

Apparent digestibility coefficients of crude protein, amino acids, crude lipid, dry matter and gross energy of ten feedstuffs for yearling Siberian sturgeon (*Acipenser baerii*, Brandt 1869)

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Abstract

Apparent digestibility coefficients (ADCs) of ten feedstuffs were investigated in yearling Siberian sturgeon, *Acipenser baerii*, (290±22 g), using 1% chromic oxide as the inert marker. The ingredients included: fish (*Clopeonella* sp) meal (FM), meat and bone meal (MBM), poultry by-product meal (PBM), spray-dried blood meal (BM), feather meal (FeM), solvent extract soybean meal (SBM), canola meal (CM), corn gluten meal (CGM), wheat gluten meal (WGM) and bakers' yeast (BY). ADC values of crude protein varied from 60% to 92.9% (FeM and FM, respectively). ADC_{CP} of FM, MBM, PBM (animal origin), WGM, and CGM (plant origin) were significantly higher than the other treatments ($p<0.05$). ADCs of amino acid in feedstuffs followed a similar trend to the ADC_{CP}, but it is not thoroughly correspondence with protein digestibility. The lowest dry matter (65.5%), and lipid (64.8%) digestibility were recorded in BM and FeM, respectively. The WGM (81.5%) exhibited the highest digestibility of gross energy (ADC_{GE}); the lowest was in CM (68.1%). According to the results of this research, FM, MBM, PBM (animal origin) and also WGM and CGM (plant origin) were the more digestible feed ingredients for yearling Siberian sturgeon.

Keywords: Aquaculture, Digestibility, Fish feeding, Ingredients, Siberian sturgeon.

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Introduction

For aquaculture to satisfy the increased demand for fish farming, continuous research must be conducted to select the appropriate alternative to fish meal.

Fish meal (FM) is the most important and one of the most expensive protein sources in the aquaculture-formulated diet. Approximately 20% of the world's fish stock is used only to produce fish meal and fish oil (FAO, 2016). The deterioration of fish meal production has boosted the cost of them; hence, efforts have been made to find alternative protein sources to feed farmed aquatic animals (Quinto *et al.*, 2018). Therefore, a suitable feedstuff ingredient has to contain some nutritional properties, including high levels of protein, low levels of anti-nutrients, indispensable amino acids, high digestibility, and acceptable palatability (Francis *et al.*, 2001; Gatlin *et al.*, 2007; Mirzakhani *et al.*, 2018).

The investigation for appropriate ingredients requires precise screening of their potential nutritional value and variability at the industrial level. The nutritional value of the diet composition depends on the digestibility of each of its constituents (Lupatsch *et al.*, 1997). Feed manufacturers require adjustable, nutritious, and cost-effective formulations based on the content and availability of feedstuffs (Lemos *et al.*, 2009). Consequently, many different types of nutrient sources are required to feed farmed fish to maintain the expected fast growth. The first stage of formulating diet is to determine the digestibility and palatability of feedstuff nutrients. Information on the apparent

digestibility coefficients (ADCs) is essential; not only does it promote information to improving the diet for growth, but it also restricts contamination through fish production (Chi *et al.*, 2017; Karapanagiotidis *et al.*, 2019).

In recent years, sturgeon farming has been expanding worldwide, due to the precious eggs (caviar) and meat. Thus, the improvement of sturgeon aquaculture production would be the most reasonable and rational alternative strategy to alleviate the fishing pressure on the natural stocks of sturgeon species (Banavreh *et al.*, 2018; Bronzi *et al.*, 2011).

Siberian sturgeon is considered as an important cultured sturgeon worldwide (Babaei *et al.*, 2011; Sharif Rohani *et al.*, 2017; Falahatkar, 2018) and has been used as a biological model to initiate studies on nutrition since the 1980s (Fontagné-Dicharry *et al.*, 2018). Moreover, despite significant global interest in sturgeon aquaculture, there is still a scarcity of information on sturgeon nutrient requirements, and the success in culturing these species of fish needs this information essentially.

Many relatively low-priced animal and plant-based feedstuffs are available, that can replace fish meal in Siberian sturgeon diets, and the investigation for suitable and cost-effective protein alternatives is ongoing. In our previous study (Mirzakhani *et al.*, 2018), protein digestibility (*in vivo*) and degree of protein hydrolysis (DPH: *in vitro* pH-stat assay) of ten selected feedstuffs for yearling Siberian sturgeon were investigated. Correlation between the

results of *in vitro* and *in vivo* was determined to evaluate the protein quality of ingredients. The rest of the previous study, the present investigation aims to evaluate the apparent digestibility coefficients (ADCs) of crude protein, lipid, energy, and amino acids in 10 animal and plant ingredients for yearling Siberian sturgeon.

