Bulgarian Journal of Agricultural Science, 28 (Suppl. 1) 2022
Agricultural Academy

Comparative biochemical characterization of Siberian sturgeon (*Acipenser baerii*), Russian sturgeon (*Acipenser gueldenstaedtii*), and hybrid (F_1 *Acipenser baerii* x *Acipenser gueldenstaedtii*) reared in net cages

Magdalena Stoyanova[1], Petya Ivanova[2], Vesela Chalova[2], Georgi Georgiev[3], Lyudmila Nikolova[3*]

[1]*University of Food Technologies, Department of Analytical and Physical Chemistry, 26 Maritsa Blvd., Plovdiv 4002, Bulgaria*
[2]*University of Food Technologies, Department of Biochemistry and Molecular Biology, 26 Maritsa Blvd., Plovdiv 4002, Bulgaria*
[3]*Agricultural University, Department of Animal Science, 12 Mendeleev Blvd., Plovdiv 4000, Bulgaria*
[*]*Corresponding author:* lnn65r@abv.bg

Abstract

Stoyanova, M., Ivanova, P., Chalova, V., Georgiev, G. & Nikolova, L. (2022). Comparative biochemical characterization of Siberian sturgeon (*Acipenser baerii*), Russian sturgeon (*Acipenser gueldenstaedtii*), and hybrid (F_1 *Acipenser baerii* x *Acipenser gueldenstaedtii*) reared in net cages. *Bulg. J. Agric. Sci.*, *28 (Supplement 1)*, 146–153

Being economically important, sturgeon farming is developing rapidly worldwide. The sturgeon meat quality is influenced by fish genotype, farming technology, and the stage of development. The study aimed at comparative biochemical characterization of meat of the Siberian sturgeon (*Acipenser baerii*), Russian sturgeon (*Acipenser gueldenstaedtii*), and hybrid (F_1 *Acipenser baerii* x *Acipenser gueldenstaedtii*) reared in net cages. It was performed with two groups of each genotype differing in their live weight. The meat of the fish with a higher live weight (group 2) was characterized by a higher dry matter and lipid contents. The fish with lower live weight (group 1) though were richer in protein. Within the studied groups, the Siberian sturgeon with a higher live weight (Ab-2) exhibited the highest total energy of meat, while the Siberian sturgeon with the smaller live weight (Ab-1) had the lowest energy content. Lysine and histidine were established in the highest amounts in studied fish. The highest content of lysine and histidine were observed in the meat of the hybrid (22.98 g/100 g protein) and Russian sturgeon (17.70 g/100 g protein) with lower weight, respectively. Saturated palmitic acid (C16:0) and unsaturated oleic (C18:1) and linoleic (C18:2) fatty acids were dominating in the fatty acid profile. Higher amounts of palmitic acid were established in parental fish than in the hybrid. The Russian sturgeon from group 2 (Ag-2) contained the highest levels of oleic (46.91 %) and linoleic (16.31 %) acids. The highest values of δ -, γ -, and α-tocopherols were established in Russian sturgeon (Ag-2, 7565.52 μg/g), the hybrid with lower live weight (Hy-1, 320485.2 μg/g), and Russian sturgeon with lower live weight (Ag-1, 7971 μg/g), respectively. The study demonstrated the influence of fish genotype and stage of development on the nutritive characteristic of their meat and could be used to obtain sturgeon meat with desired biochemical characteristics.

Keywords: sturgeon; aquaculture, proximate analysis, fatty and amino acids composition, tocopherols
Abbreviations: Ab – *Acipenser baerii;* Ag – *Acipenser gueldenstaedtii;* Hy – F_1 *Acipenser baerii* x *Acipenser gueldenstaedti*

Introduction

Satisfying the growing needs for delicatessen sturgeon products is possible only through the development of sturgeon aquaculture. Emphasizing the importance of sturgeon aquaculture, Chandra & Fopp-Bayat (2021) reported that endangered natural populations of sturgeon species will not be able to survive without artificial breeding and sustainable ranching programs.

