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Influence of macerated fenugreek (*Trigonella foenum graecum*) oil added to trout feed at the different rates on the Feed Conversion Rate (FCR), body length, blood parameters and Nitroblue Tetrazolium (NBT) values of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792)

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Abstract: In this study, 1% and 2% of macerated fenugreek oil was added to the feeds of rainbow trout with an average weight of 25.79 ± 1.5 g. At the end of the study, growth rate, blood parameters and NBT (Nitroblue Tetrazolium) level of rainbow trout were determined. The best feed ratio (FCR) was observed in the control group (0.77). Statistically significant differences were found only in MID values (P<0.05), although there was a numerical increase in all blood parameters. There was no statistically significant difference between NBT levels (P> 0.05). Although the best weight gain was in the control group as in the FCR values, the maximum elongation was measured at D1 and then at D2 (P <0.05). The best survival rate was obtained with 96.66% in D1 while the worst was observed in D2 with 60% (p<0,05).

Key words: Trout; Fenugreek; NBT; Blood parameters; Length parameters.

Introduction

Plants are used on fish in various forms as disinfectants or therapeutics in different studies (1, 2, 3, 4, 5). In addition, some researchers have investigated the sensory, chemical, microbial and physiological effects of plants on fish (6, 7). The plants are either given directly to the fish in different ways, or they are left in the water and affect the fish by altering the properties of the water (8). On the other hand, the use of plants in trout breeding as feed additives has been explored for years to ensure healthy and rapid growth of the fish. Most studies do not achieve longitudinal growth, even if the trout are grown to the nearest extent. Providing a balanced growth is the most desirable situation in aquaculture. Fenugreek, also known in traditional Ottoman Medicine, has been used as a height extender (9, 10). Fenugreek contains animal growth stimulants that are not present in other fodder legumes and therefore have the potential to reduce the use of artificial growth promoters. This and other medicinal properties of fenugreek will help reduce the dependence on synthetic drugs that are considered to be serious pollutants of water resources (11).

Fenugreek is a common plant cultured in many parts of Europe, Africa and Asia and it is very rich in terms of steroidal saponins (12). It is also used in traditional medicine due to its lactogenic, anti-diabetic, anti-microbial, anti-cancer and hypocholesterolemic properties (13, 14, 15, 16). Flavonoids and saponins in fenugreek protect the living cells against oxidative stress and stimulate the immune system. It has been reported in some fish studies that fenugreek is activating the immune system of the fish (17, 18, 19).

Analysis of blood parameters revealed that there is a valuable approach to determine the health status of the cattle raised, and these parameters provide reliable information about metabolic disorders, deficiencies and chronic stress status before being found in the clinical setting (20). Blood biochemistry parameters can be also used to detect the health of fish (21). It has been found that the fenugreek participating in the rasion causes a high amount of hemoglobin, hematocrit, lysozyme and myeloperoxidase activity in fish. It is recommended for feed additives and fish breeding to improve hematological and immune status (22). In addition, fenugreek contains phenolic and flavonoid compounds, thus helping to increase antioxidant capacity in the liver (23).

In this study, we aimed to determine the feed rate (FCR), some blood and length parameters and NBT values of the rainbow trout (*O. mykiss*) of macerated fenugreek oil added to rasion at different ratios.

Materials and Methods

Material

Experimental materials

Rainbow trout (*O. mykiss*) with an average live weight of 25.79 ± 1.5 g was used in the study. Fenugreek (*T. foenum graecum*) was kept in 1/10 sunflower oil for 15 days to obtain macerated oil. The obtained macerated oil was added to a commercial trout diet at

Table 1. Mean values of blood parameters measured at the end of 21 days feeding according to experimental groups.

	WBC (10^3/µL)	LYM (10^3/µL)	MID (10^3/µL)	GRAN (10^3/µL)	RBC (10^6/µL)	HGB (g/dl)	HCT (%)	MCV fL	MCH pg	MCHC g/dl	RDW-SD (fL)
C	47,57	44,4	1,84	0,97	1,25	6,87	16,24	129,85	55,55	42,88	64,78
D 1	54,48	50,6	2,58	1,28	1,65	9,11	21,94	133	54,98	41,42	66,92
D 2	52,77	49,15	2,42	1,17	1,51	8,21	19,71	132,5	54,11	41,54	69,08

1% and 2% by spraying method. The study groups were formed as control (C) (no fenugreek oil added to the diet), experiment 1 (D1) (1% macerated fenugreek oil added to the diet) and experiment 2 (D2) (2% macerated fenugreek oil added to the diet). Ten fish were studied in each group and three were repeated. Fishes are fed daily with feed at a rate of 2% of their body weight. Feeding was done daily for 21 days, morning and evening. Feed evaluation ratio (FCR) and mean height values were determined at the end of the study.

