Scientific Papers. Series D. Animal Science. Vol. LXVI, No. 1, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

# EVALUATION OF THE HAEMATOLOGICAL PROFILE AND BIOCHEMICAL INDICES IN THE BLOOD OF COMMON CARP (*Cyprinus carpio*), AS RESPONSE TO SUPPLEMENTING THEIR DIET WITH PHYTOGENIC COMPOUNDS

**Viorica SAVIN[1], Floricel Maricel DIMA[1], Magdalena TENCIU[1], Neculai PATRICHE[1], Marcel Daniel POPA[1], Victor CRISTEA[2]**

[1]Institute of Research and Development for Aquatic Ecology, Fishing and Aquaculture, 54 Portului Street, 800211, Galați, Romania
[2]"Dunărea de Jos" University of Galați, Food Science and Engineering Faculty, 47 Domnească Street, 800008, Galați, Romania

Corresponding author email: vio_savin@yahoo.com

***Abstract***

*The aim of this study was to evaluate the impact of different phytogenic compounds on the haematological profile and biochemical indices of carp, reared in a recirculating pilot aquaculture system. The experiment was conducted for 52 days. A basic feed, Aqua Classic type, with 46% protein and 22% lipid, was used. 5 g of different phytogenic compounds were added to form the experimental diets, as follows: V1 - control, V2 - 0.5% licorice (Glycyrrhiza glabra), V3 - 0.5% echinacea (Echinacea purpurea) and V4 - 0.5% wild thyme (Thymus serpyllum). At the end of the experiment, blood samples were taken to evaluate the haematological and blood biochemical parameters. The mean values for Ht, Hb, RBC and WBC were higher in the experimental variants than in the control. Serum protein was significantly lower (p<0.05) in variants V2 and V3 compared to the control. The serum glucose values registered significant differences between the control and the 3 experimental variants. In conclusion, the addition of phytogenic compounds in the diet has beneficial effects on the haematological and blood biochemical profile of carp (Cyprinus carpio).*

***Key words****: aquaculture, carp, phytogenic, haematological profile.*

## INTRODUCTION

Due to the high-quality proteins and lipids, it contains, fish are recognized as some of the healthiest foods, contributing to a balanced human diet. According to the FAO, fish provided about 17% of the animal protein consumed worldwide in 2017 (FAO, 2020).

Intensive or super-intensive fish farming is practiced to respond effectively to consumer demand. High fish densities per spawning unit lead to a higher incidence of stress-induced diseases and reduced immunity.

In intensive aquaculture, nutrition is one of the most important aspects. Feed quality in aquaculture is an important condition to be met (Iheanacho et al., 2017). The quantity and quality of the feed provided determines the proper growth and development of the reared fish species. Optimizing the amount of feed improves carcass biochemistry, good feed conversion and protection of fish from pathogens (Volkoff et al., 2010; Singha et al., 2020). Food can affect water quality, can help to increase the fish's resistance to disease, and the nutrients it contains can increase productivity (Heal et al., 2021; Hassaan et al., 2020).

In recent years, due to the decline of wild fish stocks, it is desirable to replace fishmeal in feed with herbal substitutes, to introduce by-products of the food industry and even medicinal plants rich in bioactive compounds (Adekoya et al., 2018; Savin et al., 2022).

There is a need to develop diets with natural and inexpensive ingredients capable of improving the physiological and biological functions of fish for high growth and better disease resistance (Salam et al., 2021).

Plant extracts rich in phytogenic compounds have been shown to improve the growth performance and immunity of aquatic animals and increase their resilience to adverse environmental conditions (Ahmadifar et al., 2020; Adel et al., 2021; Magouz et al., 2021).

The present study was conducted to determine the effect of adding phytogenic compounds to the diet of carp (*Cyprinus carpio*) on the haematological profile and its biochemical indices.

## MATERIALS AND METHODS

The experiment was conducted at the Institute of Research and Development for Aquatic Ecology, Fishing and Aquaculture Galati for 52 days in a recirculating system consisting of 4 glass aquariums, with dimensions of 100 x 40 x 25 cm and a water volume of 100 liters. Each breeding unit had a water treatment system, represented by Tetra EX 1200 Plus external filters with 5 different filter media: ceramic rings, bio balls, sponge, carbon filter and felt.
185 specimens of carp (*Cyprinus carpio*) with an average mass of 40.5±0.31 g obtained from the Experimental Laboratory of Agro-Fisheries Research Brates were randomly distributed in the rearing units. They were acclimated for 7 days during which they were fed the commercial Aqua Clasic diet with 2 mm granulation, a protein content of 46% and a fat content of 22%, purchased from Aqua Garant (Table 1).