Materials and methods

Diet preparation

The current investigation, ten different sources of feedstuff in Siberian sturgeon diets evaluated. Aquafeeds ingredients include fish (*Clopeonella* sp) meal (FM), meat and bone meal (MBM), poultry by-product meal (PBM), spray-dried blood meal (BM), feather meal (FeM), solvent extract soybean meal (SBM), canola meal (CM), corn gluten meal (CGM), wheat

gluten meal (WGM), bakers' yeast (BY). An approximation analysis of all feedstuff ingredients are shown in Table 1. The feed constituents pounded for homogeneity (<500 μm) and thoroughly mixed (Chega Co., Isfahan, Iran) with fish oil before water is added to produce stiff dough (Gatlin, 2010). Then, the experimental diet was formulated with each of the feedstuffs using a 70:30 (dry weight basis) from the reference diet (Table 2) with the Lindo program (6.1, Chicago, USA) (Mirzakhani *et al.*, 2018). Diets were pressure-pelleted using a meat grinder (CGT Company, 2mec, Italy) with a 3 mm die and placed into a hot air oven (Hootakhsh, Tehran, Iran) at 40 °C for 18 h to attain a moisture content of less than 100 g kg⁻¹. The dried diets were kept at -20 °C until used (Banavreh *et al.*, 2019).

Table 1: Proximate composition (%) and gross energy content (Kj g⁻¹) of ingredients.

| Ingredient ^a | Moisture (%) | Crude protein (%) | Crude Lipid (%) | Ash (%) | Carbohydrate ^b (%) | Gross energy ^c (Kj g ⁻¹) |
|------------------------------------|--------------|-------------------|-----------------|---------|-------------------------------|---|
| Fish meal (<i>Clopeonella</i> sp) | 7.1 | 74.5 | 6.7 | 9.7 | 2 | 20.4 |
| Meat and Bone meal | 7.4 | 53.1 | 12 | 14.9 | 12.6 | 19.4 |
| Poultry by-product meal | 7 | 64.5 | 8.7 | 10 | 9.8 | 20.4 |
| Blood meal | 4 | 88.3 | 2.2 | 3.6 | 1.8 | 22 |
| Feather meal | 6.5 | 78.1 | 6.8 | 3.5 | 5 | 23 |
| Soybean meal | 9.3 | 41.9 | 1.7 | 6.9 | 40.2 | 17.5 |
| Canola meal | 11.8 | 30.6 | 3.3 | 8 | 46.6 | 16.6 |
| Corn gluten meal | 5.7 | 76.8 | 4 | 1.7 | 11.8 | 21.7 |
| Wheat gluten meal | 4.8 | 84.3 | 1.8 | 1.5 | 7.7 | 21.9 |
| Bakers' yeast | 6.2 | 41.3 | 1.6 | 1.9 | 48.9 | 18.8 |

^a Mazandaran Animal and Aquatic feed (Manaqqa) Co. Iran

^b Carbohydrates were calculated by difference. Carbohydrate=100-(crude protein+crude lipid+ash+moisture).

^c Estimated energy was calculated based on 1 g crude protein being 23.6 Kj, 1 g crude fat being 39.5 Kj and 1 g carbohydrate being 17.2 Kj.

Table 2: Formulation and proximate composition of the reference diet used to measure apparent digestibility (APD) of test ingredients.

| Test ingredients | g kg ⁻¹ |
|---|--------------------|
| Fish meal ^a | 350 |
| Soybean meal ^a | 270.7 |
| Wheat flour ^a | 181.1 |
| Soy bean oil ^a | 55 |
| Fish oil ^b | 55 |
| Lecitine ^c | 10 |
| Dicalcium phosphate ^a | 5 |
| Mineral mix ^d | 20 |
| Vitamin mix ^e | 40 |
| BHT ^f | 0.2 |
| Choline chloride ^a | 3 |
| Cr ₂ O ₃ ^g | 10 |
| Proximate composition | |
| Moisture (%) | 9.06 |
| Crude protein (%) | 40.45 |
| Lipid (%) | 14.55 |
| Ash (%) | 10.98 |
| Carbohydrate (%) ^h | 24.95 |
| Gross energy (Kj g ⁻¹) ⁱ | 19.58 |

^aKilka (*Clopeonella* sp) meal (Mazandaran Animal and Aquatic feed (Manaqua) Co. Iran)

^bKilka oil (Manaqua Co. Iran)

^cSoybean lecithin with phosphatidyl choline (Behpak company, Iran)

^dMineral mix provided (mg Kg⁻¹): Fe: 6000, Cu: 600, Mn: 5000, Zn: 10,000, I: 600, Se: 20, Co: 100, choline chloride: 6000, Career up to 1 kg

^eVitamin mix provided (Unit Kg⁻¹): A: 1,200,000 IU, D3: 400,000 IU, E: 50,000 mg, K3: 800 mg, B9: 1000 mg, C: 30,000 mg, B1: 2500 mg, B2: 4000 mg, B6: 25,000 mg, B12: 8 mg, Biotin: 150 mg, Niacin: 35,000 mg and Inositol: 50,000 mg Career up to 1 kg

^fAntioxidant (Gluba Tiox, French)

^gChromic oxide (Sigma-Aldrich®, USA)

^hCarbohydrates were calculated by difference. Carbohydrate = 100 - (crude protein + crude lipid + ash + moisture)

ⁱEstimated energy was calculated based on 1 g crude protein being 23.6 Kj, 1 g crude fat being 39.5 Kj and 1 g carbohydrate being 17.2 Kj.

