Apparent Digestibility of Nutrients from Corn Gluten Meal in Juvenile Turbot (Scophthalmus maximus) and Effects of Bile Acid and Enzyme Preparation Supplementation: Postprint

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Abstract

This experiment aimed to investigate the apparent digestibility of dry matter, crude protein, amino acids, and gross energy from corn gluten meal in turbot juveniles, as well as the effects of supplementing corn gluten meal with bile acids (purity 30×10^{5} U/g) and papain (4×10^{5} U/g) at an activity ratio of 2:1] on the apparent digestibility of its nutrients. A basal diet was first formulated, and the experimental diets consisted of 70% basal diet and 30% test protein sources [untreated corn gluten meal (CGM), corn gluten meal with added bile acids (CGMB), and corn gluten meal with added enzyme preparation (CGME)]. All diets were supplemented with 0.1% yttrium oxide (Y_2O_3) as an external indicator. Turbot juveniles with an initial body weight of (13.00 ± 0.01) g were randomly divided into 4 groups, with 3 replicates per group and 40 fish per replicate. Feces were collected using the hindgut squeezing method after feeding the respective diets for 2 weeks. The feeding trial lasted for 10 weeks. The results showed that the apparent digestibility of dry matter, crude protein, total amino acids, and gross energy in the three differently treated corn gluten meals ranged from 25.99% to 43.34%, 48.62% to 60.72%, 48.41% to 67.67%, and 35.07% to 52.34%, respectively, and the trends in apparent digestibility of total amino acids and crude protein were consistent. Among them, the apparent digestibility of all nutrients in CGMB was the highest for turbot juveniles, significantly higher than that of CGM and CGME ($P<0.05$). The apparent digestibility of dry matter, crude protein, total amino acids, and gross energy in CGMB increased by 17.35%, 12.10%, 19.26%, and 17.27%, respectively, compared with CGM. The apparent digestibility of nutrients in CGME was also improved for turbot juveniles, with the apparent digestibility of dry matter, crude protein, amino acids, and gross energy increasing by 9.53%, 4.37%, 4.29%, and 12.25%, respectively, compared with CGM, and the effects were significant

(P<0.05). These results indicate that both bile acid and enzyme preparation supplementation can improve the apparent digestibility of nutrients from corn gluten meal in turbot juveniles.

Full Text

Nutrient Apparent Digestibility Coefficients of Corn Gluten Meal in Juvenile Turbot (Scophthalmus maximus L.) and Effects of Adding Bile Acid and Enzyme Preparation on Them

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Abstract

This experiment was conducted to investigate the apparent digestibility coefficients (ADCs) of dry matter (DM), crude protein (CP), amino acids (AA), and gross energy (GE) of corn gluten meal in juvenile turbot (Scophthalmus maximus L.), and the effects of adding bile acid (purity 30×10^{5} U/g) and papain (4×10^{5} U/g) at an activity ratio of 2:1] on the nutrient ADCs. A basal diet was formulated first, and test diets were prepared by mixing 70% basal diet with 30% test protein sources [untreated corn gluten meal (CGM), corn gluten meal supplemented with bile acid (CGMB), and corn gluten meal supplemented with enzyme preparation (CGME)]. All diets were supplemented with 0.1% yttrium trioxide (Y_2O_3) as an exogenous indicator. Juvenile turbot with initial body weight of (13.00 ± 0.01) g were randomly divided into 4 groups with 3 replicates per group and 40 fish per replicate. After feeding the corresponding diets for 2 weeks, feces were collected using the hindgut squeezing method. The feeding trial lasted for 10 weeks. The results showed that the ADCs of DM, CP, total AA, and GE in the three differently treated corn gluten meals ranged from 25.99% to 43.34%, 48.62% to 60.72%, 48.41% to 67.67%, and 35.07% to 52.34%, respectively, with the ADC of total AA showing the same trend as that of CP. Among them, juvenile turbot exhibited the highest ADCs for all nutrients in CGMB, which were significantly higher than those in CGM and CGME (P<0.05). Compared with CGM, the ADCs of DM, CP, total AA, and GE in CGMB increased by 17.35%, 12.10%, 19.26%, and 17.27%, respectively. The ADCs of all nutrients in CGME for juvenile turbot were also improved, with the ADCs of DM, CP, AA, and GE increasing by 9.53%, 4.37%, 4.29%, and 12.25%, respectively, compared with CGM, and the effects were significant (P<0.05). These results indicate that both bile acid and enzyme preparation can improve the nutrient ADCs of corn gluten meal in juvenile turbot.

