EnzoMeal as a fish meal replacer: effects on growth, digestive and oxidative status of yellow perch (Perca flavescens)

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Abstract

This experiment was conducted to develop a tandem chemical and enzymatic treatment of soybean meal (SBM) to reduce the antinutritional factors (ANFs), and to produce EnzoMeal (EZM) for fish feed. Using yellow perch (*Perca flavescens*) fingerlings, a 10-week experiment was conducted to evaluate the nutritional quality of the EZM to compare with that of fish meal (FM) and SBM. Fingerlings (297 ± 11.01 ± 0.19 g) were randomly distributed in nine treatments with three replicates and fed iso-nitrogenous diets (crude protein 41%): control (FM based protein); S$_{50}$ and S$_{100}$ (50% and 100% of FM protein replaced by SBM), and EZM$_{50}$ and EZM$_{100}$ (50% and 100% of FM protein replaced by EZM). These diets were also supplemented with an exogenous enzyme cocktail to reduce ANFs and named as S$_{50+E}$, S$_{100+E}$, EZM$_{50+E}$, and EZM$_{100+E}$, respectively. All diets were processed in a Brabender single screw extruder, using a 1:1 compression ratio, with processing temperatures of approximately 40°C. The highest (P < 0.05) growth performance and nutrient utilization parameters (protein efficiency ratio and protein productive value, PPV) were observed for the EZM$_{50+E}$ group, which were not statistically different to that for control and EZM$_{50}$ groups, and significantly (P < 0.05) higher than all other groups. The least (P < 0.05) growth performance was observed for the S$_{100}$, S$_{100+E}$ and EZM$_{100}$ groups. On the other hand, the opposite trend was observed for the feed conversion ratio. Digestive enzymes (amylase, lipase and protease) activity was higher (P < 0.05) in the intestine than pyloric caeca of fish in all groups. The highest protease activity (in intestine and pyloric caeca) was observed for the control group, which is significantly similar to EZM$_{50}$, EZM$_{50+E}$, and S$_{50+E}$ groups, and the lowest value was observed for 100% replacement of FM protein by SBM and EZM fed groups. However, inclusion of exogenous enzymes in feed showed positive effects in EZM$_{50+E}$ compared to EZM$_{50}$ for PPV and lipid productive value and amylase activity in the intestine. Activity of protein metabolism enzymes i.e., alanine transaminase and aspartate aminotransferase in the
liver were the highest in the control, which was similar (P < 0.05) to the EZM<sub>50+E</sub> and EZM<sub>50</sub> groups, whereas other groups exhibited lower activity. Antioxidant enzymes (catalase, superoxide dismutase, and glutathione peroxidase) in the liver were the lowest in the control group, which was significantly similar to the group fed the feed with 50% replacement of FM protein, whereas complete (100%) replacement of FM protein exhibited the highest activity. Conclusively, performance of the EZM<sub>50</sub> and EZM<sub>50+E</sub> groups were similar to the FM fed group, and better than SBM fed groups, and EnzoMeal has considerable potential as alternatives to fishmeal in aquafeed.

**Keywords:** EnzoMeal, antinutritional factors, exogenous enzymes cocktail, growth, protein metabolism, antioxidant enzymes, yellow perch.
Introduction

Aquaculture is the fastest-growing sector of food production. Traditionally, fish meal (FM) has been the main source of dietary protein for farmed fish. However, the increasing demand in contrast to limited supply for this finite protein source has caused a significant increase in FM prices in the last decade (Kader et al. 2010; Kumar et al. 2010a; Kumar et al. 2010b; Kumar et al. 2011a; Kumar et al. 2011b; Kumar et al. 2012a). Therefore, it is a matter of urgency that alternative protein sources for fish diets be found to support global aquaculture development (Kader et al. 2010; Kumar et al. 2011a; Kumar et al. 2011b). Several alternative proteins have been tested for fish feed, among them soybean appeared to be the best FM replacer for fish diet. It has been reported that 30–50% FM could be replaced by soybean meal (SBM). Soybean meals have a high content of available protein with a well-balanced amino acid profile, a constant composition, a reasonable price, and there is a steady supply available; however, their methionine level is low, and they also contain approximately 30% of indigestible carbohydrates including non-starch polysaccharides (NSP), and several compounds or anti-nutritional factors (ANFs; protease inhibitors, lectin, and phytate) that may disturb the digestive process (Hernandez et al. 2007; Kumar et al. 2011a; Kumar et al. 2011b; Kumar et al. 2012a; Olli et al. 1994; Storebakken et al. 2000), as many of them have been reported to hinder digestion and absorption of nutrients and restrict their potential as replacements of FM in fish diets (Bureau et al. 1998; Hernandez et al. 2007; Klein et al. 1998; Kumar et al. 2011a; Kumar et al. 2011b; Kumar et al. 2012a; McGoogan & Gatlin 1997; Olli & Krogdahl 1994; Rumsey et al. 1994).

Digestion and absorption of nutrients depends on the activity of the digestive enzymes, in particular those located in the intestine, which are responsible for the final stages of breaking down and assimilation of the food (Klein et al. 1998; Kumar et al. 2011a; Kumar et al. 2011b; Kumar et al. 2012a; Silva et al. 2010). In salmonids, higher inclusion levels of
solvent-extracted SBM led to a marked reduction in the activities of such enzymes in enterocytes of the distal intestine (Bakke-McKellep et al. 2000; Krogdahl et al. 1995; Krogdahl et al. 2003; Kumar et al. 2011a; Kumar et al. 2011b; Kumar et al. 2012a; Silva et al. 2010). This suggests that measuring the activities of intestinal enzymes may represent a sensitive tool to study the effects of differently-processed SBMs and cocktail exogenous enzymes on nutrient bioavailability and to ascertain tolerability to certain soy-ANFs in various fish species.

In addition, nutrition plays an important role in animal welfare by maintaining this fragile oxidative balance, either by supplying nutrients that enhance the antioxidant system or avoiding those that would induce an increase of reactive oxygen species (ROS) production by different physiological pathways. The oxidative stress in aquatic organisms is more profound during nutritional deficiency, elevated temperature, hypoxia and exposure to xenobiotics (Avanzo et al. 2002; Hwang & Lin 2002; Kolkovski et al. 2000; Radhakrishnan et al. 2014; Romeo et al. 2000). However, either an increase in ROS production above the level that can be removed by antioxidant defenses, or a decrease in the capacity of the antioxidant defenses, could result in oxidative damage to key molecules, including DNA, protein, and lipids (lipid peroxidation) (Halliwell & Gutteridge 1999).

While the predominant concern about the effects of various alternative plant proteins is on fish growth and feed efficiency, relatively few studies have monitored the dietary influence on the biochemical index of fish, such as changes in protein metabolism enzyme activities (Krogdahl et al. 2003; Kumar et al. 2010b; Kumar et al. 2011a; Kumar et al. 2011b; Kumar et al. 2012a), hepatic metabolism (Vilhelmsson et al. 2004) and oxidative status, which have also been used to provide an indication of disturbance by specific ingredient to the metabolic function and nutrient utilization by fish (Kumar et al. 2011a; Kumar et al. 2011b; Kumar et al. 2012a; Lin & Luo 2011).
Yellow perch (*Perca flavescens*) is a major species of the Great Lakes region, particularly in the north central region of the USA, and is a consumer favorite due to low fat content and good taste (Fallahi *et al.* 2012; González *et al.* 2006). However, commercial production of yellow perch has not rapidly developed yet, partially due to limited information on nutritional requirements. A commercially available specialized diet has not been developed for yellow perch. Therefore, it has made it essential to develop suitable complete and supplemental diets for use in yellow perch. It may well be that fish with more omnivorous feeding habits, like yellow perch, could make more efficient use of high levels of dietary SBM.

To our knowledge, no studies have been reported on the use of enzyme-treated SBMs as a FM substitute in yellow perch diets. The aims of this study were to 1) test a modified soybean meal diet (EnzoMeal) against a regular soybean meal diet and a standard fish meal diet using yellow perch, and determine the benefits and deficiencies of the EnzoMeal as a fish food ingredient; and 2) evaluate the possibility of replacing different levels of FM protein by SBM and EZM in the practical diet of yellow perch.