Experimental fish

Yearling Siberian sturgeons were obtained from the International Sturgeon Research Institute (Guilan province, Iran). They were acclimated in fiberglass tanks (350-L) and were fed the reference diet for one week before the experiment. Fish (n=198) weighing 290±22 g were then distributed randomly into 33 circular fiberglass tanks at a stocking density of 6 fish per tank. Each treatment comprises three replicates (18 fish per treatment). The flow-through water (4.5 L min⁻¹) was used from spring water during the experiments. Fish were fed with

experimental diets three times a day (at 08:00, 12:00, and 16:00) to available *ad libitum*. The feeding trial was carried out using the experimental diets under a 12-hr:12-hr light: dark cycle condition for 11 days. During the experiment, water quality consisting of water temperature, dissolved oxygen, pH value was 21±2 °C, 7.1±0.5 mg L⁻¹, and 7-8, respectively.

Digestibility

During the food processing, the amount of 10 g kg⁻¹ chromic oxide (Cr₂O₃, Sigma-Aldrich®) as an inert marker was added to the apparent digestibility

coefficients determination. The fish were hand feed with experimental diets for 11 days to the contents of the alimentary tract are completely evacuated from previous foods. From day 12, feces samples were collected from all fish in separate tanks by hand stripping 6 hr postprandial, mixed as a pooled sample, and then stored in a freezer ($-20\text{ }^{\circ}\text{C}$) until further analysis. The aforementioned procedure was as long as the sample is provided sufficiently for analysis. To prevent contamination, the fish were first tranquilized with MS_{222} (100 ppm), and the region around the anus was dried with a clean towel before the feces samples were collected (Banavreh *et al.*, 2018). Frozen ($-20\text{ }^{\circ}\text{C}$) fecal samples were lyophilized, finely ground, and stored at $-20\text{ }^{\circ}\text{C}$ until further analyses. The concentration of chromic oxide in diets and feces was determined by atomic absorption spectrophotometry (Williams *et al.*, 1962). The ADCs for test and reference diets were calculated as (Lemos *et al.*, 2009; Liu *et al.*, 2009; Tibbetts *et al.*, 2011).

$$\text{ADC (\%)} = 100 - (100 \times \% \text{Cr}_2\text{O}_3 \text{ in diet} / \text{Cr}_2\text{O}_3 \text{ in feces} \times \% \text{nutrient in feces} / \text{nutrient in diet})$$

The ADC for the test ingredients was calculated by (Lemos *et al.*, 2009; Tibbetts *et al.*, 2011):

$$\text{ADC of test ingredient (\%)} = \text{ADC test diet} + (\text{ADC test diet} - \text{ADC reference diet}) \times (0.7 \times \text{Dref} / 0.3 \times \text{Dingr})$$

Where Dref = % nutrient of reference diet; Dingr = % nutrient of test ingredient.

Proximate chemical analysis

Chemical composition (crude protein, lipid, moisture, and ash) of the feed ingredients, diets and feces were analysed according to AOAC (2005) producers: total protein content ($\text{N} \times 6.25$) using an automatic Kjeldahl system (230-Hjeltec Analyzer; Foss Tecator, Hoganas, Sweden), total lipid with an automatic Soxhlet system (2050-FOSS; Sweden) by chloroform extraction, moisture was determined by drying at $105\text{ }^{\circ}\text{C}$ for 24 h in an oven (D-63450; Heraeus, Hanau, Germany), ash by burning in a muffle furnace (Isuzu, Tokyo, Japan) at $550\text{ }^{\circ}\text{C}$ for 6 h.

For each of the feedstuffs, diets, and feces, the total amino acid composition determined. Samples were hydrolyzed in 6 N HCl for 24 hr at $110\text{ }^{\circ}\text{C}$; then, samples derivatized with *o*-phthaldialdehyde (OPA) (Antoine *et al.*, 1999). That was a two-step method of digestion and derivation. Finally, HPLC (Knauer, Berlin, Germany) was used to identify individual amino acids based on comparison with known standards (Oujifard *et al.*, 2012).

Statistical analysis

After confirming the homogeneity of variance and normality of the data using Leaven and Kolmogorov-Smirnov tests, respectively, one-way ANOVA was used to compare the treatments. Duncan's multiple range tests were applied to compare significant differences among the treatments ($p < 0.05$) with SPSS™ version 17 (Texas State University, San Marcos, TX, USA).

Results

The crude protein and crude lipid content of the heterogeneous groups of feedstuff are presented in Table 1. BM had the highest crude protein content (88.3%); Baker's yeast had the lowest (41.3%). The highest crude lipid content was in PBM (8.7%); the lowest was in WGM and Baker's yeast (1.8% and 1.6%). The ash of the feedstuffs varied from 14.9% (MBM) to 1.5% (WGM). The highest of the

carbohydrate (46.6%) was in CM, followed by SBM (40.2%). The highest of the gross energy content (21.9 KJ g⁻¹) was in WGM; the lowest was in CM (16.6 KJ g⁻¹). The reference diet ingredient (CP: 40.45% and CL: 14.55%) presented in Table 2. Proximate composition and gross energy content of the reference diets were showed in Table 3.