Key words: juvenile turbot; corn gluten meal; bile acid; enzyme preparation;

apparent digestibility coefficients

In recent years, the continuous expansion of aquaculture scale has led to increasing demand for fish meal. China' s fish meal production cannot meet the needs of the aquaculture industry and relies heavily on imports, resulting in rising feed costs. Therefore, finding suitable protein sources to replace fish meal has become a pressing priority. Corn gluten meal offers advantages such as high protein content, absence of toxic and harmful substances, and lack of anti-nutritional factors. As an alternative protein source to fish meal, it has been extensively studied in aquatic animals including rainbow trout (*Oncorhynchus mykiss*), yellowtail (*Seriola quinqueradiata*), Japanese flounder (*Paralichthys olivaceus*), European sea bass (*Dicentrarchus labrax*), and gilthead sea bream (*Sparus aurata*). Research on turbot (*Scophthalmus maximus* L.) found that corn gluten meal could replace 25% of fish meal protein, but higher replacement ratios would affect turbot growth. The reason is that corn gluten meal is rich in water-insoluble zein protein. Despite its abundant nutrients, turbot' s digestive utilization is unsatisfactory. Therefore, exploring feasible methods to improve the nutrient ADCs of corn gluten meal has become key to increasing its replacement ratio in aquafeeds, which is also the main objective of this study. Currently, two common methods are used to improve corn gluten meal utilization: exogenous additive supplementation and external hydrolysis. This study employed two exogenous additives—bile acid and compound enzyme preparation—to investigate their effects on nutrient ADCs in corn gluten meal.

Bile acid is widely used as a feed additive and constitutes an important component of bile. It is a series of steroid substances produced during cholesterol metabolism with strong surface activity that can reduce the interfacial tension between oil and water phases, promote lipid emulsification, expand the contact area between fat and lipase, and accelerate lipid digestion and absorption. Studies have found that adding bile acid to bullfrog feed improved the ADCs of DM, crude lipid, and CP. Adding bile acid to weaned piglet diets significantly increased fat intake while also increasing nitrogen intake and retention. These results suggest that bile acid can improve protein digestibility.

Enzyme preparations are efficient biological catalysts that can improve feed utilization, eliminate anti-nutritional factors, increase animal growth rate, and leave no harmful residues. They are natural and safe feed additives that have achieved great success in livestock and poultry feed applications. However, the nutritional physiology of aquatic animals and feed processing conditions differ from those of livestock and poultry, affecting the widespread application of feed enzymes in aquafeeds. Nevertheless, many studies have shown that enzyme preparations can improve aquatic animals' digestive capacity, promote nutrient utilization, and enhance growth. Chen et al. found that adding neutral protease to black carp (*Mylopharyngodon piceus* Richardson) feed significantly improved the ADC of CP. Liu et al. reported that enzyme preparations improved protein digestion and absorption in gibel carp (*Carassius auratus gibelio*). Liu et al. found that adding neutral protease to corn gluten meal significantly improved the ADCs of DM and CP in lambs.

Currently, few studies have examined the effects of bile acid and enzyme preparations on nutrient ADCs of corn gluten meal in aquatic animals. Therefore, based on investigating the nutrient ADCs of corn gluten meal in juvenile turbot, this experiment explored the effects of adding bile acid and enzyme preparations on corn gluten meal utilization, aiming to provide a scientific basis for its efficient use.

1.1 Experimental Materials

Corn gluten meal, a byproduct of corn starch processing, was purchased from Qidao Bio-Tech Co. Ltd. and contained over 60% crude protein along with small amounts of starch, cellulose, and vitamins.

Bile acid was purchased from Qingdao Hepuda Trading Co. Ltd. with purity $ $30%.

Based on feed processing technology and the characteristics of corn gluten meal and protease, this experiment used a compound enzyme consisting of neutral protease (1$×10^{5}$ U/g) and papain (4$×10^{5}$ U/g) as the enzyme preparation, with an activity ratio of 2:1.