In recent years, sturgeon breeding has shown significant development (Bronzi et al., 2011; 2019; Bronzi & Rosenthal, 2014). Bronzi et al. (2019) noted that 13 sturgeon species and four hybrids are bred for meat, with the Siberian sturgeon being the dominant species. In Bulgaria, sturgeon farming is developing rapidly, as the country ranks among the leading producers of sturgeon and caviar in the world. At the beginning of its development, sturgeon farming was based mainly on Russian sturgeon, while in recent years the share of Siberian sturgeon and various hybrids has been growing (Nikolova, 2019).

The Russian sturgeon (*A. gueldenstaedtii*) is a native species of the Black Sea basin and is one of the most valuable sturgeon species. It adapts well to the conditions of industrial cultivation, which determines the interest in it (Nikolova et al., 2018;). Siberian sturgeon (*A. baerii*) refers to slow-growing fish in their natural habitat but growth and development usually improve under farming conditions (Kudersky, 2015; Adamek et al., 2007; Nikolova & Bonev, 2020; Nikolova & Georgiev, 2021). Sturgeons are characterized by very good combinatorial ability in hybridization as hybrids are often found in natural sympatric populations (Chebanov & Galich, 2013). Hybridization in sturgeon farming is used as a method to increase production efficiency (Kudersky, 2015; Nikolova et al., 2021).

There is a huge diversity of fish species and the differences in meat quality are significant (Passi et al., 2002). According to Lopez et al. (2020), species specificity is highly related to the characteristics of sturgeon meat and caviar. In addition, the fish meat quality is influenced by several genetic and paratypical factors which justify the numerous studies conducted with different species, breeds, and hybrids bred in different technologies. Thus, by studying wild and cultivated sturgeon Czesny et al. (2000) have found that factors such as habitat, diet, and origin have a significant impact on the fatty acid composition of caviar.

In the case of farmed fish, the quality of production is directly influenced by the specific farming technology. Bronzi et al. (2019) indicated that cage-based farming accounted for about 18% of all sturgeon farming technologies worldwide. In this regard, the quality of the meat of fish of different genotypes reared in net cages is of interest. The study aimed at comparative biochemical characterization of meat of the Siberian sturgeon (*Acipenser baerii*), Russian sturgeon (*Acipenser gueldenstaedtii*), and hybrid (F_1 *Acipenser baerii* x *Acipenser gueldenstaedtii*) reared in net cages.

Materials and Methods

Sample collection

The study was conducted with male individuals of different genotypes, Siberian sturgeon (*Acipenser baerii*) (Ab), Russian sturgeon (*Acipenser gueldenstaedtii*) (Ag), and their hybrid (F_1 *Acipenser baerii* x *Acipenser gueldenstaedtii*) (Hy). The fish were cultured in net cages farm, located in a dam in southeastern Bulgaria (41°37′ N latitude and 25°20′ E longitude). Fish of different species and categories were reared in separate cages. The cages were 8×8 m in size with a depth of 6 m below the water surface. Each cage was equipped with double polyamide nets. Feeding was performed with a commercial specialized sturgeon granular extruded mixture (Table 1).

Table 1. Composition of the commercial feed

Indices	Value
Protein, %	46.00
Fat, %	15.00
Crude fibre, %	1.40
Ash, %	6.50
Total P, %	1.03
Ca, %	1.40
Na, %	0.30
Vitamin A, IU.kg^{-1}	10 000
Vitamin C, mg.kg^{-1}	520
Vitamin E, mg.kg^{-1}	200
Vitamin D_3, IU.kg^{-1}	2303
Gross energy, MJ.kg^{-1}	21.00
Digestible energy, MJ.kg^{-1}	19.20

The fish on the farm were sorted by live weight throughout the rearing period. At the end of the vegetation period, five fish from a cage with a smaller (first weight group – 1) and a higher live weight (second weight group – 2) were randomly taken from each genotype. Fish weight groups of each genotype, related codes, and live weights are presented in Table 2.

Samples of skinless fillet muscles, derived from slaughter analysis, were homogenized and further used for the evaluation of proximate composition, amino- and fatty acids profile, and the content of α-, δ-, and γ-tocopherols.

