Effect of Medical Plants on Digestive Enzymes and Growth Performance of rainbow trout (*Oncorhynchus mykiss)*

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Abstract: This study was designed to evaluate the growth and digestive enzyme activity parameters of rainbow trout juvenile *Oncorhynchus mykiss* fed diets containing different levels (0%, 0.1%, 0.5% or 1%) of *Glycyrrhiza glabra*, *Coriandrum sativum* and *Cassia angustifolia* aqueous methanolic extract as a feed additive seventy-five days. The fish with initial weight of 22.65 ± 0.07 g were divided into 30 tanks so that 10 groups would be formed and stored as 50 fish in each tank, so the experiment was started as three replications. At the end of every month, samples are collected from the digestive system for use in measuring digestive enzymes, and scales and weights for use in measuring growth rate. Digestive enzymes like pepsin, trypsin, amylase and lipase and growth parameters such as final weight, weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR). Amylase, lipase, pepsin and trypsin are different in terms of enzyme activities**.** The effect of medicinal plants gave a good indication of improvement in the effectiveness of digestive enzymes with the length of the experiment during the surrounding environment. The values related to the growth parameters were lower than the control group in the study where similar or low values were encountered.

Key words: *Glycyrrhiza glabra*, *Coriandrum sativum*, *Cassia angustifolia*, rainbow trout, digestive enzymes, growth parameters.

INTRODUCTION

Culture activities are carried out at differing intensities and systems in 190 countries where 600 different species are produced in aquatic environments (Md Aklakur, 2016). Proper nutrition is directly proportional with the activity of the digestive enzymes located in the digestive tract. For fishes, digestion begins at the stomach and ends at the anus (Smith, 1989).

The morphologic structure of the intestines, contents of the food taken and feeding habits are the primary aspects that affect the digestive enzyme activity in fishes (Ray, 1988), (Kuz'mına & Smırnova, 1992), (Sabapathy U., 1993). In addition, other factors such as age, pH and temperature also affect the activity of digestive enzymes (Kuz'mina, 1996). Use of antibiotics and disinfectants for treatment and prevention of many diseases faced in aquaculture is quite common. However, constant and erroneous use of antibiotics may cause antibiotic-resistant bacteria, environment pollution and residue accumulation in fishes (Ringo E, 2010). In order to

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prevent economic losses related to health deficiencies, commercial drugs are used commonly for treatment and prevention of disease outbreaks faced in aquaculture (Rico, 2013). Intense use of synthetic drugs constitutes many disadvantages in regards to both environment and health. Intensive use of antibiotics caused accumulation in the muscles of commercialised animals (Cabello, 2006), (Romero-Ormazábal, 2012) and formation of resistant bacteria strains (Miranda, 2002), (Seyfried, 2010). Medicinal herbs and plants are preferred to be used for fish cultures due to being a more adequate and affordable resource in treatment and combatting diseases without products causing toxicity (Madhuri, 2012). Medicinal plants contain helpful metabolites that have immense physiological effects towards supporting the wellbeing and health of the tissues of humans and other animals. Medicinal aromatic plants had been important parts of social insurance throughout the human history (Schippmann, 2002). Use of medicinal plants has advantages such as low/minimal cost, effect and efficiency, tolerance increase, extra protection, limited side effects, accessibility and recyclability (Parveen, 2012). Natural plants contain phytochemicals of various qualities and effectiveness, some of which may be listed as phenolic acids, flavonoids, tannin, lignin and other components defined by (Cowan, 1999). Such plants have effects towards assisting health and wellbeing such as antibacterial, antimutagenic, anticarcinogen, antithrombotic and vasodilator effects (Bidlack, 2000). Inclusion of plant extracts to fish food may affect the fishes' food finding capabilities by stimulating their sense of smell and thus promote them to eat more food than normal (Adams, 2005). They are also of low cost to producers and formation of resistance by pathogens is unlikely (Ribeiro, 2016); (Hashimoto, 2016). Medicinal plants promoted growth in fish and livestock reported by (Levic JG, 2008), (Kumar IV, 2014), (Reverter, 2014), and (Iheanacho S, 2017a).

