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Research Article:

Changes of amino acids and proximate compositions in freshwater farmed beluga sturgeon (*Huso huso*) caviar

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Abstract

This work is mainly concerned with the changes of amino acids and proximate compositions of Caspian Sea Beluga sturgeon (*Huso huso*) caviar after culture in freshwater. Proximate compositions did no differ between wild and farmed Beluga caviar (p>0.05). Eighteen amino acids were identified in wild and farmed Beluga caviars, but they had significant differences in some amino acids content. Isoleucine was the most abundant amino acid among total amino acids, which was 50.17±2.75 (mg/g) in wild caviar and was 26.83±0.82 (mg/g) in farmed ones (p<0.05). The essential amino acids content (EAA), in wild and farmed caviar were 132.66±6.8 and 110.16±7.35 (mg/g), nonessential amino acids (NEAA) were 107.92±5.68 and 121.79±4.08 (mg/g) and total amino acids (TAA) were 240.58±12.48 and 231.95±11.43 (mg/g) respectively (p>0.05). But the ratio of EAA/NEAA in wild samples 1.23±0 was significantly higher than farmed ones 0.9±0.03 (p<0.05). Functional amino acids (FAA) in wild and farmed samples were 96.1±8.66 and 116.8±9.65 (mg/g) (p>0.05), but the ratio of (FAA/TAA) in wild caviar was 0.40±0.02 and in farmed one was 0.50±0.02, which was significantly higher in farmed caviar (p<0.05). Delicious amino acids (DAA) were 54.9±1.89 and 54.86±2.01 (mg/g) in wild and farmed caviar and the ratio of (DAA/TAA) were 0.23±0 and 0.24±0 (p>0.05) respectively. The present study indicated that, farmed Beluga caviars were well balanced with the essential amino acids, functional amino acids and had a good EAA/NEAA ratio, so can be considered as a valuable food resource as well as the wild ones.

Keywords: Amino acids, Beluga sturgeon, Caspian Sea, Caviar, Freshwater

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Introduction

Over-fishing, water impurity and undesirable habitat are reducing sturgeon populations all over the world (Esmailnia *et al.,* 2019). Sturgeon caviar is the most famous seafood which is marketed very expensive (Saliu *et al.*, 2017). Recently, the number of species reared in aquaculture setting has increased extremely all over the world and within Iran (Matani Bour *et al.*, 2018; Kazemi *et al*., 2020; Pajand *et al*., 2020).

We have witnessed that, the discovery amino acids (AA) are not only cell signalling molecules but are also regulators of gene expression and the protein phosphorylation cascade. Additionally, AA are key precursors for syntheses of hormones and low-molecular weight nitrogenous substances with each having enormous biological importance (Wu, 2013). Fish are rich food sources for humans in terms of amino acids (Erdem *et al.*, 2009). One of the important problems in sturgeon culture is differences in amino acids profile that should be matched with the wild ones. The nutritional contribution of protein in foodstuff depends on its digestibility and ability to provide all essential amino acids (Sánchez-Alonso *et al.*, 2007). The amino acid composition of fish and its divisions are influenced by intrinsic (e.g., species, size and sexual maturity) and extrinsic factors (e.g., food resources, fishing season, water salinity and temperature Akiyama *et al.* (1997), Limin *et al.* (2006), and Özyurt and Polat (2006).

Only few data are available in literature in this field. Iwasaki and Harada (1985) analysed "proximate and amino acid composition of the roe and muscle of selected marine species", Tamaru *et al.* (1992) studied on "fatty acid and amino acid profiles of spawned eggs of stripped mullet, (*Mugil cephalus*)", Eun *et al.* (1994) worked on "chemical composition and micro flora of channel catfish (*Ictalurus punctatus)* roe and swim bladder", Wing Keong and Hung (1994) worked on "amino acid composition of whole body, egg and selected tissues of white sturgeon (*Acipenser transmontanus*)", De Silva *et al.* (2001) studied "weaning of Australian short fin glass eels (*Anguilla australis)*", Bledsoe *et al.* (2003) worked on "caviar and fish roe products", Vaccaro *et al.* (2005) analysed "fatty acid composition of a cultured sturgeon hybrid, (*Acipenser naccarii* × *A. baerii*)", Zareh *et al.* (2006) studied "quality assessment of various meat processing modes for meat from 2-year-old farmed (*Huso huso*)", Mol and Turan (2008) compared "proximate, fatty acid and amino acid compositions of various types of fish roes", Bekhit *et al.* (2009) determined the "impact of maturity on the physicochemical and biochemical properties of Chinook salmon (*Oncorhynchus tshawytscha*) roe", Ovissipour and Rasco (2011) compared "fatty acid and amino acid of domestic and wild Beluga (*H. huso*) roe and impact on fertilization ratio", Gong *et al.* (2013) studied "nutritional composition of caviar from three

