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ISSN 0792 - 156X





PUBLISHER:

Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

*The Israeli Journal of Aquaculture - Bamidgeh, IJA_67.2015.1173, 9 pages*


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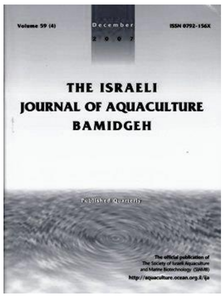


# Apparent Digestibility of Selected Feed Ingredients in Juvenile Turbot (*Scophthalmus maxima* L.)

**Yanjie Wei, Gen He*, Kangsen Mai, Wei Xu, Huihui Zhou, Lin Mei**

*Key Laboratory of Aquaculture Nutrition and Feed, Ministry of Agriculture, and the Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, P. R. China*

(Received 23.11.2014, Accepted 7.1.2015)

Key words: Digestibility; protein sources; bioavailability, Turbot.

**Abstract**

In aquaculture, replacing dietary fishmeal with other protein sources is challenging. In order to select substitute sources, it is necessary to determine the apparent digestibility coefficients, many of which have not been well characterized for fish species. We investigated the apparent digestibility of eight protein sources fed to juvenile turbot *(Scophthalmus maxima* L.*)*. These were Peruvian super red fishmeal (FM), peanut meal (PM), corn gluten meal (CGM), dehulled solvent extracted soybean meal (SBM), wheat gluten (WG), Australian beef meat and bone meal (MBM), spray-dried (pork) hemoglobin meal (SDHM) and American pet-food grade poultry byproduct meal (PBM). The apparent digestibility coefficients of dry matter (DM), crude protein (CP), gross energy (GE), and amino acids were analyzed. Results indicated that turbot utilized high-protein feedstuffs better than high-carbohydrate or high-fiber feedstuffs. This study provides valuable information on ingredient selection and evaluation of feed for turbot.

*Corresponding author. Tel. / Fax: +86 532 8203 1589. *E-mail address*: hegen@ouc.edu.cn

## Introduction

Fishmeal has been considered an indispensable protein source in aquafeeds. However, with limited supply of fishmeal and rising high cost, much attention has focused on fishmeal replacement by other proteins, especially plant protein sources (Nagel et al 2012; Bonaldo et al., 2011, Cassiano et al.,2012, Slawski et al., 2011). Although much progress has been made, plant protein sources still remain a challenge for a high level of fishmeal replacement especially for carnivorous fish where excessive fishmeal replacement has led to reduced growth (Regost, 1999; Bonaldo et al., 2011), increased feed conversion ratio (FCR) (Nagel et al 2012), reduced protein utilization (Opstvedt et al., 2003) and pathological changes in fish intestine (Storebakken et al., 2000). Many factors are involved in the limited utilization of non-fishmeal proteins by fish. A major drawback for many protein sources has been their poor digestibility by fish. Selection of protein sources with acceptable digestibility should provide the basis for high dietary utilization and low waste production. In many fish species, apparent digestibility coefficient (ADC) of major protein sources has not been fully characterized.

Turbot, a carnivorous fish, has a high protein requirement (Lee, 2003). Research has been carried out to find a suitable replacement for fishmeal for seabream (Kissil and Lupatsch, 2004) and turbot diets (Yigit et al., 2006; Ergun et al., 2008; Fournier et al., 2004; Bonaldo et al., 2011), but these studies showed that only 20%-50% fishmeal was successfully replaced suggesting that fishmeal replacement is unsatisfactory in turbot feeds. ADCs of limited types of protein sources have been available for turbot. These include feather meal, poultry meat meal, hemoglobin meal, extruded peas, extruded lupin, and rapeseed meal (Burel et al., 2000; Davies, 2009). It has been difficult to compare these results because of the differing fish sizes and feeding environments used in these studies. The objective of this study was to determine the apparent digestibility coefficients of dry matter, crude protein, energy, and amino acids availability in fish meal (FM), peanut meal (PM), corn gluten meal (CGM), soybean meal (SBM), wheat gluten (WG), meat and bone meal (MBM), spray-dried hemoglobin meal (SDHM) and poultry byproduct meal (PBM) for juvenile turbot.