Table 1. Structure and nutritional value of basic carp feed

| ***Parameters*** | ***Quantity*** |
|---|---|
| Crude protein | 46 % |
| Crude fat | 22 % |
| Cellulose | 1.5 % |
| Calcium | 1.5 % |
| Sodium | 0.35 % |
| Phosphorus | 1.15 % |
| Vitamin A | 10000 U.I. |
| Vitamin C | 250 mg |
| Vitamin E | 200 mg |

The feed ration used was 2.5%/day of body weight and food was administered twice a day. Three experimental diets and one control diet were prepared:
- Control diet (AM) - without phytogenic compounds;
- Diet 1 (A1) - with the addition of 5 g of licorice (*Glycyrrhiza glabra*) to 1 kg of basic feed;
- Diet 2 (A2) - with an addition of 5 g echinacea (*Echinacea purpurea)* to 1 kg of basic feed;
- Diet 3 (A3) - with the addition of 5 g of thyme (*Thymus serpyllum*) to 1 kg of basic feed.

The experimental diets were prepared according to the method described by Savin et al. (2022).

Blood samples were collected by tail vein puncture from 7 specimens of each experimental group using heparinized syringes to determine the haematological profile. Before blood sampling, the fish were anesthetized by bathing in a clove oil solution (2.5 ml per 10 litters of water).
According to Baker and Silverstone, 1976, red and white blood cells were counted in a Neubauer counting chamber after the blood had been diluted with Vulpian's solution for red blood cells and Turk's solution for white blood cells, respectively.
The formula used to determine the red blood cell (RBC) count was:

$$RBC \times 10^6\ \mu l^{-1} = n \times 10000,\ n = \text{number of erythrocytes in 80 squares}$$

White blood cells (WBC) were determined according to the formula:

$$WBC \times 10^3\ \mu l^{-1} = n \times 40,\ n = \text{number of white blood cells counted}$$

***Haemoglobin*** (Hb, g $dl^{-1}$) was determined using the cyanmethemoglobin method described by Kondi (1981). 20 microliters of blood collected on anticoagulant was mixed with 5 ml of Drabkin's reagent and after 30 minutes the absorbance at 540 nm was read with a Spekol 1300 spectrophotometer (Analytik Yena).
***Haematocrit*** (Ht, %) was determined by introducing blood into the capillary tubes and centrifuging horizontally in a microhematocrit centrifuge at 10,000 rpm for 2 minutes. It was expressed as a percentage, as a ratio of the erythrocyte's column to the whole blood column (Davison et al., 2023).
Based on the above, the erythrocyte constants (MCH, MCV, MCHC) were determined, according to the method described by Dacie & Lewis, 2011.
For protein and blood glucose determination, blood collected without anticoagulant was centrifuged at 4000 rpm for 5 minutes.
***Total plasma proteins*** (TP, g $dl^{-1}$) were determined by the Biuret method according to Gornâll et al. (1949).
***Blood glucose levels*** (GLU, mg $dl^{-1}$) were determined using the ortho-toluidine method described by Wedemeyer & Yasutake (1977).
***Statistical analysis.*** For statistical analysis SPSS Statistics 17.0 for Windows was used. All data

are shown as mean ± standard deviation. The Kolmogorov-Smirnov normality test followed by the t-test was performed to check the differences between the experimental variants. Comparisons between variants were assessed using Duncan's test for multiple comparisons. The results were considered statistically different at $p<0.05$.

## RESULTS AND DISCUSSIONS

Supplementation of the diet with phytogenic compounds significantly changed the haematological parameters of the fish in the groups that received diets supplemented with phytogenic compounds. Significant increases in haematocrit, haemoglobin concentration, and erythrocyte count were observed (Table 2).

The haematocrit (Ht) recorded significantly higher differences ($p<0.05$) in all the groups in which the phytogenic compounds were administered, the highest percentage being in the A3 variant (36.49±0.02), in which administered wild thyme. There were no significant differences ($p>0.05$) between the control group AM (34.81±0.50) and groups A1 (34.85±0.20) - licorice and A2 (35.19±0.01) - echinacea (Table 2).

Almarri et al., 2023 obtained similar results by supplementing the *Nile tilapia* diet with 0.5; 1; 1.5 and 2% *Annona squamosa* leaf extract. The increase in haematocrit in the groups fed with phytoadditives may indicate an improvement in the nutritional status of the fish.

