

Apparent Digestibility of Nutrients in Several Protein Sources by Juvenile Turbot: Effects of Extrusion Processing (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 Peruvian fish meal, soybean meal, sunflower meal, and corn gluten meal in turbot (*Scophthalmus maximus* L.), as well as the effects of extrusion processing on these parameters. The experimental diets consisted of 70% basal diet and 30% test protein source, with 0.1% yttrium oxide (Y_2O_3) added as an external indicator, and were prepared as normal pellets and extruded pellets, respectively. Juvenile turbot with an average body weight of (8.98 ± 0.01) g were randomly divided into 10 groups, with 3 replicates per group and 40 fish per replicate, and fed the corresponding diets according to different treatments. Fecal samples were collected using the distal intestine squeezing method from week 2 to week 6. The results showed that the apparent digestibility of dry matter, crude protein, total amino acids, and gross energy from the four protein sources in juvenile turbot ranged from 22.33%–65.50%, 48.97%–85.28%, 47.70%–84.14%, and 44.50%–81.16%, respectively. Among these, the apparent digestibility of all nutrients from fish meal was significantly higher than that from the three plant protein sources ($P < 0.05$). Among the three plant protein sources, the apparent digestibility of crude protein was highest in soybean meal, followed by sunflower meal, and lowest in corn gluten meal. The trend of apparent digestibility of total amino acids in each test protein source was essentially consistent with that of crude protein apparent digestibility. After extrusion processing, the apparent digestibility of nutrients from the four protein sources in turbot was improved, especially for the three plant protein sources, with significant effects ($P < 0.05$). The apparent digestibility of dry matter, crude protein, total amino acids, and gross energy from the four protein sources after extrusion processing ranged from 24.26%–70.87%, 57.20%–85.33%, 68.27%–87.09%, and 49.23%–84.35%, respectively. It can be concluded that extrusion processing can improve the apparent digestibility of nutrients from Peruvian fish meal, soybean meal, sunflower meal, and corn

gluten meal in juvenile turbot.

Full Text

Nutrient Apparent Digestibility Coefficients of Several Protein Sources in Juvenile Turbot (*Scophthalmus maximus* L.) and Effects of Extrusion Treatment

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Abstract: This experiment was conducted to determine the apparent digestibility coefficients (ADC) of dry matter (DM), crude protein (CP), amino acids (AA), and gross energy (GE) in Peruvian red fish meal, soybean meal, sunflower meal, and corn gluten meal for juvenile turbot (*Scophthalmus maximus* L.), and to evaluate the effects of extrusion treatment on these digestibility values. Test diets were formulated with 70% basal diet and 30% test protein source, with 0.1% yttrium trioxide (Y_2O_3) added as an external indicator. Diets were processed into both normal pellets and extruded pellets. Juvenile turbot with an average body weight of (8.98 ± 0.01) g were randomly allocated into 10 groups, with 3 replicates per group and 40 fish per replicate. Fish were fed the corresponding experimental diets for 2 weeks, after which fecal samples were collected using the hindgut squeezing method until week 6. The results showed that the ADC of DM, CP, total amino acids, and GE in the four protein sources ranged from 22.33% to 65.50%, 48.97% to 85.28%, 47.70% to 84.14%, and 44.50% to 81.16%, respectively. Fish meal exhibited significantly higher ADC for all nutrients compared to the three plant protein sources ($P < 0.05$). Among the plant proteins, soybean meal showed the highest CP digestibility, followed by sunflower meal, with corn gluten meal being the lowest. The trend for total amino acid digestibility was consistent with that of CP digestibility. Extrusion treatment improved the apparent digestibility of all nutrients from the four protein sources, particularly the plant protein sources, with significant effects ($P < 0.05$). After extrusion, the ADC of DM, CP, total amino acids, and GE ranged from 24.26% to 70.87%, 57.20% to 85.33%, 68.27% to 87.09%, and 49.23% to 84.35%, respectively. These results demonstrate that extrusion treatment can enhance the nutrient apparent digestibility of Peruvian red fish meal, soybean meal, sunflower meal, and corn gluten meal in juvenile turbot.

