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Prediction of apparent protein digestibility by *in vitro* pH-stat degree of protein hydrolysis with species-specific enzymes for Siberian sturgeon (*Acipenser baeri*, Brandt 1869)

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ABSTRACT

In the present study *in vivo* apparent protein digestibility (APD) and *in vitro* degree of protein hydrolysis (DPH; pH-stat assay) of numerous feed ingredients ($n = 10$) were investigated in juvenile Siberian sturgeon (290 ± 22 g). The ingredients included: fish meal (FM; Clopeonella), 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). APD values of the ingredients varied from 60 to 92.9% (feather meal and fish meal, respectively). APDs lower than 70% were found in blood meal (68.5%), soybean meal (66.2%), bakers' yeast (66%), canola meal (61%) and feather meal (60%). Also, the ingredients indicated APD values higher than 70%; corn gluten meal (74.3%), wheat gluten meal (76.9%), poultry by-product meal (83.1%) and meat and bone meal (85.6%). Higher DPH values (> 5%) were observed for fish meal, poultry by-product meal and wheat gluten meal; mid-range DPH values (3–5%) for corn gluten meal, meat and bone meal, soy bean meal, blood meal, bakers' yeast; and lower DPH values (< 2%) for canola meal and feather meal. Linear regressions between *in vivo* APD and *in vitro* degree of DPH with enzyme extracts in feed ingredients for Siberian sturgeon resulted in a considerable correlation (R2 = 0.89–0.99). Also, linear regressions between APD and DPH suggested a close relationship between peptide bond breakage by pyloric caeca and intestine digestive enzymes and the ADP. As a result, this may be a useful tool to provide important nutritional information.

1. Introduction

Feed ingredients are one of the important parts of research about fish nutrition. In order to consider quality of ingredients for feed formulation, there are several important factors should be noticed such as digestibilities and palatability of ingredient, and nutrient utilization and interference. (Glencross et al., 2007)Different types of ingredients are needed to meet the nutritional requirements of fish. Among these requirements, protein is the main nutrient in fish feed and plays important role in fish growth and quality (Hardy, 2010). Moreover, seafood, terrestrial animals and plants by-products are widely available as protein sources for use in fish feed. However, all of these sources have various digestibility and quality of protein and other nutrients (Hardy, 2010).

Regarding the various nutrients, determination of protein digestibility after the initial chemical analysis is considered one of the most important factors in determining the quality of the food (Cho et al., 1982). The use of *in vivo* and *in vitro* methods is a common practice to determine the digestibility of feed ingredients in different species. The *in vivo* methods are used to determine the digestibility of feed ingredients either directly (collection of whole feces) or indirectly (using markers and collecting part of the feces), which are time consuming and costly compared to the *in vitro* methods (Austreng et al., 2000; Liu et al., 2009; Tibbetts et al., 2011b; Vandenberg and De La Noüe, 2001). Therefore, determining the quality of proteins in *in vitro* condition has been more focused. Firstly, investigators used digestive enzymes such as pepsin, trypsin, chymotrypsin, amino peptidases and *in vitro* method for evaluating of protein digestibility of feed ingredients. However, each of these methods according to the type and source of used enzymes, had different outputs. Particularly, most of these methods were based on the used methods for terrestrials, while the digestive system and condition of digestive enzymes activities of the fishes have significant differences

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with mentioned animals (Dimes and Haard, 1994). These issues led to finding more accurate and appropriate *in vitro* methods for determining the quality of protein in feed ingredients thanks to the aquatic animals digestive enzymes. For instance, the pyloric caeca enzymes of Atlantic salmon *Salmo salar*, rainbow trout *Oncorhynchus mykiss* and European bass *Dicentrarchus labrax* were extracted and the feed ingredients protein digestibility *in vitro* condition were examined (Rungruangsak-Torrissen et al., 2002). Their results indicated that each fish species shows a different digestibility for the same feed ingredient. Among different method, the anticipation of *in vivo* apparent protein digestibility (APD) by *in vitro* degree of protein hydrolysis (DPH) with enzymes that were extracted from the digestive system of target species has been considered to be practical for some feed ingredients (Dimes and Haard, 1994; Lemos et al., 2000; Tibbetts et al., 2011a; Tibbetts et al., 2011b). This method could be applied to measure the quality of ingredients and feeds at large scale as it is fast, precise, safe, it does not require complex equipment and it provides specific response (by using standardized species enzymes) (Lemos et al., 2009; Yasumaru and Lemos, 2014).