**Materials and methods**

This study and all experimental procedures involving animals were performed according to the protocol approved by the Ohio State University Institutional Animal Care and Use Committee.

*Preparation of EnzoMeal and diet formulations*

The EZM was obtained by heating SBM (NUTRASOY® 7B, obtained from Archer Daniels Midland, Decatur, IL, USA) in 3% hydrogen peroxide solution using a proprietary catalyst. The product was isolated and further treated with an enzyme cocktail.
Prior to feed formulation, the proximate composition of SBM, whole wheat meal, EZM, soy protein isolate, and FM were determined. Nine isonitrogenous and isolipidic diets were formulated. Experimental diets containing crude protein 41%, crude lipid 11%, vitamin premix 2%, mineral premix 2%, and Titanium oxide (TiO2) 0.2% were prepared. TiO2 was added for digestibility measurement. Methionine was supplemented at the rate of 0.3% in 100% FM protein replacement diets. Experimental diets are as follows: control (FM based protein); S_{50} and S_{100} (50% and 100% of FM protein replaced by SBM), and EZM_{50} and EZM_{100} (50% and 100% of FM protein replaced by EZM), and SBM and EZM based diets were also supplemented with an exogenous enzyme cocktail (phytase, NSPase, and carbohydrase) and named as S_{50+E}, S_{100+E}, EZM_{50+E} and EZM_{100+E} respectively (Table 1).

**Enzyme cocktail supplementation**

Enzymes (cocktail) and buffer were mixed only with SBM and EZM and kept at room temperature for three hours to hydrolyze the carbohydrates and phytate. Thereafter, these soybean products (hydrolyzed SBM and EZM) were mixed along with other fish feed ingredients for fish diet preparation.

**2.1.2. Feed extrusion:** All ingredients were ground with a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ, USA) to an average particle size of approximately 500 μm. All components were then combined and mixed for 15 min (Kushlan Products, Inc., Goldendale, WA). After all ingredients were thoroughly homogenized, each blend was adjusted to a desired pre-extrusion moisture content of ~40% by adding adequate amounts of water, then mixed again for 15 min. The extrusion processing of each blend was performed using a single screw extruder (Brabender Plasti-Corder, Model PL 2000, South Hackensack, NJ, USA), which had a compression ratio of 3:1, a screw length-to-diameter ratio of 20:1, and a barrel length of 317.5 mm. The die assembly was conical, and tapered from an initial diameter of
6.0 mm to a diameter of 3.0 mm at the discharge opening. The length of the die was 27.0 mm, which resulted in a die length-to-diameter ratio of 9.0. A 7.5 HP (5.5 kW) motor powered the extruder. The screw speed was set to 100 rpm during extrusion, and the temperature of all extrusion zones (feed, transition, and die) were set to 40°C for the duration of processing. After extrusion, the pelletized feed blends were dried in a laboratory oven (Thelco Precision, Jovan, Winchester, VA) at 50°C for 24 h. After drying, the diets were broken by hand, sieved into proper pellet size, and were stored at −15°C. Enzymes may have been active during the feed drying process, thus hydrolyzing substrates in the feed ingredients.

**Experimental system and animals**

A mixed strain of yellow perch fingerlings (about 10-12 g) was produced in the breeding program at the Ohio Center for Aquaculture Research and Development at The Ohio State University South Centers, Piketon, OH, USA. These fingerlings (N = 297) were randomly distributed in nine groups with three replicates, each having 11 fish (11.01 ± 0.19 g) in a round tank (55 L capacity). At the start of the experiment, six fish were preserved at −20°C for analysis of the initial body composition. After fish were acclimatized for two weeks in the experimental tanks, fish were trained for different experimental diets for 1 week. The all tanks were supplied with water from a flow-through system. The system was subjected to a photoperiod of 12 h light: 12 h darkness. Water quality was monitored throughout the experiment. All the water parameters were in the optimum range (temperature 17.4 – 20.1°C, pH 7.0 – 7.5, and dissolved oxygen 6.7 – 8.4 mg l⁻¹). Water flow was adjusted to the same level for all tanks. During the experimental period, a ration totaling 3% body weight daily was hand-fed to the fish in three equal proportions at 9:00, 13:00, and 17:00 h. Fish were weighed individually at the beginning of the experiment and at every other week intervals during the experimental period to adjust the feeding level for the subsequent week. On the
designated weigh days, the fish were weighed in the morning, just prior to the 9:00 h feeding, having not had feed for 16 hours prior.

The experiment lasted for 10 weeks. At the end of the experiment, three fish per group were anaesthetized with tricaine methanesulfonate (MS222; 250 mg/L). Anaesthetized fish were carefully dissected to isolate the intestine, pyloric caeca, and liver. These samples were stored at -80°C for determination of activities of digestive, protein metabolism and antioxidant enzymes. Four additional fish per group were killed by a blow to the head with a metal rod and then stored at −20°C for chemical composition analysis. Prior to determination of the proximate composition, the fish were autoclaved at 121°C for 20 min, thoroughly homogenized using an Ultra-Turrax T25, frozen overnight and freeze-dried.

**Proximate analysis of feed ingredients, experimental diets, and whole body of fish**

All samples were ground to a fine powder by using a grinder prior to analyses. The proximate composition of the feed ingredients, experimental feeds, and of the fish carcasses was determined according to the official methods (AOAC, 1990), i.e., for moisture (oven-drying at 105°C overnight), crude protein by CN analyser (N X 6.25), fat [by extraction according to the method described by Smedes (1999) as modified by Schlechtriem et al. (2003) and ash (oven incineration at 480°C overnight).

**Growth and nutrient utilization parameters**

Growth performance and diet nutrient utilization were assessed in terms of:

- Body mass gain percentage (BMG, %) = \[\frac{(final\ body\ mass - initial\ body\ mass)}{initial\ body\ mass}\] X 100

- Specific growth rate (SGR, g/day) = \[\frac{(ln\ final\ body\ mass\ in\ g) - (ln\ initial\ body\ mass\ in\ g)}{number\ of\ trial\ days}\] X 100
Metabolic growth rate (MGR, g kg\(^{0.8}\) day\(^{-1}\)) = \frac{(Body mass gain in g)}{[(initial body mass in g / 1000)^{0.8} + (final body mass in g / 1000)^{0.8}] / 2} / number of trial days

Feed conversion ratio (FCR) = dry feed fed (g)/body mass gain (g)

Protein efficiency ratio (PER) = body mass gain (g)/crude protein fed (g)

Protein productive value (PPV, %) = \frac{[\text{final fish body protein in g} - \text{initial fish body protein in g}]}{\text{total protein consumed in g}} \times 100

Lipid productive value (LPV, %) = \frac{[\text{final fish body lipid in g} - \text{initial fish body lipid in g}]}{\text{total crude lipid consumed in g}} \times 100

Organ indices

Relative intestinal length (RIL), hepatosomatic index (HSI) and intestinal somatic indexes (ISI) are calculated as indicated below:

RIL = Intestine length (mm)/body mass (g)

HSI = Liver mass (g) X 100 / body mass (g) and

ISI = Intestinal mass (g) X 100 / body mass (g).

Enzyme assays

Digestive enzyme assay

Amylase activity was measured in the pyloric caeca and intestine by using and amylase assay kit (Kit no. # MAK009 SIGMA, Aldrich, USA). Amylase activity was determined using a coupled enzymatic assay, which resulted in a colorimetric product, proportional to the amount of substrate, ethylidene-pNP-G7, cleaved by the amylase. One unit is the amount of amylase that cleaves ethylidene-pNP-G7 to generate 1.0 μmole of p-nitrophenol per minute at 25°C. RayBiotech’s Protease Activity Assay Kit (CODE: 68AT-Protease-S100, Ray Bio, USA) was used to determine the protease activity in pyloric caeca and intestine of fish. One
unit is defined as the amount of protease that cleaves the substrate, to yield an amount of fluorescence equivalent to 1.0 nmol of unquenched fluorescein isothiocyanate labeled casein per minute at 25°C. Lipase activity was determined using a coupled enzyme reaction, which resulted in a colorimetric product proportional to the enzymatic activity present in pyloric caeca and intestine of fish. Lipase Activity was measured using an assay Kit (MAK046 Sigma Aldrich, USA). One unit of lipase is the amount of enzyme that will generate 1.0 μmole of glycerol from triglycerides per minute at 37°C.