Keywords: turbot; protein source; apparent digestibility; extrusion

Introduction

Digestibility refers to the degree to which ingested nutrients are digested and absorbed by animals. Determining the apparent digestibility of protein sources

is an important method for evaluating their nutritional value and is a necessary step in developing formulated feeds [?]. Establishing appropriate inclusion levels based on the nutritional value of protein sources can improve feed utilization, reduce production costs, and protect aquatic environments. In recent years, as the production of fish meal—the primary protein source for aquatic animals—has continued to decline while prices keep rising, the utilization of plant protein sources that are widely available and inexpensive has become a research hotspot [?]. Numerous studies have shown that fish exhibit low apparent digestibility for plant protein sources due to the presence of anti-nutritional factors and imbalanced amino acid profiles [?]. Commonly used methods to improve the digestibility and utilization of plant protein sources include physical, chemical, and biological approaches, such as heat treatment, extrusion processing, microbial fermentation, exogenous enzyme supplementation, and soaking. Extrusion processing is a widely used physical method. Under extrusion conditions, heat-labile anti-nutritional factors such as trypsin inhibitors and lectins are destroyed, and the structural state of plant proteins is altered, making nutrients more readily absorbable by fish and thereby improving their digestibility and utilization of plant protein sources [?].

Turbot (*Scophthalmus maximus* L.) belongs to the order Pleuronectiformes, family Scophthalmidae, and genus *Scophthalmus*. It is a typical benthic carnivorous fish and an important marine economic fish species in northern China. Turbot has high protein requirements [?], yet few studies have examined the digestibility of common protein sources for this species. Therefore, how to maximize the role of plant protein sources in turbot formulated feeds by improving digestibility has become an urgent issue to address. This study investigated the apparent digestibility of DM, CP, amino acids, and GE in Peruvian red fish meal (FM), soybean meal (SBM), sunflower meal (SFM), and corn gluten meal (CGM), as well as the effects of extrusion treatment on these digestibility values, to provide support for the application and development of plant protein sources in turbot formulated feeds.

Materials and Methods

1.1 Experimental Materials and Feed Preparation

Four protein sources were selected for this experiment: Peruvian red fish meal, soybean meal, sunflower meal, and corn gluten meal, all purchased from Qingdao Qihao Biotechnology Co., Ltd. The basal diet was formulated to meet the basic nutritional requirements of turbot. Test diets consisted of 70% basal diet and 30% test protein source, with 0.1% yttrium trioxide (Y_2O_3) added to all diets to determine the apparent digestibility of DM, CP, amino acids, and GE. The composition and nutrient levels of the basal diet are shown in Table 1, while the nutrient levels and amino acid composition of the test protein sources are presented in Table 2. All feed ingredients and protein sources were ground to pass through a 60-mesh sieve and mixed thoroughly by weight in ascending order before pellet production. Normal pellet feeds and slow-sinking extruded

pellet feeds were produced using an F-26 twin-screw extruder and a TSE65 twin-screw extruder, respectively. The feeds were dried in a 45°C oven for 12 hours and then stored sealed at -20°C.

1.2 Experimental Fish and Culture Conditions

Juvenile turbot were obtained from a farm in Jiaonan, Qingdao, and the feeding trial was conducted at Qingdao Jiaonan Yihai Feng Aquatic Products Co., Ltd. Prior to the experiment, fish were acclimated in the culture system for 2 weeks and fed commercial feed to adapt to the environment. Uniform-sized, healthy fish [average body weight (8.98±0.01) g] were randomly distributed into 10 groups with 3 replicates per group and 40 fish per replicate, and cultured in an indoor flow-through system. Before the formal trial, fish were fed the basal diet for 1 week, after which each group received the corresponding test diet. During the culture period, fish were fed to satiation twice daily at 06:00 and 18:00, followed by water exchange to maintain water quality. Water temperature was maintained at 19–22°C, salinity at 30‰–33‰, pH at 7.5–8.0, ammonia nitrogen below 0.1 mg/L, nitrite below 0.1 mg/L, and dissolved oxygen above 6.0 mg/L.

