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THE PHYSIOLOGICAL STATUS OF STURGEON HYBRIDS FED WITH HERBAL SUPPLEMENTS IN RECIRCULATING AQUACULTURE SYSTEM

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Abstract

Sturgeon hybrids are an important romanian aquaculture species raised for meet and caviar. Knowing the beneficial properties of medicinal herbal supplements, the objectives of present study was to evaluate the effects of sea buckthorn and thyme extract on haematological parameters in juvenile sturgeon hybrids reared in recirculating aquaculture system. The juvenile hybrids were distributed in following groups: (1) Control, untreated fish, (2) fish fed with 1% Sea buckthorn, (3) fish fed with 1% thyme/kg feed and (4) fish fed with mix 0.5% Sea buckthorn and 0.5% thyme. The blood sampling was analyzed to: RBCc, WBCc, Ht, Hb, MCV, MCH, and MCHC. The supplementation of the diet of the hybrid resulted in the improvement of the physiological state, registering differences, depending on the period of administration of phytobiotics as well as the specific action of each type of plant extract. This study provides new information for the use of medicinal herbs as supplementation to sturgeon hybrids diet; their use can increase disease resistance by improving the physiological status.

Key words: *haematological parameters, sturgeon hybrid, sea buckthorn, thyme herbal extract.*

INTRODUCTION

The sturgeons of the Black Sea and Danube River have been highly endangered due to overfishing, loss of natural habit and water pollution. In Romania the growth of sturgeons in a controlled system started 20 years ago to produce meat and caviar but also to reduce of pressure on natural wild sturgeon population. As many animal species, hybrid sturgeons are bred to get the best characteristics from both parents.

The important objective in aquaculture is to keep on fish health as well as to enhance fish performance. The intense use of antibiotics for disease control has affected the growth of fish species because it cause to antibiotic and chemical resistance (MacMillan, 2001). One of the most popular methods of controlling diseases in aquaculture is prophylactic administration of natural plant extract or essential oils by improvement the defence mechanism of fish (Citarasu, 2010).

World Health Organization (WHO) encourages supplemented diets incorporated with medicinal herbs or plants which minimize the use of chemicals in fish diet (Dada, 2015). Sea buckthorn contains many natural antioxidants in all of its parts like as sterols, tannins, vitamins, and minerals (Kumar et al., 2013). Its leaves, stems, tubers, roots as well as blossom contain a high content of ascorbic acid (vitamin C), and also carotenoids, polyphenols, flavonoids, tocopherols, alkaloids, chlorophyll derivates, amino acids and amines (Christaki, 2012).

Thyme essential oil has two major components: as percentages of the total content are the phenols, carvacrol and thymol that represent the main antioxidant components. The thyme oil has the most pronounced antimicrobial activity compared to other oils used in aquaculture (Kateryna et al., 2012).

In sturgeon culture, herbs such as *Allium cepa, Rosa canina, Aloe vera, Allium sativum, Camellia sinensis* among others have been reported to enhance appetite, immune responses and survival rate (Akrami et al., 2015; Dadras et al., 2016; Sharif et al., 2017; Lee et al., 2014; Ebrahimi et al., 2017).

Several researches have reported the beneficial effects of dietary supplemented with sea-buckthorn and thyme oil extract on different fish species but there is no documented

evidence about the effect of this oil extracts on sturgeon hybrid which is a cross between a *Acipenser gueldenstaedtii* female x *Acipenser ruthenus* male (Kucukgul et al., 2013; Yilmaz et al., 2012).
Knowing the beneficial properties of sea buckthorn and thyme oil, the aim of this study was to evaluate the effects of sea-buckthorn and thyme oil extract on the haematological responses in sturgeon hybrid to promote alternative drug to chemotherapeutics in aquaculture.

MATERIALS AND METHODS

Experimental design

The juvenile hybrid sturgeon (298.73 ± 0.7 g) from the sturgeon farm of S.C. Danube Reasearch Consulting (Tulcea, Romania), obtained by cross between a *Acipenser gueldenstaedtii* female x *Acipenser ruthenus* male, were used for this experiment.
Fish were acclimatized for 2 weeks prior to beginning the experiment, during this period they were fed with Coppens. After the acclimation period, fish were randomly distributed into 12 fiberglass tanks, in four treatment groups including V1-CNT–Control, untreated fish, fed with normal diet, V2-SB- fish fed with 1% Sea buckthorn (*Hippophae rhamnoides)/*kg feed, V3-TH- fish fed with 1% Thyme (*Thymus vulgaris*)/kg feed and SB+TH- fish fed with 0.5% Sea buckthorn and 0.5% Thyme/kg feed.

Isolation of Essential Oils and preparing diet

The oils were isolated from dried materials by extractions which carried out in a pilot-plant supercritical carbon dioxide extractor (Natex, Prozesstechnologie GesmbH, Austria, Fabr. no. 10-023/2011). The essential oils used in the study were added to feed via pulverization method. The diets were dried in drying stove for 24 hours at 85°C. Feed was given three times a day at 8:00, 14:00 and 20:00 at a rate of 2% body weight.