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commercially farmed sturgeon species in China" and Hamzeh *et al.* (2015) worked on "amino acid composition of roe from wild and farmed Beluga sturgeon (*H. huso*).

The objective of present study was to investigate and find detailed information about amino acid profiles and proximate compositions in Caspian Sea Beluga sturgeon (*H. huso*) caviar after culture in freshwater. This will be a significant contribution to preserve the wild population of this valuable species and will help sturgeon aquaculture industry, also will have a significant impact on the global market.

Materials and methods

Sturgeon caviar collection and analytical procedures

Wild Beluga caviar samples (300g) were provided by Mazandaran Sturgeon Affairs Office and farmed sturgeon caviars were collected from Sturgeon Hatchery Centers (Commercial Private Estates). Farmed samples (300g) were conducted and originated from different three individual female Beluga sturgeons. Both caviar samples packed in closed commercial glass cans (each glass can consisted 50g caviar) and stored at 0-4°C until delivered to the laboratory where the samples were analysed. Farmed Beluga sturgeons were reared in concrete tanks with freshwater constantly overflowing and important parameters of the tanks water were kept relatively constant: temperature 17-19°C, pH 6.9-7.5, oxygen level more than 7mg/L and salinity≤1ppt. Fish were fed by commercial diet (caviar Coppens, Germany) once a day, 0.3% of their biomass. The proximate compositions of the diet contained (dry matter basis), 50% crude protein, 12% crude fat, 0.6% crude fiber, 7.8% ash, 1.29% P, Vitamin A 10000 (IE/kg),Vitamin D 100 (IE/kg),Vitamin E 200 (mg/kg),Vitamin C 1000 (mg/kg), gross energy 20.4 (MJ/kg), digestible energy 18.8 (MJ/kg) and metabolisable energy 16.4 (MJ/kg).

Proximate composition

Caviar samples were analysed for proximate composition including crud protein, lipid, moisture and ash by using AOAC standard methods (Horwitz and Latimer, 2005). Briefly, crude protein content was measured by determining nitrogen content (×6.25) using automated Kjeldahl analysis (V50 Analyzer, Bakhshi, Iran). Lipid was extracted by using an automatic Soxtec system (Soxtec 6CTF, Bakhshi, Iran). Moisture was measured after drying the sample at 105°C to constant weight (SL 901, KTS, Iran). Ash was determined by incineration in a muffle furnace (KLI 14, KTS, Iran) to a constant weight at 550°C.

Amino acid analysis

For caviars amino acid analysis and determination, as described previously by Ovissipour and Rasco (2011) at first, caviar samples were defatted Wing Keong and Hung (1994) and Antoine *et al.* (1999), then the samples were freeze-dried at -60°C (Operon, FDU-7012, Gyeonggi-do, South Korea)

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before amino acid analysis. All samples were hydrolyzed with 6 mol of HCL at 110°C for 24h and derived with o-phthaldialdehyde (OPA, Antoine *et al.*, 1999). The total amino acids were analysed by HPLC unit (CECIL, CE4900, UK) using C18 (1.6µm, 2×100 mm) at the flow rate of 1mL /min using mixed methanol and 50 mM sodium acetate buffer (pH 7, Merck, Darmstadt, Germany) as the mobile phase with a fluorescence detector (Fluorescence wave length, 348-450nm). Samples were run in triplicates and average areas were calculated. The amount of amino acids was calculated by comparison with retention time and peak areas of standard amino acids, as milligrams amino acid per gram dry caviar powder sample. Cysteine, lysine and Proline were not detected in any samples.