1.3 Sample Collection

Fecal samples were collected using the hindgut squeezing method. During the acclimation period, defecation patterns of juvenile turbot were observed at different time points (1, 2, 3, 4, 5, and 6 hours post-feeding) to determine the optimal collection time, which was identified as 4 hours post-feeding. Fecal collection began 2 weeks after the start of formal feeding. Fish were anesthetized with eugenol (1:10,000), dried with gauze to remove surface moisture and exclude urine, then gently squeezed at a point 3 cm anterior to the anus to collect expelled feces, which were stored at -20°C. Fecal collection from each tank was performed at 1-week intervals to allow fish to recover to normal physiological conditions. Collection continued for 6 weeks to ensure sufficient sample quantity for analysis.

1.4 Index Determination and Calculation Methods

Moisture content was determined by drying at 105°C under atmospheric pressure, crude protein by the Kjeldahl method, crude fat by Soxhlet extraction, crude ash by combustion at 550°C, gross energy by bomb calorimetry, Y₂O₃ content by high-frequency inductively coupled plasma optical emission spectrometry, and amino acid composition by Hitachi L-8900 automatic amino acid analyzer. The formulas for calculating apparent digestibility coefficients of nutrients [?] were as follows:

$$ADC = 100 \times (1 - DY/FY)$$

$$ADC_d = 100 \times [1 - (F/D) \times (FY/DY)]$$

$$ADC_i = [(0.7 \times N_r + 0.3 \times N_i) \times ADC_t - 0.7 \times N_r \times ADC_r] / (0.3 \times N_i)$$

Where: ADC is the apparent digestibility coefficient of DM in the diet (test diet or basal diet) (%); ADC_d is the apparent digestibility coefficient of a nutrient in the diet (test diet or basal diet) (%); ADC_i is the apparent digestibility coefficient of the test protein source (%); DY is the Y₂O₃ content in the diet (%); FY is the Y₂O₃ content in feces (%); F is the content of the corresponding nutrient in feces (%); D is the content of the corresponding nutrient in the test diet (%); ADC_t is the apparent digestibility coefficient of the corresponding nutrient in the test diet (%); ADC_r is the apparent digestibility coefficient of the corresponding nutrient in the basal diet (%); N_r is the content of the corresponding nutrient in the basal diet (%); and N_i is the content of the nutrient in the test protein source (%).

1.5 Data Processing and Analysis

Experimental data were analyzed using SPSS 17.0 software for one-way ANOVA. If significant differences were detected ($P < 0.05$), Tukey's multiple comparison test was performed. All data are expressed as mean \pm standard error.

Results

2.1 Apparent Digestibility of DM, CP, Amino Acids, and GE in Four Protein Sources from Normal Pellet Feeds

As shown in Table 3, the apparent digestibility of DM in the four protein sources for juvenile turbot ranged from 22.33% to 65.50%. Fish meal showed the highest DM digestibility, significantly higher than the three plant protein sources ($P < 0.05$). Significant differences also existed among the plant protein sources ($P < 0.05$), with soybean meal being the highest, followed by corn gluten meal, and sunflower meal the lowest. The apparent digestibility of CP in the four protein sources ranged from 48.97% to 85.28%. Fish meal exhibited the highest CP digestibility, significantly different from other protein sources ($P < 0.05$). Among the plant protein sources, CP digestibility ranked as soybean meal > sunflower meal > corn gluten meal, with significant differences among them ($P < 0.05$). The apparent digestibility of total amino acids ranged from 47.70% to 84.14%, showing a similar trend to CP digestibility. Fish meal had the highest digestibility for all individual and total amino acids, with lysine showing the highest value at 91.36%, significantly higher than other protein sources ($P < 0.05$). Among plant protein sources, soybean meal had relatively high total amino acid digestibility, followed by sunflower meal and corn gluten meal. Both arginine and methionine showed relatively high digestibility in the three plant

protein sources. The apparent digestibility of GE ranged from 44.50% to 81.16%. Fish meal had the highest GE digestibility, significantly higher than the three plant protein sources ($P < 0.05$). Among plant protein sources, soybean meal showed the highest GE digestibility, followed by corn gluten meal and sunflower meal, with no significant difference between corn gluten meal and sunflower meal ($P > 0.05$).

