Effect of phytase addition and citric acid on the production parameters of feed for Common Carp (Cyprinus carpio L.)

Iveta Zugarkova, Jan Mares, Ondrej Maly, Jan Grmela
Department of Zoology, Fisheries, Hydrobiology and Apiculture
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC
xzugark1@mendelu.cz

Abstract: The aim of the experiment was to evaluate the effect of addition phytase enzyme together with citric acid to Feed conversion ratio (FCR) and a Specific growth rate (SGR) in feed for common carp. The phytase addition increases the digestibility of phytate phosphorus from plant components in feed mixtures. The addition of citric acid (CA) optimize a pH level in guts for the phytase enzyme. For the experiment was prepared five types of feed. The basic component was a commercial mixture for carp (KP1) with 10% share of soybean meal. This mixture was fed to the control group. Next 4 experimental mixtures were prepared by addition of 500 FTU, 1000 FTU, 500 FTU + 3% of CA and 1000 FTU + 3% of CA. The test was made in 10 tanks (5 groups with one replication) with 15 specimens in each group. The test lasted 72 days. Results show statistically insignificant differences of production parameters in groups 500 FTU and 1000 FTU, but differences of FCR and SGR were statistically significant in groups 500 FTU + CA and 1000 FTU + CA. FCR of these groups decreased by 20% over the control group (one–way ANOVA: F=24, df=4, P=0.002). SGR increased by 11% over the control group (one–way ANOVA: F=17.93, d.f.=4, P=0.004).

Key Word: common carp, phosphorus, fish nutrition, Feed conversion ratio

INTRODUCTION
More frequent use of plant components in fish nutrition leads to higher content of phosphorus in feed. The phosphorus in plants is deposited in the form of phytic acid (Lundová 2014). Phytic acid in plants is poorly digestible for monogastric animals and fish. It can also limit some minerals in the body bounded to phosphoric acid residues (Kalač and Míka 1997). Singh (2008) reports the highest content of phytic acid in seeds, especially in packs and bran. The most available phytic acid is stored in the seed germ.

According to Simons et al. (1990), the phytase can be found in rumens micro flora of ruminants, plants and external microorganisms. The microflora of monogastric animals and fish is poor and a digestibility of the phytic acid is low. To increase the digestibility in monogastric animals and fish are used exogenous industrial phytases. This phytases are produced by genetically modified microorganisms and extracted. The main benefit of increased phosphorus digestibility is lower phosphorus in excrements and consequent lower load on the aquatic environment (Brož 2002).

The use of phytase in feeds is complicated by its sensitivity to pH level and ambient temperature. Shah et al. (2015) states the highest phytase activity in the range of pH from 2.5 to 5.5. Fish without a stomach has neutral pH in guts and the phytase activity cannot be optimal. For the pH level optimization are added organic acids to the feed mixtures for example a citric acid. The acids decrease the pH level in the digestive tract and they slows the secretion. According to Cao et al. (2007) is the maximum tolerated temperature for phytases about 40–60 °C. During the feed processing (granulation or pelleting) is the temperature up to 100 °C, at this point phytase proteins denature and the digestibility of the phosphorus cannot be influenced.

The aim of the experiment was to evaluate the effect of phytase and citric acid in the standard feed mixture on the production parameters.
MATERIAL AND METHODS

Characteristic of experimental feeds

Basic mixture

For the basic mixture was used KP1 (Výroba krmiv spol. s.r.o., Stříbrné Hory, Czech Republic). The main component of the feed is crushed wheat, corn, wheat flour, wheat bran, extracted soybean meal, rapeseed expector and soybean oil. The mixture is enriched with a mineral–vitamin premix. Due to the low crude protein content (17.89%) was the experimental mixture enriched by addition of 10% soybean extract.

