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RESEARCH PAPER

Growth, Feed Utilization, Apparent Digestibility and Fatty Acid Composition of Beluga (*Huso huso*) Juveniles to Different Inclusion Levels of Whole Seed Meal of Faba Bean (*Vicia faba*)

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Abstract

This study was conducted to evaluate the inclusion of whole faba bean (*Vicia faba*) meal (WFBM) on growth, feed utilization, apparent digestibility, and flesh fatty acid composition of juvenile beluga (*Huso huso*) (n = 144; initial weight = 82 g) at six levels 0 (control), 5, 10, 15, 20, and 25%) in a completely randomized design (each with three replicates) in 18 tanks (250 L) for 56 days. Growth performance, final weight, weight gain, average daily growth rate, and feed efficiency of fish fed diets containing 0, 5, and 10% WFBM were higher ($P \leq 0.05$) compared to other groups; and the poorest values ($P \leq 0.05$) were attained in the fish fed with 25% WFBM. Feed conversion ratio values showed rising trend with significant differences ($P \leq 0.05$) by increasing WFBM levels in diets containing 20 and 25% WFBM (0.79-0.82). Apparent digestibility of dry matter, protein, and lipid contents relatively decreased ($P \leq 0.05$) with increasing levels of WFBM from 10 to 25%. Flesh fatty acid composition showed elevated total n-6 fatty acids ($P \leq 0.05$) whereas the content of total n-3 decreased ($P \leq 0.05$) with increasing of the incorporated WFBM level. The ratio of n-3/n-6 significantly declined ($P \leq 0.05$) with raising the incorporated WFBM level. A decreasing trend in eicosapentaenoic and docosahexaenoic acids contents and a relatively increasing trend in oleic and linoleic acids contents were obtained in treatments with high dietary WFBM levels ($P \leq 0.05$). According to the results, WFBM can be included only up to 10% of juvenile beluga diet without any adverse effects on growth, digestibility, and flesh fatty acid composition.

Keywords: *Huso huso*, whole faba bean meal (WFBM), growth performance, feed utilization, fatty acid composition.

Introduction

A large number of fish species are cultured throughout the world, but the nutritional requirements of several species have been extensively studied. One of the consequences of this lack of information is a heavy dependence on fish meal in the feeding regimes for most farmed aquaculture species. Some knowledge of nutritional requirements, information on digestibility coefficients for a wide range of ingredients, and data on maximum inclusion levels for key ingredients provide the basis for the use of least-cost formulated diets. On the other hand, rearing of most fish species rely on the quantity and quality of fish meal in diet, which influence the total cost of formulated commercial fish feed (Hardy & Barrows, 2002). Given the global needs for fish meal in aquaculture (FAO, 2014), there is an increasing demand for more insight into the potential of alternative protein sources in aqua-feeds (New & Wijkstrom, 2002). Consequently, alternative protein sources, either of animal or plant origin, with cheap and abundant availability for use in fish formulation have been in the focus of many researches over the past three decades (Pongmaneerat, Watanabe, Takeuchi, & Satoh, 1993; Watanabe *et al*., 1998; Glencross, Evans, Hawkins, & Jones, 2004; Gatlin *et al*., 2007; Lin & Luo, 2011; Zhu *et al*., 2011; Rapatsa & Moyo, 2017).

Among the vegetable protein sources, legume seeds such as lupins (*Lupinus* sp.) and faba (*Vicia faba*) beans have shown good potential as the alternatives for use in pelleted feeds (Gouveia & Davies, 1998; Farhangi & Carter, 2001; Glencross *et al*., 2004; Azaza, Wassim, Mensi, Abdelmouleh, Brini, & Kraiem, 2009; Adamidou *et al*., 2011; Ouraji, Zare Tabar, & Rahmani, 2013; De Santis *et al*., 2015, 2016). *Vicia faba* (known as faba bean or broad bean) is widely cultivated in different parts of the world particularly in the Mediterranean and Middle East regions. Faba is primarily cultivated in central and northern parts of Iran; it is used as a source of protein in human diets as well as fodder and forage crops for animals. The average crude protein, carbohydrate, crude lipid, and energy contents of faba beans are approximated to be 27-32%, 45-50%, 10-15%, and 16.2 kJ g^{-1} of dry weight, respectively. Faba bean also has a fairly balanced amino acid composition meeting many requirements for fish and relatively low concentrations of anti-nutrients with potentially less acute effects on fish compared to soybean-derived products (Knudsen, 1997; Crepon, 2006; Gaber, 2006). A number of studies have tried to determine the amounts of faba bean meal that can be included in fish diets with no adverse effects on growth performance (Gaber, 2006; Adamidou *et al*., 2009, 2011; Azaza *et al*., 2009; Ouraji *et al*., 2013). Additionally, the applicability of air-classified faba bean protein concentrate in combination with fish meal and soy protein concentrate has recently been evaluated in Atlantic salmon (*Salmo salar*) parr in freshwater (De Santis *et al*., 2015) and post-smolt in seawater (De Santis *et al*., 2016).