Haematology assay

In the experiment, six fish per group on the 6 and 12 week, at 24 h after feeding, were used for blood sampling, for the determination of haematological analysis. Blood samples (1.5 ml) were collected from the caudal vein of individual fish using a heparinized 2 ml syringe and were analyzed with routine method used in fish hematology (Svobodova et al., 1991).
Red blood cells (RBC, 10^6 mL^{-1}), hematocrit (Hct, %) and hemoglobin (Hb, g dL^{-1}) were determined by using the method by Blaxhall and Daisley, in 1973. The hematological indices of mean cell hemoglobin concentration (MCHC), mean cell haemoglobin (MCH) and mean cell volume (MCV) were calculated. For each fish two blood smears were immediately dried, fixed and colored with May-Grünwald Giemsa panoptic method. The types of leukocytes were determined based on identification characters listed by Svobodova et al. (1991). Absolute number of circulating blood leukocytes and thrombocytes were determined in relation to 1000 erythrocytes in haemograms stained with panoptic method MGG and converted to unit blood volume.

Statistical analysis

Data were subjected to statistical analysis using the SPSS software ver. 18. The results were submitted to the variance analysis (ANOVA) ($p<0.05$) and Tukey test used to determine the significant differences between haematological parameters ($p<0.05$).

RESULTS AND DISCUSSIONS

After 6 weeks of treatment, statistical analysis of data showed that there were no significant differences ($P>0.05$) of haematological indices compared to the control group.
At the end of the 12 week, the RBC levels and Ht significantly increased ($P<0.05$) in fish fed with 0.5% sea buckthorn and 0.5% thyme when compared to control. The herbal extract did not change Hb concentration ($P>0.05$) at the end of both experimental periods (Table 1).
Blood smears from the experimental groups did not reveal any swelling, shrinkage or other deformations in blood cells.
Haematological indices, including WBCs, RBCs, Hb, Hct, MCHC, MCV and MCH, are used as important diagnostic tools to assess the health and physiological status of fish (Fazio et al., 2013). According to some authors the addition of different herbal extract in fish food had significant influence on some of

haematological parameters of different fish species (Gabriel et al., 2015; Güllü et al., 2016; Quezada-Rodríguez et al., 2016; Yilmaz and Ergün, 2012).

The erythrocyte constants can provide information on the size, shape, and hemoglobin loading of red blood cells, thus reflecting their degree of function. The statistically insignificant differences ($p>0.05$) of the VEM, HEM and CHEM values recorded in the three feeding variants compared to the V1-control indicate a good physiological state of the hybrids (Table 1).

Table 1. Hematological profiles of juvenile sturgeon hybrid after feeding with sea-buckthorn and thyme oil extract for 6 and 12 weeks (mean ± SD)

Sampling time	Percent oil in feed	RBC (10^6 mL^{-1})	Htc (%)	Hb (g dL^{-1})	MCV (fL)	MCH (pg)	MCHC (g dL^{-1})
After 6 weeks	CNT	0.85±0.12	23.79±2.00	8.32±0.47	285.56±55.97	98.67±10.62	35.13±4.39
	1 SB	0.89±0.09	23.64±1.71	8.26±0.62	265.56±20.53	92.51±8.09	34.90±2.84
	1 THY	0.88±0.08	23.38±3.17	7.89±1.44	265.82±22.62	90.73±8.19	34.40±5.74
	0.5 SB + 0.5 THY	0.91±0.10	24.29±2.96	7.88±0.78	267.92±32.18	87.11±8.43	32.93±5.25
After 12 weeks	CNT	0.82±0.08	23.35±0.84	8.18±0.39	285.48±17.57	99.99±5.39	35.05±0.72
	1 SB	0.9±0.11	23.55±1.01	8.32±0.58	263.32±21.62	92.78±5.68	35.31±1.48
	1 THY	0.91±1.09	23.63±0.98	8.37±0.67	262.21±19.83	92.67±7.51	35.39±2.07
	0.5 SB + 0.5 THY	1.05±0.13*	25.04±1.04*	8.83±0.48	241.8±20.48	85.17±6.45	35.26±0.79

The improvement of the values of the main haematological indicators (RBCc and Htc) in V4 variant may be due to the synergistic effect of some compounds extracted from thyme (carvacrol and tymol) respectively from sea buckthorn (vitamins C and E, flavonoids, carotenoids). The improvement of these hematological indices can signify the ability of herbal extracts to stimulate erythropoiesis, thus increasing the capability of oxygen transport and reinforcement of defense mechanisms against physiological stress.

In folk medicine, sea buckthorn has been used since ancient times to treat anemia due to the high content of vitamin C, which together with vitamins A, E and folic acid exerts immunomodulatory properties. The evaluation of the leukocyte profile may reflect the state of the general immune system of the fish (Ellis, 1977).

Numerous studies have shown the beneficial effect of feed additive with different plant extracts in improving the general physiological state of the fish as a result of their immunostimulatory action by increasing the total number of leukocytes (Jian et al., 2004; Nya et al., 2009; Abdel-Tawwab et al., 2010; Harikrishnan et al., 2010).