## Materials and methods

*Diet preparation.* The reference diet (RF) was formulated to satisfy the protein and lipid requirements of turbot (Lee, 2003), see Table 1.

**Table 1.** Reference and test diet formulations for digestibility coefficient determination.

| *Ingredients* | *Reference diet (% Dry matter)* | *Test diet (% Dry matter)* |
|---|---|---|
| Fish meal [a] | 600.00 | 420.00 |
| Soybean meal [a] | 50.00 | 35.00 |
| Wheat meal [a] | 229.50 | 160.40 |
| Lecithin | 20.00 | 14.00 |
| Fish oil | 45.00 | 31.50 |
| Vitamin premix [b] | 20.00 | 14.00 |
| Mineral premix [c] | 20.00 | 14.00 |
| Choline chloride | 3.00 | 2.10 |
| Attractant [d] | 5.00 | 3.50 |
| Mold inhibitor | 1.00 | 0.70 |
| Antioxidant | 0.50 | 0.35 |
| $CaH_2(PO)_4$ | 5.00 | 3.50 |
| Yttrium oxide [e] | 1.00 | 1.00 |
| Test ingredient | 0.00 | 300.00 |

Eight experimental diets composed of 70% reference diet and 30% of the test ingredients (on a dry weight basis) were prepared as described by Cho and Slinger (1979). Yttrium oxide ($Y_2O_3$, 0.1%) was used as an inert marker and was incorporated into the reference and experimental diets. Proximate composition and amino acid composition of the test ingredients and diets are shown in tables 2 and 3 respectively.

**Table 2.** Proximate composition and amino acid composition of the experimental feeding ingredients[a] (% Dry matter)

| | *FM* | *PBM* | *SDHM* | *MBM* | *SBM* | *PM* | *CGM* | *WG* |
|---|---|---|---|---|---|---|---|---|
| **Proximate composition** | | | | | | | | |
| Crude protein | 75.46 | 72.82 | 99.32 | 59.66 | 53.27 | 54.97 | 60.94 | 88.33 |
| Crude lipid (%) | 8.85 | 13.31 | 0.60 | 9.50 | 2.55 | 1.70 | 4.16 | 1.60 |
| Moisture (%) | 8.61 | 5.11 | 8.40 | 7.84 | 10.79 | 7.09 | 4.92 | 7.09 |
| Ash (%) | 14.98 | 12.41 | 3.77 | 30.80 | 6.63 | 6.42 | 1.80 | 0.99 |
| Energy(KJ/Kg) | 22.07 | 22.73 | 23.01 | 17.86 | 20.04 | 20.00 | 21.73 | 23.07 |
| **Amino acid** | | | | | | | | |
| Arg | 4.07 | 4.28 | 3.87 | 3.63 | 3.41 | 5.30 | 1.76 | 2.80 |
| His | 2.23 | 1.28 | 7.00 | 0.43 | 1.20 | 1.09 | 1.21 | 1.60 |
| Phe | 2.95 | 2.53 | 6.51 | 1.69 | 2.50 | 2.57 | 3.59 | 4.08 |
| Lys | 5.39 | 3.84 | 7.57 | 1.57 | 2.72 | 1.31 | 0.86 | 1.11 |
| Val | 3.30 | 2.85 | 7.98 | 2.07 | 2.11 | 1.82 | 2.67 | 3.21 |
| Met | 1.84 | 1.36 | 0.73 | 0.39 | 0.59 | 0.41 | 1.48 | 1.09 |
| Ile | 2.78 | 2.39 | 0.44 | 1.29 | 2.02 | 1.45 | 2.23 | 2.95 |
| Leu | 5.17 | 4.39 | 12.59 | 2.62 | 3.58 | 3.11 | 9.47 | 5.56 |
| Thr | 3.07 | 2.51 | 3.06 | 1.54 | 1.93 | 1.38 | 2.02 | 2.08 |
| Ser | 2.84 | 2.63 | 4.22 | 3.01 | 2.52 | 2.44 | 3.00 | 3.81 |
| Glu | 9.61 | 8.83 | 8.52 | 5.67 | 9.32 | 10.18 | 13.43 | 31.47 |
| Gly | 4.11 | 6.09 | 4.59 | 8.88 | 2.11 | 2.88 | 1.61 | 2.79 |
| Ala | 4.50 | 4.21 | 7.85 | 3.88 | 2.12 | 1.98 | 5.12 | 2.14 |
| Cys | 0.59 | 0.77 | 0.73 | 0.80 | 0.58 | 0.57 | 1.15 | 1.69 |
| Tyr | 2.29 | 1.86 | 2.07 | 1.16 | 1.71 | 1.84 | 2.88 | 2.82 |
| Asp | 6.25 | 5.15 | 10.99 | 3.32 | 5.36 | 5.55 | 3.51 | 2.61 |
| Pro | 2.56 | 3.78 | 3.05 | 5.80 | 2.20 | 1.84 | 5.13 | 9.29 |