Beluga (*Huso huso*) appears to be a good candidate for aquaculture purposes due to its adaptability to rearing conditions, high growth rate, and resistance to high stocking densities in culture conditions (Mohseni, Ozorio, Pourkazemi, & Bai, 2008; Hosseini and Khajepour, 2013). At this time, the feeding diets used for beluga production in Iranian farmers are the same as those formulated for carps and/or salmonids in the culture conditions. Due to the recent introduction of sturgeon species in the aquaculture industry, little is known about nutritional requirements, diets and ingredients' digestibility as well as the utilization of alternative feed ingredients to replace fish meal in sturgeon aqua-feeds and the component dietary effects on flesh fatty acid composition. However, few researches have been conducted on the influence of dietary faba bean meal (FBM) on growth performance of juvenile beluga (Soltanzadeh *et al*., 2016). It has been determined that farmed sturgeon species have an abundance of *n*-3 high unsaturated fatty acid (HUFA) and a high *n*-3/*n*-6 PUFA (polyunsaturated fatty acid) ratio (Vaccaro, Buffa, Messina, Santulli, & Mazzola, 2005; Kaya, Turan, & Erdem, 2007). Therefore, the main objective of the present study was to evaluate the effects of different inclusion levels of whole faba bean seed meal (WFBM) in practical diets on growth performance, feed utilization, digestibility, and flesh fatty acid composition of juvenile beluga (*H. huso*).

Materials and Methods

Diets Formulation, Trial Design, and Feed Composition

The formulated diets contained kilka (*Clupeonella delicatula*) fish meal, whole faba bean meal (WFBM), and both wheat flour and gluten. Faba bean seed was used with no processing in the present study (such as de-hulling, heat treatment, autoclaving, and/or cooking). Proximate chemical composition of ingredients used in experimental diets is presented in Table 1.

Six iso-nitrogenous (41% crude protein) and iso-energetic (20.36 KJ g^{-1}) experimental diets were formulated using commercial ingredients (Table 2). Whole faba bean seed meal (WFBM) was included at graded levels of 0 (control), 5, 10, 15, 20, and 25% in the diets. When WFBM content was increased in the diets, the fish meal and wheat flour were gradually reduced to adjust the protein and carbohydrate contents. The experimental diets were prepared by mixing the dry ingredients using a mixer (Pars Electric Co., Tehran, Iran), then oil and water (25% v/w) were added to the dry mixture to ensure it was well homogenized. The mixed paste was extruded through an electric meat grinder and dried at 60°C for 6 h in an oven (Fan Azma Gostar, Model, BM 55). Diets were made as pellets of appropriately 2-2.5 mm in diameter. The dried pellets were dried in the oven at 60°C for 24 h, and kept in separate, tightly zip-lock plastic bags (10×20 cm), and finally stored at -20ºC until use. The diet formulation is provided in Table 2.

Fish, Experimental Procedure, and Feeding Trials

The feeding trial was conducted in the experimental facilities located at Sari Agricultural Sciences and Natural Resources University (SANRU, Sari, Iran). Juveniles *H. huso* were obtained from a local commercial hatchery (Shahid Marjani Sturgeon Hatchery Center, Golestan Province, Iran). Prior to the feeding trial, juveniles were acclimatized to the

Table 1. Chemical composition of ingredients used in experimental diets

Proximate composition (%)	Raw ingredients in the diet			
	Whole seed of faba bean meal	Fish meal [a]	Wheat gluten [b]	Wheat flour
Dry matter	90.6	91.7	92	89.3
Crude protein	27.1	67.2	76.4	11.5
Crude lipid	4.1	8.6	1.7	2.4
Ash	7.3	15.4	2.5	8.6
Crude fiber	22.4	0.9	0.8	2.8
NFE [c]	38.1	3.8	17.5	73.5

[a] Kilka fish meal (mainly *Clupeonella delicatula*, low - temperature dried fish meal), Pars Kilka Co., Miroud, Mazandaran Province, Iran.
[b] Khorke Dame Abziyane Mazandaran, Mazandaran Province, Iran.
[c] NFE: Nitrogen free extract.

Table 2. Formulation and proximate composition of experimental diets containing different levels of whole faba (*Vicia faba*) bean meal

Ingredient (%)	Levels of WFBM (%)					
	0 (Control)	5	10	15	20	25
Fish meal [a]	47	45	43	41	39	37
Faba bean meal	0	5	10	15	20	25
Wheat flour	23	20	17	14	11	8
Wheat gluten	10	10	10	10	10	10
Fish oil	5	5	5	5	5	5
Canola oil	10	10	10	10	10	10
Molasses	2	2	2	2	2	2
Vitamin mix [b]	1.5	1.5	1.5	1.5	1.5	1.5
Mineral mix [c]	1.5	1.5	1.5	1.5	1.5	1.5
Chromic oxide	5	5	5	5	5	5
Composition (% dry weight)						
Dry matter	92.56	89.95	93	87.17	83.84	91.6
Crude protein	41.45	40.84	40.23	41.22	40.73	40.5
Crude lipid	19.11	19.48	18.75	18.45	19.12	18.17
Ash	11.9	11.5	11.4	11.25	9.5	10
Total carbohydrate [d]	27.54	28.18	29.62	29.08	30.65	31.33
Gross energy [e]	20.79	20.45	20.79	19.81	19.65	20.67

