

## АКВАКУЛЬТУРА

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### COMPARATIVE STUDY ON SOME BIOCHEMICAL PARAMETERS IN THREE SPECIES OF SALMONIDAE FAMILY FARMED IN BULGARIA

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The purpose of the present project was to establish the physiological range of some blood biochemical parameters of the three most commonly used fish species of Salmonidae family in Bulgaria. The plasma biochemical profile was used as an indicator for fish welfare. At the same time the focus of this study was to identify any differences and similarities in biochemical indices in the blood of these three salmonids. Blood levels of biochemical parameters of three freshwater fishes, rainbow trout (*Oncorhynchus mychiss*), brown trout (*Salmo trutta fario*) and brook trout (*Salvelinus fontinalis*) were measured to establish the normal range, to serve as baseline data for evaluating health status. For the purposes of this project, 12 samples were obtained per each trout species from private fish farm within months October-November, when the water temperature was optimal for trout species ( $\approx 13.8^{\circ}\text{C}$ ). Blood was drawn from the vena caudalis in heparinized containers. For biochemical analysis plasma were obtained by centrifugation (5 min at 3000 rpm) of samples. Plasma was kept frozen up to the time it was analyzed. Blood biochemical parameters were determined using a semi-auto analyser (Mindray BA-88, Shenzhen, China) with commercial diagnostic kits (Giese, Diagnostics, Italy). The statistical analysis was performed using one-way analysis of variance (ANOVA). The results were processed with software Statistica v.10 (StatSoft Inc., 2002). In this study were analyzed concentrations of total protein, albumin, globulins, urea, creatinine, cholesterol, alanine aminotransferase, aspartate aminotransferase, calcium, phosphorus.

The main aquaculture fish species in Bulgaria are the rainbow trout. Our findings do not show any significant differences for ALT activity and albumin values between rainbow and brook trout. Contrary, the mean calcium concentrations between these two species were the same. The obtained values of plasma globulins and phosphorus in rainbow trout were higher than other two fish species. The concentration of urea, creatinine, total protein, cholesterol and AST activity of rainbow trout were close of those reported for the brown trout. Results, obtained in this manuscript, revealed the baseline values and provide knowledge of some biochemical parameters of three of the most used species of Salmonidae family in Bulgaria. Recent results, although showing differences can be used as an important tool for monitoring of fish health and physiology condition. The data of the present investigation were similar to those found in previous studies of ours on the same three trout types wherein differences of some immunity parameters were determined as well.

Key words: biochemical parameters, rainbow trout, brown trout, brook trout.

**Introduction.** Although aquaculture in Bulgaria comprises fresh water fish farming and marine farming, the aquaculture of freshwater fish (including trout species) is a bigger part of commercial production (almost 95% of the Bulgarian aquaculture). The main cold-water aquaculture species in Bulgaria are the trout species: rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta fario*) and brook trout (*Salvelinus fontinalis*) (Koynarski et al, 2017). The trout family includes more than 40 species, where the usual commercial production includes just a few of them. In the present work, we planned to focus on three of the most common. As a difficulty in estimating health of farmed fish is the lack of sufficient information on the normal range of biochemical blood parameters in order to determine baseline levels.

The analysis of blood biochemical parameters are very important and can be used as a diagnostic tool in piscine medicine (Tripathi, 2003). Some pathologic changes are reflected in blood plasma before the clinical signs of the disease appear. The values for some hormones, enzymes and ions may be used as indicators of health status for different animal species as well as fish (Labarrère et al., 2013).

The aim of this study is to evaluate the reference values in three of the most used species of *Salmonidae* family (rainbow trout, brown trout and brook trout) in Bulgaria to supplement the information in the existing database that used to determine the health status of *Salmonidae*. The dates of this work contribute to knowledge of the baseline of biochemical parameters of three of the most used salmons farmed in Bulgaria.

