REVIEW

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on a dissertation for the award of the educational and scientific degree "Doctor" in the scientific specialty: "Breeding of farm animals, biology and biotechnology of reproduction"; Field of higher education - 6. Agricultural sciences and veterinary medicine; Professional direction - 6.3. Animal husbandry.

Author of the dissertation: Georgi Kirilov Georgiev, part-time doctoral student at the Department of Animal Sciences, Faculty of Agronomy, at the Agricultural University - Plovdiv,

Topic of the dissertation: "Morphophysiological and biochemical characteristics of fish from the family *Acipenseridae*."

Scientific supervisors:

Prof. Dr. Lyudmila Nikolova

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Reviewer: Prof. DSc Vasil Kostadinov Atanasov, registered in the NATIONAL CENTRE FOR INFORMATION AND DOCUMENTATION - Academic degree "Doctor of Agricultural Sciences", Research area 6.3. "Stock-breeding" and Academic rank "Professor", Research area 4.3. "Biological sciences" appointed as a member of the scientific jury according to Order No. RD - 16-613/ 14.05.2025 of the Rector of the Agricultural University - Plovdiv.

1. Brief introduction of the candidate.

The doctoral candidate Georgi Kirilov Georgiev was born on October 21, 1986 and is a veterinary technician with secondary specialized education. He graduated with a bachelor's degree /2016. zoo engineer/ and a master's degree /2016 - 2017 "Animal Breeding and Reproduction"/ at the Agricultural University, Plovdiv, after which he studied under the doctoral program at the Department of "Animal Sciences", Agricultural University - Plovdiv, Faculty of Agronomy in the scientific specialty: "Breeding of farm animals, biology and biotechnology of reproduction" in Professional direction 6.3. "Animal breeding". The training was carried out in part-time form during the period 2019-2023.

2. Relevance of the problem.

Against the backdrop of the peak reached in sea and river catches, in the conditions of global warming and a changing environment, aquaculture remains the only real alternative for satisfying humanity with essential fatty and amino acids and overcoming the global shortage of wholesome foods. Natural populations of hydrobionts are depleted and cannot meet the growing needs. The situation with populations of sturgeon species is particularly critical. The reasons for this are complex: deterioration of conditions in natural habitats; hydro-construction, inconsistent with the requirements of passing fish; unsustainable practices in fisheries; poaching, etc. To save natural sturgeon populations in our country and in other countries of the European Union, a complete ban on sturgeon fishing has been introduced. In this aspect, sturgeon species are a source of delicacy products with a high market price, which is why sturgeon farming has been developing everywhere in recent decades. In Bulgaria, sturgeon

farming is also distinguished by very good development. At the same time, scientific research in our country is extremely insufficient, especially with regard to sturgeon species cultivated in industrial super-intensive farms.

The topic of this dissertation is particularly relevant, since issues related to the cultivation in industrial conditions of species and hybrids important for sturgeon farming in our country and in the world have been developed: Siberian sturgeon. Russian sturgeon, hybrid of Siberian and Russian sturgeon. The features of development, slaughter indicators and meat quality of fish with different live weight, when cultivated in a super-intensive cage farm, have been studied.

3. Aim, tasks, hypotheses and research methods.

The aim of the dissertation is to study the morphophysiological and biochemical characteristics of fish from the *Acipenseridae* family - Siberian sturgeon (*Acipenser baerii*), Russian sturgeon (*Acipenser gueldenstaedtii*), a hybrid of Siberian sturgeon x Russian sturgeon (*F1 A. baerii x A. gueldenstaedtii*), with different live weight, cultivated in a cage industrial farm.

To achieve the aim, 8 tasks and 18 subtasks have been set for implementation as follows:

I. Morphophysiological analysis of Siberian sturgeon with different live weight:

- a. Plastic signs.
- b. Morphometric and morphophysiological indices.
- c. Slaughter indicators.

II Analysis of the meat of Siberian sturgeon with different live weight:

- a. Chemical composition and energy content of the meat.
- b. Amino acid profile.
- c. Protein profile.

III. Morphophysiological analysis of Russian sturgeon with different live weight:

- a. Plastic signs.
- b. Morphometric and morphophysiological indices.
- c. Slaughter indicators.

IV. Analysis of meat of Russian sturgeon with different live weight:

- a. Chemical composition and energy content of meat.
- b. Amino acid profile.
- c. Protein profile.

V. Morphophysiological analysis of the hybrid of Siberian and Russian sturgeon with different live weight:

a. Plastic signs.

b. Morphometric and morphophysiological indices.

c. Slaughter indicators.

