ribe su zadržavale 6.1 i 8.8% fosfora više u P1 i P2 skupinama u usporedbi s pozitivnom kontrolom.