**Materials and methods.** The three trout species – rainbow, brown and brook trout were grown under intensive technology in concrete tanks in private fish farm located near the town of Peshtera, Bulgaria. For the purposes of this project, 12 samples were obtained per each trout kind, where the total number of investigated fish was 36. Samples for the study were obtained from commercial size fish (>24 months old fish) within months October-November, when the water temperature was optimal for trout species ( $\approx 13,8^{\circ}\text{C}$ ).

Blood was drawn from the *vena caudalis* in heparinized containers. The collected blood (approximately 2,0-2,5 ml) has been centrifuged at 3000 rpm for 5 min at room temperature and was immediately separated and stored at  $-20^{\circ}\text{C}$  until analysis. Plasma biochemical parameters were analysed using a semi-auto analyser (Mindray BA-88, Shenzhen, China) with commercial diagnostic kits (Giese, Diagnostics, Italy). In the present work were analyzed values of total protein (TP), albumin (Alb), globulins (Glb), alanine aminotransferase (ALT), aspartate aminotransferase (AST), calcium (Ca), phosphorus (P), cholesterol (Ch), urea (U) and creatinine (Cr). The statistical analysis was performed using one-way analysis of variance (ANOVA). The results were processed with software Statistica v.10 (StatSoft Inc., 2002). All results are presented as mean and standard deviation of the mean (Mean  $\pm$  SD).

**Results and discussion.** Statistically processed data for the evaluated biochemical indices of three freshwater fishes farmed in Bulgaria – rainbow

trout (*Oncorhynchus mychiss*), brown trout (*Salmo trutta fario*) and brook trout (*Salvelinus fontinalis*) are shown in Table 1. All results are presented as mean and standard deviation of the mean (Mean  $\pm$  SD).

Statistical analysis of the plasma alanine aminotransferase activity emphasized non-significant differences between rainbow and brook trout (28,00 $\pm$ 10,63 and 29,5 $\pm$ 9,46 U/L, respectively). Interestingly, these results were found much superior compared to the brown trout data (20,83 $\pm$ 6,11 U/L) ( $P<0,05$ ).

Furthermore, differences in aspartate aminotransferase activity between rainbow and brook trout were observed as well (378,3 $\pm$ 11,1 U/L and 532,8 $\pm$ 20,77 U/L). The levels of blood urea have very close values among rainbow and brown trout (0,59 $\pm$ 0,31 mM/L and 0,61 $\pm$ 0,17 mM/L, respectively) compared to the third species (0,92 $\pm$ 0,26 mM/L). In case of available protein profile, the differences were determined when compared to total protein concentrations.

**Table 1. Results on some biochemical parameters of rainbow trout, brown trout and brook trout. Mean and standard deviation (Mean $\pm$ SD) with minimum and maximum values in breaks**

Assessed blood parameters	Rainbow trout ( <i>Oncorhynchus mychiss</i> )	Brown trout ( <i>Salmo trutta fario</i> )	Brook trout ( <i>Salvelinus fontinalis</i> )
ALT (U/L)	28,00 $\pm$ 10,63 (10-43)	20,83 $\pm$ 6,11 (13-30)	29,50 $\pm$ 9,46 (20-47)
AST (U/L)	378,3 $\pm$ 11,1 (280-570)	405,0 $\pm$ 10,72 (263-594)	532,8 $\pm$ 20,77 (349-908)
Urea (mM/L)	0,59 $\pm$ 0,31 (0,20-1,09)	0,61 $\pm$ 0,17 (0,45-0,87)	0,92 $\pm$ 0,26 (0,65-1,38)
Total protein (g/L)	34,16 $\pm$ 4,83 (28-40)	36,83 $\pm$ 4,44 (31-42)	29,5 $\pm$ 6,34 (20-35)
Albumin (g/L)	22,33 $\pm$ 7,91 (10-30)	28,00 $\pm$ 3,16 (24-33)	20,33 $\pm$ 9,89 (11-34)
Globulins (g/L)	11,83 $\pm$ 4,21 (6-18)	8,83 $\pm$ 2,40 (5-11)	9,33 $\pm$ 6,21 (1-20)
Cholesterol (mmol/L)	0,45 $\pm$ 0,14 (0,24-0,62)	0,57 $\pm$ 0,11 (0,44-0,76)	n.d
Creatinine ( $\mu$ mol/L)	182,4 $\pm$ 47,35 (118-236)	195,5 $\pm$ 82,66 (71-320)	n.d
Calcium (mM/L)	3,26 $\pm$ 0,26 (2,88-3,58)	3,47 $\pm$ 0,40 (3,0-4,21)	3,26 $\pm$ 0,3 (2,87-3,66)
Phosphorus (mM/L)	4,47 $\pm$ 1,71 (3,00-7,73)	3,42 $\pm$ 0,52 (2,89-4,27)	4,05 $\pm$ 0,75 (3,02-4,95)