[a] Pars Kilka Co., Miroud, Mazandaran Province, Iran.
[b] Contained (g kg^{-1} mix): $MgS0_4.2H_20$, 127.5; KCl, 50; NaCl, 60; $CaHPO_4$, $2H_2O$, 727.8; $FeSO_4$. $7H_2O$, 25; $ZnSO_4.7H_2O$, 5.5; $CuSO_4$. $5H_2O$, 0.785; $MnSO_4$. $4H_2O$, 2.54; $CoSO_4$. $4H_2O$, 0.478; Ca $(IO_3)_2$. $6H_2O$, 0.295; $CrCl_3$, $6H_2O$, 0.128.
[c] Vitamin premix contained the following vitamins (each kg^{-1} diet): vitamin A, 10,000 IU; vitamin D3 2,000 IU; vitamin E, 100 mg; vitamin K, 20 mg; vitamin B_1, 400 mg; vitamin B_2, 40 mg. vitamin B_6 20 mg; vitamin B_{12}, 0.04 mg; biotin, 0.2 mg; choline chloride, 1,200 mg; folic acid, 10 mg; inositol, 200 mg; niacin, 200 mg; pantothenic calcium, 100mg.
[d] Total carbohydrate = 100 – [protein (%) + lipid (%) + ash (%)]
[e] Calculated energy content using the factors: carbohydrates, 11 kJ g^{-1}, protein, 20.9 kJ g^{-1}, and lipids, 35.1 kJ g^{-1} (Brafield, 1985).

experimental conditions in six 300- L tanks for 14 days and fed with a commercial extruded diet (41% crude protein and 14% crude lipid) to visual satiation. At the start of the experiment, similar-sized juveniles (*n* = 144; initial body weight of 82.38±0.8 g; mean ± SD) were randomly distributed into 18 circular tanks (300 L) with eight fish per tank. The dietary treatments were randomly assigned to the experimental tanks. Six triplicate experimental treatments were randomized, and the feeding trial lasted for 56 days. Fish were manually fed three times daily with at 3-4% of body weight (g feed per 100 g) at 07:00, 12:00 and 17:00 hrs, respectively. The fish were reared under indoor culture conditions with continuous aeration in each tank through an air-stone connected to a central air compressor. During the trial period, photoperiod was 12-h light/12-h dark with the light period lasting from 08:00 to 20:00 hrs. Water quality parameters such as temperature, pH, dissolved oxygen, total ammonia, nitrite, and salinity were checked and maintained at ranges of 18±1.6°C, 7.5-7.7, 6.1±0.6 mg L^{-1}, 0.2-0.3 mg L^{-1}, 0.039 mg L^{-1}, and 0.5-0.6 mg L^{-1}, respectively, during the rearing period.

Growth Performance and Survival Rate

At the end of 56-day feeding trial, fish were starved for 24 h and anaesthetized with clove oil powder before weighing and sampling. Weight gain (WG), weight gain percentage (WG%), specific growth rate (SGR), condition factor (C_f), average daily growth rate (ADGR), feed conversion ratio (FCR), feed efficiency (FE), protein conversion ratio (PCR), protein productive value (PPV), lipid productive value (LPV), lipid efficiency ratio (LER), and survival rate (SR) were calculated as follows:

$$WG = W_f - W_i$$

$$WG (\%) = [(W_f - W_i) / W_i] \times 100$$

$$SGR (\% / day) = [(Ln\ W_f - Ln\ W_i) / T] \times 100$$

$$C_f = W_f / L_f^{\ 3} \times 100$$

ADGR (g/day) = [total wet weight gain (g) / T (day)]

FCR = [total dry feed consumed (g) / total wet weight gained (g)]

FE = [total wet weight gain (g) / total feed intake (g)]

PCR = [total protein intake (g) / total wet weight gain (g)]

PPV = 100× [protein retained (g) / protein intake (g)]

LPV = 100× [lipid retained (g) / lipid intake (g)]

LER = [total wet weight gain (g) / lipid intake (g)]

$$SR (\%) = 100 \times N_f / N_i$$

Where W_f and W_i are final and initial fish weights, respectively; N_f and N_i denote final and initial numbers of fish in each replicate, respectively; L_f indicate mean final length, and T is the experimental duration in days.

Proximate Biochemical Analyses of Diets and Whole Body Fish

Three fish from each tank (9 fish per treatment) were randomly collected and kept frozen to determine the proximate whole body composition. Dry matter (at 105°C for 24 h), crude protein (nitrogen × 6.25, Kjeldahl apparatus), crude fat (extracted with petroleum ether by Soxhlet apparatus), and ash (by incineration at 600°C for 6 h) were analyzed to estimate proximate composition of both fish and diets according to the standard methods of AOAC (2005).

Apparent Digestibility Assessment

Apparent digestibility (AD) was determined by feeding fish with diets containing chromic oxide (Cr_2O_3; 0.5% of the diet) as a marker. Feces were sampled daily by siphoning method during the last 10 days of the feeding trial (Dogan & Erdem, 2010). Samples of feces from individual tanks were homogenized, dried at 105°C, and then stored at -20°C until analysis of apparent digestibility of dry matter, protein, and lipid. Chromic oxide contents in feces were determined through digesting with nitric and perchloric acids according to following formula (Zhou, Tan, Mai, & Liu, 2004):

$$AD (\%) = [1- (ID \times NF)/ (IF \times ND)] \times 100$$

Where ID represents the marker in the feed, IF is the marker in feces, and ND and NF, denote nutrient/energy in the feed and feces, respectively.