Fishes were anesthetized (Benzocaine 30 mg/L) before the blood collection. Experiments were conducted in accordance with ethical rules (Inonu University, Faculty of Medicine, Experimental Animal Ethics Committee, Protocol No:2017/A-24). Blood samples were taken from the tail veins of the fried fishes by injector and transferred to tubes containing EDTA. On the same day nitro-blue tetrazolium (NBT) activity (total oxidative radical production of neutrophils) from EDTA blood samples was determined spectrophotometrically. Full blood count was also performed with PROCAN PE-6800VET brand fully automated hematology analyze.

The measured water in the experiment was determined as temperature 14.61 ºC, pH 8.25, Conductivity 16.9 (dS/m), Hardness 11.25 A ° S, Salinity 8.4 mg/l.

Methods

Analysis of hematologic parameters

In the 21st days of the application, the blood samples of fish (n=10) randomly selected from all groups were taken. Fish were anesthetized with the 0.30 ml L^{-1} 2-phenoxyethanol for 10 minutes (24) and observed anesthesia of fish under deep sedation, including loss of swimming action and partial loss of equilibrium (25). The blood samples were taken from the caudal vena into K2 EDTA tubes (4 ml). The HCT, Hg, RBC, WBC, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), and the activity of Nitroblue-Tetrazolium (NBT) in the blood samples and analysed by fully automatic hematology analyser (PROCAN PE-6800VET Brand) in the same day.

Analysis of Nitroblue Tetrazolium (NBT) activity

The 0.1 ml of 0.2 % NBT solution was added to the microtiter plates and an equal amount of blood was added on it. After this suspension was allowed to stand at room temperature for 30 minutes, 0.05 ml of it was added to glass tubes containing 1 ml of N, N-dimethylaformamide. After centrifugation at 3000 rpm for 5 minutes (centrifuge; Nuve Brand NF800 Model), the upper layer was read on a spectrophotometer (Hach Lange Brand DR6000 Model) at 540 nm.

Length, weight measurements and determination of FCR values

The length of the fish was measured with a ruler and their weights were weighed on the precision scale. Feed Conversion Ratio was obtained by the following formula:

F.C.R. = Feed given / Animal weight gain

Statistical analysis

The data obtained were statistically tested using the SPSS statistical program at the 0.05 confidence interval by DUNCAN test ($P<0.05$). The mean values were given in the results as mean ± standart error of means. However, all results were analysed by the SPSS 24.0 Package Programme.

Results

Hematological parameters

A numerical increase in all blood parameters outside the MCHC was observed for fish in the D1 and D2 groups. On the other hand, only a statistically significant difference was found in the MID (Monocyte) parameters ($P<0,05$). No statistically significant difference was found in other blood parameters ($P>0,05$). The blood parameters measured at the end of the 21-day trial are given in Table 1.

Nitroblue Tetrazolium (NBT) activity

NBT values were measured as 0.296 ± 0.09 before study in the control group. At the end of the 21 day study; 0.353 ± 0.06 in the control group, 0.332 ± 0.03 in the D1 and 0.305 ± 0.05 in the D2. Although the obtained data show an increase compared to the values obtained before feeding, NBT activity was not significant in the case of macerated oil application due to its water-soluble phenolic compounds in fenugreek.

Feed conversion ratio

In this study, the best FCR value was obtained in the control group. Although there were numerical differences between the control and the two experimental groups, no statistically significant difference was observed ($P>0.05$). Table 2 shows FCR change values.

Length increase

The best elongation value at the end of 21 days of feeding was obtained at D1. A statistically significant difference was found between the length of the fish measured before the feeding and the length of the fish fed with the fenugreek supplemented feed ($P>0.05$)

Table 2. FCR, survival rates, mean weight and mean length change values.

	Control	D 1	D 2
FCR	0,77	1,14	1,04

Table 3. Mean length and mean weight change values.

	Control	D 1	D 2
Lenght 0 day (Average±sd)	13.08±1.5	13.02±1.4	12.94±1.9
Lenght 21 day (Average±sd)	13.68±1.6	14.39±1.4	14.30±1.9
Average Weight 0 Day	27.29±1.0	24.73±1.2	25.35±1.1
Average Weight 21 Day	40.49±1.3	35.76±1.1	34.72±1.3

Table 4. Survival rate.

	Control	D 1	D 2
Survival Rate %	73.33	96.66	60

(Table 3).