VI. Analysis of meat of the hybrid of Siberian and Russian sturgeon with different live weight:

a. Chemical composition and energy content of meat.

b. Amino acid profile.

c. Protein profile.

VII. Comparative analysis of morphophysiological indicators of Siberian sturgeon, Russian sturgeon and their hybrid with different live weight.

VIII. Comparative analysis of the protein profile of the meat of Siberian sturgeon, Russian sturgeon and their hybrid with different live weight.

The working hypotheses of the study are correctly derived and are a real prerequisite for solving the tasks set. Modern research methods adequate to the set goals and tasks were used, which shows the very good preliminary theoretical and practical preparation of the doctoral student.

4. Illustration and presentation of the results obtained.

The presented dissertation work is in the field of aquaculture and is written on 191 pages, and is very well illustrated with the help of 38 tables and 43 figures. The list of cited literature lists 277 sources, of which 77 in Cyrillic and 200 in Latin.

The development contains the standard structural components and meets the requirements of the Agricultural University - Plovdiv. The topic is relevant, as it concerns morphophysiological and biochemical characteristics of fish from the family Acipenseridae. The goal closely correlates with the title of the dissertation, and the tasks set logically form 8 work packages, the implementation of which is generally related to the study of morphophysiological and biochemical characteristics of Siberian sturgeon (Acipenser baerii), Russian sturgeon (Acipenser gueldenstaedtii), a hybrid of Siberian sturgeon x Russian sturgeon (F1 A. baerii x A. gueldenstaedtii), with different live weight, cultivated in a cage industrial farm. The object of the study is extremely promising, since sturgeon fish, as economically valuable species, are distinguished by high value of caviar, good organoleptic and dietary and prophylactic qualities of meat. In this aspect, the development is in unison with the main priority of aquaculture satisfying the growing needs of humanity for essential amino and fatty acids at a time when the world catch of hydrobionts has reached its peak. The main thesis of the work is supported by the scientific literature used - 277 authors are cited, of which 77 are in Cyrillic. The extensive literature review shows the excellent awareness of the dissertationist on the problem, his indepth knowledge and the great potential for solving important technological tasks in the cultivation of sturgeon fish and their hybrids in the conditions of superintensive technology. The methods used are adequate for the study, and the modern equipment and methodologies are suitable for solving the identified tasks. Valuable scientific data were obtained concerning both morphophysiological and slaughter indicators, as well as the biochemical composition and amino acid profile of the meat. Variational-statistical data processing was used to determine a number of important dependencies.

5. Discussion of the results and used literature.

The dissertation presents the obtained scientific and applied results in a very well-illustrated section "Results and Discussion". The logical sequence for solving the tasks set has been observed. A detailed characterization has been made of a number of morphophysiological. slaughter and biochemical indicators of female and male individuals of Russian sturgeon and a hybrid of Siberian and Russian sturgeon from different age and weight groups. The comparative analysis shows that there are reliable differences between them in a number of biological and technological characteristics. In addition, the weight group has an impact on the morphological and slaughter indicators, the chemical and biochemical composition of the meat, and on a number of indicators, the influence on individual genotypes is specific. In Russian sturgeon, fish from the lower weight group have better slaughter qualities. It has higher slaughter and consumer yields. In the Siberian sturgeon, the two weight groups have similar slaughter qualities, slightly higher in the heavier fish. In the hybrid, the fish from the lower weight group has a higher slaughter and consumer yield. The Russian sturgeon surpasses the Siberian and the hybrid in all three slaughter yields and in the whole fillet in the whole fish in the lighter weight group. In the Siberian sturgeon, the relative share of the whole fillet in the whole fish and in the cleaned carcass is the highest in the larger weight group, and the hybrid as a whole occupies an intermediate position, to the parental forms, and only in the share of the whole fillet in the cleaned carcass, it slightly surpasses them in the group of smaller fish. The meat of the fish from the lower weight group has the highest protein content in dry matter: in the hybrid - 84.5%, in the Russian - 78.5% and in the Siberian -75.0% sturgeons. In the higher weight group, the protein content is 73.5%, 64.0% and 64.1%, respectively. The meat of the studied fish is of medium fat content. The fat content in the dry matter of the fish of the lower weight group is 12.5%, 15.7% and 17.4%, respectively, for the hybrid, Russian sturgeon and Siberian sturgeon, and in the higher one -20.6%, 25.4% and 28.3%, respectively. The difference in water content between the groups is insignificant and the protein and fat content in the fresh meat is of a similar order. Proteins are biologically most complete in the meat of the Russian sturgeon and the hybrid of the lower weight group, in which the essential amino acids are 67.2% and 55.5% of the total amount, respectively. Next in line is the higher weight group of Russian sturgeon with 47.9% essential amino acids, while in the other groups their content is from 31.2% to 36.2%. The differences come mainly from the high content of isoleucine, lysine and phenylalanine in the lower weight group of Russian sturgeon, and of lysine, in the same weight group of the hybrid, with lysine having the highest content in the meat of the other groups. In addition, it has been proven that protein electrophoretic patterns can be used to differentiate sturgeon species.