Lipid Extraction and Fatty Acid Analysis

Total lipids were extracted from the fillet homogenized in chloroform-methanol (2:1, v/v) as described by Folch, Lees, & Sloane-Stanloey (1957) and determined gravimetrically in triplicate. The formation of fatty acid methyl esters (FAME) was carried out according to Desvilettes *et al.* (1994) and analyzed with a 6890 N GC–FID (Agilent Technologies, Wilmington, DE, USA) fitted with a J&W DB-Wax capillary column (30 m, 0.25 mm i.d., 0.25 mm film thickness), a split-splitless injector with Agilent tapered liner (4 mm i.d.) and a flame ionization detector. Nitrogen was used as the carrier gas. The column temperature was set at 200°C; injector and detector were maintained at 250°C and 260°C, respectively. Chem Station software was used for online data collection and processing. Individual FAME was identified by comparison with known standards (Sigma-Aldrich Corps, St Louis, MO, USA) and quantified as the area percentage of each FAME.

Statistical Analyses

All statistical analyses were performed using statistical package software SPSS 20 (IL, USA). Results are expressed as mean values±SD. Descriptive statistics of the results were calculated for each treatment. The means were tested using Levene's test for homogeneity of variances. When data were normally distributed, mean values for each parameter of different treatments were analyzed by one-way analysis of variance (ANOVA) to test the effects of experimental diets. When significant differences occurred, the means were further compared with Tukey's tests. Differences were considered significant at P≤0.05.

Results

Proximate compositions of WFBM used to formulate the experimental diets were moisture (10%), crude protein (27.1%), crude lipid (4.1%), and ash (7.3%) (Table 1). The growth response and feed utilization of beluga juveniles fed diets containing different levels of WFBM showed that the final weight, WG, ADGR, and FE of fish fed dietary levels of 0, 5 and 10% were higher (P≤0.05) compared with other treatments, but no differences were observed among these three feeding groups (P≥0.05) at the end of the feeding trials. No differences (P≥0.05) were observed in final length, C_f, and PPV among all experimental groups. FCR values showed increasing trends with increasing dietary WFBM levels of 20 and 25% (0.79 and 0.82, respectively) resulting in significant differences (P≤0.05). The highest and lowest values (0.327 and 0.295, respectively) of PCR were recorded in the control and fish fed the diets containing 25% of WFBM (P≤0.05). LPV and LER values significantly decreased as WFBM percentage elevated in the diet (P≤0.05). Also, survival rate did not differ significantly (P≥0.05) among all treatments (Table 3).

Apparent digestibility of dry matter, protein and lipid for juvenile beluga fed the experimental diets were significantly affected by dietary WFBM levels. When fish fed WFBM diet of 15, 20 and 25%, the apparent digestibility of dry matter, protein, and lipid significantly reduced (P≤0.05) compared to other inclusion levels; while no significant differences (P≥0.05) were found in fish fed diets with WFBM levels of 0, 5, and 10% (Table 4).

Fatty acid composition (% of total fatty acids) of experimental diets containing different levels of WFBM revealed higher contents of several fatty acids including 16:00, 18:1n9, 18:2n6, 18:3n3, and 22:6n3 among the different treatments (Table 5).

There was significant differences (P≤0.05) among the fatty acid profiles of all treatments in terms of unsaturated fatty acids consisting of 16:1n7, 18:1n9, 18:2n6, 22:5n3, and 22:6n3 in the fish flesh. Meanwhile, decreasing trends (P≤0.05) in EPA and DHA contents and relatively increasing trends (P≤0.05) in oleic and linoleic acids contents were obtained in treatments with high dietary WFBM levels (Table 6).

The abundance of MUFAs in the fish flesh was

Table 3. Growth response, feed utilization, and survival rate of juvenile beluga (*Huso huso*) fed diets containing different levels of whole faba (*Vicia faba*) bean meal