[a] These protein sources were obtained from Great seven Bio-Tech (Qingdao, China), except for SDHM, which was obtained from NP protein limited company (TianJin, China)

**Table 3.** Proximate composition and Amino acid composition of the experimental diets (% Dry matter)

| | *Reference diet* | *FM diet* | *PBM diet* | *SDHM diet* | *MBM diet* | *SBM diet* | *PM diet* | *CGM diet* | *WG diet* |
|---|---|---|---|---|---|---|---|---|---|
| **Proximate comp** | | | | | | | | | |
| Crude protein (%) | 530.5 | 597.75 | 589.83 | 669.33 | 550.35 | 531.18 | 536.28 | 554.19 | 636.36 |
| Crude lipid (%) | 123.07 | 112.76 | 126.08 | 87.95 | 114.65 | 93.80 | 91.25 | 98.63 | 90.95 |
| Ash (%) | 95.27 | 111.63 | 100.23 | 77.46 | 152.13 | 86.79 | 88.80 | 76.47 | 69.54 |
| Energy(KJ/Kg) | 206.82 | 208.79 | 212.96 | 213.80 | 198.35 | 204.89 | 204.77 | 209.96 | 213.98 |
| **Amino acid** | | | | | | | | | |
| Arg | 26.45 | 29.50 | 32.30 | 28.50 | 29.55 | 29.45 | 32.80 | 22.75 | 25.90 |
| His | 14.70 | 16.50 | 14.55 | 28.30 | 11.95 | 14.10 | 13.30 | 13.00 | 13.85 |
| Phe | 20.95 | 22.70 | 23.70 | 32.35 | 19.75 | 22.40 | 23.05 | 23.95 | 26.20 |
| Lys | 33.75 | 37.65 | 35.75 | 43.55 | 28.60 | 32.55 | 27.55 | 24.85 | 24.70 |
| Val | 22.15 | 23.65 | 24.70 | 35.95 | 21.60 | 22.05 | 22.40 | 22.55 | 24.15 |
| Met | 11.80 | 11.35 | 11.65 | 8.65 | 8.20 | 9.25 | 9.70 | 11.35 | 11.00 |
| Ile | 18.90 | 20.10 | 21.15 | 14.00 | 16.75 | 19.60 | 18.90 | 19.05 | 21.20 |
| Leu | 34.90 | 38.30 | 38.35 | 58.20 | 32.95 | 36.15 | 37.15 | 49.80 | 39.65 |
| Thr | 20.00 | 21.95 | 21.30 | 22.40 | 18.60 | 20.20 | 18.30 | 18.95 | 19.35 |
| Ser | 20.25 | 21.75 | 21.85 | 25.90 | 23.50 | 22.10 | 21.10 | 21.90 | 24.80 |
| Glu | 78.00 | 80.75 | 83.60 | 77.05 | 72.05 | 83.50 | 87.15 | 90.75 | 144.85 |
| Gly | 28.10 | 30.90 | 37.60 | 31.75 | 45.60 | 26.65 | 27.70 | 23.35 | 27.35 |
| Ala | 29.55 | 32.65 | 33.20 | 41.65 | 32.35 | 27.40 | 28.20 | 34.00 | 25.85 |
| Cys | 6.15 | 6.40 | 6.20 | 6.55 | 6.80 | 6.45 | 6.55 | 7.35 | 9.65 |
| Tyr | 40.50 | 44.95 | 44.10 | 57.85 | 38.35 | 45.35 | 43.70 | 36.65 | 34.60 |
| Asp | 16.15 | 17.25 | 16.90 | 16.35 | 15.15 | 16.85 | 17.20 | 18.90 | 19.30 |
| Pro | 21.40 | 22.15 | 26.65 | 22.95 | 32.25 | 22.40 | 21.75 | 28.70 | 41.45 |