MATERIAL AND METHODOLOGY

Material

Design of the Study

The design of the study is based on the feeding programme set up for the purpose of increasing the effectiveness of immuno-stimulants and antioxidant system of rainbow trout (*Oncorhynchus mykiss*) through use of various medicinal plants. After the fished were fed with control diet for a week and adaptation was realised, they were fed with test food containing three different plant extracts sprayed into the commercial food for a period of 75 days. On the 15^{th}, 30^{th}, 45^{th}, 60^{th} and 75^{th} days of the research, various immune-stimulants and antioxidant enzyme activities were examined through blood parameters.

Place of Study and the Fishes Utilised

The study was conducted at Germeçtepe Inland Water and Marine Fish Production, Application and Research Centre, Faculty of Aquaculture of Kastamonu University. Rainbow trout juveniles (*Oncorhynchus mykiss*) named by (Johann Julius Walbaum in 1792). (1500 fish) with average weight of 21±2 g were used in the study that spanned 75 days. Fishes (50 fish) selected at random from within the juvenile growing cages of the Research Centre were placed in each of the net cages with dimensions 1.5 x 1.5 x 1.5 m. The testing arranged for these 30 groups was conducted in three stages. Control group fishes were fed only with commercial fish food. Medicinal plant groups were fed with food dosed at 0.1, 0.5 and 1g.

Methodology

Obtaining the Medicinal Plants Used in the Study and Their Watered Methanolic Extracts

The licorice root (*Glycyrrhiza glabra*), coriander seed (*Coriandrum sativum*), and cassia (*Cassia acutifolla*) medicinal plants used in the study were provided from the herbalists inside Kastamonu province. The plants were dried and powdered, and kept in airtight bottles. After being powdered, they were weighed as 50 grams each, and after being mixed with 40% methanol inside brown bottles the mixtures were stored for three days. The mixture was screened at the end of 72 hours, and only the liquid part was evaporated through extraction method in the evaporator until it reached the consistency of honey. The extract that is brought to honey consistency then dissolved in distilled hot water and applied to the fish food via spraying method.

Fish Food and Feeding Programme

Commercial food used as study rations. All test foods outside of the control group were prepared by spraying the plants' prepared watered methanolic extracts into the commercial food.

*Table **(1)** Content of commercial food*

Proximate Content	Macro Elements	Trace Elements	Vitamins
Raw Protein (44%)	Phosphorus 1.10 %	$FeSo_4$ 80 ppm	A 7.400 UI
Raw Oil (21%)	Calcium 1.30 %	KI 2 ppm	D3 1.000
Ash (9%)	Sodium 0.20 %	Cu So_4 7 ppm	
Raw Fibre (3.9%)		Mn So_4 15 ppm	
		Zn So_4 110 ppm	

The amount of daily food given to the fishes was regulated based on the size of the fish and the temperature of the water used in test tanks. Fishes were given daily food based on 2% and 2.5% of their live weights. Throughout the feeding tests, the fishes were fed by hand, twice each day at 09:00 in the morning and 16:00 in the evening. Care was shown that the entire food was taken by the fish during the feeding process. Two days prior to each of the days when measurements and analyses were conducted throughout the testing, the feeding process was halted.

Digestive Enzyme Analyses

Tissue Sampling and Preparation for Analyses

Prior to tissue sampling, the fishes were kept hungry for a period of (48) hours. After anaesthesia implementation, they were kept on ice for (15) minutes, after which their stomach and front intestine tissues were taken, and cleansed of fats and food remnants. The sampled tissues were weighed at 0.1 g each, and placed with distilled water ten times their weight (1000 µl) at (+4°C) into tubes. After the tissues were fragmented adequately, they were subjected to centrifuge at 20000 G and (+4°C) for (45) minutes. Following the centrifuge, the supernatants were collected and kept at (-80°C) temperature for analysis. Readout processes of all analyses were realised by Thermo Scientific Multiscan Go device.

Because it is necessary to also know the protein values of the tissues for the calculation of digestive enzymes, tissue protein concentrations were defined through the prepared supernatants by use of (Bradford, 1976) method.

Amylase Enzyme Analysis

Absorbance readout was realised at 540 nm, and the calculations conducted as below (Bernfeld, 1955).