Parameters	Diets (% WFBM)					
	0 (Control)	5	10	15	20	25
Initial weight (g)	82.89±1	82.91±1.2	82.58±0.4	81.91±0.6	81.62±0.6	82.33±0.4
Final weight (g)	257.8±5.5 ^{a}	251.6±7.4 ^{ab}	248±3.1 ^{abc}	242.20±4 ^{bc}	239.20±4.8 ^{c}	238.10±6.1 ^{c}
Final length (cm)	39.37±3.29	39.5±1.55	38.18±4.03	37.87±4.99	37±5.76	36.18±7.34
Weight gain (g)	174.83±4.6 ^{a}	168.7±7.6 ^{ab}	165.45±3.5^{abc}	160.25±3.6 ^{bc}	157.58±4.4 ^{c}	155.75±6.3 ^{c}
Weight gain (%)	211±4.35 ^{a}	203.5±5 ^{ab}	200.31±11.2^{abc}	195.7±8.5 ^{bc}	193.1±7.5 ^{c}	189.20±5.3 ^{c}
Specific growth rate	2±0.4 ^{b}	1.98±0.3 ^{b}	2.77±0.4 ^{a}	1.94±0.2 ^{bc}	1.93±0.2 ^{bc}	1.89±0.3 ^{c}
Condition factor	0.42±0.03	0.41±0.05	0.45±0.04	0.45±0.02	0.47±0.03	0.48±0.02
Average daily growth rate	3.56±0.1 ^{a}	3.44±0.15 ^{ab}	3.37±0.07 ^{abc}	3.27±0.07 ^{bc}	3.21±0.08 ^{c}	3.18±0.12 ^{c}
Feed conversion ratio	0.70±0.02 ^{c}	0.73±0.04 ^{bc}	0.75±0.03 ^{bc}	0.76±0.01 ^{b}	0.79±0.04 ^{ab}	0.82±0.03 ^{a}
Protein conversion ratio	0.295±0.01 ^{c}	0.302±0.02 ^{bc}	0.302±0.01 ^{bc}	0.314±0.02 ^{ab}	0.318±0.01 ^{ab}	0.327±0.02 ^{a}
Feed efficiency	1.40±0.03 ^{a}	1.35±0.05 ^{ab}	1.33±0.02 ^{ab}	1.31±0.02 ^{bc}	1.27±0.01 ^{bc}	1.25±0.02 ^{c}
Protein productive value	46.9±3.01	44.58±6.73	46.18±3.78	41.28±4.63	39.43±2.01	39.08±1.58
Lipid productive value	84.92±15.64 ^{a}	80.5±3.08 ^{ab}	81.26±14.33 ^{ab}	63.94±8.45 ^{bc}	58. 2±6.78 ^{c}	58.1±8.16 ^{c}
Lipid efficiency ratio	7.34±0.17 ^{a}	6.98±0.27 ^{abc}	7.1±0.15 ^{ab}	6.98±0.22 ^{abc}	6.69±0.02 ^{c}	6.81±0.29 ^{bc}
Survival rate (%)	100	100	100	100	100	100

Mean values and standard deviation (±SD) are presented for each parameter (three groups per treatment).
Means in the same row with different letters are significantly different (P≤0.05).

Table 4. Apparent digestibility of dry matter, protein, and lipid for juvenile beluga (*Huso huso*) fed diets containing different levels of whole faba (*Vicia faba*) bean meal

Parameters	Diets (levels of WFBM, %)					
	0 (Control)	5	10	15	20	25
Dry matter	64.81±0.85 ^{a}	65.45±0.43 ^{a}	64.13±1.26 ^{a}	61.71±0.67 ^{b}	61.4±1.63 ^{b}	60.18±2.89 ^{b}
Protein	81.36±1.2 ^{a}	81.27±0.58 ^{a}	80.24±1.7 ^{ab}	80.4±2.87 ^{ab}	79.4±1.91 ^{b}	77.6±1.84 ^{b}
Lipid	76.44±0.87 ^{ab}	75.49±1.37 ^{ab}	77.95±1.23 ^{a}	71.31±0.36 ^{b}	72.77±1.3 ^{b}	68.33±1.92 ^{b}

Values are presented for each parameter (three groups per treatment).
Means in the same row with different letters are significantly different (P≤0.05).
Mean values and standard deviation (±SD) in the same row not with different superscript letters are significantly different (P≤0.05).

mainly due to the high values of oleic acid contributing approximately to 90% of the total MUFAs content in the examined fish. The fatty acid profile shows copious amounts (P≤0.05) of the two classes of unsaturated fatty acids, namely MUFAs (45.2%) and PUFAs (35.62%). When fatty acids of the diet were grouped by their saturation index, MUFAs amounted the highest (45.20%), whereas the percentage compositions of SFAs and PUFAs were recorded as 15.06% and 35.62%, respectively, of total fatty acids. Mean content of linolenic acid in the fish flesh displayed no differences (P≥0.05) among all treatments; however, linoleic acid content in the fish flesh (P≤0.05) increased with rising levels of WFBM inclusion in the diet. Increasing dietary levels of WFBM from 0 to 25%, resulted in elevated amounts of total *n*-6 in the flesh (P≤0.05), while total *n*-3 fatty acids and n-3/*n*-6 significantly decreased (P≤0.05). Furthermore, DHA content was the most abundant fatty acid contributing about 43% of total PUFAs to the fish flesh (Table 6).

Discussion

To our knowledge, this is the first study to evaluate dietary inclusion of graded levels of whole faba bean meal (WFBM) on growth performance, feed utilization, and fatty acid profile of the flesh in *H. huso* juveniles. According to the results, all juveniles survived at the end of the feeding trials; in which, a survival rate of 100% was recorded in different treatments with no morphological abnormality in fish. There were no significant differences between fish fed the control diet (WFBM-free) and those fed up to 10% of WFBM diet in terms of final weight gain, ADGR, FE, PCR, and LPV. The highest values on growth performance indices (weight gain, ADGR, and FE) were recorded in fish fed the control diet; however, there were no significant differences between the control and fish fed with 5 and 10% WFBM in the diet. All these parameters significantly decreased with increasing WFBM levels from 10 to 25% in the diet. Our results are consistent with previous findings in great sturgeon (*H. huso*) fed replaced fish meal with faba bean meal (FBM) reported by Soltanzadeh *et al.* (2016) who concluded that an optimum level of up to 10% could be recommended for beluga juveniles and that higher inclusion levels from 15 to 25% could have adverse effects on final weight gain, SGR, and FCR indices. Also, the present findings are somewhat in agreement with the results of Adamidou (2008) who successfully included WFBM up to 17% in extruded diet for sea bass (*Dicentrarchus labrax*); however, their results

