THESIS OF PhD DISSERTATION

RESEARCH OF THE HETEROSIS EFFECT DURING THE STURGEON HYBRID'S (ACIPENSER RUTHENUS L. x ACIPENSER BAERI B.) INITIAL GROWING

AUTHOR:

JENŐ KÁLDY

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UNIVERSITY OF WEST HUNGARY FACULTY OF AGRICULTURAL AND FOOD SCIENCES

UJHELYI IMRE PhD SCHOOL OF ANIMAL SCIENCES

IMPROVEMENT AND BREEDING TECHNOLOGY CONSIDERATIONS OF ANIMAL PRODUCTION PROGRAM

HEAD OF THE PhD SCHOOL: PROFESSOR Dr. FERENC SZABÓ DSc

SUPERVISOR: ASSOCIATE PROFESSOR Dr. LÁSZLÓ SZATHMÁRI PhD

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1. INTRODUCTION, OBJECTIVES

At present, in Hungary as many people die in heart and cardiovascular diseases as by other reasons caused mortalities all together. Researches suggested that n-6 and n-3 fatty acids from the family of unsaturated fatty acids significantly reduce the risk of heart and vascular diseases. In preventing heart and cardiovascular diseases, in reducing the risk of myocardial infarction and stroke and some lingering illnesses the positive effect of the n-3 type fatty acids are proved (Simopoulos, 1991).

In our country according to the newly accepted Act CII of 2013 on fish farming and fish protection the sturgeon hybrids can be placed in fish producing establishments or in fish cultivating waters since they are unable for reproduction. The act refers to Council Regulation (EC) No 708/2007 of 11 June 2007, its Annex IV mentions the sturgeon species like sterlet sturgeon (*Acipenser ruthenus*), Russian sturgeon (*Acipenser gueldenstaedtii*), bastard sturgeon (*Acipenser nudiventris*), stellate sturgeon (*Acipenser stellatus*), Siberian sturgeon (*Acipenser baeri*), common sturgeon (*Acipenser sturio*) and beluga sturgeon (*Huso huso*) and these sturgeon species' hybrids. However it is important to note, that among in the Annex listed fish species it deals only just with the sturgeon hybrids in details and allows only these hybrids' breeding and establishing.

During our work we examined the sturgeon hybrid called 'szicsege' obtained by artificial hybridization of a sterlet father with Siberian sturgeon mother.

The research aims

During our experiments we have been looking for the answer for the following questions:

- Will the growing capability of the sturgeon hybrid exceed the parent species average?
- Will the feed utilization capability of the sturgeon hybrid exceed the parent species average?
- Will the unsaturated fatty acid content of the sturgeon hybrid meat exceed the parent species ones?
- Can the unsaturated fatty acid content of the sturgeon hybrid meat be increased by vegetable oil completion?
- Does the smoke method influence the sturgeon hybrid fillet's fatty acid profile?

2. MATERIALS AND METHODS

2.1 The pre-rearing of the fish

For pre-rearing the sterlet and Siberian sturgeon spawns were kept in 300 litre space utilized, round-cornered fish farming tanks, whilst the sturgeon hybrids spawn were located in a 250 litre space utilized, round-cornered fish tube. The water distribution of all three fish rearing tanks was a recirculation system, the filtered water was recycled through the rain hood, this ensured the suitable dissolved oxygen level for the fish and the water cleaning as well. But later on it was necessary to compress air, which was solved by a 60 l/min powerful air compressor. In this way during the whole time of the experiment the water oxygen content differed between 6-8±1.5 mg/l, whilst the water temperature between 14-16±2.0 Celsius in all three fish tanks. To measure the dissolved oxygen level a Hach Lange HQ-30d luminescence dissolved-oxygen meter was used twice a day, in the morning and in the evening.

While the fish growth and the unviable individuals' death the mortality tapered off and at the time the fish changed into artificial feed, it practically ended both at the sturgeon hybrids and at the Siberian sturgeons (Figure 1).

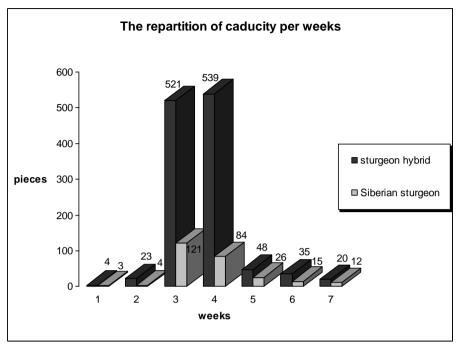


Figure 1: The repartition of sturgeon hybrid and siberian sturgeon's caducity per weeks (*Source: several study, 2010*).