Amylase = [(sample result – blank result) ^{2} x 7.712] - [1.082 x (sample-blank)] + 0.082 = Result / mg protein

Lipase Enzyme Analysis

Lipase enzyme activity calculation was conducted according to(Furne, 2005) method, at 405 nm.

$$LIPASE = [(Supernatant\text{-}Blank) \times (0.2359 + 0.0153)^2] / mg\ protein$$

Pepsin Enzyme Analysis

Pepsin Enzyme Activity calculation was conducted according to (Anson, 1938), at 280 nm.

$$PEPSIN = [(Sample\text{-}Bland) \times 1000] / [10 \times mg\ protein]$$

Trypsin Enzyme Analysis

According to (Erlanger, 1961) method, at 410 nm.

$$Absorbance\ result = [(Final\ result - initial\ result) / 10\ min]$$

Trypsin= [(Absorbance result x 1 million) / 8.800] / 2 = Result / mg protein

Calculation of the Growth Performance of Fishes

The live weights of all individual fishes were weighed at the beginning and in 15-day periods by applying anaesthesia (clove oil + 10 L water) to all the fish in the tanks on a scale with 0.1 g sensitivity and their lengths were measured by use of a fish measurement board (Bagenal, 1978). Throughout the measurement process, the average value of two tanks was taken as basis for each group.

Individual live weight increase (Weight gain g) was calculated by subtracting the live weight value at the beginning (A_1) from the live weight value at the period end (A_2) (Ricker, 1979).

$$Weight\ gain\ (g) = A_2 - A_1$$

Weight gain (g): Live weight increase

$A_{2:}$ Average individual weight at the end of the period (g)

$A_{1:}$ Average individual weight at the beginning of the period (g)

$$(SGR) = 100 \times (Ln\ Wf - Ln\ Wi / t) \quad (Ricker, 1979)$$

Specific growth rate (SGR) (%) : Specific growth rate .

Wf: Individual weight at the end of the period.

Wi: Individual weight at the beginning of the period.

t: time (days).

The amounts of fish food consumed periodically for the test groups were found out by separate calculations. Individual food consumption amount was calculated by dividing the total amount of food amount consumed at each period by the number of fishes in the tank. Food utilisation ratio (Ricker, 1979).

Feed conversion ratio (FCR) (%) = the amount of food consumed throughout the period (g) / Weight gain (g)

Daily intake rate (DIR) = ([feed intake /mean body mass] / no. days) ×100.

Average daily gain (ADG) (g/fish/day) = total gain / duration period in day.

(DIR): Daily intake rate

Weight gain: Live weight increase

Statistical Analyses

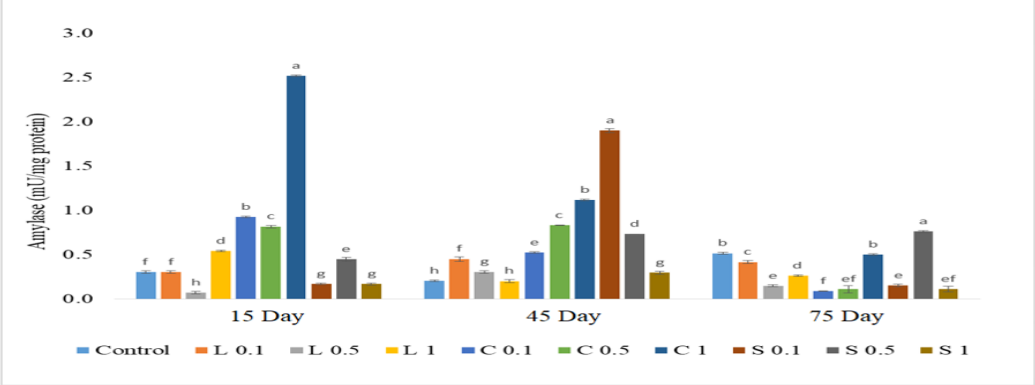
The mean and standard deviation (+Std) values of all data obtained in the test were calculated by the assistance of Microsoft Office Excel program. Variance Analysis (ANOVA) was conducted by implementing SPSS Statistics Program (SPSS 23.0) on these data. Tukey multiple comparison test was implemented on groups with difference and the significance of the difference between the values was evaluated within 95% accuracy limits. ($P < 0.05$) expression was used in the event the difference between values is found to be significant, and ($P < 0.05$) expression was used in the event the difference between values is found to be insignificant.