In conclusion, I could note that the doctoral student has entered deeply into the field of aquaculture, is able to organize correct scientific experiments and fully interpret the results obtained. In this aspect, the training has fulfilled the main goal regarding the qualification and professional growth of the candidate.

6. Contributions of the dissertation work.

The contributions of each scientific work show its usefulness, both in fundamental and in scientific-applied aspects. In this sense, Georgi Georgiev's dissertation is a large-scale study, as a result of which 10 contributions are generally exposed, which could be briefly summarized and arranged as follows:

A. Scientific contributions

1. It has been established that proteins are biologically most complete in the meat of the Russian sturgeon and the hybrid of the lower weight group, in which the essential amino acids are respectively 67.2% and 55.5% of the total amount. Next is the higher weight group of the Russian sturgeon - with 47.9% essential amino acids, and in the remaining groups their content is from 31.2% to 36.2%. The differences come mainly from the high content of isoleucine, lysine and phenylalanine in the lower weight group of the Russian sturgeon, and of lysine in the same weight group of the hybrid, with lysine having the highest content in the meat of the other groups. Original scientific contribution;

2. It was established that in the studied conditions, the weight group has an impact on the morphological and slaughter indicators, the chemical and amino acid composition of the fish meat, and for a number of indicators, the impact on individual genotypes is specific. **Original scientific contribution;**

3. It has been confirmed that protein electrophoretic patterns can be used for genetic differentiation of sturgeon species. **Confirmatory scientific contribution**;

B. Scientific and applied contributions

1. A number of morphological and slaughter indicators have been studied; the chemical, amino acid composition and protein profile of the meat of Siberian sturgeon (*Acipenser baerii* Brandt, 1869), Russian sturgeon (*Acipenser gueldenstaedtii* Brandt et Ratzeburg, 1833) and their hybrid (F1 *A. baerii* x *A. gueldenstaedtii*), with different consumable weights, cultivated in a superintensive cage farm located in the warm-water Kardzhali reservoir. **Original scientific and applied contribution for Bulgaria;**

2. It has been established that sturgeon fish cultivated in a super-intensive industrial farm have good slaughter qualities and meat quality. **Confirmatory scientific and applied contribution**;

3. It has been established that in Russian sturgeon, fish from the lower weight group have better slaughter qualities. It has higher: slaughter (90.4% vs. 86.7% in the higher weight group, p<0.01) and consumer (87.2% vs. 84.3%, p<0.05) yields; relative share of whole fillet in whole fish (49.9% vs. 48.3%) and in cleaned carcass (77.8% vs. 75.3%). Confirmatory scientific-applied contribution;

4. It was found that in the Siberian sturgeon the two weight groups have similar slaughter qualities, slightly higher in the heavier fish: slaughter yield - 86.9% versus 86.4% in the lighter ones, consumer yield - 84.2% versus 83.5%, yield for the canning industry - 61.5% versus 58.1%, relative share of the whole fillet in the whole fish - 50.8% versus 45.6%, respectively. Only the difference in the relative share of the fillet in the cleaned carcass is reliable (82.5% versus 78.4%, p<0.001). Confirmatory scientific and applied contribution;

5. It was found that in the hybrid, the fish from the lower weight group had a higher slaughter (89.5% vs. 86.5%, p<0.05) and consumer (86.6% vs. 83.8% p<0.05) yield. In the higher weight group, the relative shares of the whole fillet in the whole fish (50.0% vs. 48.0%) and in the cleaned carcass (81.4% vs. 79.0%), as well as the yield for the canning industry (61.5% vs. 60.8%). Confirmatory scientific and applied contribution;

6. It was found that in the studied conditions, the Russian sturgeon outperformed the Siberian and the hybrid in the three slaughter yields and in the whole fillet in the whole fish in the lighter weight group. In the Siberian sturgeon, the relative share of the whole fillet in the whole fish and in the cleaned carcass is the highest in the larger weight group, and the hybrid as a whole occupies an intermediate position, to the parental forms, and only in the share of the whole fillet in the cleaned carcass, it slightly surpasses them in the group of smaller fish. Original scientific and applied contribution for Bulgaria;