During the 44 day long pre-rearing period the sturgeon hybrids reached 27.5 mm average body length and 0.1 gram average weight. The Siberian sturgeon reached 28 mm average body length and 0.12 gram average weight, but the stocks' uneven growing are significant at both groups.

The fish nutrition was started in ad libitum quantity with chopped red chironomidae larva (*Chironomus spp.*) and with tubeworm (*Tubifex tubifex*). For pre-rearing, starting from the first measuring to feed the fish spawns to their size suitable, three different pellet-sized, complete, by Joosen-Luyckx GmbH produced Aqua Bio fish feed were used, which raw protein content was 58-, 50- and 45%

(Table 1). The daily feed portion was 2 % of the weight; the nutrition portion was corrected after every weighing. The fish were fed first in every 3 hours, then increasingly in wider time periods and to the end of the experiment only daily, in the morning and in the evening.

2.2 The post-rearing of the fish

The post-rearing started from their age of 60 days, at the time the fish were fed only by fish feed, and all three groups' stock number stabilized.

For post-rearing the fish were placed in 3500 litre space utilized fish tanks, in which recirculation system having water filtration and air compression with a 260 l/min powerful air compressor were in usage. The recirculation system having filter was cleaned on monthly basis, also the 25% of the whole water capacity was replaced in every 4 days.

The water temperature during the post-rearing period was between $16-22\pm1.5$ Celsius, whilst the water oxygen concentration formed between $5.0-6.8\pm1.4$ mg/l.

For measuring the dissolved oxygen level a HQ-30d luminescence dissolved oxygen meter was used on daily base, before the morning feeding.

For post-rearing nutrition Aller Aqua 0/0 size, dry fish feed was used.

 Table 1: The composition of the used nutrients during the growing

 period

	Crude protein %	Crude fat %	Crude fibre %	Crude ash %
Aqua Bio fingerling nutrient	58	12	0,5	10,5
Aqua Bio fingerling nutrient II	50	20	0,5	9
Aqua Bio rearing nutrient	45	20	1,4	9,3
Allaer Aqua adult nutrient	42	12	2,7	7

(Source: several study 2010-2011)

All three size ranges' starting and ending average body length and weight growing were determined as well as the result's dispersion, the Specific Growth Rate, $SGR = (lnW_t - lnW_0)/t \times 100$, and the *Feed Conversion Ratio*, $FCR = F/(W_t - W_0)$. (W₀: the fish average starting weight, W_t: the fish average ending weight and F: the nutrition portion per fish during the experiments period). From the results the fish's condition factor has been determined with the formula $(K=W\times L^{-3} \times 100)$. [(W: the fish average weight (g), L the fish average length (cm).]

2.3. Determination of the fatty acid content of the raw sterlet, Siberian sturgeon and the sturgeon hybrid fillet

The samples of the raw sterlet, Siberian sturgeon and sturgeon hybrid were examined in the Analytical Laboratory of the University of West Hungary, Faculty of Agricultural and Food Sciences, Institute of Animal Sciences, Department of Animal Nutrition, and in the Physiology Laboratory of the University of Pannonia, Georgikon Faculty, Institute of Animal Sciences and Husbandry by gas chromatography method.

2.4. Nutrition experiments of the sturgeon hybrid

2.4.1. The examination of the linseed-oil and nut-oil completion having fish feed's used groups

From the sturgeon hybrids two groups were formed with 36 fish in each group so that neither in their length nor in their weight was difference between the groups. Aller Aqua 0/0 size, dry fish feed was used for nutrition of the basic feed consumed group, whilst for the feeding of the examined group same size Aller Aqua was used but first with 5% linseed-oil and then with 5% nut-oil completion.

The group having the linseed-oil completed nutrition was habituated successfully for the feed during a month, in a way that each day they received 5 gram more treated feed, so for the beginning of the experiment the fish were only fed by the treated nutrition. 2.4.2. Smoking of the linseed-oil completed fillet

5 samples were sent to the in Győr based 'Előre HTSZ' collective farm's fish processing plant located in Kisbajcs. Here the fillets were smoked by Kerres Smoke-Air System 1600-EL-C.