Siberian sturgeon has recently received considerable attention and could be an important part of the fish farming in temperate zone in near future (Adámek et al., 2007). Information about the nutritional requirements and the digestibility of feed ingredients for this species is limited, so more research is necessary. In this sense, the main objective of this study was to determine the protein quality of ten various feed ingredients (animal and plant origin) for Siberian sturgeon based on *in vivo* APD and *in vitro* DPH.

2. Materials and methods

2.1. Experimental design and diet preparation

Ten feed ingredients of different types and from different sources were evaluated. The ingredients included: fish (Clopeonella) 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). Proximate composition (%) and gross energy content (Kj g^{-1}) of used ingredients in this study are shown in Table 1. All feed ingredients in the reference diet (Table 2) were mixed for 2 h to ensure homogeneity with the size of < 0.5 mm. Experimental diets were prepared by mixing each test ingredient with the reference diet (30:70 *w*/w) for 30 min. Separately; deionized water (400 ml kg^{-1}) was added and mixed to attain appropriate dough for extrusion. Dough was pelleted in (3 × 5 mm, diameter and length) and dried in a hot air oven (Hootakhsh, Tehran, Iran) at 40 °C for 12–18 h. The diets were fragmented and sieved into proper pellet size, packed and stored at −20 °C until used (Esmaeili et al., 2017).

Table 2
Formulation and proximate composition of the reference diet used to measure *In vivo* apparent protein digestibility (APD) of ingredients in Siberian sturgeon.

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
Dicalsium phosphate[a]	5
Mineral mix[d]	20
Vitamin mix[e]	40
BHT[f]	0.2
Choline chloride[a]	3
Cr2O3[g]	10
Proximate composition	
Moisture (%)	9.06
Crude protein (%)	40.45
Lipid (%)	14.55
Ash (%)	10.98
Carbohydrate (%)[h]	24.96
Gross energy (Kj g^{-1})[i]	19.58

[a] Clopeonella meal (Mazandaran Animal and Aquatic feed (Manaqua) Co. Iran).
[b] Kilka oil (Manaqua Co. Iran).
[c] Soybean lecithin with phosphatidyl choline (Behpak company, Iran).
[d] Mineral 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.
[e] Vitamin 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 to1 kg.
[f] Antioxidant (Gluba Tiox, French).
[g] Chromic oxide (Sigma, USA).
[h] Carbohydrates were calculated by difference. Carbohydrate = 100 − (crude protein + crude lipid + ash + moisture) (Babaei et al., 2017).
[i] 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 (National Research Council, 2011).

2.2. Rearing site and conditions

A total of 198 Siberian sturgeon (290 ± 22 g) were obtained from International Sturgeon Research Institute (Gilan, Iran) and randomly

Table 1
Proximate composition (%) and gross energy content (Kj g^{-1}) of ingredients were fed to Siberian sturgeon.

Ingredients	Moisture (%)	Crude protein (%)	Lipid (%)	Ash (%)	Carbohydrate (%)[b]	Gross energy (Kj g^{-1})[c]
Fish meal [a]	7.1	74.5	6.7	9.7	2	20.4
Meat and Bone meal [a]	7.4	53.1	12	14.9	12.6	19.4
Poultry by-product meal [a]	7	64.5	8.7	10	9.8	20.4
Blood meal [a]	4	88.3	2.2	3.6	1.8	22
Feather meal [a]	6.5	78.1	6.8	3.5	5	23
Soybean meal [a]	9.3	41.9	1.7	6.9	40.2	17.5
Canola meal [a]	11.8	30.6	3.3	8	46.6	16.6
Corn gluten meal [a]	5.7	76.8	4	1.7	11.8	21.7
Wheat gluten meal [a]	4.8	84.36	1.8	1.5	7.7	21.9
Bakers, yeast [a]	6.2	41.3	1.6	1.9	48.9	18.8

[a] Mazandaran Animal and Aquatic feed (Manaqua) Co. Iran.
[b] Carbohydrates were calculated by difference. Carbohydrate = 100 − (crude protein + crude lipid + ash + moisture) (Babaei et al., 2017).
[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 (National Research Council, 2011).