Table 5. Fatty acid composition (% of total fatty acids) of experimental diets containing different levels of whole faba (*Vicia faba*) bean meal

Fatty acids	Diets (levels of WFBM, %)					
	0 (Control)	5	10	15	20	25
14:0	1.68	1.20	1.30	1.28	1.41	1.16
16:0	12.45	10.25	11.45	11.17	12.05	10.83
18:0	2.91	2.35	2.88	2.82	3.28	2.93
20:0	0.86	0.66	0.60	0.67	0.54	0.58
16:1n-7	2.46	1.62	1.92	1.76	1.82	1.60
18:1n-9	43.33	46.22	44.15	44.25	46.92	46.19
18:2n-6	15.08	17.05	17.72	18.18	18.73	18.90
18:3n-6	0.38	0.45	0.42	0.42	0.51	0.48
20:4n-6	0.01	0.02	0.02	0.01	0.01	0.01
22:5n-6	2.45	2.25	2.40	2.23	1.58	1.80
18:3n-3	5.16	5.44	4.95	5.17	4.48	5.20
18:4n-3	0.88	1.04	0.94	0.88	1.41	1.08
20:3n-3	0.14	0.13	0.12	0.10	0.20	0.10
20:5n-3	0.26	0.26	0.23	0.22	0.2	0.2
22:5n-3	0.30	0.31	0.32	0.29	0.22	0.25
22:6n-3	6.81	6.43	6.80	6.10	5.82	4.95
∑ SFA[a]	17.90	16.46	16.23	16.94	17.28	16.5
∑ MUFA[b]	45.79	47.84	46.07	46.01	48.74	47.79
∑ PUFA[c]	31.47	33.38	33.92	33.60	29.16	32.97
∑ *n*-6 PUFA [d]	17.92	19.77	20.56	20.84	18.83	21.19
∑ *n*-3 PUFA [e]	13.55	13.61	13.36	12.76	10.33	11.78
n-3 HUFA [f]	7.37	7	7.35	6.61	6.24	5.4
n-3/*n*-6	0.75	0.69	0.65	0.61	0.55	0.55

Values are % of the total fatty acid expressed as the mean.
[a] Saturated fatty acids: 14:0 + 16:0 + 18:0 + 20:0.
[b] Monounsaturated fatty acids: 16:1n7 + 18:1n9.
[c] Polyunsaturated fatty acids: 18:2n6 + 18:3n6 + 20:4n6 + 22:5n6 + 18:3n3 + 18:4n3 + 20:3n3 + 20:5n3 + 22:5n3 + 22:6n3.
d *n*-6 PUFA: 18:2n6 + 18:3n6 + 20:4n6 + 22:5n6.
e *n*-3 PUFA: 18:3n3 + 18:4n3 + 20:3n3 + 20:5n3 + 22:5n3 + 22:6n3.
f *n*-3 HUFA: 20:5n3 + 22:5n3 + 22:6n3.

showed a decreasing trend in the growth rate with increasing WFBM levels from 17 to 35%. In Nile tilapia (*Oreochromis niliticus*) juveniles, Gaber (2006) and Azaza *et al.* (2009) demonstrated that de-hulled faba bean meal (FBM) could be included in diets up to 37 and 24%, respectively, with no undesirable effects on growth and feed efficiency. Accordingly, excessive intake of WFBM from 10 to 25% would have significantly depressed the growth performance of *H. huso* juveniles in the current investigation. These results are in accordance with those of Ouraji *et al.* (2013) in rainbow trout (*Oncorhynchus mykiss*) fingerlings fed with high inclusion levels of 45% dehulled faba bean meal instead of soybean meal. The findings of various researchers indicate that different fish species respond differently to the faba bean meal used in practical diets. On the other hand, it seems that the type of faba bean meal (whole and/or dehulled seed meal, and protein concentrated) used in diets can affect suitable levels of faba bean meal in the diets.

Unfortunately, the phenolic and tannins substances in diets containing faba bean were not analyzed in this study, but researches (Azaza *et al.* 2009) highlighted that the high values of total phenolic and tannins substances in diets with high faba bean levels may result in decreased feed intake by reducing the palatability. Furthermore, a low availability of methionine was found to limit the use of high levels of dietary faba bean meal in fish diets (Gaber, 2006). Hence, it is expected that incorporation of WFBM at high levels (15-25%) in juvenile beluga diet would affect lower measures of ADGR and FE as recorded in the current study.