2.5. The determination of the fillets' fatty acid composition

The sampling were done from randomly chosen, slaughtered fish in the way that after the fish skin was taken away one-one side fillet were cut from both sides. Thereafter the samples were frozen, then before the examination they were unfrozen and minced. Thereafter 4-4 and 5-5 samples were taken to the laboratories.

2.6. Calculation of the heterosis %

The heterosis rate was calculated in certain qualities point of view, that the parent population's average was subtracted from the descendant population's average, and the result was divided by the parent population's average and then multiplied by 100. In this way the expression of the heterosis effect could be stated.

2.7. Statistical evaluation

The statistical researches of the experimental results were made by StatSoft Inc. Statistica 11 program. To analyze each data row the methods and graphics of the descriptive statistics were used. Whereas for examination of the hypothesis regarding the immediate data t-test was used (Hancz, 2004), so during our experiments to compare the data two-sample t-test was used (*t-test, independent, by variables*). At major fatty acids examinations dispersion measures were also done.

3. RESULTS

3.1. The sturgeon hybrids' growth and their feed utilization

During the first 5 months the growing of the fish didn't show any significant difference, but from the 6th month the sturgeon hybrids and the Siberian sturgeons grew measurably, significantly better, than from the father species descendent sterlet stock. There was no significantly percentable difference between the growth of the sturgeon hybrid and the mother species, Siberian sturgeon during more of the 300 days either. The sturgeon hybrids' growth both in body weight and in body length has exceeded the two species' growth arithmetic average, and rather showed similarities to the mother species', Siberian sturgeon's growing rate (Figure 2).

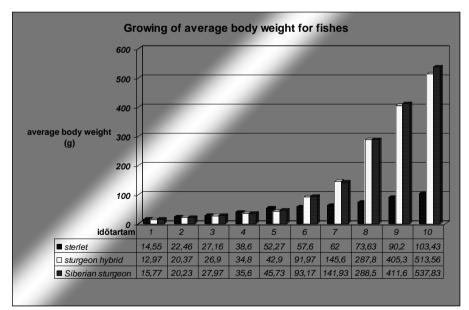


Figure 2: Growing of average body weight for fishes

(Source: several study, 2010-2011)

10 JENŐ KÁLDY: THESIS OF PhD DISSERTATION

	Sterlet t=305 nap	Sturgeon hybrid t=302 nap	Siberian sturgeon t=307 nap
SGR (%/nap)	0,64±0,33 ^a	1,57±0,66 ^b	1,28±0,76 ^b
FCR (g/g)	3,63±2,27 ^a	1,37±0,85 ^b	1,82±1,2a ^b
Initial. body length (cm)	14,53±1,95 ^a	12,97±8,39 ^a	13,12±1,88 ^a
Final body lenght (cm)	22,2±4,19 ^a	47,33±3,1 ^b	49,87±2,81 ^c
Initial condition. faktor	2,12±0,52 ^a	1,85±0,16 ^a	2,18±0,44 ^a
Final condition factor	2,22±1,24 ^a	2,43±0,43 ^a	2,58±0,72 ^a

 Table 2: The growing and feed conversion capability of fishes

Superscript letters within one column represent significant difference (at confidence level $P \le 0.05$)

(Source: several study, 2010-2011)

From Table 2 it can be well observed, that between the fish's starting and ending conditions the difference was significant neither within the same species, nor between the species.

The feed utilization capability showed significant difference in case of all three groups. The worst rate (3.63 ± 2.27) was measured at the sterlet group, whilst the stureon hybrid group showed the best rate (1.37 ± 0.85) .

The sterlet group's feed utilization capability showed the widest fluctuation (3.63 ± 2.27) , whilst the sturgeon hybrid's (1.37 ± 0.85) and the Siberian sturgeon's (1.82 ± 1.2) FCR data moved

in narrow ranges. Although it can be stated, that the sturgeon hybrid's feed utilization capability is significantly better, than the two parents groups' feed utilization capability's arithmetic average.

During the 85 day lasting nutrition experiments, in equal conditions kept, with 5% linseed-oil completed fish feed didn't amended significantly the daily growth of the sturgeon hybrids, and the feed utilization capability was significantly better neither.