1.2 Experimental Diets

A basal diet meeting the basic nutritional requirements of juvenile turbot was formulated first. Then corn gluten meal was mixed with the basal diet at a 3:7 ratio to prepare three test diets. During mixing, one test diet remained untreated (the test protein source designated as CGM), one was supplemented with 1.0% bile acid (designated as CGMB), and one was supplemented with 0.1% enzyme preparation (designated as CGME). All diets were supplemented with 0.1% yttrium trioxide (Y_2O_3) as an exogenous indicator to determine the ADCs of DM, CP, AA, and GE in CGM, CGMB, and CGME. The composition and nutrient levels of the basal diet are shown in Table 1 . All feed ingredients were ground to pass through a 60-mesh sieve. The ground ingredients were mixed stepwise from smallest to largest quantity according to the formula, then oil and appropriate water were added and kneaded before pelleting. The diets were dried in a 45°C oven for approximately 12 hours and then stored sealed at -20°C.

1.3 Experimental Fish and Culture Management

The feeding trial was conducted at Qingdao Jiaonan Yihai Feng Aquatic Products Co. Ltd. Juvenile turbot were purchased from Yantai Laizhou Farm. Before the formal experiment, juvenile turbot were acclimated in a recirculating aquaculture system and fed commercial feed to adapt to the experimental environment.

After acclimation, the fish were fasted for 24 hours, weighed, and selected for uniform size and good vigor [average body weight (13.00$±$0.01) g]. They were randomly distributed into 12 tanks with 40 fish per tank and cultured in an indoor flow-through system. Three tanks constituted one group, and each diet was randomly assigned to three groups of fish. Before the experiment, all groups were fed the basal diet for one week, after which each group was fed the corresponding test diet. During the culture period, fish were fed to apparent satiation twice daily (07:00 and 19:00). After feeding, approximately 70% of the water was exchanged to maintain good water quality. The culture period lasted 10 weeks. Water temperature was maintained at 18-20°C, salinity at 30‰-33‰, pH at 7.5-8.0, nitrite concentration $ $0.1 mg/L, ammonia concentration $ $0.1 mg/L, and dissolved oxygen concentration >7 mg/L.

Table 1 Basal diet composition and nutrient levels (dry matter basis)

Items	Content
Ingredients	
Fish meal[1]	60.00
Wheat flour	10.00
Soybean meal	18.00
Fish oil	6.00
Soybean lecithin	2.00
$Ca(H_2PO_4)_2$	0.50
Choline chloride	0.50
Vitamin premix[2]	1.00
Mineral premix[3]	1.00
Calcium propionate	0.05
Ethoxyquin	0.03
Attractant[4]	0.50
Y_2O_3	0.10
Total	100.00
Nutrient levels[5]	
GE/(MJ/kg)	20.50
CP	52.00
EE	10.50

[1]Fish meal obtained from Qidao Bio-Tech Co. Ltd. (Shandong, China).
[2]Vitamin premix provided the following per kg of diet: VB_1 0.025 g, VB_2 0.045 g, VB_6 0.020 g, VB_{12} 0.010 g, VK_3 0.010 g, inositol 0.800 g, calcium pantothenate 0.060 g, niacin acid 0.200 g, folic acid 0.020 g, biotin 0.060 g, VA 16,000 IU, VD_3 2,500 IU, VE 0.240 g, VC 2.000 g, microcrystalline cellulose 16.473 g.
[3]Mineral premix provided the following per kg of diet: $MgSO_4 \cdot 7H_2O$ 1.200 g, $CuSO_4 \cdot 5H_2O$ 0.010 g, $FeSO_4 \cdot H_2O$ 0.080 g, $ZnSO_4 \cdot H_2O$ 0.050 g, $CoCl_2$ (1%) 0.050 g, $Ca(IO_3)_2$ (1%) 0.060 g, Na_2SeO_3 (1%) 0.020 g, zeolite powder 18.485 g.

[4]Composite attractant: betaine: DMPT: glycine: alanine: inosine 5-phosphate = 4:2:2:1:1.
[5]Nutrient levels were measured values.

1.4 Sample Collection

Fecal samples were collected using the hindgut squeezing method. During the acclimation period, defecation timing after satiation was observed to determine that 5 hours post-feeding was optimal for feces collection. Feces collection began 2 weeks after formal feeding. Juvenile turbot were anesthetized with eugenol (1:10,000), then dried with gauze. After urine was expelled, feces were gently squeezed from both sides 3 cm anterior to the anus and collected in 10 mL centrifuge tubes, then stored at -20°C. Feces were collected from 2 tanks daily, with a 1-week interval between collections from the same tank to ensure fish recovered to normal physiological status.