Statistical analysis

Analysis was carried out in triplicate for each sample of two treatments and the results were presented as mean and standard deviation. An unpaired t-test using the Statistical Package for the Social Sciences (SPSS) software release 19.0.0 (SPSS Inc., Chicago, IL., USA) at a probability level of $p \leq 0.05$ were conducted.

Results

Proximate composition

The proximate composition of Beluga caviar, both wild and farmed is presented in Figure 1. Results showed that: crude protein was 26.56±0.11%, 26.37±0.65%; crude lipid was 16.06±0.16%, 16.35±0.18%; moisture was 51.26±0.21%, 51.40±0.28% and ash was 5.00±0.95%, 4.85±0.06% in wild and farmed samples respectively. The results showed that there was no significant difference between the samples ($p>0.05$).

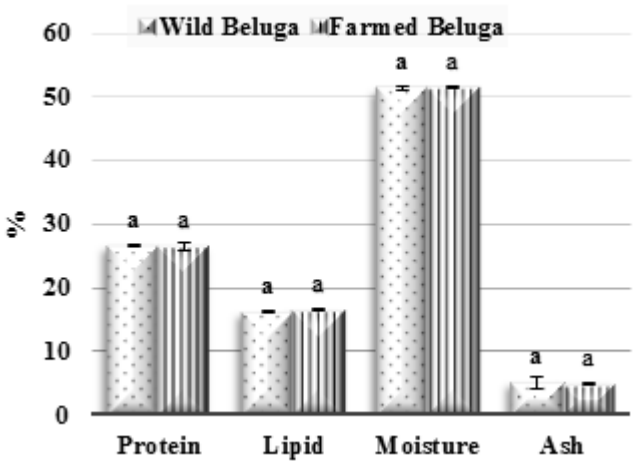

Figure 1: Proximate compositions in wild and farmed Beluga caviar (%). *Values are MEAN±SD. Within the columns, values with different superscripts are significantly different ($p \leq 0.05$). Error bars show standard deviation.

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Amino acid composition

The detailed composition of wild and farmed caviar samples is given in Table 1. According to the results, the content of essential amino acids (EAA) in wild and farmed caviars were 132.66±6.8mg/g (55.14%) and 110.16±7.35mg/g (47.49%), and nonessential amino acids (NEAA) in wild and farmed caviars were 107.92±5.68mg/g (44.86%) and 121.79±4.08mg/g (52.51%), which there were no significant difference between them (p>0.05). Also content of total amino acids (TAA) in wild and farmed samples had no significant difference with 240.58±12.48mg/g and 231.95±11.43mg/g (p>0.05, Table 2). The ratio of EAA to NEAA in wild and farmed caviars were 1.23±0 and 0.9±0.03 respectively (p<0.05, Table 2).

Table 1: Amino acids profile of wild and farmed Beluga caviar (mg/g, %). [†] EAA: Essential Amino Acids, [‡] NEAA: Nonessential Amino Acids, FAA: [§] Functional Amino Acids, [¶] DDA: Delicious Amino Acids. Lysine, cysteine and proline were not detected in any samples. ND: Not detected. Values are MEAN±SD. Within the same row, values with different superscripts were significantly different (p≤0.05).

