Effects of Non-Starch Polysaccharide Enzyme Supplementation in Diets Containing Varying Levels of Corn Dried Distillers Grains with Solubles on Growth Performance, Nutrient Digestibility, and Antioxidant Capacity of Juvenile Turbot (Scophthalmus maximus) Postprint

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Abstract

This study aimed to investigate the effects of non-starch polysaccharide enzyme supplementation in diets containing different levels of distillers dried grains with solubles (DDGS) on growth performance, nutrient digestibility, and antioxidant capacity of juvenile turbot. Seven hundred twenty juvenile turbot with an initial body weight of (13.00$±$0.01) g were randomly distributed into eight groups with three replicates per group and 30 fish per replicate. The D3, D6, D9, and D12 groups served as non-supplemented controls, fed experimental diets in which 3%, 6%, 9%, and 12% of fish meal in the basal diet was replaced by DDGS, respectively. The D3+, D6+, D9+, and D12+ groups were enzyme-supplemented treatments, fed experimental diets in which 3%, 6%, 9%, and 12% of fish meal in the basal diet was replaced by DDGS and supplemented with xylanase (activity 120,163 IU/g, inclusion rate 20 g/t) and cellulase (activity 13,424 IU/g, inclusion rate 300 g/t). The feeding trial lasted 9 weeks. The results showed that when fed diets with identical DDGS content, the enzyme-supplemented groups exhibited higher final body weight, weight gain rate, specific growth rate, and feed efficiency compared with the non-supplemented control groups, though these differences were not statistically significant ($P>0.05$). No significant differences were observed among all groups in feed intake or whole-body moisture, crude protein, and crude lipid contents ($P>0.05$), nor in condition factor, hepatosomatic index, or viserosomatic index ($P>0.05$). When fed diets with identical DDGS content, the enzyme-supplemented groups demonstrated higher apparent digestibility coefficients for

dry matter and crude protein relative to the non-supplemented control groups; specifically, apparent dry matter digestibility in the D9+ group was significantly elevated compared with the D9 group (P<0.05), apparent crude protein digestibility in the D3+ group was significantly elevated compared with the D3 group (P<0.05), and apparent crude protein digestibility in the D12+ group was significantly elevated compared with the D12 group (P<0.05). When fed diets with identical DDGS content, the enzyme-supplemented groups exhibited significantly enhanced serum superoxide dismutase and glutathione peroxidase activities (P<0.05) and significantly reduced serum malondialdehyde content (P<0.05) compared with the non-supplemented control groups. It was concluded that dietary non-starch polysaccharide enzyme supplementation in diets with varying DDGS levels improved feed utilization in juvenile turbot.

Full Text

Effects of Varying Dietary Levels of Corn Distillers Dried Grains with Solubles (DDGS) Supplemented with Non-Starch Polysaccharide Enzymes on Growth Performance, Nutrient Apparent Digestibility, and Antioxidant Capacity of Juvenile Turbot (Scophthalmus maximus L.)

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Abstract

This experiment investigated the effects of corn distillers dried grains with solubles (DDGS) diets at varying inclusion levels, supplemented with non-starch polysaccharide enzymes, on the growth performance, nutrient apparent digestibility coefficients, and antioxidant capacity of juvenile turbot (Scophthalmus maximus L.). Seven hundred twenty juvenile turbots with an initial body weight of (13.00$±$0.01) g were randomly assigned to eight experimental groups, with three replicates per group and thirty fish per replicate. The D3, D6, D9, and D12 groups served as enzyme-free controls, receiving diets in which corn DDGS replaced 3%, 6%, 9%, and 12% of fish meal, respectively. The D3+, D6+, D9+, and D12+ groups were enzyme-supplemented treatments, receiving the same DDGS replacement diets further supplemented with 20 g/t xylanase (120,163 IU/g) and 300 g/t cellulase (13,424 IU/g). The trial lasted nine weeks. Results showed that final body weight, weight gain rate, specific growth rate, and feed efficiency in enzyme-supplemented groups were higher than those in enzyme-free control groups at the same DDGS level, though differences were not statistically significant (P>0.05). Feed intake, whole-body moisture, crude protein and crude lipid contents, condition factor, hepatoso-

matic index, and viscerosomatic index were not significantly affected by dietary enzyme supplementation (P>0.05). Apparent digestibility coefficients of dry matter and crude protein in enzyme-supplemented groups were higher than those in control groups at the same DDGS level. Specifically, dry matter apparent digestibility in the D9+ group was significantly higher than in the D9 group (P<0.05), crude protein apparent digestibility in the D3+ group was significantly higher than in the D3 group (P<0.05), and crude protein apparent digestibility in the D12+ group was significantly higher than in the D12 group (P<0.05). At the same DDGS level, enzyme-supplemented groups exhibited significantly increased serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities (P<0.05) and significantly decreased serum malondialdehyde (MDA) content (P<0.05) compared to enzyme-free controls. In conclusion, supplementation with non-starch polysaccharide enzymes improved the utilization of corn DDGS diets in juvenile turbot.