Protein metabolism enzyme

BioVision’s Aspartate aminotransferase (AST) assay Kit (Catalog# K753-100, Bio Vision, USA) was used to determine the AST activity in liver of fish. The principle is amino group is transferred from aspartate to α-ketoglutarate. The products of this reversible transamination reaction are oxaloacetate and glutamate. The glutamate is detected in a reaction that concomitantly converts a nearly colorless probe to color. One unit of AST is the amount of enzyme that will generate 1.0 mmole of glutamate per minute at pH 8.0 at 37°C. Alanine transaminase (ALT) activity was quantified by using an ALT activity Assay Kit (Cayman Chemical Item Number 700260, USA). Measurement of the ALT activity was carried out by monitoring the rate of NADH oxidation in a coupled reaction system employing lactate dehydrogenase (LDH). The oxidation of NADH to NAD⁺ was accompanied by a decrease in absorbance. One unit is defined as the amount of enzyme that will cause the oxidation of 1.0 μmol of NADH to NAD⁺ per minute at 37°C.

Antioxidant enzymes

A superoxide dismutase (SOD, inhibition rate %) assay kit (Catalog # 19160 SIGMA, Sigma Aldrich, USA) was used to assess the SOD enzyme activity in the liver of fish. SOD was
assayed by utilizing Dojindo’s highly water-soluble tetrazolium salt, WST-1 (2-(4-Iodophenyl) - 3-(4-nitrophenyl)-5-(2,4-disulfophenyl) - 2H-tetrazolium, monosodium salt) that produces a water-soluble formazan dye upon reduction with a superoxide anion. Catalase enzyme activity was determined with a catalase assay kit (Cayman Chemical Item Number 707002, USA). The determination is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H$_2$O$_2$. The formaldehyde produced was measured spectrophotometrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as the chromogen. Purpald specifically forms a bicyclic heterocycle with aldehydes, which, upon oxidation, changes from colorless to a purple color. One unit is defined as the amount of enzyme that will cause the formation of 1.0 nmol of formaldehyde per minute at 25°C. A glutathione peroxidase (GPx) colorimetric assay kit (Catalog # K762-100, Bio Vision, USA) was used to determine the GPx activity in the liver. One unit is defined as the amount of enzyme that will cause the oxidation of 1.0 μmol of NADPH to NADP$^+$ under the assay kit condition per minute at 25°C.

Statistical analysis

All data were subjected to a one-way analysis of variance ANOVA and the significance of the differences between means was tested using Tukey's HSD (Honestly Significant Difference) test (P < 0.05). The software used was SAS, Version 9.3. Values are expressed as means ± standard deviation.

Results

Proximate composition of feed ingredient and experimental diets

Total crude protein content in the EZM was 58%, and SBM contains 47.5% crude protein (Table 2), which revealed that crude protein amount increased almost 22%, whereas ANFs
especially oligosaccharides, NSPs, and phytate content decreased (>50%) in EZM compared to SBM (data not shown). Heat labile antinutrients such as lectin and trypsin inhibitor were absent in EZM because we have used the roasted SBM.

The composition of experimental diets is shown in Table 1. Proximate composition of feed ingredients and experimental diets are shown in Table 2. Experimental diets contained about 41% crude protein and 15% crude lipid and were isonitrogenous and isolipidic.

Chemical composition of whole body of fish and organ indices

The effects of different inclusion levels of EZM and SBM with and without supplementations of exogenous cocktail enzymes on the body composition of yellow perch are presented in Table 3. The highest (P < 0.05) inclusion of EZM and SBM (100% FM protein replacement groups) regardless the inclusion of exogenous enzymes, exhibited higher (P < 0.05) moisture content than that of the control and other groups; whereas crude protein and lipid content exhibited an opposite trend. The highest (P < 0.05) moisture content was observed in the S100 group, which was significantly similar (P > 0.05) to the 100% replacement groups (S100+E, EZM100 and EZM100+E), whereas the lowest value was found in the control group. Whole body protein content was the highest (P < 0.05) in the EZM50+E group, which is statistically similar to EZM50, S50+E, and control groups; whereas the lowest value was observed for 100% replacement groups (S100, EZM100, and S100+E). The highest (P < 0.05) crude lipid content was observed in EZM50, which was similar to S50 and the control groups, and these values were statistically higher than other groups. Dietary soybean protein and exogenous enzyme supplementation did not significantly (P > 0.05) affect the ash content in the whole body of the fish.

Hepato somatic index, GSI, CI, and RIL of yellow perch fed different experimental diets are shown in Table 3. The hepatosomatic indexes were higher in EZM fed groups.
(except EZM_{100+E}) than regular SBM fed groups. The relative intestinal length values of plant-fed groups were significantly (P < 0.05) higher than the control group. EZM and SBM did not influence (P > 0.05) the GSI of fish among the groups.

**Fish behavior, feed intake, growth and nutrient utilization**

Based on the visual observation during the feeding period, palatability or acceptability of feed was good and the behaviour of fish was normal, except in the 100% FM replacement groups, wherein these groups exhibited lower palatability than other groups.

The effects of the two processed soybean meals (EZM and SBM) with and without the exogenous enzyme cocktail for growth performance, feed intake and nutrient utilization are summarized in Tables 3 and 4, and Figures 1A and 2. The highest (P < 0.05) growth performance (FBM, weight gain, SGR, and MGR) were observed for the EZM_{50+E} group, which were not statistically different to that for the control and EZM_{50} groups, and significantly (P < 0.05) higher than all other groups. The least (P < 0.05) growth performance was observed for the S_{100}, S_{100+E}, and EZM_{100} groups. On the other hand, the opposite trend was observed for the FCR. Feed intake was positively correlated to the growth performance (tables 3 and 4). The highest (P < 0.05) PER, PPV, and LPV values were observed for the S_{50+E} group which is statistically similar to the control and EZM_{50} groups. The lowest values for these parameters were observed in the 100% FM replacement groups. Overall, EZM fed groups exhibited higher (P < 0.05) growth performance and feed utilization than SBM fed groups; whereas dietary supplementation of exogenous enzymes both in EZM or SBM did not significantly affect the growth performance and feed utilization of yellow perch except PPV and LPV in the EZM_{50+E} group.

**Enzymes**
Digestive enzyme activities in pyloric caeca and the intestine of yellow perch are presented in Figure 3A, 3B and 3C. Digestive enzymes (amylase, lipase, and protease) activity was higher (P < 0.05) in the intestine than in pyloric caeca of fish in all groups. Amylase activity in pyloric caeca and intestine was the highest (P < 0.05) in EZM\textsubscript{50+E} whereas other groups exhibited lower (P < 0.05) value. Inclusion of exogenous enzymes in feed showed positive effects (P < 0.05) in EZM\textsubscript{50+E} compared to EZM\textsubscript{50} for the amylase activity in the intestine. The highest protease activity (in intestine and pyloric) was observed for the control group, which is significantly similar (P > 0.05) to the EZM\textsubscript{50}, EZM\textsubscript{50+E}, and S\textsubscript{50+E} groups, and the lowest (P < 0.05) value was observed for 100% replacement of FM protein by SBM and EZM fed groups. Lipase activity in the intestine was the highest (P < 0.05) in EZM\textsubscript{50+E}, which was significantly similar (P > 0.05) to the control, EZM\textsubscript{50}, and 50% replacement groups (FM replaced by SBM), whereas 100% FM replacement groups exhibited lower activities of lipase in the intestine. Amylase activity in the pyloric caeca did not differ significantly (P > 0.05) among the groups.