2.2 Apparent Digestibility of DM, CP, Amino Acids, and GE in Four Protein Sources from Extruded Pellet Feeds

As shown in Table 3, the apparent digestibility of DM in the four protein sources ranged from 24.26% to 70.87%. Compared with the normal pellet group, DM digestibility significantly increased for all protein sources except sunflower meal ($P > 0.05$), with increases of 5.37% for fish meal, 4.00% for soybean meal, and 10.72% for corn gluten meal. The apparent digestibility of CP ranged from 57.20% to 85.33%. Compared with the normal pellet group, CP digestibility significantly increased for all protein sources except fish meal ($P > 0.05$), with increases of 7.94% for soybean meal, 6.02% for sunflower meal, and 8.23% for corn gluten meal. The apparent digestibility of total amino acids ranged from 68.27% to 87.09%. Compared with the normal pellet group, total amino acid digestibility significantly increased for all four protein sources ($P < 0.05$), with fish meal, soybean meal, sunflower meal, and corn gluten meal increasing by 2.95%, 11.48%, 10.34%, and 20.57%, respectively. Except for some amino acids in fish meal that showed no significant change ($P > 0.05$), most amino acids in the four protein sources exhibited significant changes ($P < 0.05$). The apparent digestibility of GE ranged from 49.23% to 84.35%. Compared with the normal pellet group, GE digestibility significantly increased for all protein sources except fish meal ($P > 0.05$), with increases of 7.35% for soybean meal, 4.73% for sunflower meal, and 8.40% for corn gluten meal.

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

[Table content with all numerical values preserved exactly as in original]

Values in the same row with different small letter superscripts indicate significant differences ($P < 0.05$). FM represents Peruvian red fish meal, SBM represents soybean meal, SFM represents sunflower meal, and CGM represents corn gluten meal. Protein source names without subscript represent normal pellet feed groups, while those with subscript E represent extruded pellet feed groups.

Discussion

3.1 Selection of Experimental Methods

This experiment used juvenile turbot as the research subject, and the results may differ from other studies due to variations in fish species, life stages, and experimental methods. Considering the impact of fecal collection methods on

results [?] and following the requirements of Chinese aquaculture industry standards for digestibility determination, the hindgut squeezing method was selected for fecal sample collection. Test diets were formulated according to the method of Cho et al. [?], with a 7:3 ratio of basal diet to test protein source. The calculation of apparent digestibility coefficients for nutrients in test protein sources used a modified formula [?] (see section 1.4), which eliminated the influence of different nutrient contents between basal and test diets on the digestibility values of test protein sources, ensuring accurate results.

3.2 Apparent Digestibility of Nutrients in Four Protein Sources for Juvenile Turbot

The quality of protein in feed protein sources is a primary factor affecting fish nutrition, and digestibility is an important indicator for evaluating the availability of nutrients in protein sources. This experiment determined the apparent digestibility of DM, CP, amino acids, and GE in different protein sources to explore their utilization value in turbot feeds. The apparent digestibility of DM reflects the overall digestive capacity for protein sources and their nutritional value [?]. In this study, juvenile turbot showed the highest DM digestibility for fish meal, while DM digestibility for soybean meal, corn gluten meal, and sunflower meal was relatively low. Fish meal has long been considered the optimal protein source due to its balanced nutrition and easy absorption, and the DM digestibility of 65.50% measured in this study is similar to results reported for cobia (*Rachycentron canadum*) [?], large yellow croaker (*Pseudosciaena crocea*) [?], and tilapia (*Oreochromis niloticus*) [?]. Research indicates that carnivorous fish utilize DM from animal protein sources better than from plant protein sources [?], which is consistent with our results. This may be related not only to the carnivorous nature of turbot but also to the relatively high crude fiber content in plant protein sources, which accelerates chyme movement through the digestive tract, and the lack of corresponding cellulase enzymes in fish [?].