Key words: juvenile turbot; corn DDGS; non-starch polysaccharide enzymes; growth performance; nutrient apparent digestibility; antioxidant capacity

Introduction

Previous studies have demonstrated that corn DDGS is rich in yeast cells, B vitamins, unidentified growth factors produced during fermentation, and carbohydrates, conferring high feeding value. Currently, diets with low corn DDGS content have been widely applied in livestock, poultry, and aquaculture production. Due to its low cost, corn DDGS is commonly used as a dietary protein source to reduce feed costs in the feed industry. However, its application is limited by several factors. First, different raw material sources and processing techniques result in significant variations in nutritional composition and utilization rates among corn DDGS products. Second, the production process typically employs low-temperature liquefaction, where liquefying amylase is added during corn fermentation to convert starch directly into sugars. As nearly all carbohydrates in corn are fermented into alcohol, the protein and fat content become concentrated in DDGS, while non-starch polysaccharide content also increases substantially, with xylan content reaching 18% and cellulose content reaching 8%.

The digestive tract of carnivorous fish cannot secrete xylanase endogenously, and xylan can significantly affect nutrient absorption and utilization through its anti-nutritional effects. Research has shown that when xylan content is high in the digesta of farmed animals, xylan molecules interact to form a gel-like structure that increases chyme viscosity, thereby slowing intestinal transit. Xylan can also complex with digestive enzymes in the intestine, preventing enzymatic reactions with substrates and delaying digestion. Additionally, due to its high water-holding capacity, xylan forms a thick unstirred water layer on the intestinal mucosa, hindering nutrient diffusion and normal absorption. Furthermore,

slow passage of high-viscosity chyme provides a stable environment for bacterial growth and reproduction, allowing intestinal bacterial colonization and intensifying competition with the host for nutrients. The result is reduced feed flow and intake, limiting nutrient assimilation efficiency. Similarly, cellulose, in the form of cell wall structures, encapsulates nutrients, preventing contact between substrates and enzymes, increasing endogenous nutrient losses, and reducing nutrient utilization rates. Studies have shown that feed enzymes can supplement deficiencies in endogenous digestive enzymes, eliminate anti-nutritional factors such as xylan and cellulose, and significantly improve feed conversion rates and nutrient utilization. Current research on corn DDGS has focused primarily on production effects, while studies on enzyme supplementation in diets with varying DDGS contents remain scarce.

Turbot (*Scophthalmus maximus* L.), commonly known as "Duobao fish," is a valuable cold-water economic fish species. Due to its rapid growth, delicious meat, and high economic value, it has become an important aquaculture species in northern China. This study selected turbot as the research subject and added xylanase and cellulase to diets with varying corn DDGS contents. By investigating growth performance, nutrient digestibility, and antioxidant capacity, we aimed to comprehensively evaluate the application effects of non-starch polysaccharide enzymes in high-DDGS diets and provide a scientific basis for the rational development and application of enzyme preparations.

1.1 Experimental Diets

Fish meal, corn DDGS, wheat gluten meal, and wheat flour served as the main protein sources, while fish oil and soybean lecithin served as lipid sources to formulate the basal diet. Eight experimental diets were formulated: four enzyme-free control diets (D3, D6, D9, and D12) in which corn DDGS replaced 3%, 6%, 9%, and 12% of fish meal in the basal diet, respectively; and four enzyme-supplemented diets (D3+, D6+, D9+, and D12+) in which corn DDGS replaced the same proportions of fish meal and were supplemented with 20 g/t xylanase (activity: 120,163 IU/g) and 300 g/t cellulase (activity: 13,424 IU/g). Both enzymes were provided by Beijing Challenge Bio-Technology Co., Ltd. Corn DDGS was provided by Great Seven Bio-Tech (Qingdao) Co., Ltd., and its essential amino acid composition and nutrient levels are shown in Table 1 . The composition and nutrient levels of the experimental diets are shown in Table 2 .

All ingredients were ground to pass through an 80-mesh sieve and mixed sequentially according to the formulation in Table 2 from smallest to largest quantity, then thoroughly mixed with oil, and finally kneaded with 30% distilled water before pelleting. After drying and cooling, the diets were sealed in double-layered plastic bags and stored at -20 °C until use.