Dietary inclusion of EZM and SBM significantly (P < 0.05) changes the activities of protein metabolism enzymes (ALT and AST) in liver of yellow perch (Figure 4A). Activities of protein metabolism enzymes i.e., ALT and AST in liver were the highest in control, which was similar (P < 0.05) to the EZM\textsubscript{50+E} and EZM\textsubscript{50} groups whereas other groups exhibited lower activity. Activities of protein metabolism enzymes were positive correlated with growth performance and feed utilization. Alanine transaminase and AST activities in liver exhibited positive response (not significantly) with dietary inclusion of the exogenous enzyme cocktail in EZM and SBM based diets.

Antioxidant enzyme (catalase, SOD, and GPx) activities in liver were significantly altered by different experimental diets (Figures 1B and 4B). Antioxidant enzymes such as catalase, SOD, and GPx in the liver were the lowest (P < 0.05) in the control group, which was significantly similar to the 50% replacement of FM protein fed groups, whereas groups

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having complete (100%) replacement of FM protein exhibited the highest activity. Dietary supplementation of exogenous enzyme cocktail in SBM and EZM based diets did not influence (P > 0.05) the antioxidant enzyme activity in fish.

Discussion

The use of plant protein products in aquaculture diets is generally limited by their low levels of digestible protein and/or energy (Kumar et al., 2011a; Kumar et al., 2011b). The seed meals of soybeans, lupins, pea, and Jatropha represent some of those plant products that contain a considerable amount of proteins, and efforts have been made to further enhance their protein levels by processing technologies (Glencross et al. 2005; Kumar et al. 2012a; Makkar & Becker 2009; Overland et al. 2009). In the present study we have developed a method to produce soybean meal containing a high amount of protein (58%) with no ANFs (oligosaccharides, NSP, and phytate), and tested efficacy in replacing fish meal in yellow perch. To our knowledge, no studies have been reported on the use of enzyme-treated SBM as a FM substitute in yellow perch diets.

Feed intake, growth and nutrient utilization

The results of the present study clearly show that the nutritive value of complete diets for yellow perch may vary when differently processed soybean meals (roasted SBM and EZM along with or without the inclusion of exogenous enzymes) are used to replace varying amounts of FM protein. In this experiment relative to control (fish meal based diet), palatability was not adversely affected by type of SBM (regular SBM or EZM) inclusion in the diet; however, the level of SBM inclusion adversely affected the feed intake. In general, feed intake has an inverse relationship to higher FM replacement levels with plant proteins in
omnivorous and carnivorous fish (Kader et al. 2011; Kader et al. 2012; Uyan et al. 2006). In the present experiment, feed intake was lower in the 100% FM replacement groups compared to the 50% replacement groups. However, there were no significant effects of supplementation of exogenous enzymes in either EZM or SBM based diets on feed intake and growth performance. In this study, there was a decreasing trend in feed intake with increasing dietary SBM and EZM level, similar to the findings of some previous studies (Espe et al. 2006; Kaushik et al. 2004). Kaushik et al. (2004) used a regression model, while Espe et al. (2006) used a total replacement of FM, adding different ingredients to increase voluntary feed intake. The former reported that feeding diets containing only 0.5% FM to European seabass did not affect feed intake and reduce performance on high inclusion of plant ingredients, while in the latter study only one of the groups reduced feed intake, but all reduced growth as compared to the FM control. These results indicate that the effects on the growth response might directly be attributed to the partly even significantly lower feed intake of fish fed the plant protein diets (Espe et al. 2006). Hence, in our study, reduced feed intake (consequently a lower intake of essential nutrients and digestible energy) could be the main reason for reduced growth performance of yellow perch as the dietary SBM and EZM level increased.

Reduced nutrient/energy bioavailability and growth in response to complete feeds containing high levels of conventionally-processed SBM and EZM, relative to FM-based diets, has been observed in almost all carnivorous and omnivorous fish species, including yellow perch investigated to date, and various causes and mechanisms have been claimed as possible reasons. Of these, oligosaccharides and NSPs in soy preparations have been suggested to lead to reduced bioavailability of all nutrients and energy through mechanisms involving a binding action with bile salts and/or by an obstructing action on digestive enzymes coupled with changes in digesta viscosity and transit rate (Francis et al. 2001; Tibaldi et al. 2006). This is supported by findings that soy-protein derivatives obtained
through processes, which ensure partial (50%) removal of non-starch carbohydrate fractions, like EZM or the enzyme-treated SBMs here tested, resulted in higher nutritive value relative to regular SBM when used at 50 and 100% replacement levels (Bureau et al. 1998; Hernandez et al. 2007; Kumar et al. 2010a; Kumar et al. 2010b; Kumar et al. 2011a; Kumar et al. 2011b; McGoogan & Gatlin 1997; Olli & Krogdahl 1994; Rumsey et al. 1994).

Deficiencies in minerals or essential amino acids may also have impacted the ability of perch to utilize soy products at levels greater than 500 g/kg (Brown et al. 1997; Kasper et al. 2007; Ketola 1975). In the present study, the use of the EZM with zero oligosaccharides to substitute up to 50% FM protein in the diet resulted in nutrient utilization (FCR, PER, PPV and LPV) values and growth performance, which did not differ from those measured in fish fed the control diet (Refstie et al. 1999). Our results are in concurrence with many studies (Denstadli et al. 2007; Glencross et al. 2005; Kaushik et al. 2004; Mambrini et al. 1999; Overland et al. 2009; Vielma et al. 2000), which have shown that soy, lupin, and pea protein isolates and soy protein concentrate can replace 50-75% of FM protein in rainbow trout and Atlantic salmon diets without impairing the growth performance and nutrient utilization. Médale et al. (1998) observed that total replacement of FM by soy protein concentrate led to a significant decrease in feed intake and resulted in poor growth partially due to methionine deficiency in the soy protein concentrate based diet.

Soybean meal fed groups exhibited lower growth performance compared to EZM fed groups. Hence, there seems to be some evidence that a higher intake of soy non-starch carbohydrates lowers the nutritive value of yellow perch diets, but the extent to which this depressive effect is attributable to soy oligosaccharides or NSPs could not be established in this experiment.

The weight gain data indicate that SBM/EZM inclusion had no positive impact on production when combined with supplemental exogenous cocktail enzymes (NSPase, phytase
and carbohydrase) but there is increasing trend (not significantly) towards growth and nutrient utilization of feed. Our results concur with other reports suggesting no adverse effects when these enzymes are added to feeds (Allan et al. 1998; Kocher et al. 2003; Stone et al. 2003). However, research with other species has shown a positive impact when a supplemental exogenous cocktail enzyme has been included in aquafeeds wherein a multienzyme (amylase, protease, β-glucanase, β-glucosidase, cellulose and combination of these enzymes) in feed significantly improved weight gain, FCR, and other nutritional parameters (Baas & Thacker 1996; Castañón et al. 1997; Cowieson et al. 2006; Denstadli et al. 2011). The mechanism by which exogenous multienzymes enhance nutrient digestion and utilization in plant proteins have not been identified, but disruption of cell wall integrity and the breakdown of the highly viscous NSP and carbohydrate were thought to be the major factors involved (Sinha et al. 2011). The supplemental exogenous enzymes had been shown to decrease digesta viscosity, feed and protein utilization, and improve digestibilities of amino acids, protein, and lipid in fish (Denstadli et al. 2011).

**Biochemical composition**

Efficient protein synthesis requires sufficient availability of all essential amino acids (Dabrowski & Guderly 2002; Kumar et al. 2011a; Kumar et al. 2011b). Unbalanced amino acid concentrations in a diet resulted in increased protein degradation (Langar et al. 1993; von der Decken & Lied 1993), and thereby increased protein turnover (Martin et al. 2003). The plant protein (SBM) based diets lower nitrogen retention in salmon and trout because these diets have less digestible energy and an amino acid profile that is suboptimal for muscle growth (Cheng et al. 2003; Kumar et al. 2011a; Kumar et al. 2011b; Pack et al. 1995; Refstie et al. 2000). A similar pattern was found in the present study. Crude protein content of whole yellow perch was lower in groups fed soy-based protein feeds with 100% FM replacement
than in the control and in the groups fed 50% FM protein, regardless the inclusion of exogenous enzymes. However, interestingly, crude protein content in the whole body was higher in the partially (50% replacement) soy based protein fed groups than in the 100% replacement groups. Similarly, Barrows et al. (2008) and Cheng et al. (2003) also found that the body protein content increased significantly when SBM replaced FM in trout diet. This indicates that the combination of 50% FM and 50% soy based protein diets contain optimum digestible energy and balanced amino acid profile optimal for yellow perch muscle growth.