On the other hand, several studies in *Cyprinus carpio*, *Oncorhynchus mykiss*, *Oreochromis* sp. reported that some herbal extracts presented lower hematological indices and to a certain extent cause anemia in fish especially at higher dosage (Pakravan et al., 2011; Haghighi et al., 2014; Gabriel et al., 2015).

Therefore, optimization of herbal extracts based not only on growth and feed utilization parameters but also on blood parameters such hematology is essential for aquaculture (Gabriel, 2019).

Microscopic blood smears analysis led to the identification of the following categories of leukocytes: lymphocytes 87.54-94.48% (the fish's leukocyte system is of lymphocytic type), monocytes 0.96-2.16%, neutrophyles 2.66-9.59% and eozinophyles 0.63-3.56%. Basophilic granulocyte leukocytes have not been identified on blood smears.

The cytological study of blood smears provides important diagnostic information, reflecting the physiological state of the fish (Docan, 2014). In this study, significant increases have been

reported in the leukocyte and eozinophyle cells in V2 group, respectively monocyte and neutrophyle cells in V4 group, after 6 weeks feeding (Table 2).

Table 2. Leukocyte profiles of juvenile sturgeon hybrid after feeding with sea-buckthorn and thyme oil extract (mean ± SD)

Sampling time	Percent oil in feed	Leukocyte ($x10^3$ cel/µl)	Limphocyte ($x10^3$ cel/µl)	Monocyte ($x10^3$ cel/µl)	Neutrophyle ($x10^3$ cel/µl)	Eozinophyle ($x10^3$ cel/µl)
After 6 weeks	CNT	61.33±5.86	55.89±4.93	0.78±0.14	2.96±0.34	1.7±0.82
	1 SB	67.71±8.17*	61.53±7.02	0.75±0.15	5.01±0.93*	0.41±0.11
	1 THY	64.87±11.07	60.59±11.71	0.68±0.29	3.24±0.86	0.37±0.07
	0.5 SB + 0.5 THY	64.41±10.21	56.7±9.74	1.34±0.25*	5.89±0.46*	0.47±0.19
After 12 weeks	CNT	58.13±5.18	54.76±4.77	0.91±0.11	1.92±0.33	0.58±0.13
	1 SB	62.65±7.86	59.04±6.12	0.65±0.14	2.59±0.39*	0.42±0.09
	1 THY	62.55±9.91	58.93±9.09	0.63±0.10	1.88±0.11	1.04±0.18*
	0.5 SB + 0.5 THY	64.93±2.02*	60.92±2.70	0.88±0.23	1.97±0.24	1.09±0.21*

At the end of 12 weeks feeding significant increases have been reported in the leukocyte and eozinophyle cells in V4 group and neutrophyle cells in V2 group. These elevations determined in the haematological indicators support the findings obtained by the investigators concerning the subject.

After 6 weeks of experiment, was observed a significant increase ($p<0.05$) with 10.4% of the total number of leukocytes in the case of V2 group compared with V1 group (Table 2). This increasing tendency of the total number of leukocytes compared to the control group is maintained at the end of the 12 weeks, but only in the mixed diet group the total number of leukocytes increased significantly ($p<0.05$) by 11.70% compared to the V1 group.

The increases in lymphocytes, neutrophyles and eozinophyles, which are the basic elements of the defense system, showed the effect of sea buckthorn and thyme herbal extract in body defenses and this was also confirmed in the other studies.

Considering that a reduction in the number of leukocytes could be the consequence of nutritional errors, the results obtained in this study suggest that the plant extracts have contributed to the improvement of the physiological state of the hybrids from groups V2, V4 (Cain et al., 2003).

The leukocytes are important indicators of the nonspecific defence reaction, the increase of the total number of leukocytes registered at the end of the experimental period in V4 group, suggests the beneficial effect, combined, their immunomodulatory (Pedro et al., 2005).

Leukocytes perform major functions in the body which, via monocytes, are involved in triggering the immune response (Bektas and Ayik, 2009) and neutrophyles contribute to the activation of nonspecific immunity.

Scientists have intensified efforts to exploit natural products such as herbs in developing alternative dietary supplements that enhance growth performance, health and immune system of cultured fish, as these products are inexpensive, safer, effective, and can be easily prepared and are biodegradable (Syahidah et al., 2015).

CONCLUSIONS

In this study, the effects *of sea buckthorn and thyme oil extract* administered as a mixture with the feed, on the hematological profile in sturgeon hybrid were detected. The supplementation of the diet of the hybrid resulted in the improvement of the physiological state, registering differences, depending on the period of administration of phytobiotics as well as the specific action of each type of plant extract.

The analysis of the blood metabolic profile highlights the synergistic action of the herbal

extract on the physiological state of the hybrids: intensification of the haematopoiesis (increase of the RBCc and Htc in V4 group) and improvement of the non-specific defences (increase of WBCc).

Conclusively, in this study the use of sea buckthorn and thyme extract herbal as feed supplements on sturgeon hybrid, can improving the physiological status and immunity by stimulating the production of blood cells and other hematological indices.

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