The effect of supplementation of feed with exogenous enzymes on the growth of common carp (*Cyprinus carpio*)

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Summary

Supplementary feed exogenous enzymes have improved the growth rates of various food animals. In this research, the effect of Endofeed W, a multienzyme feed supplement, was investigated on the growth of carp. Accordingly, 134 fish (33.1 ± 0.8 g) were randomly allocated to 4 experimental groups. During the first stage of the experiment (10 days), groups 1-3 received 1, 2 and 3 g Endofeed W per kg diet, respectively. The fourth group (control) received a placebo. All fish were weighed and redistributed for the second stage of the experiment, during which the test groups received 0.25, 0.5 and 1 g Endofeed W per kg diet, respectively. During the first stage of the experiment, the multienzyme supplement reduced the fish weights, dose dependently, being statistically significant with the highest dose ($P<0.05$). During the second stage of the experiment, a rather similar weight loss, especially with higher doses of the supplement, was observed. However, the differences were not significant ($P>0.05$). The feed conversion rates were evidently higher in groups receiving Endofeed W. The present study suggests the enzyme supplement, Endofeed W, is not only ineffective in improving the growth and feed conversion rates of carp, it may even exert negative effects with higher doses.

Key words: Growth supplement, Enzyme supplement, *Cyprinus carpio*, Feed conversion rate, Endofeed W

Introduction

Plant cells are strengthened by cell walls that are mainly indigestible to vertebrate enzymes. The long fiber-like molecules of cellulose are cemented by pectin, hemicellulose and lignin together. Cellulose is composed of non-branching chains of glucose monomers joined by β[1-4] glycosidic bonds, in contrast to the α[1-4] linkages in starch. Pectin and hemicellulose are composed of various proportions of several sugars and sugar acids. None of the cell wall components can be hydrolyzed by the vertebrate digestive enzyme, amylase. However, cellulose, hemicellulose and pectin can be hydrolyzed by a complex of microbial enzymes known as cellulase. In the absence of cellulase, the plant cell walls cannot be digested. Furthermore, the encased cell components cannot be exposed to digestive enzymes. These will result in the reduced digestibility of plant based feeds (Chesson, 1993; Dudley-Cash, 1997; Cunningham and Klein, 2007).

There is evidence suggesting soluble, high molecular weight non-starch polysaccharides (NSPs) contained within plant cell walls increase digesta viscosity, thereby reducing digestive enzyme access to other nutrients (Castanon *et al*., 1997; Bedford, 2000; Francis *et al*., 2001). This can result in reduced feed efficiency and lower growth rates in fish and crustaceans (Watanabe, 2002). Supplementary exogenous enzymes have been shown to reduce the antinutritive effects of viscous NSPs (Choct and Annison, 1992; Bedford and Classen, 1993).

The use of exogenous enzyme supp-

lements containing cellulase, hemicellulase, etc has shown beneficial effects in improving the growth/feed conversion rate in different domestic animals including chickens (Bedford and Classen, 1993; Choct *et al*., 1995; Saleh *et al*., 2005), pigs (Omogbenigun *et al*., 2004), Atlantic salmon (Carter *et al*., 1994), larval gilthead sea breams (Kolkovski *et al*., 1993) and tiger prawns (Buchanan *et al*., 1997).

Various commercial enzyme mixtures are available and are routinely supplemented in poultry feed. These products contain endoglucanse and endoxylanase enzymes, which hydrolyze the β[1-4] bonds in cellulose and xylan products, respectively, within the digestive tract. The aim of this study was to investigate the effectiveness of Endofeed W, a commercial multienzyme product routinely used in poultry farms, on the growth performance and feed efficiency in carp.

Materials and Methods

Experimental tanks

The experiments were carried out in four glass aquaria. The volume of water in these tanks was 150 liters each. Water exchange was carried out every other day. Proper aeration was achieved using air pumps. The level of oxygen in the water was 5.8-6 ppm. The temperature of the water was maintained at 22°C throughout the experiments. The tanks were randomly allocated to four different experimental groups.

Experimental fish and handling

The fish were clinically examined and were sampled for probable microbial or parasitic infections after arrival and were diagnosed as healthy. All fish were disinfected using NaCl 3% solution for 15 min and were allowed to acclimate for 5 days before the start of the experiment. The experiment was conducted in two consecutive steps.