Amino acid	Wild Beluga	Farmed Beluga
Isoleucine (Ile) [†]	50.17±2.75 [a] (20.85)	26.83±0.82 [b] (11.57)
Leucine (Leu) [†§]	17.56±1.16 [a] (7.3)	18.38±1.21 [a] (7.92)
Threonine (Thr) [†]	3.43±0.86 [a] (1.43)	5.49±0.66 [a] (2.37)
Valine (Val) [†]	12.86±2.04 [a] (5.35)	7.65±1.15 [a] (3.3)
Phenylalanine (Phe) [†]	12.24±1.52 [a] (5.09)	11.09±0.38 [a] (4.78)
Methionine (Met) [†§]	3.9±0.22 [a] (1.62)	7.05±1.23 [a] (3.04)
Tryptophan (Trp) [†§]	11.96±1.67 [a] (4.97)	7.14±1.93 [a] (3.08)
Arginine (Arg) [†§]	15.45±0.81 [a] (6.42)	14.59±2.06 [a] (6.29)
Histidine (His) [†]	5.09±1.72 [a] (2.12)	11.94±0.87 [a] (5.15)
Lysine (Lys) [†]	ND	ND
Tyrosine (Tyr) [‡§]	5.82±0.43 [a] (2.42)	10.78±1.33 [a] (4.65)
Aspartic acid (Asp) [‡§¶]	11.99±1.11 [a] (4.98)	18.88±1.16 [b] (8.14)
Glutamic acid (Glu) [‡§¶]	12.6±0.77 [a] (5.24)	20.65±1.05 [b] (8.9)
Glycine (Gly) [‡§¶]	7.31±1.65 [a] (3.04)	11.33±0.99 [a] (4.88)
Alanine (Ala) [‡¶]	23±1.64 [a] (9.56)	4±0.8 [b] (1.72)
Serine (Ser) [‡]	13.77±1.3 [a] (5.72)	16.18±0.2 [a] (6.98)
Asparagine (Asn) [‡]	18.26±0.7 [a] (7.59)	23.64±0.91 [b] (10.19)
Glutamine (Gln) [‡§]	9.51±2.14 [a] (3.95)	8±0.68 [a] (3.45)
Citrulline (Cit) [‡]	5.66±0.09 [a] (2.35)	8.33±1.06 [a] (3.59)
Cysteine (Cys) [‡§]	ND	ND
Proline (Pro) [‡§]	ND	ND

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Table 2: Comparison of amino acid series in wild and farmed Beluga caviar (mg/g). EAA: Essential Amino Acids, NEAA: Nonessential Amino Acids, TAA: Total Amino Acids, FAA: Functional Amino Acids, DAA: Delicious Amino Acids. Values are MEAN±SD. Within the same row, values with different superscripts were significantly different ($p \leq 0.05$).

Amino acid	Wild Beluga	Farmed Beluga
∑EAA	132.66±6.8 [a]	110.16±7.35 [a]
∑NEAA	107.92±5.68 [a]	121.79±4.08 [a]
EAA/NEAA	1.23±0 [a]	0.9±0.03 [b]
TAA	240.58±12.48 [a]	231.95±11.43 [a]
FAA	96.1±8.66 [a]	116.8±9.65 [a]
DAA	54.9±1.89 [a]	54.86±2.01 [a]
FAA/TAA	0.4±0.02 [a]	0.5±0.02 [b]
DAA/TAA	0.23±0 [a]	0.24±0 [a]

There were significant differences ($p<0.05$) between the amount of isoleucine (Ile), aspartic acid (Asp), glutamic acid (Glu), alanine (Ala) and asparagine (Asn) in Caviar samples (Table 1). Among total amino acids, the most abundant amino acid in both samples was isoleucine (Ile) which was 50.17±2.75mg/g (20.85%) in wild samples and 26.83±0.82mg/g (11.57%) in farmed samples ($p<0.05$). In wild Beluga caviar samples the highest concentration of amino acids after isoleucine (Ile) were alanine (Ala) 23.00±1.64mg/g (9.56%) and asparagine (Asn) 18.26±0.7mg/g (7.59%) and in farmed Beluga caviars after isoleucine (Ile) the highest concentration of amino acids were asparagine (Asn) 23.64±0.91mg/g (10.19%), glutamic acid (Glu) 20.65±1.05mg/g (8.90%) and aspartic acid (Asp) 18.88±1.16mg/g (8.14%, $p<0.05$). Functional amino acids (FAA) in wild and farmed samples were 96.1±8.66mg/g (39.95%) and 116.8±9.65mg/g (50.36%) which did no differ between them ($p>0.05$). But their ratio to total amino acids (FAA/TAA) in wild samples was 0.40±0.02 and in farmed one was 0.50±0.02, which was significantly higher in farmed caviar ($p<0.05$). Delicious amino acids (DAA) were 54.9±1.89mg/g (22.82%) and 54.86±2.01mg/g (23.65%) in wild and farmed caviar respectively and their ratio to total amino acids (DAA/TAA) were 0.23±0 and 0.24±0 ($p>0.05$). Lysine, cysteine and proline were not detected in any samples.

Discussion

The proximate compositions of wild and farmed Beluga caviars had no significant difference ($p>0.05$). The results were within the range reported for sturgeon caviars by other researchers, like Rehbein (1985), Sternin and Doré (1993), Gessner *et al.* (2002), Caprino *et al.* (2008) and Mol and Turan (2008).