1.5 Index Determination and Calculation Methods

In this experiment, DM content was determined by the 105°C constant pressure drying method, crude ash by the 550°C incineration method, CP by the Kjeldahl method, ether extract (EE) by the Soxhlet extraction method, GE by bomb calorimetry, Y_2O_3 content by high-frequency inductively coupled plasma optical emission spectrometry, and AA content by Hitachi L-8900 automatic amino acid analyzer.

The ADCs of nutrients in diets (basal or test diets) and test protein sources (CGM, CGMB, or CGME) were calculated using the following formulas:

$$\mathrm{ADC} = 100 \times \left(1 - \frac{M_d}{M_f}\right)$$

$$\mathrm{ADC}_d = 100 \times \left[1 - \left(\frac{N_f}{N_d}\right) \times \left(\frac{M_d}{M_f}\right)\right]$$

$$\mathrm{ADC}_i = \frac{\mathrm{ADC}_t \times (0.7 \times N_r + 0.3 \times N_i) - 0.7 \times N_r \times \mathrm{ADC}_r}{0.3 \times N_i}$$

Where:
ADC = apparent digestibility coefficient of DM in diets (basal or test diets) (%);
M_d = Y_2O_3 content in diets (basal or test diets) (%);
M_f = Y_2O_3 content in feces (%);
ADC_d = apparent digestibility coefficient of a nutrient in diets (basal or test diets) (%);
N_d = content of the corresponding nutrient in diets (basal or test diets) (%) or GE value (J/mg);
N_f = content of the corresponding nutrient in feces (%) or GE value (J/mg);
ADC_i = apparent digestibility coefficient of a nutrient in test protein sources

(CGM, CGMB, or CGME) (%);
ADC_t = apparent digestibility coefficient of the corresponding nutrient in test diets (%);
ADC_r = apparent digestibility coefficient of the corresponding nutrient in basal diet (%);
N_r = content of the corresponding nutrient in basal diet (%) or GE value (J/mg);
N_i = content of the corresponding nutrient in test protein sources (CGM, CGMB, or CGME) (%) or GE value (J/mg).

1.6 Data Analysis and Processing

Experimental data were analyzed by one-way ANOVA using SPSS 17.0 software. If differences were significant ($P<0.05$), Tukey' s multiple comparison test was performed. Data are expressed as means ± standard error.

2 Results and Analysis

The nutrient ADCs of the three differently treated corn gluten meals in juvenile turbot are shown in Table 2 . The apparent digestibility coefficient of DM ranged from 25.99% to 43.34%. CGMB showed the highest DM ADC, which was significantly different from CGME and CGM ($P<0.05$) and increased by 17.35% compared with CGM. Additionally, CGME also showed significantly higher DM ADC than CGM ($P<0.05$), with a 9.53% increase.

The apparent digestibility coefficient of CP ranged from 48.62% to 60.72%. CGMB exhibited the highest CP ADC, which was significantly different from CGME and CGM ($P<0.05$) and increased by 12.10% compared with CGM. CGME also showed significantly higher CP ADC than CGM ($P<0.05$), with a 4.37% increase.

The apparent digestibility coefficient of total AA ranged from 48.41% to 67.67%, showing the same trend as CP ADC. Among the three differently treated corn gluten meals, CGMB showed the highest ADCs for all individual AAs and total AA. For essential AAs, lysine showed the highest ADC at 78.98%, which was significantly higher than in CGME and CGM ($P<0.05$). CGME ranked second in total AA ADC, which was significantly higher than in CGM ($P<0.05$). Compared with CGM, the total AA ADC in CGMB and CGME increased by 19.26% and 4.29%, respectively.

The apparent digestibility coefficient of GE ranged from 35.07% to 52.34%. CGMB showed the highest GE ADC, which was significantly different from CGME and CGM ($P<0.05$) and increased by 17.27% compared with CGM. CGME also showed significantly higher GE ADC than CGM ($P<0.05$), with a 12.25% increase.