Results

1- Digestion Enzyme Activities

Amylase activity

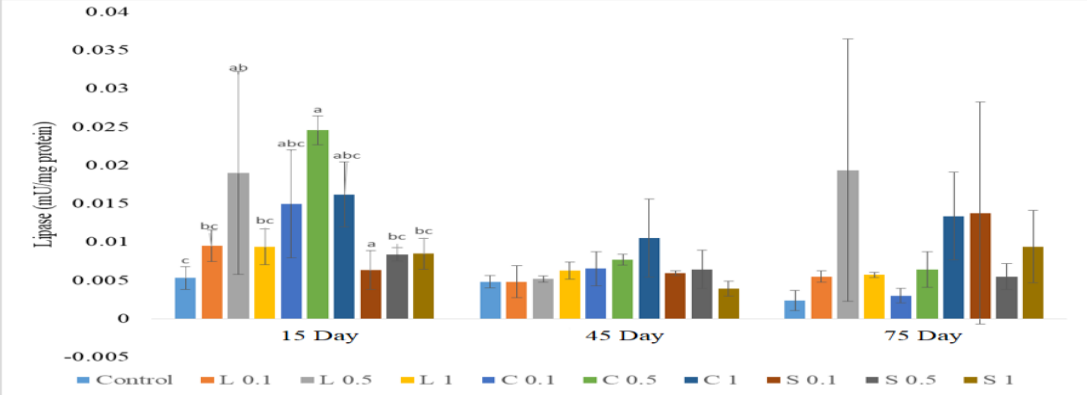
On the 15th day, amylase activity displayed the same value for the control group and 0.1% L group (0.31±0.02 mU/mg), while the lowest value was observed for 0.5% L (0.08±0.02 mU/mg) group and the highest value was observed for 1% C (2.52±0.01 mU/mg) group (Graph **(1)**). On the 45th day, the control group displayed the lowest level in amylase values (0.21±0.01 mU/mg), while 0.1% S group (1.90±0.02 mU/mg) was observed to have the highest amylase value among all test groups (Graph **(1)**). On the 75th day, the highest level of amylase values was observed to belong to 0.5% S group (0.76±0.01 mU/mg), while the control group (0.52±0.01 mU/mg) and 1% C (0.51±0.01 mU/mg) group displayed higher amylase values compared to those of the remaining test groups (Graph **(1)**).



Graph **(1)** The changes that occurred in the amylase activities of rainbow trout fed with Licorice root (L), Coriander seed (C) and Cassia leaves (S) watered methanol extract for the duration of 75 days (mU/mg protein).

Lipase Activity

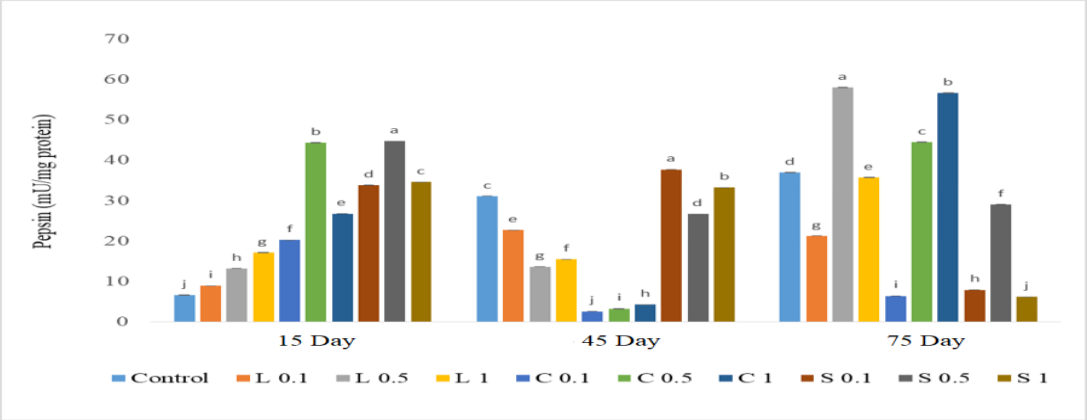
On the 15th day, the highest lipase activity was observed in 0.5% C group, while the lowest values were observed in the control group and 0.1% S group ($P < 0.05$) (Graph **(2)**). On the 45th day, the highest lipase activity was observed in 1% C group, while the lipase values of the other groups displayed no difference that is statistically significant ($P < 0.05$) (Graph **(2)**). The group with the highest lipase activity was observed to be 0.5% L, while the lipase activity values were observed to be low in the control group and 0.1% C group ($P < 0.05$) (Graph **(2)**).