Table 3: Proximate composition (%) and gross energy content (KJ g⁻¹) of the reference and test diets^a used to determination of digestibility coefficients for Siberian sturgeon (*Acipenser baerii*).

| Diets | Moisture (%) | Crude protein (%) | Crude Lipid (%) | Ash (%) | Carbohydrate ^b (%) | Gross energy ^c (KJ g ⁻¹) |
|------------------------------------|--------------|-------------------|-----------------|---------|-------------------------------|---|
| Reference | 9.06 | 40.45 | 14.55 | 10.98 | 24.96 | 19.58 |
| Fish meal (<i>Clopeonella</i> sp) | 7.36 | 52.06 | 12.17 | 11.14 | 17.36 | 20.08 |
| Meat and Bone meal | 9.74 | 43.03 | 12.86 | 12.56 | 21.38 | 18.91 |
| Poultry by-product meal | 8.16 | 46.46 | 13.28 | 10.42 | 21.33 | 19.88 |
| Blood meal | 8.06 | 53.34 | 12.32 | 8.64 | 17.65 | 20.49 |
| Feather meal | 7.68 | 52.43 | 12.23 | 9.12 | 18.47 | 20.39 |
| Soybean meal | 9.04 | 40.96 | 11.82 | 9.72 | 28.37 | 19.21 |
| Canola meal | 9.82 | 36.28 | 12.67 | 10.81 | 30.38 | 18.79 |
| Corn gluten meal | 7.46 | 52.14 | 11.86 | 8.24 | 20.35 | 20.5 |
| Wheat gluten meal | 6.71 | 53.38 | 11.19 | 7.92 | 20.58 | 20.58 |
| Bakers' yeast | 8.96 | 40.5 | 11.84 | 8.54 | 30.07 | 19.41 |

^aTest diets (70% reference diet+30% test ingredient)

^bCarbohydrates were calculated by difference. Carbohydrate =100-(crude protein+crude lipid +ash+moistur).

^cEstimated energy was calculated based on 1 g crude protein being 23.6 KJ, 1 g crude fat being 39.5 KJ and 1 g carbohydrate being 17.2 KJ.

The lowest concentration of Lys amino acid was found in CGM (4.9%) WGM (5.1%), PBM and CM (5.2%) (Table 4), and the highest value observed in FM (21.2%). The sulfur amino acid, such as

Met in FM (23.8%), was found in high concentration. Also, the lowest Lys and Met in the test diets were observed in PBM (7.8% and 6.9%, respectively) (Table 5).

Table 4: Amino acids composition of the test ingredients for Siberian sturgeon (*Acipenser baerii*; g kg⁻¹).

| Essential amino acids | Test ingredients ^a | | | | | | | | | |
|---------------------------|-------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | FM | MBM | PBM | BM | FeM | SBM | CM | CGM | WGM | BY |
| Arg | 48.9 | 32.5 | 36.6 | 51.2 | 57.1 | 24.4 | 16.8 | 25.2 | 36.5 | 17.9 |
| His | 11.2 | 3.6 | 4.2 | 29.6 | 7.3 | 6.2 | 4.9 | 9.5 | 12.5 | 4.9 |
| Leu | 69.1 | 43.2 | 50.7 | 92.8 | 69.1 | 34.9 | 25.6 | 93.6 | 83.7 | 33.4 |
| Ile | 50.3 | 29.1 | 33.9 | 42.2 | 45.5 | 26.4 | 16.8 | 42.8 | 53.6 | 25.6 |
| Val | 66 | 52.2 | 60.8 | 89.6 | 66.5 | 32.1 | 27.4 | 57.7 | 65.4 | 34.1 |
| Lys | 21.2 | 6.4 | 5.2 | 26.9 | 18.2 | 7.6 | 5.2 | 4.9 | 5.1 | 11.1 |
| Met | 23.8 | 4.1 | 3.3 | 6.7 | 10.9 | 3.3 | 2.9 | 15.7 | 18.5 | 2.9 |
| Thr | 93.4 | 11.1 | 60.7 | 13.3 | 49.5 | 3.2 | 2.3 | 8.1 | 12.6 | 8.6 |
| Phe | 29.6 | 18.4 | 22.3 | 45.3 | 50.6 | 14.5 | 10.9 | 41.0 | 50.1 | 15.1 |
| Non-essential amino acids | | | | | | | | | | |
| Ala | 53.6 | 37.0 | 41.3 | 88.4 | 45.1 | 16.9 | 19.7 | 88.3 | 38.3 | 32.7 |
| Asp | 64.4 | 44.8 | 52.7 | 68.6 | 62.3 | 45.3 | 22.7 | 56.8 | 44.8 | 43.2 |
| Glu | 76.8 | 55.1 | 64.9 | 67.6 | 82.1 | 77.7 | 51.8 | 123.9 | 171.4 | 58.2 |
| Gly | 22.3 | 86.5 | 83.4 | 89.3 | 72.7 | 55.7 | 35.7 | 67.0 | 87.9 | 47.5 |
| Ser | 53.4 | 69.2 | 70.3 | 69.5 | 90.9 | 30.7 | 23.5 | 67.0 | 88.8 | 35.0 |
| Tyr | 23.2 | 12.0 | 14.6 | 23.9 | 22.1 | 10.5 | 7.1 | 30.4 | 30.6 | 10.1 |
| Total amino acids | 707.2 | 504.1 | 594.9 | 814.9 | 749.9 | 389.4 | 273.3 | 731.9 | 799.8 | 380.3 |