3.2. The fatty acid profile of the raw sturgeon hybrid's and the Siberian sturgeon's muscles

During our examinations we declared the raw sturgeon hybrid and the raw Siberian sturgeon fillets' fatty acid composition. Regarding the saturated fatty acids (SFA) significantly lower level was measured in case of the Siberian sturgeon fillet, meanwhile regarding the monounsaturated fatty acids (MUFA) the lower level also in case of the Siberian sturgeon fillet was found. Although the concentration of the polyunsaturated fatty acids (PUFA) in case of the Siberian sturgeon was significantly better.

Among the unsaturated fatty acids in case of the alphalinolenic acid (ALA) there was no significant difference between the raw sturgeon hybrid and the raw Siberian sturgeon fillets (4.72 ± 0.39) and 5.36 ± 0.01). Although from the alpha-linolenic acid (ALA) resulting eicosapentaenoic acid (EPA) showed significant difference (5.81 ± 0.85) and 4.37 ± 0.01). Meanwhile in case of the docosahexaenoic acid (DHA) the difference wasn't significant. In case of the linoleic acid (LA) the difference also was significant.

It is important to note, that in point of the human nutrition the sturgeon hybrid fillet showed better values.

We can declare that between the raw sturgeon hybrid and the row Siberian sturgeon fillets the sturgeon hybrid's fatty acid composition is more favorable, than the row Siberian sturgeon's fatty acid composition, because the Siberian sturgeon consists of to the n-6 fatty acids belonging linoleic acid (LA) in the higher level (22.13 ± 0.14), which shows up in the human nutrition point of view in a less appropriate ratio of n-6/n-3 fatty acids. Whilst the sturgeon hybrid fillets holds linoleic acid (LA) in a smaller concentration (6.79 ± 0.91), therethrough in the human nutrition point of view the ratio of n-6/n-3 fatty acids is more appropriate.

The father species, the sterlet's muscles fatty acid profile wasn't examined; however we can compare our examinations with on literature based data. However it needs to be noted, that these kind of comparison must be handled carefully because of the different conditions (age, nutrition, method of research).

3.3. With linseed-oil and with nut-oil completed feed consumed group's fatty acid composition

From the experiments it can be stated, that there was no significant difference between the linseed-oil completion consumed sturgeon hybrids and the nut-oil completion consumed sturgeon hybrids regarding the saturated fatty acids $(21.6\pm0.38 \text{ and} 21.48\pm0.53)$. Although the difference is significant also between the quantity of the monounsaturated (MUFA) (35.18±0.65 and 39.8±0.92) and of the polyunsaturated (PUFA) fatty acids (29.53±0.99 and 32.37±1.1).

There was a significant difference between the two examined groups regarding the alpha-linolenic acid (ALA) as well, in this case the nut-oil fed group's fillet's alpha-linolenic acid (ALA) concentration is higher (4.62 ± 0.52 and 5.67 ± 0.19) (Figure 9 and 10). This can be also explained with, that the 12% of the nut-oil fatty acid composition is alpha-linolenic acid (ALA).

We can state that regarding both EPA and DHA a higher concentration was measured at the linseed-oil fed group (EPA: 5.23 ± 0.46 and 4.06 ± 0.21 , DHA: 8.57 ± 0.71 and 7.41 ± 0.46). The reason is that from the nut-oil the fatty acids from the n-6 group develop in a bigger quantity, than to the n-3 group belonging fatty acids.

During our examinations we stated that in total with the nut-oil completed feed increased the sturgeon hybrid fillet's MUFA and PUFA contents more, though it pushed the ratio of n-6/n-3 fatty acids to an unfavorable direction (0.48 ± 0.05 and 0.74 ± 0.06). The nut-oil complement increases the linoleic acid (LA) content significantly, but this means the increasing of the quantity of the fatty acids from the n-6 group. In case of the to the essential fatty acids belonging EPA and DHA content the linseed-oil complement provided the better result.