Graph **(2)** The changes that occurred in the lipase activities of rainbow trout fed with Licorice root (L), Coriander seed (C) and Cassia leaves (S) watered methanol extract for the duration of 75 days (mU/mg protein).

Pepsin Activity

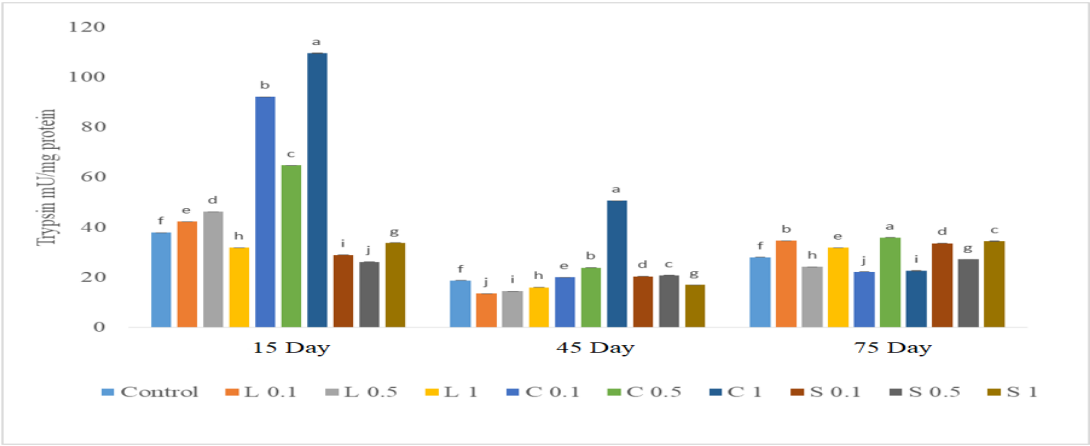
15th day control group pepsin activity (6.64±0.04) was observed to have a lower value compared to the other groups. The highest pepsin activity values compared to other test groups excluding 0.5% C (44.31±0.06) group were observed in 1%, 1% and 0.5% S groups respectively with the incrementally sorted calculations of 33.84±0.02, 34.63±0.00, and 44.70±0.02 (Graph **(3)**). 45th day lowest pepsin activity was observed in 0.1%, 0.5% and 1% C groups with the values 2.60±0.03, 3.26±0.05, 4.29±0.03, respectively. The highest pepsin activity occurred to be in 0.1% S group with a value of 37.70±0.03. Among other test groups, the highest pepsin activity occurred in 0.1%, 0.5% and 1% S groups (Graph **(3)**). Based on the 75th day data, it was observed that pepsin activity in 0.1% L, 1% L, 0.1% C, 0.1% S, 0.5% S and 1% S groups occurred to be 21.28±0.01, 35.76±0.03, 6.41±0.04, 7.91±0.02, 29.08±0.03, and 6.20±0.03, and the highest activity belonged to 0.5% L with a value of 58.02±0.03 (Graph **(3)**).



Graph **(3)** The changes that occurred in the pepsin activities of rainbow trout fed with Licorice root (L), Coriander seed (C) and Cassia leaves (S) watered methanol extract for the duration of 75 days (mU/mg protein).

Trypsin Activity

On the 15th day, the groups that displayed lower trypsin activity compared to the control group (37.85±0.01) were observed to be 1% L, 0.1% S, 0.5% S and 1% S, respectively. The values were 31.76±0.03, 28.91±0.00, 26.17±0.02 and 33.79±0.02, and the highest trypsin values were observed in the groups of 1%, 0.1% and 0.5% C respectively (Graph (4)). On the 45th day, 0.1% C, 0.1% S, 0.5% S, 0.5% C and 1% C groups displayed increase compared to the control group (18.69±0.04) in terms of trypsin activity, with the respective values of 19.94±0.00, 20.35±0.02, 20.72±0.02, 23.84±0.02 and 50.65±0.01 (Graph (4)). On the 75th day, 1% L, 0.1% S, 1% S, 0.1% L and 0.5% C groups displayed increase compared to the control group (27.93±0.01) in terms of trypsin activity, with the respective values of 31.76±0.04, 33.60±0.01, 34.45±0.03, 34.61±0.01 and 35.83±0.00 (Graph (4)).