1.2 Experimental Fish and Culture Management

Juvenile turbot used in the experiment were obtained from Huangshui Aquatic Company in Haiyang City, Shandong Province, and were from the same batch of artificially bred seedlings that year. The culture trial was conducted at the aquaculture base of Yihai Feng Aquatic Company in Qingdao City, Shandong Province. Prior to the experiment, the fish were acclimated to the culture system for two weeks using commercial feed.

After acclimation, 100 fish were randomly selected and weighed, and the average weight was taken as the initial body weight [(13.00$±$0.01) g]. Uniform-sized, healthy, and undamaged juvenile turbot were randomly distributed into 24 culture tanks, with 30 fish per tank. Each experimental diet was randomly assigned to three tanks, and fish were fed to apparent satiation twice daily at 07:00 and 19:00. One hour after feeding, residual feed was collected, tanks were cleaned, and water was exchanged to maintain water quality. Feed intake and residual feed were recorded for each tank. Any dead fish were recorded for number, weight, and symptoms. Fish were cultured in an indoor flow-through system with continuous aeration for 24 h, covered windows, and lights off. Water temperature was maintained at 19–22 °C, and pH was 7.5–8.0. The culture period lasted nine weeks.

1.3 Sample Collection

At the beginning of the experiment, 20 fish were randomly selected and stored at -20 °C as initial samples. At the end of the nine-week culture trial, fish were fasted for 24 h before weighing, and the number and weight of fish in each tank were recorded. From each tank, five fish were randomly sampled and stored at -20 °C for body composition analysis; another three fish were anesthetized, weighed, and measured for body length before dissection to obtain viscera and liver weights for calculating hepatosomatic index, viscerosomatic index, and condition factor; and five additional fish were randomly selected from each tank for blood collection via caudal vein using 2 mL centrifuge tubes. Blood was drawn slowly to prevent hemolysis due to mechanical damage, allowed to coagulate at room temperature for 1 h, then kept at 4 °C for 4 h, and centrifuged at 3,500 r/min for 10 min at 4 °C to collect serum. Serum samples were snap-frozen in liquid nitrogen and transferred to -80 °C for antioxidant index determination.

1.4 Analysis Methods

1.4.1 Body Composition Analysis The proximate nutrient contents of protein sources, diets, and fish bodies were determined using AOAC (1993) methods. Moisture content was determined by drying samples at 105 °C to constant weight; crude protein content was measured using an automatic Kjeldahl analyzer (Kjeltec 8400, FOSS, Germany); crude lipid content was determined by Soxhlet extraction using petroleum ether as the extractant; and gross energy

was measured using an oxygen bomb calorimeter (PARR 6400, PARR, USA).

1.4.2 Nutrient Apparent Digestibility Yttrium oxide (Y_2O_3) was added to diets at 1% as an indicator to determine apparent digestibility coefficients of dry matter and crude protein. Feces collection began four weeks after feeding the experimental diets and was performed by siphoning four hours after each feeding. Feces were stored at -20 °C until sufficient quantities were collected for digestibility analysis. Yttrium content was determined using the method of Furukawa et al., where diets and feces were digested with perchloric acid and analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES, Vistampx, Varian, USA).

1.4.3 Serum Antioxidant Indices Analysis Serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities and malondialdehyde (MDA) content were determined using commercial assay kits (Nanjing Jiancheng Bioengineering Institute).

1.5 Calculation Formulas

Survival rate (SR, %) = 100 × final fish number / initial fish number
Weight gain rate (WGR, %) = 100 × (final body weight - initial body weight) / initial body weight
Specific growth rate (SGR, %/d) = 100 × (ln final body weight - ln initial body weight) / culture days
Feed intake (FI, %/d) = 100 × feed consumption / [(initial body weight + final body weight) / (2 × culture days)]
Feed efficiency (FE) = fish weight gain / feed consumption
Hepatosomatic index (HSI, %) = 100 × liver weight / body weight
Viscerosomatic index (VSI, %) = 100 × viscera weight / body weight
Condition factor (CF, %) = 100 × body weight / body length^3
Nutrient apparent digestibility (%) = 100 × [1 - (Y_2O_3 in diet / Y_2O_3 in feces) × (nutrient in feces / nutrient in diet)]

1.6 Statistical Analysis

All data were expressed as mean ± standard error (mean ± SE) and analyzed using one-way ANOVA with SPSS 17.0 software. If significant differences were detected, Tukey' s test was used for multiple comparisons, with significance level set at $P < 0.05$.