Phytase enzyme

For the experiment was selected Phyzyme XP 10.000 TPT (Danisco Animal Nutrition, Denmark) suitable for use on plant–based feeds. Phytase in this preparation is produced by bacteria E.coli. It contains 10.000 FTU per gram in bulk or liquid form. The preparation has increased resistance to proteolytic enzymes and higher relative activity over a wide range of pH. The thermo stability of the enzyme is increased by TPT technology (Thermo Protective Technology) up to 95 °C and thus is suitable for granulation.

Experimental feed

The basic mixture was made by mixing the KP1 with soybean meal and binder (Pellet-Dur, Röthel GmbH, Gudensberg, Germany). To the basic mixture were added 500 FTU (FyThase Unit) or 1000 FTU phytase enzymes. Dry feed was homogenized for 2 hours using a kitchen robot (Kitchen Aid Heavy Duty 5kpm5, United States). The mixture was moistened after homogenization by 40 °C water and processed to dough. The citric acid for acidified feed was dissolved in the water. The dough was adjusted to pellets by meat grinder (Kitchen Aid Heavy Duty 5kpm5, United States) and dried at 40 °C in a hot air sterilizer (STERICELL 11, BTM Medical Technology s.r.o.). After cooling, the granules were stored in dry plastic boxes.

Table 1 Composition of compound feeds

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>F500</th>
<th>F1000</th>
<th>F500C3</th>
<th>F1000C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>KP1</td>
<td>90%</td>
<td>90%</td>
<td>90%</td>
<td>87%</td>
<td>87%</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>10%</td>
<td>10%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>binder</td>
<td>0.5%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>phytase</td>
<td>500 FTU</td>
<td>1 000 FTU</td>
<td>500 FTU</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>Citric acid</td>
<td>3%</td>
<td>3%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Test characteristics

The experiment was made in ten glass tanks (160 l) in a recirculating system (RAS) at the Department of Fisheries and Hydrobiology MENDELU in Brno (Czech Republic). The RAS included a mechanical and biological filter, a UV lamp and the water was vigorously aerated. The test was conducted for 72 days. To each tank was stocked 15 specimens of a common carp (Cyprinus carpio L.) on an average weight of 134.4 g (W) and average total length of 199.5 mm (TL). The fish were adapted to environmental conditions and feeds prior to the start of the experiment for 14 days. Each group had one repeating.

The fish were individually measured (TL) and weighed (W) at the beginning of the test. Fish was fed daily at 8:00, 13:00 and 18:00. The feed dose was 3% of total weight of each group. Control weighting and measurements were performed every 14 days and then were adjusted the feed rates. Final measurements and weighting was performed in the end of the test. Total amount of used feed was determined. The total increment and individual increment (g and %), FCR (feed consumption / total increment), SGR ([(lnWt – lnW0) / t] * 100) and their relative ratio were calculated for the production parameters.

The water temperature (°C), dissolved oxygen (mg/l), oxygen saturation (%) and pH were measured optically (HQ40D, HACH, LANGR GmbH, Germany) twice a day. Chlorides (Cl−), ammoniacal nitrogen (N–NH4+) and nitrites (N–NO2−) were monitored daily using a spectrophotometer (PhotoLab 6600 UV–VIS).

Potential impacts of different diet on all studied characteristics were evaluated by a One-Way ANOVA procedure using statistical software Statistica 13.3 (TIBCO Software, 2017).
RESULTS AND DISCUSSION

Production parameters

There were no water chemistry fluctuations that could affect feeding take during the feed test. The observed parameters varied in values suitable for carp according to Svobodová et al. (2007).