Digestibility is one of the most important factors in evaluating the suitability of feed ingredients for a target fish species. In this study, the apparent digestibility coefficients (ADCs) of dry matter, protein, and lipid were significantly reduced when fish fed with high-WFBM diet (15-25%). Also, the results of the decreased digestibility further confirmed our data on feed utilization that fish fed WFBM diet from 15 to 25% exhibited the lowest SGR and FE values. Accordingly, the ADCs in the present study are consistent with those reported by other researchers in juvenile beluga (Khajepour and Hosseini, 2012), Nile tilapia (Gaber, 2006), and European sea bass (Adamidou *et al.*, 2009). This study showed that the digestibility of protein was negatively affected by increasing dietary WFBM levels, which was accordance with those reported by Borquez *et al.* (2011) in rainbow trout. The protein digestibility values of WFBM for beluga were in the range of 77-80% in the current investigation. These values were

Table 6. Fatty acid composition (% of total fatty acids) of flesh from beluga (*Huso huso*) fed with diets containing different levels of whole faba (*Vicia faba*) bean meal

Fatty acids	Diets (levels of WFBM, %)					
	0 (Control)	5	10	15	20	25
14:0	1.08±0.14	1.05±0.04	1.11±0.02	1.12±0.01	1.02±0.05	1.01±0.06
16:0	10.91±1.02	10.99±0.08	11.47±0.43	11.29±0.4	11.18±0.38	10.55±0.08
18:0	2.41±0.02	2.48±0.77	2.11±0.23	2.36±0.23	2.77±0.16	2.10±0.01
20:0	0.55±0.1	0.57±0.01	0.53±0.02	0.56±0.08	0.59±0.01	0.49±0.04
16:1n-7	1.82±0.21 [a]	1.79±0.02 [ab]	1.89±0.06 [a]	1.85±0.08 [a]	1.59±0.01 [b]	1.72±0.13 [ab]
18:1n-9	42.83±0.03 [b]	43.43±1.6 [ab]	43.97±0.6 [ab]	42.57±0.8 [b]	43.23±0.1 [ab]	44.45±0.19 [a]
18:2n-6	15.06±0.03 [d]	16.07±0.83 [c]	16.95±0.6 [bc]	17.28±0.8 [b]	17.66±0.1 [ab]	18.42±0.01 [a]
18:3n-6	0.06±0.05 [b]	0.05±0.04 [b]	0.13±0.03 [a]	0.10±0.02 [ab]	0.13±0.01 [a]	0.12±0.01 [a]
20:4n-6	0.21±0.05	0.21±0.21	0.23±0.05	0.27±0.12	0.25±0.01	0.16±0.05
22:5n-6	2.51±0.05 [a]	2.28±0.04 [b]	2.13±0.06 [c]	2.29±0.05 [b]	1.91±0.01 [d]	1.92±0.01 [d]
18:3n-3	4.12±0.11	4.29±0.6	4.40±0.24	4.30±0.48	4.17±0.23	4.55±0.11
18:4n-3	1.31±0.16	1.31±0.19	1.37±0.06	1.18±0.01	1.25±0.07	1.31±0.01
20:3n-3	0.67±0.09	0.71±0.14	0.69±0.03	0.73±0.01	0.75±0.04	0.76±0.02
20:5n-3	0.65±0.09 [ab]	0.78±0.21 [a]	0.5±0.07 [b]	0.55±0.18 [ab]	0.60±0.06 [ab]	0.40±0.06 [b]
22:5n-3	0.47±0.02 [a]	0.40±0.03 [b]	0.34±0.01 [c]	0.39±0.03 [bc]	0.35±0.04 [bc]	0.36±0.02 [bc]
22:6n-3	10.63±0.68 [a]	9.23±0.8 [b]	8.30±0.25 [c]	9.24±0.4 [b]	8.22±0.25 [c]	7.73±0.11 [c]
∑ SFA[a]	14.96±1.26	15.10±0.82	15.23±0.62	15.34±0.72	15.57±0.47	14.17±0.17
∑ MUFA[b]	44.65±0.24 [b]	45.22±1.6 [ab]	45.87±0.6 [ab]	44.43±0.68 [b]	44.83±0.1 [ab]	46.18±0.05 [a]
∑ PUFA[c]	35.74±1.1 [ab]	35.37±0.2 [ab]	35.07±0.26 [b]	36.36±0.44 [a]	35.36±0.4 [ab]	35.79±0.1 [ab]
∑ *n*-6 PUFA	17.85±0.16 [d]	18.63±0.62 [c]	19.45±0.43 [b]	19.95±0.6 [ab]	19.98±0.1 [ab]	20.64±0.07 [a]
∑ *n*-3 PUFA	17.88±0.94 [a]	16.74±0.79 [b]	15.62±0.17 [cd]	16.40±0.15 [bc]	15.37±0.5 [cd]	15.14±0.14 [d]
n-3 HUFA	11.75±0.4 [a]	10.41±0.6 [b]	9.14±0.3 [b]	10.18±0.4 [b]	9.17±0.5 [c]	8.49±0.6 [c]
n-3/*n*-6	1±0.04 [a]	0.90±0.07 [b]	0.80±0.02 [cd]	0.82±0.03 [c]	0.77±0.03 [cd]	0.73±0.01 [d]