Results

2.1 Growth Performance

As shown in Table 3 , when non-starch polysaccharide enzymes were added to diets with different DDGS contents, the final body weight, weight gain rate, specific growth rate, and feed efficiency of juvenile turbot in enzyme-supplemented

groups were higher than those in enzyme-free control groups at the same DDGS level, though differences were not statistically significant ($P > 0.05$). Feed intake and whole-body moisture, crude protein, and crude lipid contents showed no significant differences among groups ($P > 0.05$). Similarly, condition factor, hepatosomatic index, and viscerosomatic index showed no significant differences ($P > 0.05$).

2.2 Body Composition

As shown in Table 4 , after adding non-starch polysaccharide enzymes to diets with different DDGS contents, no significant differences were observed in whole-body crude protein, moisture, or crude lipid contents among all groups ($P > 0.05$).

2.3 Condition Factor, Hepatosomatic Index and Viscerosomatic Index

As shown in Table 5 , after adding non-starch polysaccharide enzymes to diets with different DDGS contents, no significant differences were found in hepatosomatic index, viscerosomatic index, or condition factor among all groups ($P > 0.05$).

2.4 Nutrient Apparent Digestibility

As shown in Table 6 , when non-starch polysaccharide enzymes were added to diets with different DDGS contents, the apparent digestibility coefficients of dry matter and crude protein in enzyme-supplemented groups were higher than those in enzyme-free control groups at the same DDGS level. Specifically, dry matter apparent digestibility in the D9+ group was significantly higher than in the D9 group ($P < 0.05$), crude protein apparent digestibility in the D3+ group was significantly higher than in the D3 group ($P < 0.05$), and crude protein apparent digestibility in the D12+ group was significantly higher than in the D12 group ($P < 0.05$).

2.5 Serum Antioxidant Indices

As shown in Table 7 , after adding non-starch polysaccharide enzymes to diets with different DDGS contents, the activities of serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in enzyme-supplemented groups were significantly higher than those in enzyme-free control groups at the same DDGS level ($P < 0.05$), while serum malondialdehyde (MDA) content was significantly lower ($P < 0.05$).

Discussion

In this study, the addition of non-starch polysaccharide enzymes to diets with varying DDGS contents increased the specific growth rate and feed efficiency of juvenile turbot, consistent with results reported by Daskiran et al. and Wu

et al. that exogenous non-starch polysaccharide enzymes can improve feed conversion rates in monogastric animals. After appropriate supplementation with xylanase and cellulase, the xylan and other non-starch anti-nutritional factors in the basal diet can be effectively degraded into absorbable nutrients, improving dietary energy values and palatability, and increasing weight gain rates and feed utilization. The possible reason for improved growth performance without changes in feed intake is that non-starch polysaccharide enzymes enhance growth performance primarily by reducing chyme viscosity, improving intestinal environment, and increasing nutrient utilization rather than by increasing feed intake. Additionally, due to the limited culture period, the improvement in growth performance may not have been fully manifested, and differences might become more pronounced with extended culture time, warranting further investigation.

In this study, the addition of non-starch polysaccharide enzymes to diets with varying DDGS contents resulted in higher apparent digestibility coefficients of dry matter and crude protein in enzyme-supplemented groups compared to enzyme-free control groups at the same DDGS level. This finding is similar to Mathlouthi et al.' s research showing that supplementation with xylanase and β-glucanase in rye-based diets significantly improved nutrient apparent digestibility and apparent metabolizable energy in broiler chickens. This is because the addition of exogenous xylanase and cellulase improves intestinal absorption conditions, reduces bacterial growth and reproduction rates, supplements endogenous enzyme deficiencies, stimulates endogenous enzyme secretion, and enhances endogenous digestive enzyme activity, thereby facilitating nutrient digestion and absorption and improving digestibility.

This study demonstrated that adding non-starch polysaccharide enzymes to diets with varying DDGS contents significantly increased serum superoxide dismutase and glutathione peroxidase activities while significantly decreasing serum malondialdehyde content, thereby improving the antioxidant capacity of juvenile turbot. Zhong et al. reported that serum superoxide dismutase, lysozyme activity, and anti-reactive oxygen species capacity in Nile tilapia were significantly improved after feeding enzyme-supplemented diets. Huang et al. found that dietary xylanase supplementation significantly increased serum lysozyme and superoxide dismutase activities in gibel carp, which is important for enhancing non-specific immunity in fish. This is primarily because exogenous enzyme supplementation improves protein and other nutrient utilization, promotes synthesis of related immune factors, and generates more superoxide anion (O_2^-) and other free radicals, correspondingly increasing antioxidant enzyme activities such as superoxide dismutase and thereby enhancing the body' s antioxidant capacity.

In conclusion, adding non-starch polysaccharide enzymes to diets with varying DDGS contents can improve growth performance, nutrient apparent digestibility, and antioxidant capacity in juvenile turbot.

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Note: Figure translations are in progress. See original paper for figures.