Table 2 Apparent digestibility coefficients of nutrients in tested protein sources (n=3) (%)

<table>
<tr><th>Items</th><th>CGM</th><th>CGMB</th><th>CGME</th></tr>
<tr><td>DM</td><td colspan="3">25.99$±0.65c|43.34±2.31a|33.81±0.71b||*
CP
|48.62±1.16c|60.72±0.39a|52.99±0.73b||
GE
|35.07±0.31c|52.34±0.20a|47.32±0.55b||
EssentialAAs
*|||||Thr|37.32±0.16a|63.95±0.14c|50.08±0.31b||Val|42.91±0.15a|66.29±0.08c|51.19±0.
*Non−
essentialAAs*
*|||||Asp|32.61±0.12a|64.41±0.29c|51.15±0.41b||Ser|47.35±0.17a|67.90±0.59c|60.19±0.
TAA
*|48.41±0.54c|67.67±0.88a|52.80±$0.86b</td></tr>
</table>

Values in the same row with different small letter superscripts indicate significant difference ($P<0.05$).

3 Discussion

3.1 Experimental Diet Preparation and Method Selection

Under the premise of meeting the basic nutritional requirements of juvenile turbot, this experiment adopted the method of Cho et al., where test protein sources replaced 30% of the basal diet to prepare test diets, resulting in a 7:3 ratio of basal diet to test protein source. Since Cho et al.' s calculation method does not consider the influence of nutrients from the test feed ingredient on the digestibility of nutrients in the test diet, this experiment adopted the improved calculation method of You et al. based on Cho et al.' s method (see formulas in section 1.5) to calculate the nutrient ADCs in test protein sources, thereby improving the accuracy and reliability of the results.

The research subject of this experiment was juvenile turbot, and the results may differ from other studies due to factors such as fish species, growth stage, and experimental methodology. Since the main purpose was to determine nutrient ADCs in test protein sources, and considering the influence of feces collection methods on results while referring to Chinese aquaculture industry standards for determining nutrient digestibility in fish, the hindgut squeezing method was selected for fecal sample collection.

To investigate the effects of bile acid and enzyme preparation on nutrient ADCs of corn gluten meal, three test diets were prepared and the nutrient ADCs of three differently treated corn gluten meals were determined.

3.2 Nutrient Apparent Digestibility of Corn Gluten Meal in Juvenile Turbot

The quality of protein in feed protein sources is the primary factor determining its nutritional value, and digestibility is an important indicator for evaluating the availability of nutrients in feed protein sources. This experiment determined the ADCs of DM, CP, AA, and GE in differently treated corn gluten meals from protein and energy perspectives to explore their utilization value in turbot feed. The ADC of DM reflects the overall digestibility level of feed ingredients, which is related to cellulose content and the absorption degree of nutrients such as protein and fat. In this experiment, the DM ADC of CGM in juvenile turbot was 25.99%, similar to results reported by Wei et al. and Yang et al. in turbot. The low DM ADC may be related to the crude fiber in CGM accelerating chyme movement in the digestive tract, lack of corresponding cellulase in fish, and high zein content that is difficult to digest and utilize.

Protein is an important nutrient for fish, and the digestibility of dietary protein is a crucial indicator for judging raw material availability. The CP ADC of CGM was determined to be 48.62% in this experiment, similar to results from Wei et al. and Yang et al. in turbot, but differing significantly from studies on rainbow trout, cobia (*Rachycentron canadum*), Jian carp (*Cyprinus carpio* var. Jian), and Pacific white shrimp (*Litopenaeus vannamei*), possibly due to differences in fish species.

Amino acids are the basic units of protein, and protein requirements are essentially amino acid requirements. The AA ADC directly reflects protein quality in protein sources. The total AA ADC of CGM was 48.41% in this experiment, similar to the CP ADC, consistent with findings in silver perch (*Bidyanus bidyanus*), black carp, and Jian carp.

The GE ADC reflects fish' s comprehensive utilization ability of protein, fat, and carbohydrates in test ingredients. The GE ADC of CGM was 35.07% in this experiment, similar to Wei et al.' s result (36.08%) but different from Yang et al.' s result (46.28%) in turbot, possibly due to corn gluten meal quality. The GE ADCs of corn gluten meal in Atlantic cod (*Gadus morhua*) and largemouth bass (*Micropterus salmoides*) were 82.70% and 76.50%, respectively, showing large differences from this experiment, which may be related to fish species, processing methods, and corn gluten meal quality.

