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EFFECTS OF ADDING GARLIC, THYME AND SAGE POWDER AND OIL TO JUVENILE TROUT FEED

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Abstract

In this study, powder and oil forms of medicinal and aromatic plants sage (*Sage officinalis*), thyme (*Origanum onites*) and garlic (*Allium sativum*) were added to the feed of juvenile rainbow trout (*Oncorhynchus mykiss*) to improve growth performance and their antioxidant enzyme activity. For this purpose, 5% (0.05 mg/kg) of the powder forms of sage, thyme and garlic and 1% (0.01 mg/kg) of the oil forms of the same products were added to the trial feeds. The used of water phsico-chemical properties as temperature; 8ºC, dissolved oxygen; 9.6 ppm. pH; 6.5. In the study, live weight gain (41.32±2.47), specific growth rate (3.72±0.47), and feed conversion rate (1.04±0.097) were best obtained in the garlic oil (SY group) ($p<0.05$). Among the antioxidant enzyme activities, malondialdehyde (MDA) was insignificant between the groups ($p>0.05$), while catalase activity was determined best in the thyme group, and superoxide dismutase (SOD) was best in the garlic groups ($p<0.05$).

Keywords: Rainbow trout, thyme, garlic, sage, growth and antioxidant activity

Introduction

Fish, which is a source of protein originating from aquaculture, is very important, especially for individuals with low and high incomes, as it is extremely nutritious with its various features, especially in terms of protein, essential oils and nutritional components, and is easily and available. Due to the rapid increase in the world population, health problems caused by malnutrition are increasing rapidly. Especially with the pandemic, easy availability of food has become even more important. For that, foods produced within the country with less dependence on foreign sources have gained more importance. With the rapid increase in the world

population, various studies and production models are needed to meet the demand for food and quality nutrition that we will encounter in the future while preserving natural resources. Aquaculture is an issue that is taken seriously in our country's policies, not only to meet the food demand but also to provide employment and added value. Especially with its family business structure, it has provided great benefits with the employment and value it has generated almost everywhere in our country, from east to west (Yaldız et al., 2005).

Recently, under the influence of restrictive and restrictive factors, the aquaculture sector has been focusing on herbal solutions that serve the same purposes, instead of the medicaments (drugs and chemicals that have therapeutic, protective and growth-enhancing effects) that they have to use in production. In vivo studies conducted on fish, it has been found that they also have anti-stress effects (Bulfon et al., 2015).

Zakes et al. (2008) examined the growth performance, body composition, fatty acid profile and histological characteristics of liver and midgut tissues of two different medicinal plant species, *Astragalus radix* and *Lonicera japonica*, in the juvenile pikeperch (*Sander lucioperca*) fish species and the growth rate of the said plant species in fish. They found that it increased the feed conversion rate and affected body composition, fatty acid profile and fat metabolism of fish. Giannenas et al. (2012) examined the intestinal microbiota, antioxidant effect and growth performance of rainbow trout (*Oncorhyncus mykiss*) fed with fish feeds containing carvacrol and thymol. They reported that carvacrol and thymol, if added to the feed of rainbow trout in small amounts, have a positive effect on growth performance, antioxidant effect and immunity, and positively affect feed conversion. Ahmadifar et al. (2011) examined the effect of adding thymol and carvacrol to the feed of juvenile rainbow trout on hematological parameters, growth performance and tissue composition, and reported that the number of lymphocytes increased, provided better growth in fish compared to the control group.

Türkiye has a very rich flora, especially in terms of plants containing essential oils. The Mediterranean Region is the gene center of the *Labiatae* family, which includes the majority of medicinal and aromatic plants. *Origanum, Thymbra, Thymus* and *Satureja* genera, which are cultivated and commercialized in Türkiye, are important thyme species within this family. The rate of carvacrol varies between 5-75% in the *Thymus* genus, 1-45% in *Satureja*, and 50-80% in Origanum species. *Origanum vulgare* is a species that is exported and has economic value. It is an Eastern Mediterranean element and is distributed in a wide area such as the European side, Western-South Anatolia, Izmir, Aydın and Muğla. In studies conducted with the *Origanum vulgare* plant, 1-7% yield and 23-80% carvacrol were detected in its essential oil. The plant has many different uses (Yaldız et al., 2005).