Comparing the with vegetable oil completed feed consumed groups with the basic feed consumed one we got to the result, that in case of the saturated fatty acids (SFA) there was a significant difference regarding both the linseed- (21.6±0.38), and nut-oil (21.48 ± 0.53) complement to the basic feed consumed group (23.64 ± 0.56) . In case of the monounsaturated fatty acids (MUFA) there was also a significant difference between the basic (37.8 ± 1.38) and the completed feed consumed groups $(35.18\pm0.65 \text{ and } 39.8\pm0.92)$. Although, whilst the quantity of the saturated fatty acids was bigger at the basic feed consumed group, till then we found the highest ratio of the monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids at the nut-oil completed feed received group. In case of the polyunsaturated (PUFA) fatty acids there was no significant difference between the linseed-oil completed and the basic feed consumed groups, whilst between the nut-oil completed and the basic feed received group the difference was significant. Though the linseed-oil completion significantly decreased the quantity of SFA to the basic feed received group, but didn't increased significantly either the quantity of MUFA or the PUFA to the basic feed consumed group.

Regarding the linol acid and the alpha-linolenic acid the nut-oil completed feed received group $(12.2\pm1.01 \text{ and } 5.92\pm0.17)$ significantly exceeded the results of the basic feed consumed group $(6.79\pm0.91 \text{ and } 4.72\pm0.39)$. At the same time the linseed-oil nutrient completion received group exceeded the results of the basic feed consumed group (4.72 ± 0.39) in case of the linol acid (8.19 ± 0.38) , but not in case of the alpha-linolenic acid (4.62 ± 0.52) . In case of the EPA

and DHA fatty acids neither feed completion could increase these kind of fatty acids' quantity to the basic feed consumed group, moreover in case of EPA fatty acid the nut-oil nutrient completion consumed group's result (4.1 ± 0.21) was significantly lower, than at the basic feed received group (5.81 ± 0.85).

3.4. The smoked sturgeon hybrid fillet's fatty acid composition

Regarding the saturated fatty acids there is a significant difference between the two groups' in the fillet measured rates $(28.69\pm2.29 \text{ and } 21.6\pm0.38)$. This is because the smoked fillets consist of palmitic acid in a significantly bigger quantity (22.81±2.31), than the raw linseed-oil having fillets (15.75 ± 0.29) . Although neither in case of the monounsaturated (MUFA) (35.04±1.2 and 35.18±0.65) nor of the polyunsaturated (PUFA) fatty acids (28.84±2.04 and 29.53±0.99) statistical significance couldn't be proved between the two groups. In case of linol acid and linolenic acid the raw fillets consisted significantly more, than the smoked fillets. Although in case of the EPA the smoked fillet had a significantly bigger quantity (6.83±1.26 and 5.23±0.46), whilst in case of the DHA there was no significant difference (8.44±0.68 and 8.57±0.71). Regarding the ratio of n-6/n-3 there wasn't a significant difference between the two groups either (0.42±0.06 and 0.48±0.05).

3.5. The expression of the heterosis effect in the sturgeon hybrids

The first quality we examined was the growing, in which we observed, that the rate of the heterosis effect was 28.55±24.66%, in this way although the sturgeon hybrid exceeded the average of the two parent species, but couldn't overpass the efficiency of the bigger growing mother species, the Siberian sturgeon. Regarding the Specific Growth Rate (SGR) the rate of the heterosis effect was 97.66±82.87%, which means that in the daily growth the heterosis effect expressed almost fully. Although both data show a wide dispersion, which is characteristic by the sturgeon species, so the uneven growing is also significant at the sturgeon hybrids.

The second quality we examined was the Feed Conversion Ratio (FCR), about we can declare, that almost 50% was the rate of the heterosis effect; the dispersion is also wide in this case, which shows, that regarding this quality within the population the difference is quite big between each of the individuals.

The rate of the heterosis effect regarding the saturated fatty acids (SFA) was 8.8%, whilst in case of the monounsaturated (MUFA) fatty acids was 3.6% and in case of the polyunsaturated (PUFA) fatty acids was 9.68%.

In case of the human nutrition point of view important EPA the rate of the heterosis effect was 24.94%, whilst in case of the DHA it was 75.84%.

4. CONCLUSIONS, RECOMMENDATIONS

According to the author's oppinion, the sturgeon hybrid can be a valuable fish both of the fishing lakes and of the fish grower lakefarms, for example in a carp-sturgeon hybrid-pike polyculture, but even as itself in the sturgeon hybrid-predator polyculture. Based on the environmental protection law the colonisation of sturgeon hybrid is bounded to environmental protection permission, because one of the parental species, the Siberian sturgeon is not autochthonous in Hungary. At present the growing of sturgeon hybrid in fish grower lakefarms is allowed only in lakes, what are closed from every other living water, to ensure that the foreign species can not get into the natural waters. This way the danger of sexual parasitism doesn't arise. Ludvig et al. (2008) already proved, that after getting into to Danube the Siberian sturgeon spawn with other sterlet species, reducing the number of species identical individuals, moreover in the domestic rivers even independent Siberian sturgeon populations might appear. But because of the sterility of sturgeon hybrid it is not dangerous to the otherwise seriously endangered domestic sterlet population.