In the present study, exogenous enzyme supplementation did not enhance nutrient utilization and nutrient deposition in yellow perch. Our results are in contrast with other studies, which showed that inclusion of exogenous enzymes in soy based diets increased protein synthesis in fish muscle (Ai et al. 2007; Danicke et al. 2003).

In this study, moisture content exhibited an inverse relationship with lipid content. A similar trend has been reported in a study by Hasan et al. (1997), wherein FM protein was replaced by plant protein such as mustard, sesame, linseed, copra, and groundnut oil cakes. Our study also showed that complete (100%) replacement of FM protein by soy protein exhibited lower lipid content in fish than the control group. This suggests that fish were utilizing the dietary lipid for energy purposes and the contribution/utilization of energy from the nitrogen-free extract was minimal. In addition, the change in the efficiency of lipid digestion and absorption from the diet may have contributed to the reduction in whole body total fat level. Partial (50%) replacement of FM protein by soy protein showed higher lipid content in fish than that of 100% FM replacement groups. Similarly, Hasan et al. (1997) and Mazurkiewicz (2009) observed that partial FM protein replaced by plant protein in fish diet exhibited higher lipid deposition. There is evidence that partial replacement of FM by plant protein sources such as corn gluten meal and soy protein concentrates increases hepatic lipogenic enzyme activities in seabass that leads to higher whole body lipid amounts (Dias
Another possible reason for higher lipid retention could be a higher supply of some of the dispensable amino acids such as glutamic acid in excess by the plant protein fed diets (Barrows et al. 2008; Kumar et al. 2011a; Kumar et al. 2011b). These authors also indicated the involvement of possible metabolic or endocrine mechanisms in eliciting such differences in whole body lipid deposition (Kumar et al. 2011a; Kumar et al. 2011b). In the present study, a higher value of HSI in the partial FM protein replacement groups suggests higher lipid deposition in liver. Hepatosomatic index values of above 1, as observed here, are common in yellow perch.

Digestive enzyme activities and relative intestinal length (RIL)

Digestion and absorption of nutrients depends on the activity of the digestive enzymes, in particular those located in the pyloric caeca and intestine, which are responsible for the breaking down and assimilation of the feed (Klein et al. 1998). The study of digestive enzymes in fish shows a wide range of potential benefits, suggesting that determining of the properties, function and optimal conditions for nutrients (carbohydrate, protein and lipid) hydrolysis of digestive enzymes would provide a more accurate measurement for carbohydrate, protein and lipid digestibility (Klein et al. 1998; Silva et al. 2010). Therefore, digestive enzyme activities are considered as predictors of potential feed utilization and growth differences in fish (Lin & Luo 2011).

The digestive enzymatic activity measured in the pyloric caeca was significantly lower than that in the intestinal digestive tract of yellow perch (Figure 2) because high enzymatic activity in the intestine plays an important role in absorption of the digested nutrients. Bowyer et al. (2013) observed the similar results that the lower digestive enzyme activity in the pyloric caeca than the distal intestine, it appears as a symptom of reduced functionality of the distal intestine and, thus, reduced capacity for reabsorption of digestive
secretions in salmon fed SBM based diets (Bowyer et al. 2013; Krogdahl et al. 2003). However, our results are in contrast with other studies (Harpaz & Uni 1999; Harpaz et al. 2005a; Harpaz et al. 2005b; Krogdahl et al. 1999), wherein they have observed more of the high enzymatic activity in the pyloric caeca than in the intestine.

In the present study, higher (>50% FM protein replacement) inclusion levels of soy protein (SBM and EZM) led to a marked reduction in the activities of digestive enzymes (amylase, protease, and lipase; except lipase activity in pyloric caeca); and these enzymatic activities were found to decrease with lowering SGR and increasing FCR, which show that digestive processes were affected in both the intestine and pyloric caeca with increasing dietary soy protein (SBM and EZM) levels. Current results suggest that enzyme capacity of fish could be improved using nutrients (low dietary inclusion levels of soy protein, 50% FM protein replacement) that stimulate enzyme secretion. Similar results were observed in Atlantic cod and Atlantic salmon (Bakke-Mckellep et al. 2000; Bowyer et al. 2013; Bureau et al. 1998; Hidalgo et al. 1999; Krogdahl et al. 1995; Krogdahl et al. 2003; Lemieux et al. 1999). However, high percentages of soy protein (100% replacement of FM by SBM and EZM) led to decreased digestive enzyme activity, and major reasons could be the presence of heat stable antinutrients (phytate, oligosaccharides, and NSPs) in soy based diets. Phytate is known to inhibit activities of digestive enzymes such as pepsin, trypsin, and alpha-amylase (Alarcon et al. 1999; Kumar et al. 2010a; Kumar et al. 2011a; Kumar et al. 2011b; Robaina et al. 1995), or to form complexes with minerals (Sugiura et al. 1999; Teskeredzic et al. 1995) and proteins (Moyano et al. 1999), thereby modifying digestion processes and impairing intestinal absorption. High content of oligosaccharides and NSP in SBM (15% NSP) and EZM (7% NSP) leads to a significant reduction in the activities of digestive enzymes in the intestine and pyloric caeca (Baeverfjord & Krogdahl, 1996; Bakke-Mckellep et al. 2000; Bureau et al. 1998; Krogdahl et al. 1995; Krogdahl et al. 2003; Tibaldi et al. 1999).
2006) and even to diminishing of the carrier-mediated nutrient transport/absorption ability (Nordrum et al. 2000; Tibaldi et al. 2006). The activity of the intestinal enzymes could provide further insight on possible effects of differently modified SBMs on nutrient bioavailability and on the sensitivity of yellow perch intestinal mucosa functions to certain soy protein ANFs.

There was no significant effect of soy protein (SBM and EZM) levels on amylase activity in pyloric caeca of yellow perch. Similar observation was reported by various researchers (Lopez-Lopez et al. 2005; Lin & Luo et al. 2011) in other fish species. It is suggested that animals have the capacity to adapt their digestive physiology in response to changes in their nutrient requirements or dietary profile to some extent.

The exogenous enzymes (mixture of NSPase, phytase, and carbohydraz) had been shown to decrease digesta viscosity and improve digestibilities of amino acids, protein, lipid, and starch in domestic animals and fish (Ai et al. 2007; Bedford 1995; Classen 1996; Cowieson et al. 2006; Sinha et al. 2011; Kumar et al. 2012b). Also, addition of those enzymes will enhance the utilization of phytate, NSP, and oligosaccharides, which result in higher utilization of protein. However, endogenous digestive enzymes, important for digestion of nutrients, showed contradictory tendency; for example, secretion of pancreatic juice and endogenous digestive enzyme activities increased with increasing viscosity due to viscous NSP (Danicke et al. 2003; Ikegami et al. 1990). Supplementation of exogenous enzymes significantly decreased the activities of protease, chymotrypsin, amylase, and lipase in fish (Danicke et al. 2003; Li et al. 2004). However, in our study, supplementation of exogenous enzymes (mixture of NSPase, phytase, and carbohydraz) did not change digestive enzyme activity (except EZM50+E group) in pyloric caeca and intestine. The exact mechanism is not clearly understood in fish. More studies should be conducted to examine the
mechanism by which feed utilization could be improved with exogenous enzyme supplementation.

Overall, our study suggests that measuring the activities of digestive enzymes may represent a sensitive tool to study the effects of differently-processed SBMs on nutrient bioavailability and to ascertain tolerability to soy-oligosaccharides in yellow perch. The lower activity of digestive enzymes in soy protein (SBM and EZM) fed groups were correlated with lower nutrient utilization parameters (PER, PPV, and LPV).