In the first step of the experiment, 134 fish (body weight: 33.1 ± 0.8 g) were randomly allocated to 4 different experimental groups. Three groups were considered as the test groups (T1-T3) and received 1, 2 and 3 g kg^{-1} Endofeed W in their feed, respectively. The fourth group received a placebo and served as the control. All fish were weighed and measured for their total body length at the first day of the experiment (Table 1). The fish were weighed again at the end of the experiment at day 10. It is noteworthy that no mortality occurred during this period.

Table 1: Initial weights and lengths of the fish at day 1 of the first experiment. Data are represented as mean ± SEM

Groups	Control	Endofeed W (g kg^{-1})		
		1	2	3
Weight (g)	33.9±1.9	31.3±1.9	34.0±1.7	33.2±1.5
Length (cm)	13.7±0.5	14.0±0.3	14.2±0.2	14.1±0.2

The fish from the first experiment (n = 102) were redistributed and were randomly allocated to 4 experimental groups. The 3 test groups (T1-T3) received Endofeed W at 0.25, 0.5 and 1 g kg^{-1} feed, respectively. All fish were weighed at day 1. The fish had mean weights of 42.9 ± 2.4, 43.1 ± 2.6, 44.4 ± 2.4 and 43.1 ± 2.4 g in the control and the test groups, respectively. There were no significant differences among different groups in this regard (one-way ANOVA). All fish were weighed again and their total body lengths were also measured at the end of the experiment at day 60.

The diets and feed treatment

During the first experiment, the fish received daily feed at a rate of 7% of their body weights. Thirty percent of the total weight of the feed was based on cracked wheat, and the reminder (70%) was composed of formulated pellets (Table 2). In the second experiment, the fish received daily amounts of feed at 6% and 5% of their body weights for the first and the second months of the experiment, respectively. In this experiment, the same commercial pellets (90%) were mixed with cracked wheat (10%).

Endofeed W, a commercial multienzyme supplement for poultry (GNC Bioferm Inc, Canada) was used in this research. The product contained different digestive enzymes including xylanase (≥1200 U/g), β-

Table 2: the formula of the commercial diet used for the carps

Composition	Quantity	Ingredients	Percent
Humidity	7.1%	Wheat bran	34
Protein	27%	Soybean meal	15
TDN	56%	Cottonseed meal	14
Fat	6.8%	Molasses	4
Proxide	4.1%	Chicken meal	20
Ash	8.3%	Bentonite	3
Calcium	4.7%	Wheat flour	10
Fiber	6.5%		
Crude energy	3648 C		

glucanase (≥440 U/g), cellulase and hemicellulase. The dry enzyme powder was thoroughly mixed with cracked wheat and then with moistened pellets. The pellets were then covered with kitchen oil (1 ml/d), so that the enzymes are not washed out in the aquarium. The same procedure was used for the placebo, but no enzyme was added.

The enzymatic/cellulase activity of Endofeed W was assessed at acidic (pH = 5), neutral (pH = 7) and alkaline (pH = 9) pH using the diet or methyl cellulose as the substrates. Briefly, the supplement was incubated with either of the two substrates (diet or methyl cellulose) for 2 h at 45°C (n = 3 each). The decline in freezing point and glucose concentration were measured as indicators of enzyme activity. The supplement showed significant enzymatic/ cellulase activity according to the drop in freezing point, as well as the glucose level at all tested pH values following incubation (results not shown).

Statistical analysis

Statistical analysis and drawing the figures were performed using GraphPad Prism v4.0 (GraphPad Software, USA). Unless otherwise mentioned, all data are represented as mean ± SEM. Statistical comparisons of the weights and lengths were performed using analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test.

Results

At the end of the first stage of the experiment, a trend of reduction in the mean weights was observed. This reduction was dose dependent and was significant with the highest dose of the supplement (Fig. 1). At day 30 of the second stage of the experiment, the mean weights in groups receiving 0.5 or 1 g Endofeed W per kg feed were 6.6% lower compared to the control, however, the differences were not statistically significant (Fig. 2). Even higher differences of 19.5 and 15.6%, in the above mentioned groups, respectively, existed after 60 days, although not significant (Fig. 3).