Arg, arginine; His, histidine; Leu, leucine; Ile, isoleucine; Val, valine; Lys, lysine; Met, methionine; Thr, threonine; Phe, phenylalanine; Ala, alanine; Asp, aspartic acid; Glu, glutamic acid; Gly, glycine; Ser, serine; Tyr, tyrosine;

FM, fish (*Clopeonella*) meal; MBM, meat and bone meal; PBM, poultry by-product meal; BM, spray-dried blood meal; FeM, feather meal; SBM, Soybean meal; CM, canola meal; CGM, corn gluten meal; WGM, wheat gluten meal; BY, bakers' yeast

^a Mazandaran Animal and Aquatic feed (Managua) Co. Iran

Table 5: Amino acids composition of diets for Siberian sturgeon (*Acipenser baerii*; g kg⁻¹).

| Essential amino acids | Test diets ^a | | | | | | | | | | |
|---------------------------|-------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | RD | FM | MBM | PBM | BM | FeM | SBM | CM | CGM | WGM | BY |
| Arg | 23.7 | 18.1 | 21.8 | 27.2 | 30.3 | 32.6 | 23.9 | 21.6 | 24.7 | 27.2 | 21.5 |
| His | 5.6 | 7.1 | 4.9 | 5.3 | 12.4 | 5.7 | 5.8 | 5.5 | 7.5 | 7.9 | 5.6 |
| Leu | 33.6 | 37.9 | 33.3 | 38.1 | 51.9 | 42.3 | 24.6 | 30.9 | 52.1 | 49.1 | 33.2 |
| Ile | 24.7 | 34.3 | 24.4 | 26.9 | 28.4 | 29.9 | 25.2 | 22.3 | 30.8 | 32.8 | 25.3 |
| Val | 31.8 | 39.8 | 35.6 | 41.2 | 45.2 | 42.6 | 31.9 | 30.5 | 39.4 | 41.3 | 32.3 |
| Lys | 9.7 | 13.9 | 8.9 | 7.8 | 15.3 | 11.7 | 9.1 | 8.4 | 8.1 | 8.5 | 10.1 |
| Met | 9.3 | 13.1 | 7.4 | 6.9 | 7.9 | 9.3 | 7.5 | 7.4 | 11.4 | 11.8 | 7.5 |
| Thr | 33.7 | 51.1 | 27.2 | 41.2 | 26.3 | 37.8 | 24.6 | 24.2 | 26.6 | 27.6 | 26.4 |
| Phe | 10.8 | 15.8 | 13.3 | 13.1 | 20.7 | 21.5 | 11.9 | 11.2 | 21.6 | 23.5 | 12.4 |
| Non-essential amino acids | | | | | | | | | | | |
| Ala | 23.3 | 36.6 | 27.1 | 28.2 | 40.6 | 28.8 | 21.7 | 22.0 | 43.9 | 28.7 | 25.3 |
| Asp | 34.7 | 47.9 | 37.4 | 39.4 | 41.4 | 43.4 | 38.7 | 31.2 | 44.1 | 36.7 | 36.6 |
| Glu | 58.9 | 62.7 | 54.6 | 58.6 | 59.5 | 62.3 | 63.7 | 56.5 | 80.8 | 90.6 | 59.2 |
| Gly | 22.8 | 25.3 | 42.3 | 40.3 | 41.8 | 37.4 | 33.4 | 27.1 | 19.2 | 41.9 | 31.0 |
| Ser | 27.1 | 35.6 | 35.5 | 38.6 | 37.6 | 45.2 | 28.7 | 25.7 | 22.1 | 46.5 | 29.8 |
| Tyr | 10.9 | 16.2 | 10.9 | 11.3 | 13.5 | 14.1 | 10.9 | 9.9 | 19.6 | 17.1 | 10.9 |
| Total amino acids | 360.6 | 455.4 | 384.6 | 424.1 | 472.8 | 464.6 | 361.5 | 334.4 | 451.9 | 491.3 | 367.1 |

Arg, arginine; His, histidine; Leu, leucine; Ile, isoleucine; Val, valine; Lys, lysine; Met, methionine; Thr, threonine; Phe, phenylalanine; Ala, alanine; Asp, aspartic acid; Glu, glutamic acid; Gly, glycine; Ser, serine; Tyr, tyrosine;

RD, reference diet; FM, fish (*Clopeonella*) meal; MBM, meat and bone meal; PBM, poultry by-product meal; BM, spray-dried blood meal; FeM, feather meal; SBM, Soybean meal; CM, canola meal; CGM, corn gluten meal; WGM, wheat gluten meal; BY, bakers' yeast

^a Test diets (70% reference diet + 30% test ingredient)