The rainbow and brown fish had higher protein levels (34,16 $\pm$ 4,83 g/L and 36,83 $\pm$ 4,44 g/L, respectively) compared to total protein of brook trout (29,5 $\pm$ 6,34 g/L). Analysis of the plasma albumin concentrations pointed that the two species have roughly similar values, namely rainbow and brook trout. Globulins levels

revealed a very close values between brown and brook fish ( $8,83 \pm 2,40$  g/L and  $9,33 \pm 6,21$  g/L). Phosphorus registered concentrations that show differences between the three species studied. Calcium level of rainbow and brook trout were the same ( $3,26$  mM/L). Virtually identical were the calcium values obtained from brown trout ( $3,47 \pm 0,40$  mM/L) and difference between them proved to be insignificant. The reference intervals of cholesterol obtained in our investigation were very close in rainbow and brown trout ( $0,45 \pm 0,14$  mmol/L and  $0,57 \pm 0,11$  mmol/l), respectively. The mean reference values for creatinine concentration in rainbow and brown trout in this study were  $182,4 \pm 47,35$   $\mu$ mol/L and  $195,5 \pm 82,66$   $\mu$ mol/L, respectively. We have no data of brook trout about cholesterol and creatinine values.

In this manuscript some biochemical parameters of three freshwater fishes, rainbow trout (*Oncorhynchus mychiss*), brown trout (*Salmo trutta fario*) and brook trout (*Salvelinus fontinalis*) were measured. Many biotic and abiotic factors are capable to influence blood chemical profile of fish. Similarly, some environmental variables had an influence on the distribution of the main fish species; especially altitude and water velocity (S  arez et al., 2011). The present study aimed to describe the variation of blood plasma chemistry indices of these three trout species, farmed in our country.

The determination of plasma enzymes activities is a useful diagnostic tool for terrestrial mammalian physiological condition. As in mammals, after functional damage of tissues of fish, some enzymes can be detected in plasma. Also, in lower vertebrates like fish as recently used enzyme activities to monitor impaired metabolic function of the cell (Akinrotimi et al., 2018). In fish, the most commonly used enzymes to indicate environmental pollution, toxicity and can be seen as non-specific indicators of damage are alanine and aspartate aminotransferases (Kopp et al., 2010). Among trout species studied, ALT activity shows close levels but in terms of AST's activity, they show very high values. High levels of AST have been reported by some other authors (Edsall, 1999; Talas et al., 2013). The obtained data for plasma ALT and AST in rainbow trout are similar of those reported by Cakici and Aydin (2006), Talas et al., (2013). The survey values regarding activity of AST show evident differences between three studied fish species but the most important are between rainbow and brook trout with the highest value being measured at brook fish. Higher AST levels could be a consequence of enhanced liver production or increased enzyme leakage due to impaired permeability and disruption of cellular membranes. The plasma ALT concentration of brook trout in our study is significantly higher than that of studied fish in Kopp et al., (2013) research and also the AST values in our investigation are lower than in their study. On the other hand, this author has obtained results for the levels of calcium, phosphorus and urea of brook trout, which are very similar to those found in this project. In the literature available to us, there is insufficient information about the normal range of the plasma chemistry of this species. We hope our study will provide additional information on variation of these biochemical indices in healthy brook trout. The results of rainbow trout which we received about plasma total protein, albumin and globulins are similar of those reported by Cakici and Aydin