Table 2. Characteristics of two weight groups of each genotype

Genotype	Weight group	Group code Genotype / weight group	n	Body weight*
Siberian sturgeon	1	Ab-1	5	2823.00±126.044[a]
	2	Ab-2	5	4273.20±110.288[a]
Russian sturgeon	1	Ag-1	5	3010.80±72.950[b]
	2	Ag-2	5	4910.00±100.300[b]
Hybrid	1	Hy-1	5	2796.60±129.839[c]
	2	Hy-2	5	4934.80±192.539[c]

[a, b, c] *The values with the same letters in the rows for a given genotype are statistically different: ($p<0.001$); * mean ± standard error of the mean*

Proximate analysis

The crude protein content was established by the Kjeldahl method (AOAC, 1990) with a conversion coefficient of 6.25. Total lipids were evaluated as described by Bligh & Dyer (1959). Carbohydrates were determined by Dubois et al. (1956). Ash content was determined by a standardized method (ICC Standard №104/1, 1990). The energy content of fish flesh was calculated based on chemical composition evaluated assuming that proteins and fats provide 23.90 kJ/g and 39.75 kJ/g energy, respectively (Hadjinikolova et al., 2008). Data are presented on a dry weight basis. The dry weight was determined by drying the samples to a constant weight at a temperature of 105°C.

Amino acid analysis

Samples were hydrolyzed with 6 N HCl at 105°C for 24 h followed by neutralization and filtration (Blackburn,1968). The hydrolysates were derivatized by using AccQ-Fluor TM Reagent kit (Waters Corporation, Milford, MA, USA) following the manufacturer's instructions. The amino acid analyzes were performed on high-performance liquid chromatography (ELITE LaChrome, Hitachi High Technologies America, Inc., San Jose, CA, USA) equipped with a C18 AccQ-Tag (3.9 mm × 150 mm) reversed-phase chromatographic column and a diode array detector.

Fatty acid analyses

Fish oil for fatty acid analysis was extracted as described by Bligh & Dyer (1959). The fatty acids were analyzed as fatty acid methyl esters after transesterification of oil samples (ISO 5509:2000). The fatty acid composition was determined by gas chromatography (GC; 17 A Shimadzu) equipped with a flame ionization detector and a 3.0 m × 0.32 cm steel column. Nitrogen was used as carrier gas (flow rate 1 ml/min) (ISO 5508:1990).

Extraction and determination of tocopherols

One gram of fish oil was accurately weighed into Erlenmeyer flask and 15 ml of 0.5M potassium hydroxide dissolved in ethanol was added. The flask was heated on a water bath for 30 min at 70°C. After extraction of tocopherols, 15 ml 1% sodium chloride and 15 ml portions of n-hexane-ethylacetate (9:1, v/v) were added. The organic phase was evaporated to dryness at 40°C and the residue was dissolved in 1 ml methanol. After passing through a 0.45 µm filter, the samples were injected for chromatographic analysis. Qualitative and quantitative determination of tocopherols were performed by using Elite LaChrome (Hitachi) HPLC system equipped with diode-array detector (DAD) and ELITE LaCHrome (Hitachi) software. Separation of the tocopherols was performed by the Supelco Discovery HS C18 column (5 µm, 25 cm × 4.6 mm), operated at 30°C, with a mobile phase consisting of methanol: water (98:2, v/v). The detection of compounds was carried out at 285 nm and the flow rate was 0.8 ml/min. (Stoyanova, 2020). Standards of α-tocopherol, γ- tocopherol and δ tocopherol were used for the development of standard calibration curves with a linearity range of 10- 500 µg/cm^3.

Statistical analyzes.

Presented data are the mean ± standard deviation of three independent experiments ($n = 3$). Data were analyzed by one-way analysis of variance (ANOVA) using Statgraphics Centurion statistical program (version XVI, 2009) (Stat Point Technologies, Ins., Warrenton, VA, USA). Mean differences were established by Fisher's least significant difference test for paired comparison with a significance level $p < 0.05$.