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Ovissipour and Rasco (2011) found no difference in proximate compositions between wild and farmed Beluga caviars, the content of protein, lipid, moisture and ash in wild samples were 15.11±0.97, 14.87±1, 64.1±2.1 and 4.2±0.65 and in farmed caviars were, 14.56±0.2, 14.55±0.6, 64.83±0.46 and 4.51±0.91, respectively (p>0.05).

As results showed in this study, the content of isoleucine and alanine was significantly higher in wild Beluga caviar, while asparagine, glutamic acid and aspartic acid were significantly higher in farmed sample (p<0.05). Differences in some amino acid contents can be due to species, age, diet and environmental conditions (Mol and Turan, 2008).

Table 3 shows results of other researchers. Aspartic acid, glutamic acid, serine and leucine had higher contents among total amino acids in research reports on sturgeon caviar and other fish roe, such as Iwasaki and Harada (1985), Bledsoe *et al.* (2003), Mol and Turan (2008), Bekhit *et al.* (2009), Ovissipour and Rasco (2011) and Gong *et al.* (2013). Iwasaki and Harada (1985) worked on 18 marine species and stated that "glutamic acid, leucine and aspartic acid were generally found to be the major components of roes". Bekhit *et al.* (2009) found more leucine among EAAs in mature and immature (*Oncorhynchus tshawytscha*) roes. Hamzeh *et al.* (2015) stated that glutamine, serine, alanine, methionine and lysine contents were significantly higher in wild Beluga sturgeon caviar than farmed ones (p<0.05).

Table 3: Amino acids composition in wild and farmed Beluga caviar and different sturgeon caviars (mg/g). EAA: Essential Amino Acids, NEAA: Nonessential Amino Acids, TAA: Total Amino Acid, FAA: Functional Amino Acids, DAA: Delicious Amino Acids, ND: Not detected.

Amino acid	**Wild Beluga (*Huso huso*)**	**Farmed Beluga (*Huso huso*)**	**Wild Beluga (*Huso huso*)**	**Farmed Beluga (*Huso huso*)**	***Acipenser gueldenstaedti***	***Acipenser persicus***	***Huso huso***
Isoleucine (Ile)	50.17	26.83	73.4	73.3	1.63	2.58	3.60
Leucine (Leu)	17.56	18.38	8.9	8.8	3.67	2.85	3.17
Threonine (Thr)	3.43	5.49	65.6	64.8	3.80	3.15	3.33
Valine (Val)	12.86	7.65	90.1	86.6	32.96	6.81	29.04
Phenylalanine (Phe)	12.24	11.09	30.7	30.5	2.27	1.14	3.41
Methionine (Met)	3.9	7.05	23.1	24.6	25.60	27.21	27.30
Tryptophan (Trp)	11.96	7.14	-	-	1.80	52.74	3.68
Arginine (Arg)	15.45	14.59	86.7	90.3	3.10	10.47	2.45
Histidine (His)	5.09	11.94	37.0	35.0	1.05	2.72	0.36

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Table 3 (continued):