Graph **(4)** The changes that occurred in the trypsin activities of rainbow trout fed with Licorice root (L), Coriander seed (C) and Cassia leaves (S) watered methanol extract for the duration of 75 days (mU/mg protein).

2- Growth

Whereas the initial weights of all groups were similar to each other. the control group's final weight, consumed food, live weight increase (GW) and specific growth rate (SGR) percentage values were observed to be higher compared to all test groups ($P < 0.05$), while the food conversion rates (FCR) were observed to be similar in all groups including the control group ($P < 0.05$).

Table 4.4. Values of initial weight, final weight, consumed food, live weight increase (WG), food conversion rate (FCR) and specific growth rate (SGR) based on groups.

	Initial Weight	Final Weight (g)	Consumed Food	WG (%)	FCR	*SGR* (%)
Control	**22.758 ± 0.05**	**87.727 ± 0.61***	**6520.68 ± 0.60***	**285.11 ± 0.73***	**1.00 ± 0.01**	**2.25 ± 0.01***
MK0.1	**22.369 ± 0.07**	**76.187 ± 0.61**	**5366.55 ± 0.61**	**240.73 ± 0.44**	**1.00 ± 0.01**	**2.04 ± 0.01**
MK0.5	**22.673 ± 0.05**	**75.034 ± 0.68**	**5259.13 ± 0.68**	**232.01 ± 0.77**	**1.00 ± 0.01**	**2.00 ± 0.01**
MK1	**22.737 ± 0.04**	**75.240 ± 0.60**	**5201.46 ± 0.61**	**231.45 ± 0.65**	**0.99 ± 0.01**	**2.00 ± 0.01**
K0.1	**22.583 ± 0.06**	**76.645 ± 0.53**	**5428.13 ± 0.55**	**239.44 ± 0.66**	**1.00 ± 0.01**	**2.04 ± 0.01**
K0.5	**23.069 ± 0.09**	**75.056 ± 0.71**	**5241.30 ± 0.73**	**240.24 ± 0.49**	**0.99 ± 0.01**	**2.04 ± 0.01**
K1	**22.021 ± 0.08**	**73.373 ± 0.79**	**5099.25 ± 0.79**	**233.20 ± 0.47**	**0.99 ± 0.01**	**2.01 ± 0.01**
SM0.1	**22.905 ± 0.08**	**76.105 ± 0.75**	**5261.48 ± 0.73**	**232.31 ± 0.82**	**0.99 ± 0.01**	**2.00 ± 0.01**
SM0.5	**22.317 ± 0.06**	**76.242 ± 0.63**	**5405.17 ± 0.63**	**241.55 ± 0.63**	**1.00 ± 0.01**	**2.05 ± 0.01**
SM1	**23.110 ± 0.07**	**75.639 ± 0.65**	**5304.52 ± 0.63**	**227.26 ± 0.62**	**1.01 ± 0.01**	**1.98 ± 0.01**

Discussions

1- Digestive Enzyme

Amylase

Alpha-amylase secreted into the intestine and pyloric caeca by the pancreas is a digestive enzyme with a key role in the digestion of carbohydrates. In this study, it was found out that amylase enzyme displayed significant increases compared to that of the control group for the 0.5% L group in all samplings during the 15^{th}, 30^{th} and 75^{th} days. Amylase values of the other groups were generally observed to be lower than that of the control group at the end of the 75^{th} day. The increase in amylase activities for Indian carp (*Labeo rohita*) tested with food containing mulberry leaves reported by (Mondal, 2012) and in contrast to this, (Xu, 2012) observed decrease in amylase activities of sturgeon fish (*A. schrenckii*) tested with soy protein isolate.

Lipase

Produced by pancreatic tissues, lipase takes up role in the digestion of lipids (Brix, 2002), and is contained in the stomach, pancreas, liver, pyloric caeca and intestine of fishes (Tramati C., 2005). According to the results of the study as of the 75^{th} day, the lipase activities of test groups were observed to be higher than that of the control group. Increases occurring in serum lipase amounts

may provide preliminary insight into problems that may occur in pancreas (Mehmetoğlu, 2007). That food containing mulberry leaves increased lipase activity of Indian carps (*Labeo bata*) (Mondal, 2012). lipase activities of the yellowfin bream tested with food with soy flour additive has provided no significant change (Ehsani, 2014).