Allium is the largest and most important representative of the *Alliaceae* family and includes 700 species widely distributed in the northern hemisphere, primarily in North America, North Africa, Europe and Asia (Tsiaganis et al. 2006). It is known that garlic (*Allium*) species also have antimicrobial effects thanks to their organosulfuric components. Many articles show that the inhibitory effect of extracts and essential oils from plants prevents the growth of many bacteria and fungi (Kocić-Tanackov et al. 2012). Including their neurophysiopharmacological use in studies investigating the effects of Allium species also sheds light on the different uses of these plants (Abdel-Gawad et al., 2014; Abdel-Latif & Khalil**,** 2014). The beneficial effects o zamanof these plants on health are attributed to their high content of biologically active phytomolecules such as phenolic compounds, especially flavonoids and various organosulfur compounds (Fredotović et al., 2017).

Sage (*Salvia officinalis* L.) is a valuable medicinal and aromatic plant from the *Lamiaceae* family. Species in the *Salvia* genus are generally rich in essential oils and are pharmacologically important because they have antioxidant, antimutagenic and antimicrobial effects. Although the *Salvia* genus, which belongs to the *Lamiaceae (Labiatae)* family, has approximately 900 taxa distributed throughout the world, the ones with the highest commercial value are *Salvia officinalis* L., also called medicinal sage or Dalmatian sage. Medicinal sage leaves contain 0.5-2.5% essential oil. The most important components of medicinal sage essential oil are α and β-thuyon, 1,8-cineole and camphor. In its essential oil, the α-thuyon ratio varies between 1-45%, the β-thuyon ratio varies between 1-40% and the camphor ratio varies between 0.4-44% (Rahman et al., 2022).

The use of antibiotics as feed additives has been completely banned in the European Union as of January 1st 2006. With this ban, the use of medicinal plant species as feed additives has become a good alternative to control bacterial diseases by protecting the health of the gastro-intestinal microflora, to support growth performance and to reduce mortality indicated by Hermann et al. (2003), Goda (2008), Keser & Bilal (2008), Abdel-Tawwab et al. (2010), Yılmaz et al., (2011), Diler et al., (2017) and Terzioğlu & Diler (2016). In recent studies on the aquaculture sector, it has been determined that medicinal plants are effective on growth parameters in fish (Ji et al., 2007; Aly & Mohamad 2010; Immanuel et al., 2009; Oskoii et al., 2012). The use of various herbal products to increase the growth and health of fish in aquaculture is a very popular approach indicated by Ji et al. (2007), Erol-Florian et al. (2011) and Diler et al. (2017).

In recent years, the addition of essential oils obtained from medicinal plants to feeds to increase the growth performance of fish has come to the fore (Hammer et al., 1999). It is known that garlic (*Allium spp*.) species also have antimicrobial effects thanks to their organosulfuric components. Species in the Salvia genus are generally rich in essential oils and have antioxidant and antimicrobial properties. *Origanum vulgare* L., one of the Origanum species, also contains antimicrobial effective carvacrol (70-80%) and terpene (terpinene, cymen) components, which are seen in countries in the Mediterranean climate zone and are available in our country (Abdel-Tawwab et al., 2010).

In this study, powder and oil forms of medicinal and aromatic plants such as thyme, sage and garlic were used on juvenile rainbow trout and their effects on growth, feed utilization with enzyme and antioxidant activities were investigated.

Material and Method

The experiment was carried out in a private enterprise in Isparta Aksu. The used of spring water phsico-chemical properties as temperature; 8.0ºC, dissolved oxygen 9.6 ppm, pH 6.5. The research was carried out with fish weighing an average of 17 g in the facility, each tank count of fish 70 amount and 400 liter per section. They were taken to the trial ponds and adapted for 2 weeks. During this period, the trial groups were fed twice, in the morning and in the evening, with commercial trout feed without anything added. The trial groups were named and coded as follows (Table.1).

Table.1 Design of trial

AT (1) %5	**AY(1) %1**	**ST(1) %5**	**SY(1) %1**	**KT(1) %5**	**KY(1) %1**	**CO(1)**
AT(2) %5	**AY(2) %1**	**ST(2) %5**	**SY(2) %1**	**KT(2) %5**	**KY(2) %1**	**CO(2)**
AT(3) %5	**AY(3) %1**	**ST(3) %5**	**SY(3) %1**	**KT(3) %5**	**KY(3) %1**	**CO(3)**

Where; AT; Sage powder AY: Sage oil ST: Garlic powder SY: Garlic oil
KT: Thme powder KY: Thyme oil C0: Control group

During the trial, the length and weight of the experimental group fish were measured every month, and the effects of the powder and oil forms of medicinal plants on growth, feed utilization, survival rate and condition factor, and their antioxidant activities were determined. In the experiment, fish weight measurements were made with a digital scale with 0.001 g precision, and total length measurements were made with a measuring ruler with 1 mm divisions. An anesthetic agent (phenoxyethanol) was used in measurement, weighing and experimental fish infection applications to ensure ease of operation and to prevent damage to the fish.