All ingredients were ground into fine powder through 80 μm mesh. Ingredients of each diet were blended thoroughly first by hand and then mechanically. Lecithin was dissolved in oil and then mixed with all ingredients. Water was then added into the mixture to produce stiff dough which was pelleted using experimental feed mill (F-26 (II), South China University of Technology, China) and dried for about 12 h in a ventilated oven at 45 °C, and stored in freezer at -20 °C until use.

*Fish and experimental conditions.* Juvenile turbot, *Scophthalmus maxima,* (6.39±0.02 g) were obtained from a local hatchery farm. After being acclimated to the reference diet in the laboratory for 2 weeks, fish were randomly distributed into twenty seven 200-L cylindrical fiberglass tanks (three tanks for each diet) with 40 fish per tank. Fish were fed to visual satiety twice daily (06:30 and 18:30) with one of the nine experimental diets. Sand-filtered seawater was supplied to rearing tank. The feeding experiment lasted 5 weeks. Seawater temperature and salinity were monitored daily. During the experimental period, temperature, salinity, pH, and dissolved oxygen were all suitable for turbot.

*Fecal collection.* Stripping was a preferred method of fecal collection for plant protein digestibility trials because of poor fecal stability (Glencross et al., 2005). During the acclimation period, discharged feces were observed 1-5 hours after meals. To provide enough time for digestion, we chose 5 hr post-feeding as collection time. Diets were fed twice daily (06:30 and 18:30) to apparent satiation for 7 days prior to fecal collection. Manual stripping of fish was accomplished by gently applying pressure to the lower abdominal region of turbot under anesthesia into a plastic weighing pan. Care was taken to exclude urinary excretion from the collection. After stripping, fish were given a salt bath (15–20 ppm) for 10–15 min to reduce handling stress before being returned to culture tanks. Stripping for fecal material was only performed every five-days to keep stress levels of the fish to a minimum. During the entire period, the process was repeated seven times to obtain triplicate fecal samples per feed ingredient for calculation of ACDs. Fecal samples for a given tank were dried overnight at 50 $^{o}$C, pooled and stored at -20 $^{o}$C until analysis.

*Chemical analysis.* Dry matter and ash analysis of ingredients, diets, and feces were performed according to standard methods (AOAC, 1995). Yttrium content of diets and feces were determined by inductively coupled plasma original emission spectrometer (ICP-OES) [IRIS Advantage (HR), Thermo Jarrell Ash, Woburn, USA]. Crude protein was determined by the Kjeldahl method after acid digestion using a Kjeldahl System (1030-Auto-analyzer, Tecator, Sweden). Amino acids in ingredients, diets, and fecal material were analyzed by amino acid analyzer (Biochrom 30, GE) following acid hydrolysis (AOAC 1995). Total energy was determined in the reference diet by adiabatic bomb calorimetry (Parr1281; Parr Instrument Company Inc., Moline, IL, USA).