Protein is an important nutritional component for fish, and determining the apparent digestibility of CP in protein sources to evaluate protein quality is meaningful for rational feed formulation and improving fish growth performance [?]. In this experiment, the apparent digestibility of CP in fish meal for juvenile turbot was 85.28%, significantly higher than that in soybean meal (62.90%), sunflower meal (61.17%), and corn gluten meal (48.97%). Similar studies are abundant, such as those on silver perch (*Bidyanus bidyanus*) [?], spotted steed (*Hemibarbus maculatus* Bleeker) [?], black seabream (*Spurus macrocephalus*) [?], and large yellow croaker [?], which reported CP digestibility values for fish meal and soybean meal as 86.20% and 76.96%, 96.77% and 92.14%, 91.35% and 89.64%, and 89.30% and 84.50%, respectively—all showing higher digestibility for fish meal than soybean meal. Studies on CP digestibility of sunflower meal in fish are limited, but research in mammals indicates relatively high CP digestibility for sunflower meal [?]. The CP digestibility of 61.17% for sunflower meal in this study suggests it can be well utilized by juvenile turbot. Corn gluten meal

has the advantages of high protein content, low fiber, and few anti-nutritional factors. Many studies have shown high CP digestibility for corn gluten meal in fish, such as rainbow trout (*Oncorhynchus mykiss*) (97.30%) [?], cobia (94.42%) [?], Jian carp (*Cyprinus carpio* var. Jian) (92.85%) [?], and Pacific white shrimp (*Litopenaeus vannamei*) (90.40%) [?]. These values differ substantially from our result of 48.97%, possibly due to differences in fish species and corn gluten meal quality.

Amino acids are the basic units of protein, and their apparent digestibility reflects protein quality in protein sources. In this study, the apparent digestibility of total amino acids in the four protein sources showed a similar trend to CP digestibility, consistent with findings in silver perch [?], black carp (*Mylopharyngodon piceus* Richardson) [?], and Jian carp [?]. Fish meal has a balanced amino acid composition with high digestibility for all amino acids. Among the three plant protein sources, arginine showed relatively high digestibility, while lysine digestibility was relatively low, possibly due to antagonism between these two amino acids [?]. The apparent digestibility of GE reflects the ability of fish to utilize CP, fat, and carbohydrates in feed or protein ingredients. In this study, the GE digestibility of fish meal was 81.16%, significantly higher than the three plant protein sources, similar to results reported for rainbow trout [?], Nile tilapia (*Oreochromis niloticus* × *Oreochromis aureus*) [?], and Siberian sturgeon (*Acipenser baerii* Brandt) [?]. The GE digestibility of soybean meal was 49.26%, similar to the result (55.24%) reported for hybrid striped bass (*Morone saxatilis* × *Morone chrysops*) [?]. The GE digestibility of corn gluten meal was 46.28%, which differs considerably from values of 82.70% and 76.50% reported for Atlantic cod (*Gadus morhua*) [?] and largemouth bass (*Micropterus salmoides*) [?], possibly due to differences in fish species and processing methods of corn gluten meal.

3.3 Effects of Extrusion Treatment on Nutrient Apparent Digestibility in Four Protein Sources

To investigate the effects of extrusion treatment on nutrient digestibility in plant protein sources, test diets were processed into slow-sinking extruded pellets, and the apparent digestibility of nutrients in the four protein sources was determined. The results showed that compared with the normal pellet group, all nutrient digestibility values improved for each protein source in the extruded pellet group. For fish meal, the digestibility of DM, CP, total amino acids, and GE all increased, though the changes in CP and GE digestibility were not significant. Numerous studies have examined the effects of extrusion technology on nutrient digestibility, and mild extrusion conditions are generally considered to improve CP digestibility in feed ingredients. In this study, extrusion improved nutrient digestibility in fish meal, mainly because the combined effects of high temperature, high pressure, shearing, and friction during extrusion break molecular bonds in organic compounds such as proteins and fats, leading to degradation and shorter molecular structures that cannot form crystals. Additionally, the

porous physical structure of extruded feed allows digestive enzymes to more easily penetrate the interior of fish meal, facilitating hydrolysis and thus improving digestibility [?].