Mean values and standard deviation (±SD) in the same row not with different superscript letters are significantly different (P≤0.05).
[a] Saturated fatty acids: 14:0 + 16:0 + 18:0 + 20:0.
[b] Monounsaturated fatty acids: 16:1n7 + 18:1n9.
[c] Polyunsaturated fatty acids: 18:2n6 + 18:3n6 + 20:4n6 + 22:5n6 + 18:3n3 + 18:4n3 + 20:3n3 + 20:5n3 + 22:5n3 + 22:6n3.
d *n*-6 PUFA: 18:2n6 + 18:3n6 + 20:4n6 + 22:5n6.
e *n*-3 PUFA: 18:3n3 + 18:4n3 + 20:3n3 + 20:5n3 + 22:5n3 + 22:6n3.
f *n*-3 HUFA: 20:5n3 + 22:5n3 + 22:6n3.

not in accordance with those reported by Allan *et al.* (2000) and Booth *et al.* (2001) who pointed out the ADC values of protein in excess of 90% for a range of faba beans products (whole beans, and de-hulled) in Australian silver perch, *Bidyanus bidyanus*, even at an inclusion level of 30%. In accordance with Siddhuraju and Becker (2001) and Gan *et al.* (2017), decreased protein digestibility in beluga juveniles of the current study may be attributed to the formation of phenolics-protein or phenolics-protein enzyme complexes, and/or to the negative decreasing effects of supplemented dietary legume protein on the intestinal enzymatic activities such as trypsin and lipase. The nutritional value and digestibility of faba bean meal also proved favorable (> 80%) for Atlantic salmon (Aslaksen *et al*., 2007), common carp (Grabner and Hofer, 1985), rainbow trout (Grabner and Hofer, 1985; Gomes *et al*., 1995), European sea bass (Adamidou *et al*., 2009), and more recently, a higher level (> 90%) for grass carp (Gan *et al*., 2017).

Sturgeons require both *n*-3 and *n*-6 fatty acids in their diets (Badiani *et al*., 1997; Deng *et al*., 1998; Sener *et al*., 2005; Zhu *et al*., 2011). It has been determined that farmed sturgeons to have an abundance of *n*-3 highly unsaturated fatty acid (*n*-3 HUFA) and a high *n*-3/*n*-6 PUFA ratio (Garcia-Gallego *et al*., 1999; Vaccaro *et al*., 2005; Kaya *et al*., 2007). It has been reported that foods with a relatively higher content of *n*-3 PUFA, lower contents of *n*-6 PUFA, and with a high *n*-3/*n*-6 ratio are most beneficial for human health (Kinsella *et al*., 1990; Jankowska *et al*., 2005). Fatty acid composition of flesh in beluga reflected the same composition in diets as already shown in this species by Sener *et al.* (2005), Hosseini and Abedian Kenari (2010), Zhu *et al.* (2011), and Nikzad Hassankiadeh *et al.* (2012). Despite our observation that the quantity of lipids contributed by WFBM was relatively low, the fatty acid compositions of practical diets and fish flesh were affected by WFBM levels in the diets.

Our results showed that when the amounts of WFBM were increased in the diets, total *n*-6 PUFAs, particularly 18:2n6, significantly rose in flesh of the fish. Unlike *n*-6 PUFAs, total *n*-3 PUFAs in the flesh of juvenile *H. huso* was inversely related to the levels of WFBM in the diet. The results of this study also showed that the MUFAs content, especially 18:1n9, elevated in fish flesh with rising levels of WFBM in diet. Generally, major fatty acids of legumes (plant protein sources) are palmitic, oleic, and linoleic acids (Yoshida *et al*., 2009). Therefore, it is expected that the amounts of these fatty acids should increase with the inclusion of WFBM in the diets as was recorded in the current study. Nevertheless, the results revealed that with increasing levels of WFBM in the diets, the SFA levels particularly palmitic acid (16:0) in the fish flesh were similar to those in the control, probably as a consequence of minimum variations of SFA contents in the experimental diets. A higher level of DHA in the flesh of juvenile *H. huso* compared to its level in the diet is in agreement with those reported by Hosseini and Abedian Kenari (2010) and Nikzad Hassankiadeh *et al.* (2012) who suggested such a result to be a selective retention of DHA in the flesh and the essentiality of DHA in this fish even though the amount in the feed is low. Similar results were previously reported for the white sturgeon (*A. transmontanus*) (Xu *et al*., 1993; Deng *et al*., 1998), Russian Sturgeon (*A. gueldenstaedtii*) (Sener *et al*., 2005). In the present investigation, feeding sturgeon diets rich in high contents of WFBM resulted in relatively high levels of DHA in the fish flesh. These results showed that the beluga (as a sturgeon fish) has the ability to elongate and desaturate 18:2n-6 and 18:3n-3 to 20:4n-6, 20:5n-3 and 22:6n-3 fatty acids (Deng *et al*., 1998; Sener *et al*., 2005; Abedian Kenari *et al*., 2009).

In conclusion, the results of this study show that WFBM can be used up to 10% in the diets of juvenile beluga with no adverse impacts on growth, survival, digestibility, and flesh fatty acid composition. However, more studies should be carried out on the use of whole faba bean meal in beluga diet by using various processing techniques such as heat treatment, and protein concentrate to inactivate anti-nutritional factors in faba bean in future.

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Author's Contributions

All authors of the manuscript contributed equally to the writing of the manuscript and were involved in the overall planning and supervision of the work.

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