Table 2. The effect of phytogenic compounds from licorice (A1), echinacea (A2) and wild thyme (A3) on haematological indices of *Cyprinus carpio*

| Experimental diet | Haematocrit (%), mean ± sd | Haemoglobin (g/dl) mean ± sd | RBC ($*10^6$/µl) mean ± sd | WBC ( $*10^3$/µl) mean ± sd | MCV (µm³) mean ± sd | MCH (pg) mean ± sd | MCHC (g/dl) mean ± sd |
|---|---|---|---|---|---|---|---|
| *AM* | 34.81±0.50 | 9.72±0.17 | 1.71±0.02 | 51.69±0.18 | 203.80±4.83 | 56.92±1.26 | 27.94±0.52 |
| *A1* | 34.85±0.20 | 9.81±0.02 | 1.92±0.02 | 52.07±0.13 | 181.65±1.88 | 51.16±0.44 | 28.16±0.16 |
| *A2* | 35.19±0.01 | 9.79±0.02 | 1.89±0.02 | 51.75±0.01 | 186.63±1.64 | 51.91±0.41 | 27.82±0.04 |
| *A3* | 36.49±0.02 | 9.83±0.08 | 1.90±0.02 | 50.73±0.04 | 191.91±1.93 | 51.70±0.76 | 26.94±0.20 |

Haemoglobin concentration increased slightly but not significantly ($p>0.05$) in groups A1, A2, and A3, respectively, compared to the AM control. The highest haemoglobin concentration was recorded in the group where wild thyme was administered, A3 (9.83±0.08), followed by the group with echinacea A2 (9.79±0.02), respectively the group where licorice was administered, A1 (9.81±0.02). The control group had the lowest haemoglobin concentration (9.72±0.17) (Table 2).

Compared to the control group, fish fed with phytogenic compounds had a significantly higher red blood cell (RBC) count ($p<0.05$) (Figure 1). The highest number of erythrocytes was in the case of fish from the group fed with licorice A1 (1.92±0.02), followed by A3 - wild thyme (1.90±0.02), A2 - echinacea (1.89±0.02), the lowest value being recorded in fish from the control group (1.71±0.02). Statistical analysis showed that there were also significant differences between A2 and A3 ($p<0.05$) versus A1, but no significant differences were found between A2 and A3 ($p=0.139$).

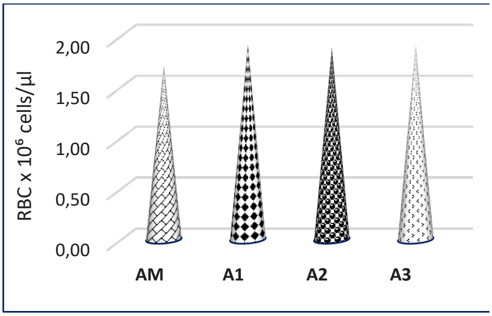


Figure 1. Variation in red blood cell count (RBC)

Regarding the number of white blood cells (WBC), there were insignificant differences ($p = 0.425$) between the control group and group A2, slightly increased in this group, in which the diet was supplemented with echinacea. In group A1 (52.07±0.13) the highest value of WBC was recorded, the differences being significant, compared to control group AM (51.69±0.18). Instead, the number of WBC decreased significantly in group A3 (50.73±0.04) compared to the control (Figure 2).

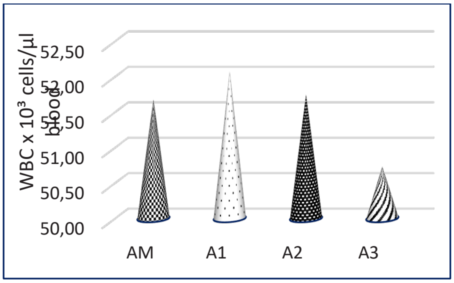


Figure 2. Variation in white blood cell count (WBC)

The increase in the number of erythrocytes results in better oxygen transport, which results in improved fish health (Sattanathan et al., 2023). Also, the increase in the number of WBC in the groups treated with licorice and echinacea indicates that these plants, through their bioactive compounds, have immunostimulating effects. Kondera et al. (2021), also reported increased leukocyte counts in common carp fed thyme oil in their daily diet.

*Cyprinus carpio* fed for 56 days with 0.5-2% thyme, showed an increase in the number of leukocytes and a better resistance to fungal infections (ALsafah & AL-Faragi, 2017).