Table 2 Increment from collections

<table>
<thead>
<tr>
<th></th>
<th>( W_{\text{total}} )</th>
<th>( \bar{x} )</th>
<th>( W_{\text{total}} )</th>
<th>( \bar{x} )</th>
<th>Increment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start</td>
<td>( g )</td>
<td>( g )</td>
<td>( g )</td>
<td>( g )</td>
<td>( g )</td>
</tr>
<tr>
<td>End</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2 000</td>
<td>67</td>
<td>4 015</td>
<td>134</td>
<td>2 015</td>
</tr>
<tr>
<td>F500</td>
<td>2 008</td>
<td>67</td>
<td>3 815</td>
<td>127</td>
<td>1 807</td>
</tr>
<tr>
<td>F1000</td>
<td>1 992</td>
<td>66</td>
<td>3 795</td>
<td>127</td>
<td>1 803</td>
</tr>
<tr>
<td>F500C3</td>
<td>2 013</td>
<td>67</td>
<td>4 348</td>
<td>145</td>
<td>2 335</td>
</tr>
<tr>
<td>F1000C3</td>
<td>2 036</td>
<td>68</td>
<td>4 493</td>
<td>150</td>
<td>2 457</td>
</tr>
</tbody>
</table>

Legend: \( W_{\text{total}} \) – total weight of group, number – number of specimens, \( x \) – average weight of specimen. Number of carps both at the beginning and at the end was 30.

Table 2 shows the fish growth (g and %) based on the weight of the groups (\( W_{\text{total}} \)) at the beginning and at the end of the test. The lowest growth was achieved in groups with 500 FTU and 1000 FTU phytase enzymes. The decrease over the control group was 10%. The citric acid addition groups showed an increase of approximately 16% respectively 21% over the control group. According to Nwanna and Schwarz (2007), the use of inorganic phosphorus in feed mixtures increases the average daily weight of common carp unlike the feed with the addition of the Ronozyme P phytase enzyme at 1000, 2000 and 4000 FTU.

The most favourable values of FCR and SGR were obtained in groups F500C3 and F1000C3 regardless of the phytase level in the feed. The FCR of both feeds with citric acid was decreased by 20% over the control group (one–way ANOVA: \( F = 24.09, df = 4, P = 0.002 \)), as shown in the table 3. The worst results were reported by F500 which increased by 3% over the control group. Čítek et al. (1998) states the FCR between 45 for plant glycid feeds, especially wheat and barley.

The SGR increase of 11% over control group in groups F500C3 and F1000C3 (one–way ANOVA: \( F = 17.93, df = 4, P = 0.004 \)). The citric acid–free groups showed worse results than the control group, as can be seen in the table 3. The differences did not reach a statistically significant level.

Danwitz (2016), in his study with \textit{Psetta maxima} L., used feed based on fishmeal and rapeseed oil. An increase in FCR and a decrease in SGR was achieved using 1000 FTU and 2000 FTU enzymes Natuphos® 5000 G. In contrast, Khajehpour et al. (2012) reported decrease in FCR and increase in SGR of the common carp fed by plant–based feed with 500 FTU enzyme Natuphos® 5000 G.
Table 3 Production parameters – statistically significant differences are red

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>F500</th>
<th>F1000</th>
<th>F500C3</th>
<th>F1000C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCR</td>
<td>2.44</td>
<td>2.52</td>
<td>2.46</td>
<td>1.97</td>
<td>1.97</td>
</tr>
<tr>
<td>SGR</td>
<td>0.99</td>
<td>0.91</td>
<td>0.91</td>
<td>1.09</td>
<td>1.12</td>
</tr>
<tr>
<td>FCR / SGR</td>
<td>2.46</td>
<td>2.77</td>
<td>2.70</td>
<td>1.81</td>
<td>1.76</td>
</tr>
</tbody>
</table>

CONCLUSION

The aim of this experiment was to find out whether the addition of phytase enzyme and citric acid in feed mixtures had an impact on fish production parameters. The best increment was achieved in fish fed by feed with phytase and 3% citric acid. The growth of F1000C3 group increased by 21% over control group. The groups with CA were also significantly better in FCR by 20% and SGR by 11% over the control group. Feed mixtures with the addition of phytase and without the citric acid showed worse results than the control group. Questionable is the use of minerals bounded to the residues of the phosphoric acid. The addition of phytase to the fish feed is an appropriate choice due to the increasing use of plant components for fish nutrition and not only for the reduction of the negative impact to the environment.

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REFERENCES