*Digestibility determinations and statistical analyses.* The ADCs of the diets were derived from the equation:

$$\text{ADC }(\%) = 100 \left[ 1- ( M_i / M_f ) ( C_f / C_i ) \right]$$

where $C_i$ and $C_f$ are the concentrations (%DM) of nutrient in the diet and feces, respectively, and $M_i$ and $M_f$ are the concentrations (%DM) of the marker in the diet and feces, respectively. The ADC of a nutrient in an ingredient ($ADC_{Ingr}$) added to the reference diet was calculated by difference, assuming no associative effects between the added ingredient and the reference diet. The apparent digestibility of the test feed ingredient used the nutrient contribution of the test ingredient rather than its weight contribution (Forster, 1999).

$$ADC_{ingr} (\%) = (ADC_{com}-(ADC_{Ref} (1 - SR_{Nut}))) / SR_{Nut}$$

where $ANC_{Com}$ is the ADC (%) of the nutrient in the combined diet, $ADC_{Ref}$ is the ADC (%) of the nutrient in the reference diet, and $SR_{Nut}$ is the substitution rate (as decimal) for the nutrient in question.

Calculation of $SR_{Nut}$ is as follows:

$SR_{Nut} = (N_{Test} SR_{Wt}) / ((N_{Test} SR_{Wt}) + (N_{Ref} (1 - SR_{Wt})))$

where $N_{Test}$ is the concentration (%) of the nutrient in the test ingredient, $N_{Ref}$ is the concentration (%) of the nutrient in the reference diet, and $SR_{Wt}$ is the substitution rate of the ingredient in the reference diet on a weight basis (in decimal : 0.3).

*Statistical analysis.* Statistical evaluation of the data was conducted using the computer software application SPSS16.0 for Windows. All data in this study are presented as means ± SD of three replicates and analyzed by one-way ANOVA to test the effects of experimental treatments. Differences among means were considered significant at $P \leq 0.05$. Turkey's test was subsequently used to identify the significant differences among the treatment mean values.

## Results

The proximate composition and amino acid composition of the test ingredients are presented in Tables 2 and 3. Apparent digestibility coefficients for dry matter, crude protein, and energy of the test ingredients in juvenile turbot were summarized in Table 4.

**Table 4.** ADC (%) of dry matter, crude protein, gross energy of feedstuffs for turbot

| *Ingredients* | *Apparent digestibility coefficients（ADCs %）* | | |
|---|---|---|---|
| | *Dry matter* | *Protein* | *Energy* |
| FM | $84.40±2.38^{e}$ | $87.68±0.55^{d}$ | $96.46±0.86^{f}$ |
| PBM | $56.02±3.23^{c}$ | $77.09±1.66^{c}$ | $68.33±3.03^{cd}$ |
| SDHM | $65.98±1.79^{d}$ | $86.03±2.72^{d}$ | $71.52±2.70^{d}$ |
| MBM | $30.34±2.60^{ab}$ | $73.93±2.59^{c}$ | $61.76±3.99^{c}$ |
| SBM | $31.03±2.64^{ab}$ | $64.53±1.60^{b}$ | $48.23±2.16^{b}$ |
| PM | $33.94±1.82^{b}$ | $71.55±3.08^{c}$ | $50.83±3.20^{b}$ |
| CGM | $25.63±3.11^{a}$ | $48.50±3.73^{a}$ | $36.08±1.07^{a}$ |
| WG | $70.20±3.81^{d}$ | $85.16±2.13^{d}$ | $85.85±0.88^{e}$ |

Values are means ± S. D. (n=3) of three replicates and values within the same Column with different letters are significantly different ($P$<0.05).

For dry matter digestibility, fishmeal was highest (84.40%) while corn gluten meal (CGM) was lowest (25.63%). After statistical analysis, the order of the ADCs of dry matter was FM $^{e}$>WG $^{d}$ >SDHM $^{d}$ > PBM $^{c}$ >PM $^{b}$ > SBM $^{ab}$ >MBM $^{ab}$ >CGM $^{a}$. Similarly, the order of protein ADCs was: FM $^{d}$ >WG $^{d}$>SDHM $^{d}$> PBM $^{c}$ >MBM $^{c}$ > PM $^{c}$ > SBM $^{b}$ >CGM $^{a}$. In addition, the order of energy ADCs was: FM $^{f}$ > WG $^{e}$ > SDHM $^{d}$ > PBM $^{cd}$ > MBM $^{c}$ > PM $^{b}$ > SBM $^{b}$ > CGM $^{a}$. Shared letters denoted no significant difference ($p$> 0.05). In general, ingredients with high protein content showed better ADCs.