Results and Discussion

Proximate analysis

Results from the proximate analysis and energy content of fish samples are presented in Table 3. The meat of the fish with a higher live weight (group 2) was characterized by higher dry matter content. The difference of approximately 6% in the dry matter content of the parent fish is more profound compared to the hybrid, where the fish of group 2 had 2% higher dry matter than the samples with a lower weight. Variations in the protein amount were also observed but in reversed order. Fish with lower live weight, Ab-1, Ag-1, and Hy-1, had a higher amount of crude protein compared to fish with higher live weight, Ab-2, Ag-2, and Hy-2. This group though possessed a higher amount of total lipids. The meat of Siberian sturgeon from the second weight group (Ab-2) was the richest in lipids (28.32%), while the most proteinous meat belonged to the F1 hybrid from the first weight group

(84.47%). A negative correlation between protein and fats was established by Dhaneesh et al. (2012) who evaluated the nutritional properties of commercially important fish species of Lakshadweep Archipelago, India. Inversely proportional contents of proteins and lipids in fish muscles was reported by González-Fandos et al. (2004) for rainbow trout and López-Huerta et al. (2018) for native fish *Dormitator latifrons* as well. The trend in mineral content was similar to that of protein. It decreases with increasing the live weight of the fish, as the lowest value of this indicator within all studied groups was observed for the Siberian sturgeon with a higher live weight (Ab-2). No clear trend in carbohydrate content variation as related to fish weight was established (Table 3).

Inversely related content of proteins and lipids in fish muscles is the most probable explanation for the higher total energy content of all studied fish from group 2 being richer in fats than in proteins (Table 3). Within the studied groups, the Siberian sturgeon with a higher live weight (Ab-2) exhibited the highest total energy of meat, while the Siberian sturgeon with the smaller live weight (Ab-1) had the lowest energy content. Lipids, along with carbohydrates and proteins, comprise the major macronutrient classes that provide energy to cells. Fats, however, deliver more calories per gram compared to carbohydrates and proteins (Dunn, 2013). As a consequence, the energy content, calculated on a protein basis, was lower for fish from group 2 than for the fish from group 1 (Table 3).

Table 3. Proximate analysis and energy content of Siberian sturgeon (*Acipenser baerii*), Russian sturgeon (*Acipenser gueldenstaedtii*), and hybrid (F$_1$ *Acipenser baerii* x *Acipenser gueldenstaedtii)* meat.

Sample	Dry weight, %	Content, %				Energy content	
		Crude protein	Total lipids	Carbohydrates	Ash	Total energy, kJ.100^{-1}g	Protein based energy, %
Ab-1	21.41±1.10	75.01±0.79	17.38±0.43	0.79±0.15	4.66±0.28	2329.07±75.75	70.34±3.25
Ab-2	27.06±0.21	64.12±0.16	28.32±1.83	0.64±0.11	3.77±0.05	2658.08±3.75	57.65±0.14
Ag-1	20.45±0.25	78.47±2.15	15.65±0.87	0.56±0.09	6.25±0.25	2497.45±51.36	75.09±2.06
Ag-2	26.64±0.14	63.98±1.25	25.42±0.21	0.78±0.09	4.48±0.34	2539.69±29.77	60.21±1.17
Hy-1	20.07±0.01	84.47±3.13	12.52±1.15	0.98±0.08	6.37±0.36	2516.44±74.77	80.22±2.97
Hy-2	22.97±0.75	73.54±0.78	20.55±0.16	0.86±0.07	5.59±0.11	2609.72±18.97	68.70±0.73

Table 4. Amino acid profile of Siberian sturgeon (*Acipenser baerii*), Russian sturgeon (*Acipenser gueldenstaedtii*), and hybrid (F$_1$ *Acipenser baerii* x *Acipenser gueldenstaedtii*) meat.