3.3 Effect of Bile Acid Addition on Nutrient Apparent Digestibility of Corn Gluten Meal

To investigate the effect of bile acid on nutrient ADCs of corn gluten meal, 1% bile acid was added to corn gluten meal to prepare CGMB, which replaced 30% of the basal diet to formulate the bile acid-supplemented test diet, and nutrient ADCs in CGMB were determined. Results showed that compared with CGM, all nutrient ADCs in CGMB were significantly improved, with DM, CP, total AA, and GE ADCs increasing by 17.35%, 12.10%, 19.26%, and 17.27%, respectively. Previous studies have reported that bile acid improves animal growth and feed utilization. Maita et al. confirmed that dietary deoxycholic acid increased weight gain in Japanese eel (*Anguilla japonica*). Deshimaru et al. found that dietary bile acid significantly improved growth rate and feed efficiency in yellowtail (*Seriola quinqueradiata*). Domestic scholars obtained similar results in studies on rainbow trout and gibel carp. Additionally, Wang found that adding bile acid to turbot feed increased CP ADC by 1.5% and crude fat ADC by 3.0%. Hu et al. demonstrated in bullfrogs that bile acid addition improved ADCs of DM, CP, and crude fat, similar to this experiment' s results. Studies on pigs showed that dietary bile acid significantly increased nitrogen intake and retention. Current explanations for bile acid' s growth-promoting mechanisms include: (1) bile facilitates lipid emulsification, improving fat digestibility and thus DM ADC; and (2) bile acid has bactericidal and bacteriostatic effects in the intestine, improving gut health, increasing protease activity, and enhancing nutrient ADCs to promote nutrient absorption. Most previous studies determined nutrient ADCs in complete feeds without corn gluten meal, whereas this experiment focused on nutrient ADCs in corn gluten meal itself. Due to its high content of water-insoluble zein, corn gluten meal utilization by juvenile turbot is unsatisfactory, but all nutrient ADCs were significantly improved after bile acid addition. Given bile acid' s strong surface activity, it may act as a carrier for insoluble proteins in corn gluten meal during digestion, promoting utilization of these proteins and thereby improving nutrient ADCs. The specific mechanism requires further investigation. In conclusion, bile acid addition effectively enhances the application value of corn gluten meal in turbot feed and shows research prospects as an additive in corn gluten meal-containing diets.

3.4 Effect of Enzyme Preparation Addition on Nutrient Apparent Digestibility of Corn Gluten Meal

To investigate the effect of enzyme preparation on nutrient ADCs of corn gluten meal, based on Chen et al.' s research, this experiment used a compound enzyme preparation with papain and neutral protease at a 2:1 activity ratio as the exogenous enzyme to study its effects. Results showed that enzyme preparation addition significantly improved the ADCs of DM, CP, and GE in corn gluten meal, although some individual AA ADCs showed downward trends, the total AA ADC was significantly improved. Many studies have reported that protease addition significantly improves nutrient ADCs in feed ingredients. Liu et al. found that dietary neutral protease greatly improved DM and CP ADCs of corn gluten meal in lambs. Zhong et al. reported that dietary compound enzyme increased feed total digestibility and CP ADC in Nile tilapia (*Oreochromis nilotica*). Lin et al. found that dietary compound enzyme significantly improved CP, GE, crude fat, and DM ADCs in plant-based diets for hybrid tilapia (*Oreochromis niloticus* × *O. aureus*), similar to this experiment' s results. Feed enzyme addition can enhance protease digestion of feed nutrients, especially in young animals. Dietary exogenous enzymes can supplement endogenous enzyme deficiencies, breaking down conventional nutrients into small molecules that facilitate intestinal absorption, with more pronounced effects. In this experiment, corn gluten meal was the main protein source besides fish meal in the diet formulation. Corn gluten meal is rich in insoluble zein, and protease preparations can degrade these proteins to varying degrees, comprehensively improving nutrient ADCs in corn gluten meal.

These results demonstrate that both bile acid and enzyme preparation supplementation can significantly improve the ADCs of DM, CP, total AA, and GE of corn gluten meal in juvenile turbot, enhancing its nutritional value as an alternative protein source in aquafeeds.

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