The apparent amino acid availability coefficients of the tested ingredients by juvenile turbot are presented in Table 5. In general, amino acid availability reflected crude protein digestibility.

Availability for all amino acids was good and balanced in the fishmeal diet, ranging between $73.67_{Asp}$-$101.25_{Cys}$%. However, there were major differences in the availability of various amino acids for most non-fishmeal protein sources. WG ranged between $69.77_{Lys}$-$111.45_{Met}$%; SDHM ranged between $50.83_{Ile}$-$93.21_{Cys}$%; PBM ranged between $67.71_{Met}$-$96.97_{Phe}$%; MBM ranged between $59.16_{Ile}$-$92.29_{Cys}$%; PM ranged between $46.97_{Gly}$-$107.46_{Leu}$%; SBM ranged between $59.88_{Thr}$-$107.65_{Cys}$%; CGM ranged between $27.20_{Thr}$-$64.12_{Lys}$%. Therefore, differential amino acid availability and imbalanced amino acid profiles both contributed to the low level of performance of non-fishmeal proteins.

**Table 5.** Apparent amino acid (AA) availabilities (%) of the ingredients for turbot

| *AA* | *FM* | *PM* | *CGM* | *SBM* | *WG* | *MBM* | *SDHM* | *PBM* |
|---|---|---|---|---|---|---|---|---|
| Arg | 93.68±0.24$^{f}$ | 78.44±0.96$^{e}$ | 41.01±1.19$^{a}$ | 85.26±0.46$^{e}$ | 86.68±1.12$^{e}$ | 82.25±1.06$^{d}$ | 70.52±0.96$^{b}$ | 91.61±0.25$^{f}$ |
| His | 97.64±1.00$^{f}$ | 58.28±1.14$^{a}$ | 55.21±2.99$^{a}$ | 72.41±1.15$^{bc}$ | 70.70±2.12$^{b}$ | 82.33±2.86$^{d}$ | 77.52±1.06$^{cd}$ | 90.19±0.37$^{e}$ |
| Ph | 96.37±1.42$^{d}$ | 86.10±0.31$^{c}$ | 48.90±3.27$^{a}$ | 74.21±0.74$^{b}$ | 92.76±0.77$^{d}$ | 72.47±3.47$^{b}$ | 81.22±1.33$^{c}$ | 96.97±1.36$^{d}$ |
| Lys | 86.97±1.02$^{e}$ | 58.16±2.29$^{a}$ | 64.12±3.91$^{b}$ | 77.05±0.62$^{d}$ | 69.77±0.75$^{c}$ | 77.42±118$^{d}$ | 78.31±1.12$^{d}$ | 89.59±0.41$^{e}$ |
| Val | 74.56±1.21$^{b}$ | 90.95±4.84$^{c}$ | 35.17±4.30$^{a}$ | 66.94±1.78$^{b}$ | 88.61±1.33$^{e}$ | 72.47±3.18$^{b}$ | 71.64±1.12$^{b}$ | 85.23±0.82$^{c}$ |
| Me | 90.84±1.50$^{cd}$ | 104.55±6.76$^{d}$ | 32.05±9.23$^{a}$ | 64.84±11.21$^{b}$ | 111.45±2.21$^{d}$ | 68.45±8.88$^{b}$ | 71.95±3.15$^{bc}$ | 67.71±10.70 |
| Ile | 73.50±0.47$^{d}$ | 93.28±1.25$^{e}$ | 34.83±3.67$^{a}$ | 67.80±0.74$^{d}$ | 87.25±1.23$^{e}$ | 59.16±3.95$^{c}$ | 50.83±2.35$^{b}$ | 87.16±0.76$^{e}$ |
| Leu | 82.15±0.32$^{be}$ | 107.46±2.00$^{e}$ | 54.70±4.01$^{a}$ | 76.69±0.67$^{b}$ | 89.34±0.90$^{d}$ | 78.28±3.71$^{b}$ | 79.33±1.13$^{bc}$ | 85.25±0.77$^{cd}$ |
| Thr | 75.90±1.06$^{de}$ | 59.42±4.67$^{b}$ | 27.20±4.04$^{a}$ | 59.88±0.94$^{b}$ | 82.47±2.40$^{e}$ | 66.86±4.23$^{bc}$ | 71.30±2.41$^{cd}$ | 71.23±0.79$^{cd}$ |