Amino acid	Amino acid content, g/100 g protein					
	Siberian sturgeon	Russian sturgeon	Hybrid			
	Ab-1	Ab-2	Ag-1	Ag-2	Hy-1	Hy-2
Non-essential amino acids						
Asp	12.26	4.08	15.95	12.27	5.56	7.71
Ser	5.75	2.06	5.05	5.26	1.95	3.22
Glu	8.32	3.56	7.09	6.57	4.88	4.72
Gly	1.35	1.08	3.25	1.95	2.12	2.11
His	18.29	10.38	17.70	13.15	10.92	14.86
Arg	6.51	5.32	5.99	7.84	10.34	5.08
Ala	10.99	9.83	13.99	12.96	14.73	11.35
Pro	3.75	2.97	4.17	4.69	4.97	3.59
Cys	2.03	2.63	3.90	0.08	0.13	2.47
Tyr	0.73	0.87	5.60	6.76	6.09	0.86
Essential amino acids						
Thr	5.13	4.09	4.62	7.50	6.15	4.50
Val	2.99	2.83	3.50	0.73	6.88	3.08
Met	4.17	4.38	4.10	4.35	2.15	2.96
Lys	11.13	11.21	17.86	19.43	22.98	13.18
Ile	3.26	4.13	22.59	7.78	8.48	2.70
Leu	1.04	0.36	1.59	1.46	1.56	0.92
Phe	5.58	9.24	12.92	6.62	7.25	3.88

Amino acid profile

The amino acid composition of muscle meat of the studied fish is presented in Table 4. Fish is a high-value protein product. Our study indicated that the studied species and hybrids were rich in essential amino acids. The threonine content varied from 4.09 g/100 g protein (Ab-2) to 7.5 g/100 g protein (Ag-2). In the Russian sturgeon, Ag-2 group had a higher amount of threonine than Ag-1, while in Siberian sturgeon the reversed trend was observed. Within all groups studied, lysine was in the highest amount. Lysine is an essential amino acid supplementation of which is dependent on food. Plant proteins are considered incomplete concerning essential amino acids where lysine is either the first or second limiting amino acid (Han et al., 2021). Thus, the sturgeon protein, investigated in this study, appears a natural source able to suffice the need of a human organism for this amino acid.

From non-essential amino acids, alanine and histidine were in the highest amounts in all studied fish species and groups. The highest amounts of alanine were found in the hybrid with lower live weight (Hy-1). Relatively higher amounts of this amino acid were found in Russian sturgeon. Histidine is considered dispensable amino acid since its deficiency in the body can be compensated for a long time by catabolism of hemoglobin and carnosine (Clemens et al., 1984; Cho et al., 1984). Histidine, however, is considered an essential amino acid for young infants since its omission from the diets reduces weight gain and nitrogen retention (Snyderman et al., 1963). Holeček (2020) underlined its unique physiological role by participating in proton buffering, metal ion chelation, and scavenging of reactive oxygen and nitrogen species. Histidine supplementation was shown to treat atopic dermatitis and reduce obesity (Tan et al., 2017; DiNicolantonio et al., 2018).

Fatty acid composition

The fatty acid composition of studied fish is presented in Table 5. From the group of saturated fatty acids, C16:0 (palmitic acid) was found to be in the highest amount in the Siberian sturgeon, Russian sturgeon, and their F_1 hybrid. Osman et al. (2007) and Celik et al. (2008) also reported palmitic acid as one of the prevailing saturated fatty acids in finfish and rainbow trout meat, respectively. From unsaturated fatty acids, the monounsaturated C18:1(oleic acid) and polyunsaturated C18:2 (linoleic) fatty acids were dominating in the fatty acid profile. Oleic acid is an essential fatty acid and is an important component of phospholipids (Dou et al., 2020). It is also a precursor of synthetic prostaglandins which are beneficial to skin repair. Eicosapentaenoic acid (EPA) (C20:5 n-3) and docosahexaenoic acid (DHA) (C22:6 n-3) were established as well (Table 5). These compounds are conditionally essential fatty acids that should be provided by food under specific conditions (Dou et al., 2020).