The total body length, measured at day 60, was not significantly different, although a dose dependent decreasing trend existed in carp receiving supplementary enzymes (Fig. 4). There was an extremely significant correlation between the weight and the length of the body (Fig. 5).

The feed conversion rates were dose-dependently higher in enzyme receiving carp in both stages of the experiment (Table 3). At the end of the first experiment, the feed conversion rates in groups receiving 2 and 3 g Endofeed W per kg feed were approximately 2 and 4 times higher than that of the control group. The feed conversion rates were between 1.5 to 2.2 times that of the control group at days 30 and 60 of the second experiments.

Table 3: The feed conversion rates of different experimental groups. The rates are calculated at the end of the first experiment (day 10) and following 30 and 60 days of the second experiment

	Control	Test 1	Test 2	Test 3
Day 10	1.79	1.87	3.89	6.94
Day 30	4.13	3.88	6.59	9.24
Day 60	5.0	5.5	8.2	7.5

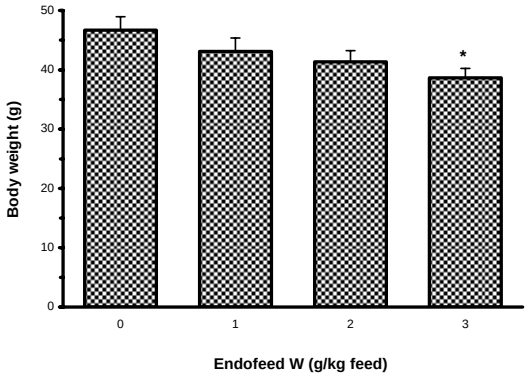

Fig. 1: The body weight of the carp at the end of the first experiment (day 10). Data are represented as mean ± SEM. The asterisk indicates statistical significant difference (P<0.05, ANOVA) compared to the control group

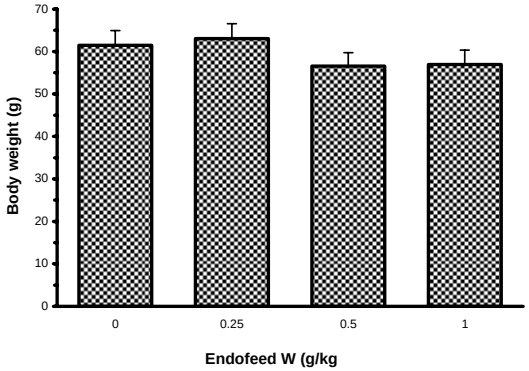

Fig. 2: The weights of the carp at day 30 of the second experiment. Data are represented as mean ± SEM. ANOVA: n.s.

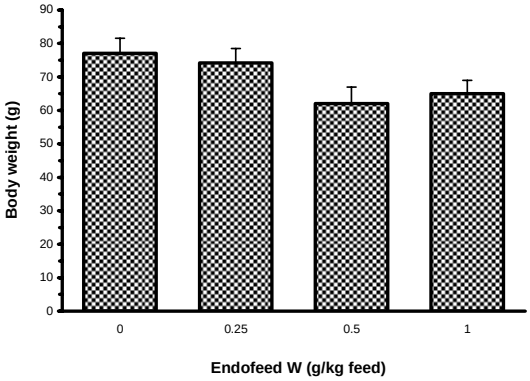

Fig. 3: The body weights of the carp at day 60 of the second experiment. Data are represented as mean ± SEM. ANOVA: n.s.

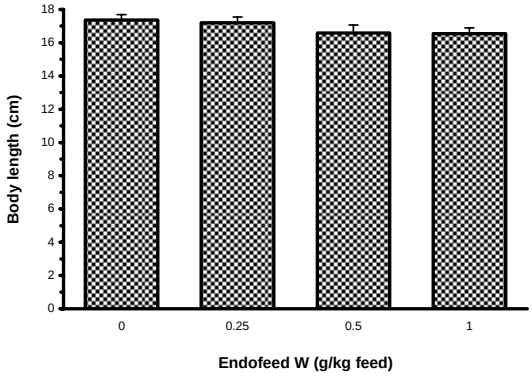

Fig. 4: The body lengths of the carp at the end of the second experiment (day 60). Data are represented as mean ± SEM. ANOVA: n.s.