It is well known that carnivorous and omnivorous fish require longer time to digest plant protein based diets compared to animal protein based diets (Buddington et al. 1997; Kumar et al. 2011a; Kumar et al. 2011b). A direct relationship between the amount of dietary plant protein and RIL has been reported earlier in fish (Kramer & Bryant 1995; Kumar et al. 2011a; Kumar et al. 2011b). In yellow perch, soy protein (SBM and EZM) based diets exhibited higher RIL than the control group. RIL value increases as the soy protein (SBM and EZM) inclusion increases in the fish diets (Overland et al. 2009). From a physiological view point, a longer RIL would facilitate an increase in digestibility and retention time by enhancing contact time of the digestive enzymes and the feed components, resulting in an increase in their digestion and absorption (Kumar et al. 2011a; Kumar et al. 2011b). Omnivorous fish such as yellow perch species showed compensation mechanisms, such as an increase in RIL, and as a result increase in digestive activity, to achieve a digestive balance and growth rates similar to those observed for the FM fed group.

Protein metabolism enzymes

Alanine aminotransferase and AST are two enzymes for protein metabolism, mostly active in the liver, which are quantitatively important in transamination of amino acids. Alanine aminotransferase and AST activities in liver were significantly different among the groups
but supplementation of exogenous enzymes in soy based diets did not change the aminotransferase (ALT and AST) activity in fish. In the present study these enzyme activities were positively correlated with growth performance and feed utilization. In the present study, the activity of protein metabolism enzymes ALT and AST in liver was reduced with increasing dietary SBM and EZM levels, which indicated the utilization of dietary protein decreased and the liver was damaged to a certain extent (Lin et al. 2007; Luo 2011). Aminotransferases, such as ALT and AST, catabolize amino acids and transfer amino groups to alpha-keto acids (reversible catalysis) but when the available amino acids are deficient, the keto acids may be reduced, thereby reducing the activity of ALT and AST (Lin and Luo, 2011). Decreasing liver AST and ALT activity and a tendency to lower liver ALT and AST activity in fish given diets containing high level of soy protein compared to the low level of soy protein based diets, indicate reduction in substrates available for transamination as a consequence of reduced dietary levels of these amino acids (Lin & Luo 2011).

Antioxidant enzymes

Reactive oxygen species (ROS), which include hydroxyl radical, superoxide anion, hydrogen peroxide, and singlet oxygen, are physiologically generated in a series of biochemical reactions within cellular compartments and increase in physiological conditions that result in oxidative stress (Dirks et al. 1982; Radhakrishnan et al. 2014). The increased levels of ROS may lead to irreversible cell damage and eventually to cell death. Antioxidant enzymes play a crucial role in the defense against oxidative cell damage, through catalyzing the breakdown of superoxide anion to oxygen and hydrogen peroxide (McCord & Fridovich, 1988; Radhakrishnan et al. 2014). In normal cells, there exists a delicate balance between the prooxidant forces and antioxidant defenses known as redox balance. Previously some
researchers have reported that the overwhelming of antioxidant defenses of cells by prooxidants leads to oxidative stress (Radhakrishnan et al. 2014).

The nutrient metabolism alterations induced by NSPs (from SBM and EZM) in fish nutrition, like those observed in Nile tilapia, *Oreochromis niloticus*, and African catfish, in which intestinal volatile fatty acid (VFA) production was increased by NSPs (Amirkolaie et al. 2006; Enes et al. 2012; Leenhouwers et al. 2007; Sinha et al. 2011), which may also induce a modification in normal cellular oxidative balance. To minimize ROS related cell damage, fish and other aerobic organisms possess antioxidant defense enzymatic mechanisms, including the key enzymes with antioxidant activity (SOD, CAT and GPx) (Enes et al. 2012; Halliwell & Gutteridge, 2007; Martinez-Alvarez et al. 2005).

In the present study, the activities of antioxidant enzymes (SOD, CAT and GPx) in partial (50% FM replaced by soy protein) replacement groups did not show any significant increase when compared with the control group; whereas 100% replacement groups exhibited higher antioxidant enzyme activity than other groups. Antioxidant enzyme activities in the liver increased as dietary soy protein increased, which suggests a rise in O$_2^-$ generation related to increased levels of NSP and oligosaccharides in soy based diets. It is known that NSP (from SBM and EZM) tends to increase digesta viscosity and thus delay gastric emptying rate in fish (Amirkolaie et al. 2005; Enes et al. 2012; Leenhouwers et al. 2006; Sinha et al. 2011; Storebakken 1985). This delay of digesta passage through the gut may stimulate NSPs fermentation by intestine microbiota, thus leading to an increase of VFA production. These VFA might have been mobilized for energy production, leading to a rise of O$_2^-$ levels and thus an increase of antioxidant enzyme activity (Choct & Kocher 2000; Enes et al. 2012). Another possibility for the suggested rise of O$_2^-$ levels would be an increase in intestinal mucosal cell turnover and consequently, the need of energy supply for the process in fish fed increasing levels of SBM (NSP) (Enes et al. 2012; Jin et al. 1999; Sinha et al. 2011).
Similar results were observed in other studies wherein plant-containing diets increased the hepatic glutathione redox status as well as antioxidant enzyme activities in fish (Enes et al., 2012). This result indicated that high inclusion of soy protein (SBM and EZM) may exert oxidative stress on yellow perch. In order to eliminate excess free radicals, the fish increased antioxidant enzyme activities to protect the body under normal function (Enes et al. 2012; Valko et al. 2007). In liver, this was evident by the increase of the GPx activity in a dose-dependent manner with plant protein increment. This higher antioxidant activity could be due to some of the plant constituents such as flavonoids present in soy based diets. Little information about such mechanisms in fish was found in the literature, therefore further research is warranted.

In the present study, dietary supplementation of exogenous enzymes did not significantly change the activities of antioxidant enzymes (SOD, CAT, and GPx) in fish, whereas, Zhu et al. (2014) found that supplementation of exogenous enzymes significantly reduced SOD and CAT activities in fish. This suggests that exogenous enzymes could protect the fish body from cell oxidative damage when fed mainly with plant source-protein. Another reason is that the enhancement of aerobic metabolism due to dietary phytase supplementation improves the gastrointestinal digestive enzymes (Nwanna, 2007; Zhu et al. 2014), and could lead to the increased risk of oxidative stress (Martinez-Alvarez et al. 2005; Zhu et al. 2014). However, the real mechanisms underlying the dietary exogenous enzymes actions remain largely speculative and further research is required.

Conclusions

EnzoMeal (EZM) containing 580 g kg\(^{-1}\) crude protein and no anti-nutritional factors, has been evaluated as an alternative for fishmeal in the diet of yellow perch with significant success. Higher growth performance and feed utilization was observed for 50% replacement of FM by
EJM fed groups compared to 100% replacement of FM by SBM and EJM fed groups. The activity of protein metabolism enzymes AST and ALT was positively correlated with growth performance and nutrient utilization parameters, whereas antioxidant enzymes (SOD, CAT, and GPx) exhibited an opposite trend. The supplementation of exogenous enzymes (mixture of NSPase, phytase, and carbohydrase) did not exhibit any positive effects on growth performance, nutrient utilization, digestive physiology, protein metabolism, and oxidative status in yellow perch. EnzoMeal with high protein and no ANFs has considerable potential as alternatives to fishmeal in aquafeed. It can be used as one of the promising fishmeal replacers in high-protein fish feeds and substitute 50% - 100% of FM protein without sacrificing fish yield.

Acknowledgments

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*Aquaculture, 319*, 391–397.


(Salmo salar L.) and rainbow trout (Oncorhynchus mykiss). Comp. Biochem. Physiol., B **125**, 317–335.


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41


http://dx.doi.org/10.1016/j.jobaz.2013.12.003


1 Table 1 Composition of the experimental diets (g kg\(^{-1}\) dry matter basis of ingredients) for yellow perch (*Perca flavescens*) fingerlings.