Preparation of trial feeds

Preparation of trial feeds was done by adding powder and oil forms of medicinal plants to commercially used trout feeds (the trade mark Abalioglu). Addition of powder and essential oil to the feed was made by spraying method. In order to preserve the effectiveness of the essential oil components, the feeds were prepared weekly and stored in capped glass bottles at +4ºC. Commercial trout food; Trout feed containing sufficient levels of protein (50% HP), fat (17% HY) and digestible energy rate (4200 kcal/kg) was used in order to be suitable for the development of the trout fry and juvenile (Lovell, 1998). The fish were fed with number 2, 3, and 4 extruded trout food depending on their size, also all trial feeds were stored in plastic containers until used.

Methods:

Eight weeks after the start of feeding, blood samples were collected from the caudal vein (six fish from each group) and placed in heparin centrifuge tubes. The collected blood samples were centrifuged at 700 rpm for 30 minutes at 4ºC. After centrifugation, plasma was collected and stored at −20 °C for future analysis.
For antioxidant activity, plasma superoxide dismutase activity (SOD, Misra and Fridovich, 1972), plasma catalase activity (CAT, Aebi (1984), lysozyme activity and malondialdehyde enzyme activity (MDA, Chen et al. 1998). were analysed. (Zheng et al. 2009).

Calculation of growth Growth Performance of Rainbow Trout

In the research, growth in live weight and length, it was evaluated by calculating absolute proportional and specific growth. The following formulas were used to calculate growth parameters (Çetinkaya, 1995; Hoşsu B., Korkut A.Y., Vural A., 2001).

Weight Gain (WG) *= Average final weight - Average initial weight*
Specific growth rate (SGR) ***(%/day)***
= [Ln (Final Body Weight g) - Ln (Initial Body Weight g)] / Number of trial days x 100
Feed Conversion Ratio (FCR) *= Total feed consumed (g) / Total live weight gain (g)*
Survival Rate (SR, %) *= (Ns / Nb) x 100*

Where; survival rate (SR) was calculated by dividing the number of fish remaining in the tank at the end of the experiment (Ns) to the number of fish at the beginning of the experiment (Nb).

Statistical Calculations

The data obtained in the experiment (such as experimental infection values, growth values, feed conversion rates) were evaluated with the Anova test in the SPSS 16.0 package program (SPSS Inc, Chicago, IL, USA). When comparing the importance levels of various parameters examined in the experiment, the results are given as mean value and standard error. The distinction between groups was determined by analysis of variance and Duncan's multiple comparison test, and the significance level was selected as P = 0.05 (Özdamar, 2001).

Results

The results obtained in the research regarding feed utilization and fish growth are summarized in Table 2 and shown Fig 1.

Table 2. Growth performance of trial groups

	K0	AT	AY	ST	SY	KT	KY
Initial weight (g)	20.14±0.17	20.18±0.74	20.24±0.81	20.36±0.56	20.13±0.31	20.24±0.32	20.33±0.68
Final weight (g)	54.66±0.21	55.71±2.79	54.85±1.32	58.41±4.28	61.45±1.45	57.44±2.41	55.11±1.07
Weight Gain (WG)	34.78±1.61	35.52±1.05	34.61±0.97	38.05±1.02	41.32±2.47	37.21±0.87	34.51±1.35
Specific Growth Rate (SGR)	3.54±0.79	3.57±0.15	3.54±0.54	3.64±0.08	3.72±0.47	3.62±0.25	3.55±0.69
Feed Evaluation Ratio (FER)	0.85±0.303	0.83±0.007	0.81±0.004	0.91±0.091	0.95±0.028	0.87±0.134	0.80±0. 097
Feed Conversion Rate (FCR)	1.24±0.078	1.21±0.095	1.24±0.033	1.14±0.018	1.04±0.097	1.16±0.052	1.25±0.056
Survival Rate (%, SR)	88.71±2.858	90.95±1.64	89.04±1.64	93.33±5.01	95.23±1.85	92.38±5.04	89.09±3.59