The values of ADCs for crude protein, crude lipid, gross energy, and dry matter of the feedstuffs are displayed in Table 6. The digestibility of dry matter fluctuated from 65.6% (BM) to 84.3% (FM). ADC values of dry matter of FM, MBM, PBM, SBM, CGM, and WGM were significantly higher than those of BM, FeM, CM, and BY ($p<0.05$). ADC_{CP} value of FM (92.9%) was significantly higher than for other ingredients ($p<0.05$), while lowest ADC_{CP} was showed in FeM and CM ($p<0.05$). ADC_{CP} lower than 70% were found in BM (68.5%), SBM (66.2%), BY (66%), CM (61%), and FeM (60%). Also, CGM (74.3%), WGM (76.9%), PBM (83.1%) and MBM (85.6%), were the feedstuffs that indicated ADC_{CP}

higher than 70%. ADC_{CP} of FM, MBM, PBM (animal origin), WGM, and CGM (plant origin) were significantly higher than those of other feedstuffs ($p<0.05$). ADC_{CL} of FeM (64.8%) was significantly lower than other feedstuffs ($p<0.05$), and FM, MBM, PBP, BM, CGM, WGM, and Bakers' yeast showed significant higher ADC_{CL} than FeM (64.8%). ADC_{GE} of animal feedstuffs such as FM (88.7%) was significantly the other feedstuffs ($p<0.05$) and followed by PBP (78.4%), BM (72.1%), MBM (71.8%), and Bakers' yeast (76.7%). In the plant feedstuffs, WGM (81.5%) and CGM (80.4%) were higher significant SBM (73.3%) and CM (68.1%).

Table 6: Apparent digestibility coefficients of dry matter, crude protein, crude lipid and gross energy in the test ingredients for Siberian sturgeon (*Acipenser baerii*) .

| Ingredient | ADC _{DM} | ADC _{CP} | ADC _{CL} | ADC _{GE} |
|------------------------------------|-------------------------|-----------------------|------------------------|------------------------|
| Fish meal (<i>Clopeonella</i> sp) | 84.3±1.3 ^a | 92.9±1.3 ^a | 89.7±0.6 ^a | 88.7±0.6 ^a |
| Meat and Bone meal | 80.4±1.1 ^b | 85.6±0.9 ^b | 81.1±0.8 ^b | 71.8±1.6 ^{cd} |
| Poultry by-product meal | 81.7±0.5 ^b | 83.1±0.8 ^b | 78.1±1.2 ^c | 78.4±1.1 ^b |
| Blood meal | 65.6±0.8 ^d | 68.5±0.4 ^d | 69.1±0.6 ^d | 72.1±0.9 ^{cd} |
| Feather meal | 67.6±0.4 ^d | 60±1.0 ^e | 64.8±0.8 ^e | 74.5±1.8 ^c |
| Soybean meal | 83.3±1.1 ^a | 66.2±1.7 ^d | 66.9±0.6 ^{de} | 73.3±0.3 ^c |
| Canola meal | 76.4±1.5 ^c | 61.0±2.6 ^e | 66.3±0.8 ^{de} | 68.1±1.6 ^d |
| Corn gluten meal | 81.1±0.6 ^b | 74.3±2 ^c | 77.1±1.3 ^c | 80.4±1.4 ^b |
| Wheat gluten meal | 82.3± 1.3 ^{ab} | 76.9±1.3 ^c | 76.1±0.2 ^c | 81.5± 1.7 ^b |
| Bakers' yeast | 82.1±0.8 ^{ab} | 66.0±3 ^d | 67.1±0.2 ^d | 76.7±1.1 ^{bc} |

ADC_{DM}, apparent digestibility coefficient of dry matter; ADC_{CP}, apparent digestibility coefficient of crude protein; ADC_{CL}, apparent digestibility coefficient of crude lipid; ADC_{GE}, apparent digestibility coefficient of gross energy.

Mean±SE (n=2); values within the same column with different superscript letters are significantly different ($p<0.05$).

ADCs for nine essential amino acids (EAAs) and six non-essential amino acids (NEAAs) in test ingredients were shown in Table 7. The Lys and Met value of FM were significantly higher than the PBM, BM, FeM, SBM, CM, CGM, WGM, and BY ($p< 0.05$). Based on data from Table 7, the lowest

digestibility of Σ EAAs and Σ NEAAs of animal-origin was observed in the FeM. The highest value of the digestibility of Σ EAAs and Σ NEAAs in plant feedstuffs was observed in CG, followed by WG and the lowest in CM.

Discussion

Nowadays, in aquaculture, there are many efforts to replace the aquafeed protein from ingredients that are continuously available and relative to the low cost (Gatlin *et al.*, 2007; Dadgar *et al.*, 2010; Dadgar *et al.*, 2014). Ascertaining the digestibility coefficient is an imperative necessity to screen potential feed ingredients for formulating nutritious feed for a species (Jannathulla *et al.*, 2018). Factors such as the type of feed production (extruded and pelletized), ANFs in feedstuffs such as fiber, which increase the intestinal evacuation rates and trypsin inhibitor, phytic acid, tannin, saponin, glucosinolate, reduce ADCs and conclusively reduces the growth (Becker and Makkar, 1999; Mandal and Ghosh, 2010; Safari *et al.*, 2016; Banavreh *et al.*, 2018; Jannathulla *et al.*, 2018).