Fish is one of the main sources of polyunsaturated fatty acids for humans. Lipids in fish differ from those in mammals in that they contain up to 40% long-chain polyunsaturated fatty acids (Secci & Parisi, 2015). Fish fats contain particularly valuable fatty acids such as eicosapentaenoic

Table 5. Fatty acid composition of Siberian sturgeon (*Acipenser baerii*), Russian sturgeon (*Acipenser gueldenstaedtii*), and hybrid (F_1 *Acipenser baerii* x *Acipenser gueldenstaedtii*) meat.

Fatty acid	Fatty acid content, %					
	Siberian sturgeon		Russian sturgeon		Hybrid	
	Ab-1	Ab-2	Ag-1	Ag-2	Hy-1	Hy-2
C12:0	1.16±0.14	1.09±0.19	1.43±0.13	1.25±0.11	1.57±0.10	1.39±0.24
C14:0	0.26±0.09	0.22±0.04	0.90±0.24	0.33±0.15	0.14±0.05	0.32±0.14
C16:0	15.68±0.12	17.06±0.08	17.23±0.14	16.19±0.18	15.74±0.23	15.19±0.19
C16:1	3.02±0.16	3.50±0.37	3.24±0.25	0	2.78±0.82	2.59±0.36
C17:0	0.13±0.03	0.15±0.06	0.11±0.06	0.13±0.03	0.14±0.04	0.14±0.08
C17:1	0.57±0.20	0.33±0.12	0.26±0.12	0.35±0.11	0.40±0.23	0.35±0.09
C18:0	5.88±0.16	6.15±0.04	5.94±0.14	6.43±0.34	5.55±0.47	6.49±0.39
C18:1	43.74±0.21	46.87±0.16	44.31±0.29	46.91±0.07	42.79±0.04	44.59±0.80
C18:2	15.28±0.13	14.41±0.33	14.32±0.26	16.31±0.27	14.60±0.04	15.79±0.55
C20:0	1.91±0.08	2.59±0.52	2.06±0.16	1.90±0.09	1.85±0.21	2.12±0.37
C20:4	2.56±0.32	1.31±0.24	2.23±0.15	1.62±0.19	3.52±0.26	2.63±0.23
C20:5	2.11±0.09	1.40±0.02	1.45±0.29	2.54±0.22	2.56±0.19	1.98±0.38
C22:5	2.43±0.02	1.58±0.16	2.24±0.20	2.85±0.05	3.13±0.19	1.78±0.24
C22:6	5.27±0.30	3.35±0.08	4.32±0.18	3.19±0.05	5.23±0.09	4.66±0.23

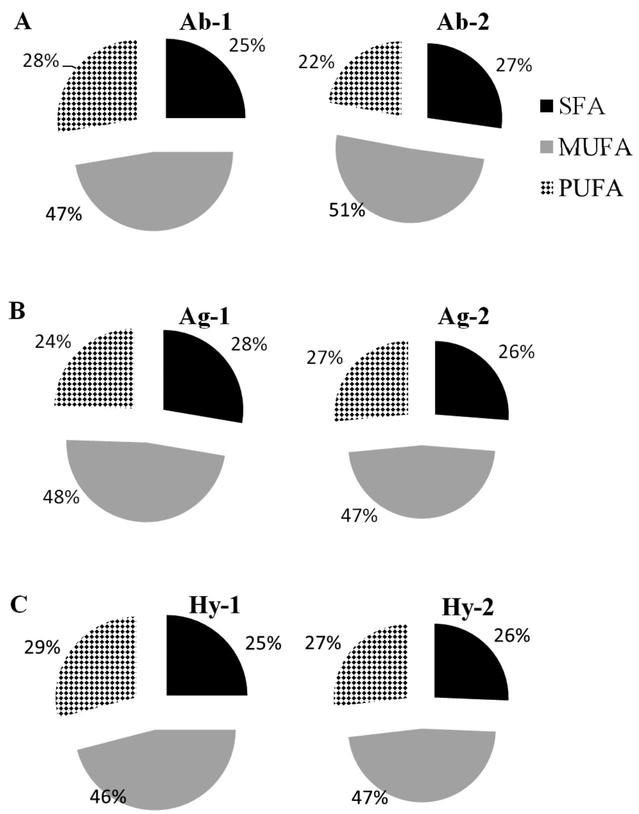

Fig. 1. Ratio of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) in Siberian sturgeon (*Acipenser baerii*) (A), Russian sturgeon (*Acipenser gueldenstaedtii*) (B), and hybrid (F_1 *Acipenser baerii* x *Acipenser gueldenstaedtii*) (C) meat.