7. It has been established that the meat of fish from the lower weight group has the highest protein content in dry matter: in the hybrid - 84.5%, Russian - 78.5% and Siberian -75.0%. In the higher weight group, the protein content is respectively - 73.5%, 64.0% and 64.1%. The meat of the studied fish belongs to the medium-fat. The fat content in the dry matter of fish from the lower weight group is 12.5%, 15.7% and 17.4% respectively for the hybrid, Russian sturgeon and Siberian sturgeon, and in the higher one – 20.6%, 25.4% and 28.3% respectively. The difference in water content between the groups is insignificant and the content of proteins and fats in the fresh meat is in a similar order. **Original scientific and applied contribution**;

In addition, **10 conclusions and 3 valuable recommendations** for fish farming practice have been formulated in the dissertation work.

7. Critical remarks, questions and recommendations.

With the exception of some technical inaccuracies, I have no significant remarks on the dissertation work and the abstract /in the final version, histidine and arginine are attributed to the conditionally essential amino acids/. However, the interpretation of the rich experimental data in a biochemical aspect could be a little more thorough. For example, the doctoral student did not note the reason for the reciprocal values of tyrosine and phenylalanine when interpreting the results for the amino acid profile of meat. In fact, it would be good to take into account their total pool, since essential phenylalanine is a precursor of conditionally essential tyrosine, which is obtained by hydroxylation of phenylalanine in the fish organism. The data on the influence of the live weight of sturgeon fish on the content of essential amino acids are extremely useful, which is interesting for consumers. In the case of threonine and methionine, as well as in the case of lysine, higher amounts were recorded in fish with a larger live mass. It would be good to explain this fact in a little more detail, as well as to make recommendations to producers and consumers of sturgeon fish. The meat of the hybrid also contains high levels of lysine, with the difference in the indicator in favor of smaller fish reaching as much as 74.4%. In the latter, the content of isoleucine (3.1 times), phenylalanine (1.9 times), valine (2.2 times), threonine (36.7%) and leucine (69.6%). The share of essential amino acids constitutes 55.5% of the total amount, which is a good attestation of the usefulness of the meat from a dietary and prophylactic point of view. The efforts of the doctoral candidate to interpret the information about parvalbumins with a molecular mass of about 11 kDa, which are responsible for more than 95% of allergies when eating fish, are commendable. In this aspect, I could qualify this dissertation as a useful development of a fundamental-applied nature.

My question to the doctoral candidate is: What is his personal participation in this large-scale dissertation?

I recommend continuing biochemical studies on the amino acid composition and fatty acid profile of sturgeon meat, since studies in this area are really scarce.

8. Published articles and citations.

4 scientific articles have been published in reputable journals related to the dissertation - 3 in Bulgarian Journal of Agricultural Science /refereed and indexed journal in world-renowned databases of scientific information/, 1 in Agricultural Sciences /refereed and indexed journal in world-renowned databases of scientific information/. In one of the publications, Georgi Georgiev is the lead author, and in the others, he is in second and third place, which is a good certificate for his participation and contribution to the developments. This brings a total of 43.5 points to the doctoral student /with 30 points required/, which significantly exceeds the requirements for such developments.

The presented abstract in a synthesized form objectively reflects the structure and content of the dissertation work.

Conclusion

The notes and recommendations made do not at all diminish the merits of the presented dissertation work, which meets the requirements of the Bulgarian Agricultural and Scientific Research Act. The topic and the biological species have been successfully selected. The scientific experimental activity has been organized and conducted with skill and competence. The results have been obtained and processed with modern methods and equipment. The discussion is up-to-date and in line with the contemporary scientific achievements in the field. The formulated conclusions, contributions and recommendations are valuable for fish farming practice and European consumers. In addition to in-depth theoretical knowledge, the dissertation candidate has also demonstrated enviable skills in analyzing and discussing the obtained results. In this aspect, Georgi Georgiev's training in the doctoral program has achieved the desired result and the materials presented in the procedure exceed the minimum national requirements. I highly appreciate the dissertation work, the abstract and the publications related to it and I strongly recommend that the members of the Honorable Scientific Jury vote for the award of the ONS "Doctor" to Georgi Kirilov Georgiev in the Scientific specialty "Breeding of farm animals, biology and biotechnology of reproduction", professional direction 6.3 "Animal husbandry".

Vasil Kostadinov Atanasov Digitally signed by Vasil Kostadinov Atanasov Date: 2025.06.20 06:44:28 +03'00'

20.06.2025. Signature:

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