| Ser | 77.65±0.98$^{c}$ | 63.45±2.98$^{b}$ | 41.22±3.82$^{a}$ | 69.35±0.84$^{b}$ | 91.29±1.22$^{d}$ | 75.83±2.60$^{c}$ | 79.35±1.40$^{c}$ | 69.32±0.68$^{b}$ |
| Glu | 80.92±1.27$^{c}$ | 80.829±2.82$^{c}$ | 45.49±5.30$^{a}$ | 68.18±0.78$^{b}$ | 94.40±0.80$^{d}$ | 71.92±4.01$^{b}$ | 67.17±3.32$^{b}$ | 86.76±0.94$^{cd}$ |
| Gly | 77.44±1.50$^{e}$ | 46.97±1.90$^{a}$ | 42.07±5.85$^{a}$ | 60.46±1.29$^{b}$ | 95.46±3.05$^{d}$ | 74.25±2.74$^{c}$ | 72.36±2.62$^{c}$ | 77.49±0.49$^{c}$ |
| Ala | 82.08±0.40$^{c}$ | 95.44±3.99$^{d}$ | 47.89±4.60$^{a}$ | 71.94±0.89$^{b}$ | 81.86±2.36$^{e}$ | 78.31±2.40$^{bc}$ | 79.79±1.09$^{c}$ | 80.95±045$^{c}$ |
| Cy | 101.25±1.45$^{cd}$ | 94.81±4.36$^{b}$ | 62.41±0.47$^{a}$ | 107.65±3.30$^{d}$ | 101.26±6.26$^{c}$ | 92.29±5.86$^{b}$ | 93.21±2.98$^{b}$ | 68.86±1.55$^{a}$ |
| Tyr | 81.05±1.14$^{cd}$ | 83.31±3.52$^{cd}$ | 48.68±8.32$^{a}$ | 75.70±4.41$^{bc}$ | 92.97±0.91$^{d}$ | 79.13±7.56$^{c}$ | 62.43±3.50$^{b}$ | 78.08±2.42$^{c}$ |
| As | 73.67±0.83$^{ed}$ | 58.29±1.99$^{b}$ | 39.51±3.43$^{a}$ | 61.46±1.07$^{b}$ | 76.97±2.59$^{d}$ | 60.26±3.25$^{b}$ | 75.11±2.57$^{ed}$ | 69.93±0.56$^{c}$ |
| Pro | 79.94±080$^{de}$ | 84.78±3.00$^{e}$ | 46.20±3.07$^{a}$ | 70.16±0.86$^{b}$ | 93.82±1.10$^{f}$ | 72.80±2.03$^{bc}$ | 68.88±3.70$^{b}$ | 78.28±0.96$^{cd}$ |

Values are means ± S. D. (n=3) of three replicates and values within the same row with different letters are significantly different ($P$<0.05).

## Discussion

The importance of digestibility coefficient availability has been well acknowledged for ingredient evaluation and selection in aquaculture feeds (Glencross et al., 2007). The ADC of nutrients may be influenced by fish species and size, water salinity, and temperature (Watanabe et al., 1996; Kim et al., 1998; Førde-Skjærvik et al., 2006). In addition, fecal collection method is directly related to the values obtained for ADC. When comparing fecal stripping and collections methods, it has been suggested that high levels of fecal carbohydrates from plant proteins decreases fecal integrity and increases the dissolution of the fecal matter when expelled into water, thereby effectively reducing the fecal nutrient collected and consequently inflating the digestibility value determined (Glencross et al., 2005). Therefore fecal stripping was the preferred fecal collection method for plant protein sources (Glencross et al., 2005). We observed a similar phenomenon and therefore chose the fecal stripping method for ADC determination in our experiments.