It is a recent scientic result, that the 5% linseed-oil completion, however increased the metabolizable energy content of fish nutrient from 18,8 MJ/kg dry material to 20,4 MJ/kg dry material, this energy surplus was not reflected in the better growing of fishes, or in the nutrient utilisation.

During the experiments we didn't succeed to increase the unsaturated fatty acid of sturgeon hybrid musculation with the mixing JENŐ KÁLDY: THESIS OF PhD DISSERTATION of 5% phytogenic oils (linseed-oil, nut-oil) into the fish nutrient. This kind of nutrient completion didn't relevantly increase the quantity of n-6 and n-3 fatty acid and also didn't improve the n-6/n-3 fatty acid rate.

Based on the examination results it can be declared, that it is not needed to complete the szicsege nutrient with phytogenic oils, because neither the 5% linseed-oil, nor the 5% nut-oil completion resulted the wished success from the improvement of sturgeon hybrid meat quality point of view. According to my oppinion it is not needed to continueu the experiments with further phytogenic oils, or with the higher mixing ratio of them, because the this way completed nutrient doesn't have a positiv effect neither on the growing, nor on other properties, however it makes the fish nutrient to be fed relevantly more expensive.

The improvement of sturgeon hybrid as meat stock with selection methods would be necessary, as it already exists in some significant tok growing countries. In the improvement work even genetic methods could be applied, with their help the improvement work would be faster and easier to control.

During the experiment we used hybrids originated from Siberian sturgeon mother and sterlet father, but there would be a need also for the creation of sturgeon hybrids coming from Siberian sturgeon father and sterlet mother, because in certain properties they might show differences in comparison to the other variant of sturgeon hybrid, and the scientic survey of these properties are inevitable from the sturgeon hybrid growing point of view. Based on the author's statement only the crossing of sterlet and Siberian sturgeon has a practical significance, because by the growing, hybridization and rearing of every other sturgeon species there are strict instructions and complicated permission procedures.

5. NEW SCIENTIFIC RESULTS

- I stated, that the analysed sturgeon hybrid variant grows better, than the average of the two parental species, but its increase doesn't exceed the increase of the mother species Siberian sturgeon. It also can be seen in the SGR value, what was 1,57% /day by the sturgeon hybrid. It is significantly higher, than by the sterlet (0,64 %/day), while by the Siberian sturgeon it is also higher, altough the difference is not significant (1,28 %/day). The extent of the heterosis effect in point of growth is 28,55%, while in point of SGR it is 97,66%, so in this property the heterosis effect was expressed.
- 2. The sturgeon hybrid's fodder realization (1,37 g/g) is significantly better, than the sterlet's fodder realization (3,63 g/g), however it doesn't deviate significantly from the Siberian sturgeon's fodder realization (1,82 g/g), but it exceeded the average of the two parental species. The extent of the heterosis effect was 48,09%.
- 3. I concluded, that the fatty acid composition of the green sturgeon hybrid musculation is more favourable, than the fatty acid composition of the sterlet and the Siberian sturgeon musculation, specially considering the EPA and DHA quantity. Altough the n-6/n-3 fatty acid rate is better by the sterlet, than by the sturgeon hybrid, the fatty acid composition is more favourable by the sturgeon hybrid. The n-6/n-3 rate was significantly worse by the Siberian sturgeon than by the

sturgeon hybrid. The extent of the heterosis was the highest in point of the multified saturated fatty acids (9,68 %), while in point of the EPA it was 24,94% and in point of the DHA it was 75,84%.

4. The fumigation, as a processing procedure didn't influence statistically verifiably the fatty acid composition of the fish meat neither in point of the EPA, nor in point of the DHA, nor in point of n-6/n-3 fatty acid rate, the sturgeon hybrid meat preserved its favourable fatty acid composition.

6. LIST OF PUBLICATIONS

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