(EPA, C20:5 n3) and docosahexaenoic (DHA, C22:6 n3). It has been shown that daily human consumption of 250-500 mg EPA + DHA significantly reduces the risk of ischemic disease and sudden cardiac death (EFSA, 2010). Simopoulos (1991) points out that EPA is a powerful factor in reducing blood clots in human blood.

The ratio of saturated, monounsaturated, and polyunsaturated fatty acids in studied fish is presented in Figure 1. In Siberian sturgeon, as in the hybrid, heavier fish had a higher percentage of saturated and monounsaturated fatty acids, while the ratio of polyunsaturated fatty acids was higher in the fish from group 1 having lower weights. In Russian sturgeon, the trend was opposite. The content of saturated and monounsaturated fatty acids was higher in the fish from group 1.

Tocopherol composition

The tocopherol composition of studied fish is presented in Table 6. The highest values of δ -, γ -, and α-tocopherols were established in Russian sturgeon (Ag-2, 7565.52 µg/g), the hybrid with lower live weight (Hy-1, 320485.2 µg/g), and Russian sturgeon with lower live weight (Ag-1, 7971 µg/g), respectively. Alpha-tocopherol is the most important and most active isomeric form of vitamin E (Feki et al., 2001). Along with δ – and γ –tocopherols, it is involved in the prevention of biomolecules from the deleterious effect of free radicals. They are also involved in the stabilization of biological membranes. Data presented here indicate Siberian sturgeon (*Acipenser baerii*), Russian sturgeon (*Acipenser gueldenstaedtii*), and their F_1 hybrid as good sources of tocopherols.

Conclusions

The study demonstrated the influence of fish genotype and stage of development on the nutritive characteristic of the meat of Siberian sturgeon (*Acipenser baerii*), Russian sturgeon (*Acipenser gueldenstaedtii*), and hybrid (F_1 *Acipenser baerii* x *Acipenser gueldenstaedtii*) reared in net cages. The fish with higher live weight had a higher dry matter and lipid contents. In contrast, fish with lower live weight were richer in crude protein. As expected, fish with a higher lipid content achieved a higher energy value. Lysine, which is either the first or second limiting amino acid in plant proteins, was established in the highest amount in studied fish. Sat-

Table 6. Tocopherol composition of Siberian sturgeon (*Acipenser baerii*), Russian sturgeon (*Acipenser gueldenstaedtii*), and hybrid (F_1 *Acipenser baerii* x *Acipenser gueldenstaedtii*) meat.

Fish sample	Tocopherol content, µg/g		
	δ-tocopherol	γ- tocopherol	α-tocopherol
Ab-1	19179.51±0.51	59339.84±0.29	2347.83±1.49
Ab-2	1606.1±0.87	8689.18±0.87	519.17±0.76
Ag-1	6752.16±3.27	149129.5±2.05	7971.5±1.25
Ag-2	7565.52±1.37	122129.9±0.90	1022.85±0.13
Hy-1	17427.52±2.09	320485.20±1.68	4148.85±1.22
Hy-2	6286.46±3.68	140785.80±1.23	6647.12±1.13

urated palmitic acid (C16:0) and unsaturated oleic (C18:1) and linoleic (C18:2) fatty acids were dominating in the fatty acid profile. The results demonstrated that sturgeon meat can be a significant source of tocopherols. The study could be useful in the production of Siberian sturgeon (*Acipenser baerii*), Russian sturgeon (*Acipenser gueldenstaedtii*), or hybrid (F_1 *Acipenser baerii* x *Acipenser gueldenstaedtii*) meat with desired nutritive characteristics.

Acknowledgments

This study was supported by the „Centre of Research, Technology Transfer and Protection of Intellectual Property Rights“ of Agricultural University – /project 06-17/.

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