The mean corpuscular volume (MCV) varied between 181.65±1.88 $\mu m^3$ in A1 and 191.91±1.93 $\mu m^3$ in A3, the highest value being 203.80±4.83 $\mu m^3$ in AM (Figure 3).

Mean corpuscular haemoglobin (MCH) recorded values of 56.92±1.26 pg in AM and lower in fish given phytogenic compounds; 51.91±0.41 pg. in A2, 51.70±0.76 pg. in A3, respectively 51.16±0.44 pg in A1, the differences being significant ($p<0.05$) (Figure 4).

Mean corpuscular haemoglobin concentration (MCHC) was insignificantly increased in A1 (28.16±0.16) compared to control.

A significant decrease in this value was observed in the group fed with wild thyme, A3 (26.94±0.20) (Figure 5).

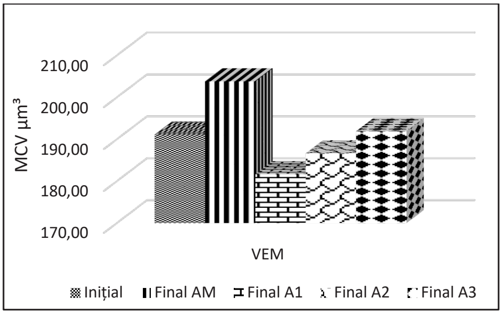


Figure 3. Variation of mean corpuscular volume (MCV)

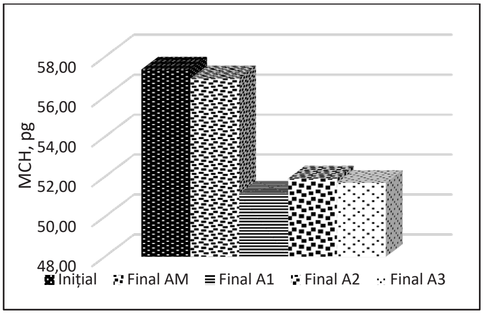


Figure 4. Variation of mean corpuscular hemoglobin (MCH)

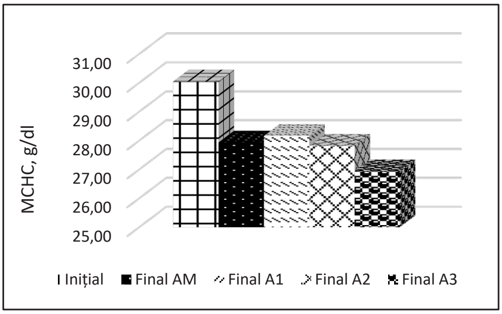


Figure 5. Variation of Mean corpuscular hemoglobin concentration (MCHC)

glucose in fish and can be a stress limiting factor (Bulfon et al., 2015).

Glucose values in this study were significantly lower in fish treated with the addition of licorice (91.14±0.02) and thyme (93.57±0.01) ($p<0.05$) compared to the control group (94.46 ±0.11) (Figure 6). The hypoglycemic effect of licorice glycyrrhizin is known, thus justifying the lower blood sugar values in the experimental lot A1 (Zhang et al., 2020).

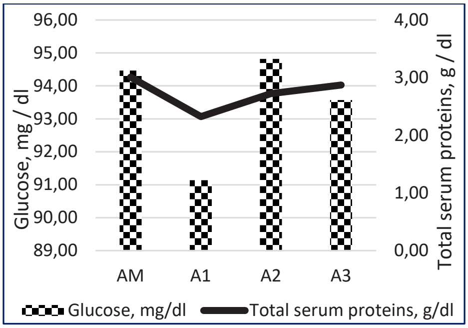


Figure 6. Evolution of glucose concentration and total serum proteins

Other authors have also reported decreases in blood glucose in fish treated with licorice. Sirakov et al. (2019) observed that the mean blood glucose values of rainbow trout fed licorice were 3.96% lower than those of the control group.

Total blood proteins were significantly lower in groups A1 (2.32 ± 0.02) and A2 (2.72 ± 0.01), while in group A3 (2.87 ± 0.05) the differences were insignificant (Figure 6).

These results were in contradiction with those of Sattanathan et al. (2023), who obtained an increase in the value of total serum proteins when a mixture of algae was included in the diet of *Labeo rohita*.

## CONCLUSIONS

The results of our research generally indicate a positive response of hematological and biochemical indices to the supplementation of carp with various phytogenic compounds from licorice, echinacea and wild thyme. However, more research is needed to determine optimal concentrations of additives in other fish species.