The present results indicated that the highest value of ADC_{DM} was observed in the diets containing FM (84.3%) and SBM (83.3%). In this frame, Liu *et al.* (2009) stated that the ADC_{DM} (ICP-AES chromic oxide analyses method) of FM and SBM in juvenile Siberian sturgeon (8.38±0.20 g) was 79.9% and 76.9%, respectively. A comparison of these findings shows that the ADC_{DM} of these two feedstuffs in the Siberian sturgeon improves with the increasing fish weight. This can be due to the improvement of digestive enzymes; however, the lowest value obtained in BM (65.6%) and FeM (67.7%). Liu *et al.* (2009) postulated that the ADC_{DM} value of FFeM (Fermented feather meal) and HFeM (Hydrolysed feather

meal) in juvenile Siberian sturgeon was 82.6% and 77.2%, respectively. Improving the digestibility of ADC_{DM} value in Liu *et al.* (2009) study can be attributed to the biotechnological processes, such as fermentation and hydrolysis because it breaks macronutrients and enhances the digestibility of the feedstuff. Differences in ADC_{DM} of high-carbohydrate plant feedstuffs can result from differences in the chemical composition of starch in plant products. In this regard, the difference ADC_{DM} value of gluten (wheat and corn) with canola meal was quite tangible. This study showed that the ADC_{DM} of baker's yeast is high, which indicates that yeast carbohydrates lack nonstarch polysaccharide (NSP).

Protein is one of the most important factors in aquafeeds. In the present study, the ADC_{CP} value of the FeM and FM varied from 60% to 92.9%, respectively. The disparity observed in the ADC of crude protein between the ingredients could be ascribed to the variations in protein solubility and dispersibility indices (Jannathulla *et al.*, 2018). Meanwhile, Gaylord *et al.* (2008) stated that the ADC_{CP} of FeM in rainbow trout was 87%. The low nutritional value of FeM could be owing to high levels of poorly digestible keratin protein (Tibbetts *et al.*, 2011; Mirzakhani *et al.*, 2018), the presence of disulfide bonding (Moran *et al.*, 1966) and the presence of indigestible amino acid processing products, namely lysinoalanine and lanthionine (Tibbetts *et al.*, 2011). Also, the experimental strategy of placing the

test ingredient into a reference diet (presently at 30%) resulted in inclusions higher than the recommended levels for many fish species, for ingredients such as blood meal and feather meal and poultry by-product meal (Lemos *et al.*, 2009; Tibbetts *et al.*, 2011). High dietary levels of some ingredients may produce a decrease in palatability (Gatlin *et al.*, 2007) and feed intake, leading to increased metabolic fecal nitrogen and, hence, decreased ADC (Bureau *et al.*, 2002; Lemos *et al.*, 2009). Brunson *et al.* (1997) suggested that high levels of indigestible material in feedstuffs could have reduced the protein digestibility of these ingredients by decreasing gut transit time and physically guarding protein in the digestive from enzyme degradation.

This study shows that the processed plant proteins (WGM, CGM) have a higher digestibility than untreated plant proteins (SBM and CM). The appearance of antinutritional factors (ANFs) in plant protein can reduce the digestibility of feed ingredients, especially for proteins (Yun *et al.*, 2014; Hardy, 2010; Collins *et al.*, 2013; Gatlin *et al.*, 2007; Jannathulla *et al.*, 2018; Ma *et al.*, 2018;). Antinutrients in SBM and CM (such as phytic acid, trypsin inhibitor, lectins, saponins, glucosinolates, and fiber) can be decreased the protein digestibility by establishing of complex compounds with proteins, mineral (Becker and Makkar, 1999; Gatlin *et al.*, 2007; Ma *et al.*, 2018), and interfere with digestive enzymes (e.g., protease, amylase, etc.) (Cheng *et al.*, 2010;

Safari *et al.*, 2016). Additionally, SBM and CM contain a lot of fiber (not measured in our study) that can reduce digestion by fasting the rate of gastrointestinal passage in monogastric animals (Krogdahl *et al.*, 2003; Sajjadi and Carter, 2004; Romarheim *et al.*, 2006; Nguyen *et al.*, 2015; Ren *et al.*, 2018).

Liu *et al.* (2009) postulated that the ADC_{CP} values of fish meal (85.4%), meat and bone meal (83.9%), poultry by-product (86.1%), hydrolyzed feather meal (88.2%), fermented feather meal (79.9%), solvent extracted cottonseed meal (83.7%) and soybean meal (91.9%) in *Acipenser baerii* (8.38 g). Organoleptic properties of experimental diets may affect feeding utilization efficiency. The experimental diets of the present study were prepared via the cold pellet method. In this regard, the extrusion technique can positively affect the digestibility and bioavailability of plant-based diets due to high pressure and temperature during processing (Safari *et al.*, 2016; Ahmed *et al.*, 2018). Also, Bureau *et al.* (1999) acknowledge that the optimization and standardization of the processing conditions in the feather meal lead to improvement of the digestibility of this feedstuff in rainbow trout. The pressure and temperature required for extrusion can be lead to eliminate the antinutrients in plant-based diets. Noncompliance with the digestibility of plant protein sources in our study with Liu *et al.* (2009) research can be due to differences in the food production methods and the fish size.