(2006). Moreover, previous study performed by Bernet et al., (2001) lends support to our finding about total protein levels and urea derivate in brown trout during autumn season. Creatinine, generated from muscle metabolism, produced from creatine and filtered mainly by the kidney. This phenomenon allows this molecule to be used as an indicator of kidney function. Increased level of creatinine means renal dysfunction or renal disease (Kulkarni and Pruthviraj, 2016). It should also be taken into account that the environment, diet, age of fish may affect the different values for creatinine. In our investigation, in all studied fish species observed the creatinine concentration found to be high as compared to other fishes reported. They were not consistent with data reported by other authors (Cakici and Aydin, 2006; Charoo et al. 2013; Talas et al, 2013) who report lower creatinine levels than those found by us. Conversely, Esmaili et al., (2017) observed higher values of ours. In other hand, plasma transaminase concentrations in our study were comparable with the same authors. Cholesterol is a lipid that is located in the cell membranes of all tissues of the body and is transported into the blood of all animals. The reference intervals of cholesterol obtained in this study were significantly lower those values reported by other authors (Talas et al., 2013; Cakici and Aydin, 2016) but were similar compared to results show by Esmaili et al., (2017). Wallaert and Babin (1994) reported seasonal variations and reproductive cycle affecting lipid levels during spawning in trout. On the other hand, cholesterol values are responsive to changes in nutritional status, too (Kopp et al., 2013).

In our survey, the determined blood chemistry indices of the studied trout species are similar of those reported by other authors to the fresh water fish despite the presence of certain differences. The present results are similar to those found in previous studies of ours on three types of trout (Koynarski et al., 2017) wherein also differences in some immunity parameters were detected.

**Conclusion.** The data from this preliminary study exhibit information for some biochemical parameters of rainbow, brown and brook trout farmed in Bulgaria. Current results, revealed the baseline values and provide knowledge of blood indices of the three of the most used species of *Salmonidae* family in our country.

## СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ НЕКОТОРЫХ БИОХИМИЧЕСКИХ ПАРАМЕТРОВ ТРЕХ ВИДОВ РЫБ СЕМЕЙСТВА ЛОСОСЕВЫХ В БОЛГАРИИ

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Целью настоящего проекта было установить физиологический диапазон некоторых биохимических параметров крови трех наиболее часто используемых видов

рыб семейства лососевых в Болгарии. Биохимический профиль плазмы использовался как показатель благосостояния рыб. В то же время внимание фокусировалось на выявлении любых различий и сходств в биохимических показателях крови этих трех видов лососевых. Проводились измерения уровня биохимических параметров у трех видов пресноводных рыб: радужной форели (*Oncorhynchus mychiss*), кумжи (*Salmo trutta fario*) и американской палии (*Salvelinus fontinalis*) – чтобы установить нормальный диапазон, который послужил бы базовыми данными для оценки состояния здоровья рыб. В соответствии с целями этого проекта 12 образцов были получены на каждый вид форели из частной рыболовной фермы в течение месяцев октябрь-ноябрь, когда температура воды была оптимальной для данных видов ( $\approx 13,8^\circ \text{C}$ ). Кровь отбирали из хвостовой вены в гепаринизированные контейнеры. Для биохимического анализа плазму получали методом центрифугирования (5 мин при 3000 об / мин) образцов. Плазма сохранялась в замороженном состоянии до начала анализа. Биохимические параметры крови определяли с помощью полуавтоматического анализатора (Mindray BA-88, Shenzhen, China) с диагностическими наборами (Giese, Diagnostics, Italy). Статистический анализ проводился с использованием одностороннего анализа дисперсии (ANOVA). Результаты были обработаны благодаря программному обеспечению Statistica v.10 (StatSoft Inc., 2002). Основным видом аквакультуры в Болгарии являются радужная форель. Наши результаты не показывают каких-либо существенных различий в отношении активности АЛТ и альбумина между радужной и кумжи. Напротив, средние концентрации кальция между этими двумя видами были одинаковыми. Полученные значения глобулинов плазмы и фосфора в радужной форели были выше, чем у других двух видов рыб. Концентрация мочевины, креатинина, общего белка, холестерина и активности АСТ радужной форели была близка к показателям кумжи. Результаты, полученные в этой рукописи, выявили базовые значения и предоставили знания о некоторых биохимических параметрах трех наиболее используемых видов семейства лососевых в Болгарии. Недавние результаты, хотя и демонстрирующие различия, могут быть использованы в качестве важного инструмента для мониторинга состояния здоровья рыб и физиологического состояния.