Survive rate

The best survival rate was obtained with 96.66% in D1 while the worst was observed in D2 with 60% ($P<0.05$) (Table 4).

Discussion

In our study, the increase in MCV values of the fenugreek applied groups was higher in the 1% group, but both groups showed an upward image. The MCV increase is a macrocytosis indicator with a decrease in the MCHC value in both treatment groups. This is the evidence that fenugreek oil stimulates renewal and the production of new erythrocytes. The parallel increase in MCV values of lenght values revealed that oxygenation and blood production were increased by stimulation. In another study of fenugreek applied on *Sparus aurata* L., the increases in RBC value were reported to increase in all fenugreek applied groups, although not statistically significant, as in our study (26). Yilmaz et al. (27) found that there was no significant change in the RBC, HGB, HCT values of *Dicentrarchus labrax*. In the same way Roohi et al. (28) did not observe a statistical change in these values of carp.

In addition to this, another study done by Gültepe et al. (29) found an increase in RBC, HGB, HCT values of fenugreek applied tilapia. A similar study by Acar et al. (30), found an increase in RBC and HGB values.

Monocytes are groups of cells that increase at the time of chronic stress (31, 32). In our study, the monocyte levels in the fenugreek oil application groups were higher than the control group. Monocyte levels in phagocytic and lysosomal function-induced immune systems are as high as in our study groups (29, 33). Monocyte increase, one of the representatives of the cellular immune response, was also found to be high in other studies performed with fenugreek (34). In another study where fenugreek oil was applied, it was found that the number of monocytes increased. This state that the substances contained in the fenugreek oil suppress ACE (The enzyme that enables the conversion of angiotensin 1 to angiotensin 2 is a vasoconstrictor) activity (35). This suppression increases the nutrient and oxygenation levels of the tissues by increasing vessel expansion.

The RDW-SD value was found to be higher in the two experimental groups than in the control. This elevation is indicative of the number of large erythrocytes, indicating that the fenugreek oil increases the formation

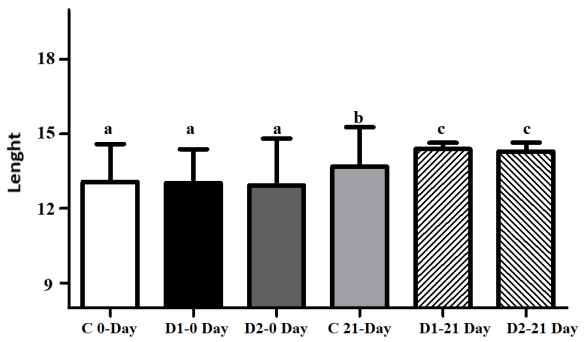

Figure 1. Statistical differences with length groups before and after study.

of new erythrocytes. Take Güllü et al. (36) in a similar experiment found that RBC, HGB and HCT values of the group fed with fenugreek-supplemented diet were not different from those of the control group.

In this study, feed conversion rate in experimental groups decreased compared to control group. There was no statistically significant difference between FCR values (Control: 0.77; D1: 1.14; D2: 1.04) in this study. ($P>0.05$). Lawal et al. (37), In an FCR study on fenugreek-fed tilapia fish, no significant difference was found between the experimental groups and the control group. In a study on Cyprinus carpio, it was observed that the rate of feed evaluation decreased in a similar way to this study (28). Öz et al. (38) investigated the effect of black black cumin oil on trout. It has been observed that the feed conversion rate of trout fed with feed of black cumin oil has decreased. On the other hand Black black cumin oil addition increased feed consumption and daily feed consumption of fish for each.

Araee et al. (39) reported that fenugreek rats were effective on bone marrow haematopoietic stem cells, In this study, fish fed with fenugreek oil extended their length more than control group. In the experimental groups, the weight values were less increased compared to the control group, on the contrary, the lengths of the experimental groups were increased more than the control groups.

NBT activity was not significant in the case of macerated oil application due to its water-soluble phenolic compounds in fenugreek. Kumar et al. (40) found that NBT activity was at the highest level in a study used by fenugreek.

As a result, it is possible to say that the addition of fenugreek macerated oil to trout feeds gives positive results. Macerated fenugreek oil reveals numerical differences in all parameters. However, the most statistically significant result was that survival rates at Experiment Group 1 and fish fed with fenugreek fat-fed diet had a longer prolongation of their size. It may be possible to obtain more striking results by developing this research in future studies.

Authors' contributions

All of the responsibilities and contributions belong to correspounding author.

Conflict of interest disclosure

There is no conflict of interest of this manuscript.

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