Pepsin Activity

Pepsin activity enables digestion of protein inside the stomach (Alarcón, 1999). On the 75th day of the study, it was determined that licorice root and coriander increased pepsin activity compared to the control group. Increases in the pepsin activity of rainbow trout they tested with food with lupine, mango, stinging nettle additives (Awad, 2012) and likewise, (Lazzari, 2010) observed an increased pepsin activity in their study conducted on *Rbamdia quelen*.

Trypsin Activity

Trypsin is a pancreatic protease that enables the activation of itself and other proteases secreted from the pancreas (Hjelmeland, 1984). According to the study results, it was determined that licorice root, coriander and cassia generally caused increases in trypsin values. Trypsin had increased intestine protease activity in *Labeo rohita* species fed with food with guar and cottonseed additives reported by (Iqbal, 2016). Trypsin activity increased in *Oreochromis niloticus* species they fed with food containing aloe vera powder published by (Gabriel, 2017). Soy flour added food decreased trypsin activity of the sea bream fishes (*Pagrus major*) subject to test (Murashita, 2015).

2- Growth Performance

The initial weights of all groups were similar to each other. The control group's final weight, consumed food, live weight increase (WG) and specific growth rate (SGR) percentage values were observed to be higher compared to all test groups ($P < 0.05$), while the food conversion rates (FCR) were observed to be similar in all groups including the control group ($P > 0.05$). Plant-based protein sources are driven directly out of the body either through metabolic processes or through excrement due to reasons such as their unbalanced amino acid profiles, non-plant based contents and unease of digestion (Siddiqui, 2014). reported that the plant-based sources they tested for their effects on immunity had no effect on the growth of Japanese flounder (*Paralichthys olivaceus*) (Ashida T., 2005). That ginger, mistletoe and stinging nettle tested as food for trout have kept the growth parameters at optimum level (A. Düğenci, 2003). Study of koi (*Cyprinus carpio*) tested with food with turnip, grape and carrot additives that there were increases in the low final weight, weight gain (WG), (SGR) and (FCR) parameters concluded by (Azab, 2016). Growth performance and food conversion at high levels for the Nile tilapia (*Oreochromis niloticus*) fed with cumin seeds (*Carum carvi*) (Ahmad, 2011). Growth performance and feed evaluation in carp (Cyprinus carpio) (Abbasi Ghadikolaei, 2017).

Conclusion

Medicinal plants have a positive effect on digestive enzymes, but from this study the growth and its indicators are low, and for this we recommend that research be conducted on this approach to improve growth.

المستخلص: الدراسة أعدت لتقييم معايير النمو ونشاط إنزيمات الهضم لسمك Rainbow trout اليافع (*Oncorhynchus mykiss*) الذي تم تغذيته بغذاء محتوي على مستويات مختلفة (0٪، 0.1٪، 0.5٪ أو 1٪) من المستخلص الميثانولي المائي لكل من Glycyrrhiza glabra و Coriandrum sativum و Cassia angustifolia كإضافة غذائية لمدة خمسة وسبعين يوما. تم تقسيم الأسماك التي يبلغ وزنها الأولي 0.07 ± 22.65 جم على 30 حوض ووزعت هذه الاحواض إلى 10 مجموعات وكل حوض يحتوي على 50 سمكة، لذلك بدأت التجربة بثلاث مكررات. في نهاية كل شهر تم جمع عينات من الجهاز الهضمي لاستخدامها في قياس إنزيمات الجهاز الهضمي ومقاييس والأوزان لاستخدامها في قياس معدل النمو. إنزيمات الجهاز الهضمي مثل البيبسين والتريبسين والأميلاز والليباز ومعاملات النمو مثل الوزن النهائي، وزيادة الوزن (WG)، ومعدل النمو المحدد (SGR)، ونسبة تحويل العلف (FCR). الأميليز والليباز والبيبسين والتربسين تختلف من حيث نشاطها الأنزيمي. تأثير النباتات الطبية أعطى مؤشرا جيدا على تحسين فعالية الإنزيمات الهاضمة على طول فترة التجربة الميدانية في البيئة المحيطة. كانت القيم المتعلقة بمعلومات النمو الأسماك المعاملة أقل من مجموعة التحكم في الدراسة حيث تم العثور على قيم متشابهة أو منخفضة.

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