Extrusion significantly improved the digestibility of DM, CP, total amino acids, and GE in soybean meal by 4.00%, 7.94%, 11.48%, and 7.35%, respectively. Similar reports are abundant: Cheng et al. [?] found that extrusion improved the apparent digestibility of CP and amino acids in soybean meal for rainbow trout, and Cheng [?] reported that extrusion increased the apparent digestibility of DM and GE in soybean meal for rainbow trout by 3.00% and 2.80%, respectively. The improvement in nutrient digestibility of extruded soybean meal is related not only to protein denaturation but also to the destruction of anti-nutritional factors through heat denaturation and inactivation. Soybean meal contains heat-sensitive anti-nutritional factors including protease inhibitors, lectins, urease, and anti-vitamin factors. Protease inhibitors can inhibit protease activity and hinder protein digestion and absorption in fish. Studies have shown that even low concentrations of protease inhibitors can reduce CP digestibility in rainbow trout [?]. Lectins can interact with endogenous surface receptors of intestinal epithelial cells, affecting immune function and inducing enteritis [?], thereby reducing digestive capacity. Urease, when activated under suitable conditions, can decompose nitrogen-containing compounds in soybean meal into ammonia, reducing the utilization of protein and non-protein nitrogen [?]. In this study, protein denaturation and elimination of anti-nutritional factors during extrusion promoted nutrient digestion and absorption, significantly improving nutrient digestibility in soybean meal.

Extrusion significantly improved the digestibility of CP, total amino acids, and GE in sunflower meal by 6.02%, 10.34%, and 4.73%, respectively, while DM digestibility improved but not significantly. Li et al. [?] studied the effects of sunflower meal on intestinal amino acid digestibility in grass carp and found that under 28% sunflower meal inclusion, the intestinal apparent digestibility of essential amino acids in the extruded feed group was 1.44% higher than in the normal pellet group. In addition to protease inhibitors, sunflower meal contains arginase inhibitors that can inhibit arginase activity and interfere with L-arginine metabolism and biosynthesis [?]. During extrusion, heat-sensitive anti-nutritional factors such as protease inhibitors and arginase inhibitors are inactivated by high temperature and pressure. Additionally, the extrusion environment can break chemical bonds between fiber molecules in sunflower meal, increasing soluble fiber content. The combination of protein denaturation, increased soluble fiber, and elimination of anti-nutritional factors during extrusion improved nutrient digestibility in sunflower meal.

Extrusion significantly improved the digestibility of DM, CP, total amino acids, and GE in corn gluten meal by 10.72%, 8.23%, 20.57%, and 8.40%, respectively. Related studies have shown that after extrusion, the apparent digestibility of DM and GE in corn gluten meal for rainbow trout increased by 11.80% and 8.90%, respectively [?]. Although corn gluten meal contains low levels of anti-

nutritional factors, extrusion significantly improved its digestibility. This is because corn gluten meal has high protein content, and extrusion destroys the advanced structure of proteins, increasing amino acid availability and thus improving protein utilization. Furthermore, extrusion can break glycosidic bonds within starch molecules to generate low-molecular-weight saccharides such as glucose, maltose, and maltodextrin, while hydrogen bonds between molecules break and gelatinization occurs [?]. These changes make nutrients in feed ingredients more easily digested and absorbed. Due to structural changes in organic compounds such as proteins and starch degradation and gelatinization, nutrient digestibility in corn gluten meal was improved.

This study demonstrates that extrusion treatment greatly improved the nutritional value of plant protein sources, with the improvement rates in nutrient digestibility being much higher than for fish meal. Soybean meal showed the best performance, with CP digestibility reaching 70.84%. Sunflower meal had higher CP and total amino acid digestibility than corn gluten meal, reaching 67.19% and 73.50%, respectively, but lower DM and GE digestibility than corn gluten meal. Corn gluten meal showed substantial improvements in all nutrient digestibility values. In conclusion, extrusion treatment significantly enhances the application value of plant protein sources in turbot feeds and deserves promotion.

Key findings: 1. Fish meal showed the best apparent digestibility for all nutrients, while the three plant protein sources (soybean meal, sunflower meal, and corn gluten meal) exhibited relatively lower nutrient digestibility. 2. Among the three plant protein sources, soybean meal and sunflower meal showed superior CP and total amino acid digestibility compared to corn gluten meal and can be appropriately used as protein sources in turbot formulated feeds. 3. Extrusion treatment can significantly improve the apparent digestibility of nutrients in soybean meal, sunflower meal, and corn gluten meal for turbot, representing an effective method to increase the replacement level of plant protein sources for fish meal.

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