The digestibility of FM obtained in the current study was generally comparable with the results obtained for turbot (Davies, 2009). The order of ADCs of dry matter was FM $^{e}$>WG $^{d}$ >SDHM $^{d}$ > PBM $^{c}$ >PM $^{b}$ > SBM $^{ab}$ >MBM $^{ab}$ >CGM $^{a}$. The ADC of dry matter for corn gluten meal was only 25.63%. In general, digestibility is correlated with protein content. FM has high protein digestibility but low carbohydrate digestibility for turbot. The carbohydrate content of these proteins was predominantly non-starch polysaccharides (NSP) and constituted limited nutritional value for most monogastric animals. Fish lack the enzymes to digest them. This also lowers dry matter and energy ADCs of plant protein sources (NRC, 1993). In the present study, there was a tendency in turbot to digest dry matter and energy in feedstuffs of animal origin more efficiently than dry matter and energy in feedstuffs of plant origin. Similar results have been reported for other carnivorous species such as rainbow trout (Cho et al., 1982). It has been suggested that low values could partly be due to the short gastro-intestinal tract of turbot and low rearing temperatures (Davies, 2009).

Protein ADCs of SDHM and WG were generally comparable to that of FM. All these protein sources have high protein content. The protein digestibility of blood meal was previously found to be around 90% in sea bass (Da Silva and Oliva-Teles, 1998) and 74.8% in turbot (Davies, 2009). In our study the protein ADC of SDHM was 86.03%. The variations may result from different fish species and different fish sizes. Wheat gluten (WG) also exhibited high protein digestibility in the current study. It was reported that the digestibility of protein in WG was as high as 100% (Glencross and Hawkins, 2004). WG is high in protein, low in fiber, and is known to contain no anti-nutritional factors. In turbot WG has been seen to be a good partial substitute for FM (Fournier et al., 2004). The protein digestibility of PBM and MBM was lower than FM, but can be effectively digested by turbot. Protein ADC for PBM in turbot was 78.4% (Davies, 2009), which was comparable with our results. PBM is an effective animal protein source (Falaye et al., 2011) with bioethical considerations (Davies, 2009). It has been successfully included in many fish diets (Guo et al, 2007; Masagounder et al., 2009). The protein ADC of MBM in turbot was low compared with cobia (Zhou, 2004), showing differential utilizations between species.

Protein ADCs of SBM, PM and CGM were lower, especially CGM, which gave the lowest ADC compared with FM. Results showed that only 30% of FM could be replaced by CGM in turbot diets. Results from our study suggested that poor performance of CGM might be due in part to its low protein digestibility (Regost, 1999). Amino acid imbalance and anti-nutritional factors were possible causes of low ADC of plant feedstuffs (Luo et al., 2009).

Amino acid availability in this study generally reflected the protein ADCs. FM had the highest AA availability and CGM was lowest among all the protein sources tested in this study. WG and PBM showed AA availability comparable to that of FM in most amino acids, a result which was similar to a previous report (Guimaraes, 2008). In the present study, fishmeal showed good and balanced AA availability. However, most non-fishmeal protein sources showed differing availability between amino acids. This phenomenon has been previously reported (Gaylord, 2004). Imbalance in AA composition and digestibility would contribute to low effectiveness of non-fishmeal proteins in aquafeeds.

In conclusion, there are major protein sources that can potentially be used in turbot diets. The proper combination of protein sources is more likely to meet the nutritional needs of fish than a single non-fishmeal protein source (Kissil and Lupatsch, 2004). The availability of ADCs of different protein sources should provide the basis for rational formulation and better utilization of diets by turbot.

## Acknowledgments

This study was supported by Special Fund for Agro-scientific Research in the Public Interest (201303053) to G.H., 973 program (2014CB138602) to K.M., and Shandong Provincial Natural Science Foundation (JQ201206) to G.H. Gen He and Kangsen Mai designed this study. Huihui Zhou and Wei Xu provided essential reagents and materials. Yanjie Wei and Lin Mei conducted research and Yanjie Wei analyzed data. Gen He and Yanjie Wei wrote the manuscript. All authors have read and approved the final manuscript.

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