<table>
<thead>
<tr>
<th>Experimental diets*</th>
<th>Control</th>
<th>S50</th>
<th>S50+E</th>
<th>S100</th>
<th>S100+E</th>
<th>EZM50</th>
<th>EZM50+E</th>
<th>EZM100</th>
<th>EZM100+E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menhaden fish meal(^a)</td>
<td>480.0</td>
<td>240.0</td>
<td>240.0</td>
<td>-</td>
<td>-</td>
<td>240.0</td>
<td>240.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Wheat meal(^b)</td>
<td>340.8</td>
<td>219.0</td>
<td>219.0</td>
<td>90.0</td>
<td>90.0</td>
<td>284.0</td>
<td>284.0</td>
<td>218.0</td>
<td>218.0</td>
</tr>
<tr>
<td>Soybean meal (SBM)</td>
<td>-</td>
<td>325.0</td>
<td>325.0</td>
<td>650.0</td>
<td>650.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EnzOMeal (EZM)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>269.0</td>
<td>269.0</td>
<td>538.0</td>
<td>538.0</td>
</tr>
<tr>
<td>Soy protein isolate(^c)</td>
<td>-</td>
<td>16.0</td>
<td>16.0</td>
<td>28.0</td>
<td>28.0</td>
<td>7.0</td>
<td>7.0</td>
<td>12.0</td>
<td>12.0</td>
</tr>
<tr>
<td>Menhaden oil(^d)</td>
<td>50.0</td>
<td>78.0</td>
<td>78.0</td>
<td>107.0</td>
<td>107.0</td>
<td>78.0</td>
<td>78.0</td>
<td>107.0</td>
<td>107.0</td>
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<tr>
<td>Soybean oil(^e)</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Vitamin premix(^f)</td>
<td>30.0</td>
<td>30.0</td>
<td>30.0</td>
<td>30.0</td>
<td>30.0</td>
<td>30.0</td>
<td>30.0</td>
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<tr>
<td>Mineral premix(^g)</td>
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<td>40.0</td>
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<tr>
<td>TiO(_2)(^h)</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
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<tr>
<td>Methionine(^i)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.0</td>
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<td>-</td>
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<td>3.0</td>
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<tr>
<td>Enzyme cocktail(^j)</td>
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<td>+</td>
<td>-</td>
<td>+</td>
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<td><strong>1000</strong></td>
<td><strong>1000</strong></td>
<td><strong>1000</strong></td>
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</table>

3 Treatments*
4 Control - Fish meal (major source of protein)
5 S50 - 50% fish meal protein replaced by SBM
6 S50+E - 50% fish meal protein replaced by SBM + enzymes (cocktail)
7 S100 - 100% fish meal protein replaced by SBM
8 S100+E - 100% fish meal protein replaced by SBM + enzymes (cocktail)
9 EZM50 - 50% fish meal protein replaced by EZM
10 EZM50+E - 50% fish meal protein replaced by EZM + enzymes (cocktail)
11 EZM100 - 100% fish meal protein replaced by EZM
12 EZM100+E - 100% fish meal protein replaced by EZM + enzymes (cocktail)
13
14 *IPC 740; International Proteins Corp., Minneapolis, Minnesota, USA.
15 *Whole wheat meal; Bob’ s Red Mill Natural Foods, Inc. Milwaukie, OR, USA.
16 *Solaes, LLC, St. Louis, USA.
17 *Virginia Prime; Omega Protein, Inc., Reedville, Virginia, USA.
18 *Product number OF1870E; Consumers Supply Distributing, Sioux City, Iowa, USA.
19 *Test Diet; Land O’Lakes Purina Feed, Richmond, Indiana. Pantothenic acid, 4,601 mg/kg; niacin, 5,000 mg/kg; riboflavin, 3,000 mg/kg; vitamin B12, 200 μg/kg; vitamin B6, 17,500 mg/kg.
20 *Test Diet; Land O’Lakes Purina Feed. Calcium, 8.00%; phosphorus, 8.00%; potassium, 5.00%; magnesium, 1.33%; sodium, 3.48%; chloride, 2.80%; fluorine, 144 mg/kg; iron, 1,600 mg/kg; zinc, 1.091 mg/kg; manganese, 276 mg/kg; copper, 126.2 mg/kg; cobalt, 248.1 mg/kg; iodine, 114.68 mg/kg; chromium, 8.0 mg/kg; molybdenum, 5.61 mg/kg; and selenium, 0.02 mg/kg.
21 and Sigma Aldrich, Saint Louis, MO, USA.
Table 2 Proximate compositions of feed ingredients and experimental diets (% dry matter basis).

<table>
<thead>
<tr>
<th>Feed ingredients</th>
<th>Dry matter</th>
<th>Crude protein</th>
<th>Crude lipid</th>
<th>Nitrogen free extract</th>
<th>Ash</th>
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<tr>
<td>Fish meal</td>
<td>92.45</td>
<td>67.05</td>
<td>10.77</td>
<td>4.45</td>
<td>10.18</td>
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<tr>
<td>Soybean meal</td>
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<td>47.45</td>
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<td>EnzoMeal</td>
<td>91.05</td>
<td>58</td>
<td>1.87</td>
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<td>6.28</td>
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<td>Soy protein isolate</td>
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<td>91.03</td>
<td>0.74</td>
<td>0.77</td>
<td>4.05</td>
</tr>
<tr>
<td>Whole wheat meal</td>
<td>90.19</td>
<td>11.85</td>
<td>2.15</td>
<td>74.53</td>
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<table>
<thead>
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<th>Experimental diets*</th>
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<th></th>
<th></th>
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</tr>
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<tbody>
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<td>Control</td>
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<td>14.48</td>
<td>27.41</td>
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<tr>
<td>S50</td>
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<td>41.94</td>
<td>14.56</td>
<td>30.49</td>
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<tr>
<td>S50+E</td>
<td>94.84</td>
<td>40.48</td>
<td>15.29</td>
<td>30.47</td>
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<td>S100</td>
<td>94.41</td>
<td>41.01</td>
<td>14.34</td>
<td>33.23</td>
<td>5.83</td>
</tr>
<tr>
<td>S100+E</td>
<td>95.91</td>
<td>42.16</td>
<td>14.52</td>
<td>33.52</td>
<td>5.71</td>
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<td>42.30</td>
<td>14.9</td>
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<td>8.17</td>
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<tr>
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<td>95.27</td>
<td>41.52</td>
<td>15.35</td>
<td>29.71</td>
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<td>41.91</td>
<td>15.09</td>
<td>31.94</td>
<td>4.56</td>
</tr>
<tr>
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<td>42.03</td>
<td>15.27</td>
<td>33.23</td>
<td>4.21</td>
</tr>
</tbody>
</table>

* See footnotes to Table 1.
Table 3
Chemical composition of whole body (at the start and at the end of the experiment, g kg\(^{-1}\) wet basis ± SD), relative intestinal length (RIL, mm g\(^{-1}\)), hepato somatic index (HSI) and feed intake of yellow perch (Perca flavescens) fingerlings of different experimental groups.