Ключевые слова: биохимические параметры, радужная форель, кумжа, американская палия.

## **ПОРІВНЯЛЬНЕ ДОСЛІДЖЕННЯ ДЕЯКИХ БІОХІМІЧНИХ ПАРАМЕТРІВ ТРЬОХ ВИДІВ РИБ РОДИНИ ЛОСОСЕВИХ У БОЛГАРІЇ**

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Метою цього проекту було встановлення фізіологічного діапазону деяких біохімічних параметрів крові трьох найбільш часто використовуваних видів риб родини лососевих у Болгарії. Біохімічний профіль плазми використовувався як показник добробуту риб. У той же час увага фокусувалася на виявленні будь-

яких відмінностей і подібностей у біохімічних показниках крові цих трьох видів лососевих. Проводилися вимірювання рівня біохімічних параметрів у трьох видів прісноводних риб, таких як: райдужна форель (*Oncorhynchus mychiss*), кумжа (*Salmo trutta fario*) і американська палія (*Salvelinus fontinalis*) – щоб встановити нормальний діапазон, який послужив би базовими даними для оцінки стану здоров'я риб. Відповідно до цілей цього проекту 12 зразків були отримані на кожен вид форелі з приватної рибоводної ферми протягом жовтня-листопаду, коли температура води була оптимальною для даних видів ( $\approx 13,8^{\circ}\text{C}$ ). Кров відбирали з хвостової вени в гепаринізовані контейнери. Для біохімічного аналізу плазму отримували методом центрифугування (5 хв. при 3000 об / хв.) зразків. Плазма зберігалася в замороженому стані до початку аналізу. Біохімічні параметри крові визначали за допомогою напівавтоматичного аналізатора (Mindray BA-88, Shenzhen, China) з діагностичними наборами (Giese, Diagnostics, Italy). Статистичний аналіз проводився з використанням одностороннього аналізу дисперсії (ANOVA). Результати були оброблені завдяки програмному забезпеченню Statistica v.10 (StatSoft Inc., 2002). Основним видом аквакультури в Болгарії є райдужна форель. Наші результати не показують будь-яких істотних відмінностей у ставленні активності АЛТ і альбуміну між райдужною фореллю та кумжею. Навпаки, середні концентрації кальцію між цими двома видами були однаковими. Отримані значення глобулінів плазми і фосфору в райдужній форелі були вище, ніж в інших двох видів риб. Концентрація сечовини, креатиніну, загального білка, холестерину і активності АСТ райдужної форелі була близька до показників кумжі. Результати, отримані в цьому рукописі, виявили базові значення і надали знання про деякі біохімічні параметри трьох найбільш використовуваних видів родини лососевих у Болгарії. Нещодавні результати, хоча і демонструють відмінності, можуть бути використані в якості важливого інструменту для моніторингу стану здоров'я риб і фізіологічного стану.

Ключові слова: біохімічні параметри, райдужна форель, кумжа, американська палія.

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