In the present study, the trend of digestibility of EAA and NEAA was similar to that of apparent digestibility coefficients. The average digestibility of EAA and NEAA of FM and FeM was 90.6%, 71.8 %, and 87.9%, 61.6%, respectively. Although the amino acid contents in FM are not the highest among these ingredients, the most individual amino acid in FM had the highest digestibility, which is in line with the results of Liu *et al.* (2009). The highest value of the digestibility of amino acids was related to Arg in FM (94%), which coincided with Chi *et al.* (2017) finding on cobia. The apparent digestibility coefficients of amino acid varied from 64.8% for Lys in WGM to 90.4% for Lys in FM and 68.1% for SBM in Met to 91.1% for FM in Met. Liu *et al.* (2009) corroborated that the digestibility of Lys and Met amino acid for SBM in juvenile Siberian sturgeon was 92.8% and 88.6%, respectively. One of the reasons for this difference can be correlated with the method used to collection faces, which may lead to the estimated nutrient digestibility, be lower than their actual values. However, nutritional studies in fishes showed that ADCs of nutrients in the Guelph system, as one of the fecal collection methods, were 6–10% higher than those in the stripping method (Glencross *et al.*, 2007). The stripping methods because the low length of time food remains in the gut can influence its digestibility (Heinitz *et al.*, 2016). Specifically, formulate feed and prognostication fish performance, it is essential to base both the commendation and feed formulations

on digestible rather than total amino acid contents, particularly in protein arrogate and decrease of aquaculture excretion (Mirzakhani *et al.*, 2018).

The deficiency of a digestible EAA will lead to reduced utilization of the dietary protein (Lemme *et al.*, 2004; Liu *et al.*, 2009). As many modern aquaculture feeds are now is formulated on a digestible amino acid basis (Glencross *et al.*, 2007).

ADC_{CL} in the FM diet was well digested (89.7%) by Siberian sturgeon, while the lowest digestibility observed in the FeM (64.8%). Lupatsch *et al.* (1997) stated a strong influence of fatty acid composition, and thus the melting point of the fat on lipid digestibility in gilthead seabream (*Sparus aurata*). The earlier study by Austreng (1978) showed that lipid digestibility coefficients were affected by fatty acid chain length, unsaturated value, the content of incorporation in dietary fat and other fatty acids ratio, and melting point. Fish oil is typically high in polyunsaturated fatty acids (Abedian Kennari *et al.*, 2007), but FeM has saturated fatty acids (fatty acid not assay in this investigation). The findings of our study showed that the ADC_{CL} in plant feedstuffs on the yearling Siberian sturgeon is low, which is consistent with the results of Safari *et al.* (2016) in the great sturgeon (*Huso huso*).

Differences in ADC_{GE} among feedstuffs were similar to differences in ADC_{DM} but tended to be more pronounced. With few exceptions, feedstuffs that differed significantly in ADC_{DM} also differed in ADC_{GE}.

Brunson *et al.* (1997) acknowledged that in aquatic animals, the ADC_{GE} of plant feedstuffs was inversely related to fiber and also in some species to starch content. Fiber encompasses effectively no digestible energy for fishes (monogastric animals), and starches may be digested with diverging levels of efficiency, depending on the chemical composition of the starch and the digestive capability of the species to which it fed. The results of this study showed that ADC_{GE} is low for untreated plant feedstuffs (i.e., CM and SBM) and untreated animal feedstuffs (BM and PBP). Although the energy digestibility of the treated plant feedstuffs proteins (i.e., WGM and CGM), as well as the FM, is high in yearling Siberian sturgeon. The apparent digestibility of the gross energy of bakers' yeast was mid-range in the feedstuffs. Thus significant differences in energy digestibility of feedstuffs that embraced high levels of carbohydrate and low levels of protein and lipid (i.e., CM feedstuff) seemed to be associated with the nature of the starch present rather than its quantity, corroborating results reported by Chu *et al.* (2015).

ADC_{GE} indicated, however, that energy-yielding compounds in animal protein feedstuffs, which are being principally in the form of highly-digestible proteins and lipids, were well utilized by yearling Siberian sturgeon.

Considerable variability in the coefficients of apparent digestibility of dry matter, lipid, protein, and amino acids in ten feedstuffs was determined. These variables should be considered in

the substitution of total protein by amino acids for the more realistic and cost-effective formulation of sturgeon feeds. This research shows that there is an appropriate level of essential amino acids (especially lysine and methionine) in the feedstuffs. Still, the digestibility of the essential amino acids in the Siberian sturgeon is low. To more specifically formulate feed, it is essential to base both the commendation and feed formulations on digestible rather protein than total amino acid contents, particularly in protein replacement and reduction of aquaculture excretion. However, in the current trial, animal protein such as FM, MBM, and PBM and plant protein concentrate such as CGM and WGM exhibited high apparent protein digestibility coefficients for yearling Siberian sturgeon (290 ± 22 g). Also, FM and plant protein concentrate (CGM and WGM) displayed high apparent energy digestibility coefficients, when compared to other feedstuffs. It recommended that the pre-processing technique be carried out to improve the digestibility of the feedstuff.

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