<table>
<thead>
<tr>
<th>Moisture</th>
<th>Crude protein</th>
<th>Crude lipid</th>
<th>Ash</th>
<th>Calcium</th>
<th>Phosphorus</th>
<th>Magnesium</th>
<th>Potassium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial fish</td>
<td>73.79 ± 0.55</td>
<td>14.55 ± 0.58</td>
<td>3.60 ± 0.34</td>
<td>4.69 ± 0.88</td>
<td>1.69 ± 0.01</td>
<td>2.16 ± 0.01</td>
<td>0.02 ± 0.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Moisture</th>
<th>Crude protein</th>
<th>Crude lipid</th>
<th>Ash</th>
<th>RIL</th>
<th>HSI</th>
<th>GSI</th>
<th>Feed intake (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>67.96 ± 1.19(^b)</td>
<td>16.32 ± 0.71(^{ab})</td>
<td>9.02 ± 0.35(^{ab})</td>
<td>3.86 ± 1.52</td>
<td>0.49 ± 0.03(^c)</td>
<td>1.79 ± 0.37(^{ab})</td>
<td>2.49 ± 0.05</td>
<td>36.96 ± 0.86(^a)</td>
</tr>
<tr>
<td>S(_{50})</td>
<td>68.03 ± 1.38(^b)</td>
<td>16.95 ± 0.86(^{ab})</td>
<td>7.27 ± 3.16(^{ab})</td>
<td>4.79 ± 0.57</td>
<td>0.64 ± 0.02(^a)</td>
<td>1.32 ± 0.41(^c)</td>
<td>2.07 ± 0.22</td>
<td>36.45 ± 0.77(^a)</td>
</tr>
<tr>
<td>S(_{50}+E)</td>
<td>70.86 ± 2.80(^{ab})</td>
<td>17.77 ± 1.05(^{ab})</td>
<td>6.20 ± 1.26(^b)</td>
<td>5.09 ± 1.68</td>
<td>0.66 ± 0.01(^a)</td>
<td>1.61 ± 0.41(^b)</td>
<td>2.43 ± 0.64</td>
<td>35.62 ± 2.93(^a)</td>
</tr>
<tr>
<td>S(_{100})</td>
<td>73.63 ± 1.19(^a)</td>
<td>14.67 ± 0.89(^{b})</td>
<td>5.15 ± 0.91(^{c})</td>
<td>3.67 ± 0.71</td>
<td>0.61 ± 0.05(^{ab})</td>
<td>1.41 ± 0.21(^b)</td>
<td>2.75 ± 0.28</td>
<td>18.32 ± 0.85(^b)</td>
</tr>
<tr>
<td>S(_{100}+E)</td>
<td>73.37 ± 2.76(^{ab})</td>
<td>15.16 ± 2.24(^{b})</td>
<td>7.64 ± 1.34(^{b})</td>
<td>4.92 ± 0.41</td>
<td>0.64 ± 0.04(^a)</td>
<td>1.54 ± 0.05(^{b})</td>
<td>2.84 ± 0.29</td>
<td>18.98 ± 0.78(^b)</td>
</tr>
<tr>
<td>EZM(_{50})</td>
<td>69.15 ± 1.29(^{ab})</td>
<td>17.97 ± 0.53(^a)</td>
<td>9.65 ± 0.26(^{a})</td>
<td>4.28 ± 1.02</td>
<td>0.55 ± 0.05(^{b})</td>
<td>1.91 ± 0.06(^{a})</td>
<td>2.20 ± 0.24</td>
<td>37.34 ± 1.06(^a)</td>
</tr>
<tr>
<td>EZM(_{50}+E)</td>
<td>69.56 ± 0.84(^{ab})</td>
<td>18.04 ± 0.84(^a)</td>
<td>5.87 ± 0.25(^{b})</td>
<td>4.41 ± 0.47</td>
<td>0.59 ± 0.04(^{b})</td>
<td>2.13 ± 0.06(^{a})</td>
<td>2.25 ± 0.32</td>
<td>39.09 ± 0.77(^a)</td>
</tr>
<tr>
<td>EZM(_{100})</td>
<td>72.86 ± 1.97(^{ab})</td>
<td>14.59 ± 1.52(^{b})</td>
<td>4.30 ± 0.19(^{c})</td>
<td>5.62 ± 0.91</td>
<td>0.61 ± 0.02(^{ab})</td>
<td>2.05 ± 0.63(^{a})</td>
<td>2.64 ± 0.77</td>
<td>19.46 ± 0.62(^b)</td>
</tr>
<tr>
<td>EZM(_{100}+E)</td>
<td>71.21 ± 2.73(^{ab})</td>
<td>14.42 ± 1.16(^{b})</td>
<td>4.96 ± 0.52(^{c})</td>
<td>3.96 ± 0.79</td>
<td>0.60 ± 0.06(^{ab})</td>
<td>1.29 ± 0.04(^{c})</td>
<td>2.40 ± 0.24</td>
<td>21.67 ± 2.13(^b)</td>
</tr>
</tbody>
</table>

* See footnotes to Table 1

Values are mean (n = 3) ± standard deviation. Mean values in the same column with different superscript differ significantly (P < 0.05).
Table 4

Growth performance of Yellow perch (*Perca flavescens*) fingerlings fed with the experimental diets for ten weeks.

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Initial weight (g)</th>
<th>Final weight (g)</th>
<th>Weight gain (g)</th>
<th>Body mass gain (%)</th>
<th>Metabolic growth rate (g kg(^{-0.8}) day(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.96 ± 0.06</td>
<td>39.96 ± 2.42(^{ab})</td>
<td>29.00 ± 2.41(^{ab})</td>
<td>264.65 ± 21.88(^{ab})</td>
<td>8.03 ± 0.38(^{ab})</td>
</tr>
<tr>
<td>S(_{30})</td>
<td>11.04 ± 0.07</td>
<td>33.13 ± 2.30(^{cd})</td>
<td>22.09 ± 2.25(^{cd})</td>
<td>199.98 ± 19.43(^{cd})</td>
<td>6.80 ± 0.42(^{c})</td>
</tr>
<tr>
<td>S(_{50})+E</td>
<td>10.91 ± 0.05</td>
<td>36.90 ± 4.88(^{bc})</td>
<td>25.99 ± 4.65(^{bc})</td>
<td>237.11 ± 74.87(^{bc})</td>
<td>7.42 ± 1.49(^{bc})</td>
</tr>
<tr>
<td>S(_{100})</td>
<td>10.98 ± 0.07</td>
<td>22.04 ± 1.65(^{c})</td>
<td>11.06 ±1.59(^{c})</td>
<td>100.71 ± 13.95(^{c})</td>
<td>4.24 ± 0.45(^{c})</td>
</tr>
<tr>
<td>S(_{100})+E</td>
<td>10.99 ± 0.11</td>
<td>22.51 ± 2.54(^{c})</td>
<td>11.52 ± 2.38(^{c})</td>
<td>104.85 ± 23.82(^{c})</td>
<td>4.35 ± 0.76(^{c})</td>
</tr>
<tr>
<td>EZM(_{50})</td>
<td>11.16 ± 0.14</td>
<td>40.02 ± 0.63(^{ab})</td>
<td>28.85 ± 0.66(^{ab})</td>
<td>258.50 ± 7.28(^{ab})</td>
<td>7.96 ± 0.12(^{ab})</td>
</tr>
<tr>
<td>EZM(_{50})+E</td>
<td>11.06 ± 0.13</td>
<td>45.38 ± 1.70(^{c})</td>
<td>34.32 ± 1.78(^{c})</td>
<td>310.28 ± 18.54(^{c})</td>
<td>8.79 ± 0.27(^{c})</td>
</tr>
<tr>
<td>EZM(_{100})</td>
<td>10.71 ± 0.14</td>
<td>24.91 ± 0.85(^{c})</td>
<td>14.20 ± 1.18(^{c})</td>
<td>133.02 ± 15.78(^{de})</td>
<td>5.16 ± 0.38(^{de})</td>
</tr>
<tr>
<td>EZM(_{100})+E</td>
<td>11.18 ± 0.12</td>
<td>27.89 ± 1.71(^{de})</td>
<td>16.72 ± 1.61(^{de})</td>
<td>149.54 ± 13.48(^{de})</td>
<td>5.64 ± 0.35(^{de})</td>
</tr>
</tbody>
</table>

* See footnotes to Table 1.

Values are mean (n = 3) ± standard deviation. Mean values in the same column with different superscript differ significantly (P < 0.05).
Figure legends

Fig. 1. (A) Specific growth rate (%/day) and (B) antioxidant enzyme (Glutathione peroxidase) activity in liver of yellow perch (*Perca flavescens*). Values are mean (n = 3) ± standard deviation. Mean values with different superscript differ significantly (P < 0.05).

Fig. 2. Nutrient utilization parameters (A) feed conversion ratio and protein efficiency ratio, (B) protein productive value and lipid productive value of yellow perch (*Perca flavescens*). Values are mean (n = 3) ± standard deviation. Mean values with different superscript differ significantly (P < 0.05).

Fig. 3. Digestive enzymes (A) amylase (B) protease and (C) lipase activity in pyloric caeca and intestine of yellow perch (*Perca flavescens*). Values are mean (n = 3) ± standard deviation. Mean values with different superscript differ significantly (P < 0.05).

Fig. 4. (A) Protein metabolism enzymes (alanine amino transferase, ALT and aspartate transferase, AST) activity in liver, (B) antioxidant enzymes (superoxide dismutase and catalase) activity in liver of yellow perch (*Perca flavescens*). Values are mean (n = 3) ± standard deviation. Mean values with different superscript differ significantly (P < 0.05).