## REFERENCES

Adekoya, A., Porcadilla, M., Varga, D., & Kucska, B. (2018). Replacing fish meal with alternative protein sources in common carp's feed. *Acta Agraria Kaposváriensis, 22*(2), 18-24. https://doi.org/10.31914/aak.2283

Adel, M., Omidi, A.H., Dawood, M.A.O., Karimi, B., & Shekarabi, S.P.H. (2021). Dietary Gracilaria persica mediated the growth performance, fillet colouration, and immune response of Persian sturgeon (*Acipenser persicus*). *Aquaculture*, 530, 735950

Ahmadifar, E., Yousefi, M., Karimi, M., Fadaei Raieni, R., Dadar, M., Yilmaz, S., Dawood, M.A.O., & Abdel-Latif, H.M.R. (2021). Benefits of dietary polyphenols and polyphenol-rich additives to aquatic animal health: an overview. *Reviews in Fisheries Science & Aquaculture, 29*(4), 479-511. https://doi.org/10.1080/23308249.2020.1818689

Almarri, S.H., Khalil, A.A., Mansour, A.T., & El-Houseiny, W. (2023). Antioxidant, Immunostimulant, and Growth-Promoting Effects of Dietary *Annona squamosa* Leaf Extract on Nile Tilapia, *Oreochromis niloticus*, and Its Tolerance to Thermal Stress and *Aeromonas sobria* Infection. *Animals*, 13, 746. https://doi.org/10.3390/ani13040746

ALsafah A. H., & AL-Faragi J. K. (2017). Influence of thyme (*Thymus vulgaris*) as feed additives on growth performance and antifungal activity on *Saprolegnia spp.* in *Cyprinus carpio* L. *Journal of Entomology and Zoology Studie*s, 5, 1598–1602. https://www.entomoljournal.com/archives/2017/vol5issue6/PartV/5-5-317-340.pdf

Baker, F. J., & Silverton, R. E. (1976). *Introduction to Medical Laboratory Technology Butterworth* London UK pp. 575. file:///D:/Downloads/Introduction%20to%20Medical%20Laboratory%20Technology%20(%20PDFDrive%20).pdf

Bulfon, C., Volpatti, D., & Galeotti, M. (2015). Current research on the use of plant-derived products in farmed fish. *Aquaculture Research*, 46, 513–551. 10.1111/are.12238

Dacie, J.V., & Lewis, S.M. (2011). Practical Hematology. 11th edition, New York: Churchill Livingstone, 41.

Davison, W.G., Cooper, C.A., Sloman, K.A., & Wilson, R. (2023). A method for measuring meaningful physiological variables in fish blood without surgical cannulation. *Scientific Reports*, 13, 899. https://doi.org/10.1038/s41598-023-28061-w

FAO (2020). *The State of World Fisheries* and Aquaculture 2020. Sustainability in action. Rome. https://doi.org/10.4060/ca9229en

Gornâll, A. G., Bardawill C. J., &. David, M. M. (1949). Determination of serum proteins by means of the biuret reagent. *Journal of Biological Chemistry*, 177, 751. https://www.sciencedirect.com/science/article/pii/S0021925818570216 accesat 10.02.2023

Hassaan, M.S., Mohammady, E.Y., Soaudy, M.R., Palma, J., Shawer, E.E. & El-Haroun, E. (2020). The effect of dietary sericite on growth performance, digestive enzymes activity, gut microbiota and haematological parameters of Nile tilapia, *Oreochromis niloticus* (L.) fingerlings. *Animal Feed Science and Technology,* 262, 114400. https://doi.org/10.1016/j.anifeedsci.2020.114400

Heal, R. D., Hasan, N. A., & Haque, M. M. (2021). Increasing disease burden and use of drugs and chemicals in Bangladesh shrimp aquaculture: a potential menace to human health. *Marine Pollution Bulletin,* 171, 112796. https://doi.org/10.1016/j.marpolbul.2021.112796

Iheanacho, S.C., Nworu, S.A., Ogueji, E.O., Nnatuanya, I., Mbah, C.E., Anosike, F., Okoye, C., Ibrahim, U.B., Kogi, E., & Haruna, M. (2017). Comparative assessment of proximate content Organoleptic quality of African catfish (*Clarias gariepinus*) processed by smoking and solar drying method. *African Journal of Agricultural Research, 12*(38), 2824-2829.