Amino acid	Wild Beluga (*Huso huso*)	Farmed Beluga (*Huso huso*)	Wild Beluga (*Huso huso*)	Farmed Beluga (*Huso huso*)	*Acipenser gueldenstaedti*	*Acipenser persicus*	*Huso huso*
Lysine (Lys)	ND	ND	90.1	90.5	55.95	26.47	40.29
∑EAA	132.66	110.16	506.7	504.4	123.08	123.60	116.63
Tyrosine (Tyr)	5.82	10.78	3.16	2.7	1.50	8.15	4.42
Aspartic acid (Asp)	11.99	18.88	103.3	105.3	33.48	30.11	29.58
Glutamic acid (Glu)	12.6	20.65	153.4	164.6	48.54	45.30	47.97
Glycine (Gly)	7.31	11.33	53.8	51.7	2.85	2.39	2.35
Alanine (Ala)	23	4	27.0	26.7	8.14	3.01	1.63
Serine (Ser)	13.77	16.18	130.3	124.7	30.10	10.08	23.28
Asparagine (Asn)	18.26	23.64	-	-	0.75	2.77	0.47
Glutamine (Gln)	9.51	8	-	-	-	-	-
Citrulline (Cit)	5.66	8.33	-	-	-	-	-
Cysteine (Cys)	ND	ND	-	-	1.60	2.42	4.32
Proline (Pro)	ND	ND	-	-	0.65	3.07	1.38
∑NEAA	107.92	121.79	471.1	475.8	119.47	107.30	115.40
EAA/NEAA	1.23	0.9	1.076	1.060	1.00	1.23	0.99
TAA	240.58	231.95	978.8	980.3	242.55	230.90	232.03
FAA	96.1	116.8	432.36	448.0	122.79	184.71	126.62
DAA	54.9	54.86	337.5	348.3	93.01	80.81	81.53
FAA/TAA	0.4	0.5	0.44	0.46	0.51	0.80	0.55
DAA/TAA	0.23	0.24	0.34	0.36	0.38	0.35	0.35
References	Present study	Present study	(Ovissipour & Rasco 2011)	(Ovissipour & Rasco 2011)	(Mol & Turan 2008)	(Mol & Turan 2008)	(Mol & Turan 2008)

Aspartic acid, glycine and glutamic acid are known to have important role in wound repair (Chyun and Griminger, 1984). Zhao *et al.* (2010) stated that, glutamine is the major free amino acid in body and in critical sickness; the efflux of muscle glutamine serves as an important ammonia carrier to the splanchnic area and the immune system.

In this study there were no significant difference between essential amino acids (EAA), nonessential amino acids (NEAA) and total amino acids (TAA) contents in wild and farmed samples (p>0.05). Similarly, Ovissipour and Rasco (2011) and Hamzeh *et al.* (2015)

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reported that there were no significant differences in EAA, NEAA and TAA contents between wild and farmed Beluga sturgeon caviars (p>0.05).

In present study, the ratio of EAA/NEAA was 1.23±0 and 0.9±0.03 in wild and farmed samples, that was significantly higher in wild caviar (p<0.05). In all food sources the ratio of EAA/NEAA is an important factor, consequently, the greater ratio indicates the better food quality. Iwasaki and Harada (1985) stated that, this ratio is higher in marine fish rather than fresh water fish. For example, De Silva *et al.* (2001) found the content of EAA/NEAA in (*Cyprinus carpio*) roe (0.8) and Iwasaki and Harada (1985) found it in (*Thunnus thynnus*) roe (1.06).

Some evidences suggest that "some amino acids are important regulators of key metabolic pathways and necessary for maintenance, growth, nutrient utilization and immunity in organisms" Wu (2009). "These amino acids are called functional amino acids (FAA) that include arginine, cysteine, glutamic acid, leucine, proline, tryptophan, glycine, glutamine, tyrosine, methionine, taurine and aspartic acid" Wu (2013).

In present study, functional amino acids in wild and farmed caviar showed no significant difference (p>0.05) but the ratio of FAA/TAA was significantly greater in farmed samples (p<0.05), so it can be said that farmed Beluga caviar is a good source of functional amino acids.

Delicious amino acids (DAA) which include, aspartic acid, glutamic acid, glycine and alanine, are well known for their fresh tasting. In this study, DAA in wild and farmed samples and the ratio of DAA/TAA had no significant difference (p>0.05). Gong *et al.* (2013) measured FAA, DAA and their ratios to TAA in (*Acipenser baerii*, AA), (*Acipenser Schrenckii*, AS), and (*Huso dauricus* × *A. schrrenkii*, HS) and stated that, they all had good FAA and they were similar in DAA. The caviars contained higher contents of DAA compared to other fish roes (Park *et al.*, 2015).

In conclusion, present study indicated that, wild and farmed Beluga caviars had no significant difference in contents of essential amino acids (EAA), nonessential amino acids (NEAA) and total amino acids (TAA), but EAA/NEAA ratio showed significant differences. The farmed Beluga caviar had a good EAA/NEAA ratio and functional amino acids and can be considered as a valuable food resource as well as the wild ones and is a good substitution for the wild caviar. This will help to protect valuable wild Beluga sturgeons from elimination and will have a significant impact on sturgeon aquaculture industry.

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