Discussion

The current research was performed using different concentrations of Endofeed W added to different diets (regarding the percentage of cracked wheat added), in order to investigate the possible effects of exogenous carbohydrase enzymes on the growth rate and feed conversion rate of the carp. Lower doses of Endofeed W (0.25-1 g kg^{-1} feed) showed no significant effects on the carp, while higher doses of the supplement (2-3 g kg^{-1} feed) reduced weight gain in a dose dependent manner, although the reduction was not significant.

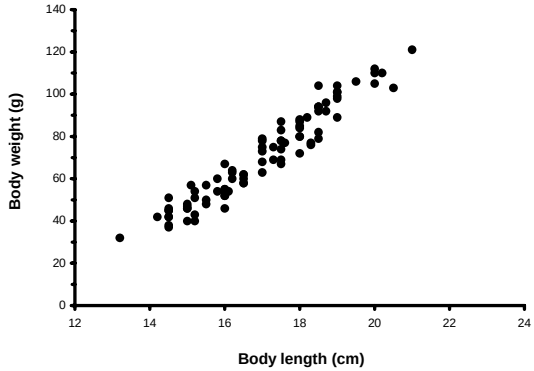

Fig. 5: The correlation between the weights and the lengths of the carps at day 60. P<0.0001, R^2: 0.928

The influence of feed supplementary carbohydrase enzymes on the growth of carp fingerlings has been studied by Bogut *et al.* (1995). In this report, a multienzyme preparation containing amylase, protease, β-gluconase, β-glucosidase and cellulase, at a rate of 1.5 mg/kg feed, significantly improved weight gain and nutritional parameters. This research has been presented in the form of an abstract, and the necessary full details have not been published. Thereby, authentication of this report is not possible. Besides, the enzymes used in this study were partly different from the current research.

Although most studies on other species indicate that exogenous supplementary carbohydrase enzymes improve weight gain and feed conversion rate (Bedford, 1995; Castanon *et al*., 1997; Wyatt *et al*., 1997; Bedford, 2000; Cowieson *et al*., 2003; Saleh *et al*., 2005), conflicting reports are also present suggesting no or even adverse effects when these enzymes are added to animal feeds (Kocher *et al*., 2000; Stone *et al*., 2003).

The process of digestion is not fully understood in fish. Hydrolyzing enzymes for non-starch polysaccharides such as β-glucanase or β-xylanase are scarce or

nonexistent. Cellulase activity has not been detected in some fish species such as *Chanos chanos* (Chiu and Benitez, 1981), *Salmo gairdneri* (Kitamikado and Tachino, 1960) and *Cyprinus carpio* (Bondi and Spanhof, 1954). It is, therefore, difficult to interpret the conflicting results.

It has been claimed that high enzyme levels could liberate excessive amounts of monosaccharides, and thereby induce hyperglycaemia (Stone *et al*., 2003). In contrast, low levels of enzymes may increase viscosity of the digesta by increasing the soluble non-starch polysaccharides via solubilizing the insoluble carbohydrate fraction, resulting in reduced digestibility and absorption (Irish and Balnave, 1993; Castanon *et al*., 1997). In this research, different concentrations of Endofeed W (0.25-3 g kg^{-1} feed) were used, and interestingly, higher amounts of the supplement even decreased weight gain. Therefore, inappropriate enzyme levels seem unlikely to justify the results.

It has been suggested that commercial carbohydrase enzymes still contain small quantities of protease activity and this protease activity may reduce the effectiveness of the commercial enzymes (Saleh *et al*., 2005). In fact, carbohydrases may be digested by proteases present in enzyme preparations.

The use of supplementary enzymes may result in the liberation of galactose and xylose from non-starch polysaccharides. It is noteworthy that most fish species are intolerant to these carbohydrates, and excessive amounts of these monosaccharides are detrimental to fish growth performance and health (Stone, 2003). In fact, a remarkable reduction in growth and feeding activity has been observed in carp fed diets containing 30% galactose (Shikata *et al*., 1994). This mechanism, therefore, seems likely to justify the current results. More studies, however, are required to verify this hypothesis.

In conclusion, the present research studied the effect of the commercial feed supplementary multienzyme, Endofeed W, on the growth of carp. The product did not exhibit any beneficial effects on the growth performance and feed conversion rate of the fish, however, it did rather negatively influence these parameters with higher doses.

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