Kondera, E., Bojarski, B., Ługowska, K., Kot, B., & Witeska, M. (2021). Hematological and Hematopoietic Effects of Bactericidal Doses of Trans-Cinnamaldehyde and Thyme Oil on *Cyprinus carpio* Juveniles. *Frontiers in Physiology*, 12, 771243. doi: 10.3389/fphys.2021.771243

Kondi, V. (1981). *Clinical laboratory - Hematology*, Bucharest, RO: Medical Publishing House.

Magouz, F.I., Mahmoud, S.A., El-Morsy, R.A.A., Paray, B.A., Soliman, A.A., Zaineldin, A.I., & Dawood, M.A.O. (2021). Dietary menthol essential oil enhanced the growth performance, digestive enzyme activity, immune-related genes, and resistance against acute ammonia exposure in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 530, 735944.

Perez-Velazquez, M., Gatlin, D. M., González-Félix, M. L., García-Ortega, A., de Cruz, C. R., Juárez-Gómez, M. L., & Chen, K. (2019). Effect of fishmeal and fish oil replacement by algal meals on biological performance and fatty acid profile of hybrid striped bass (*Morone crhysops* ♀ × *M. saxatilis* ♂). *Aquaculture*, 507, 83–90. https://doi.org/10.1016/j.aquaculture.2019.04.011

Salam M.A., Rahman M.A., Paul S.I., Islam F., Barman A.K., Rahman Z., Shaha, D.C., Rahman, M. M., & Islam, T. (2021) Dietary chitosan promotes the growth, biochemical composition, gut microbiota, hematological parameters and internal organ morphology of juvenile *Barbonymus gonionotus*. *PLoS ONE*, 16(11). e0260192. https://doi.org/10.1371/journal.pone.0260192

Sattanathan, G., Liu, W.C., Padmapriya, S., Pushparaj, K., Sureshkumar, S., Lee, J.W., Balasubramanian, B., & Kim, I.H. (2023). Effects of Dietary Blend of Algae Extract Supplementation on Growth, Biochemical, Haemato-Immunological Response, and Immune Gene Expression in *Labeo rohita* with *Aeromonas hydrophila* Post-Challenges. *Fishes*, 8, 7. https://doi.org/10.3390/fishes8010007

Savin, V., Mocanu, E., Dima, F., Patriche, N., Popa, M.D., & Cristea. V. (2022). Influence of phytogenic additives on growth parameters and meat biochemistry in Cyprinus Carpio. *Scientific Papers: Animal Science and Biotechnologies, 55*(2), 91-96. https://www.spasb.ro/index.php/spasb/article/view/2857

Singha, K. P., Shamna, N., Sahu, N. P., Sardar, P., Harikrishna, V., Thirunavukkarasar, R., Chowdhury, D. K., Maiti, M. K., & Krishna, G. (2021). Optimum dietary crude protein for culture of genetically improved farmed tilapia (GIFT), *Oreochromis niloticus* (Linnaeus, 1758) juveniles in low inland saline water: effects on growth, metabolism and gene expression. *Animal Feed Science and Technology*, 271, 114713. https://doi.org/10.1016/j.anifeedsci.2020.114713

Sirakov, I., Velichkova, K., Stoyanova, S., & Staykov, Y. (2019). Growth performance, biochemical blood parameters and meat quality of rainbow trout (*Oncorhynchus mykiss* W.) fed with licorice (*Glycyrrhiza glabra* L.) supplemented diet. *Trakia Journal of Sciences*, 4, 284-291. DOI: 10.15547/tjs.2018.04.004

Volkoff, H., Hoskins L.J., & Tuziak S.M. (2010). Influence of Intrinsic Signals and Environmental Cues on the Endocrine Control of Feeding in Fish: Potential Application in Aquaculture. *Gen Comp Endocrinol,* 167, 352–359. https://doi.org/10.1016/j.ygcen.2009.09.001

Wedemeyer G. A., & Yasutake W. T. (1977). Clinical methods for the assessment of the effects of environmental stress on fish health. *Fish and Wildlife Service Technical Paper* no. 89. Government Printing Office, Washington, D.C.

Zhang, W., Li, T., Zhang, X.J., & Zhu, Z.Y. (2020). Hypoglycemic effect of glycyrrhizin acid, a natural non-carbohydrate sweetener, on streptozotocin-induced diabetic mice. *Food Funct., 11*(5), 4160-4170. doi: 10.1039/c9fo02114k. Epub 2020 Apr 29. PMID: 32347846.