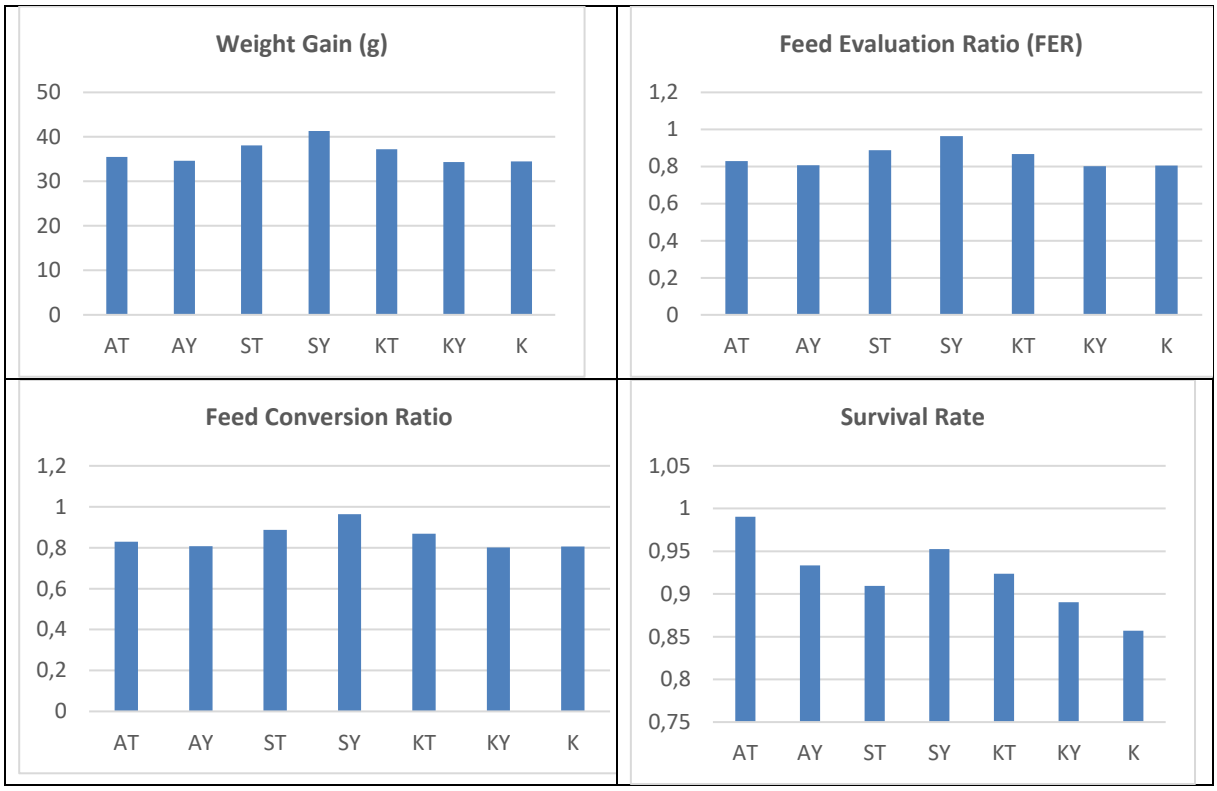

Fig 1. Graphical distribution of growth parameters data of the trial groups *

Where; AT; Sage powder, AY: Sage oil, ST: Garlic powder, SY: Garlic oil, KT: Thyme powder KY: Thyme oil C0: Control group

It is observed that live weight gain is better in groups supplemented with garlic oil, garlic powder and thyme powder. Garlic oil is also quite effective compared to other groups in terms of feed conversion rate and feed evaluation ratio. When examined in terms of survival rate, it is understood that the sage powder form gives the best results in the groups to which garlic oil, sage oil, thyme powder, garlic powder, and thyme oil are added, respectively. The highest number of deaths occurred in the control group. As can be understood from here, it can be said that medicinal plants increase the survival rate in powder and oil form. Sage powder and oil, garlic powder and oil did not create a significant difference in terms of catalase enzyme activity compared to the control group ($P>0.05$). However, thyme powder and oil showed statistically significantly higher catalase activity than both the control and sage powder group ($P<0.05$).

None of the forms of sage, garlic and thyme added to the feed caused any difference in MDA compared to the control group (Fig.2) ($P>0.05$).

All feed additives used throughout the experiment showed similar SOD values to the control group ($P>0.05$). However, a statistically significant difference was detected between sage forms; The powder form showed significantly higher SOD activity than the oil form ($P<0.05$).

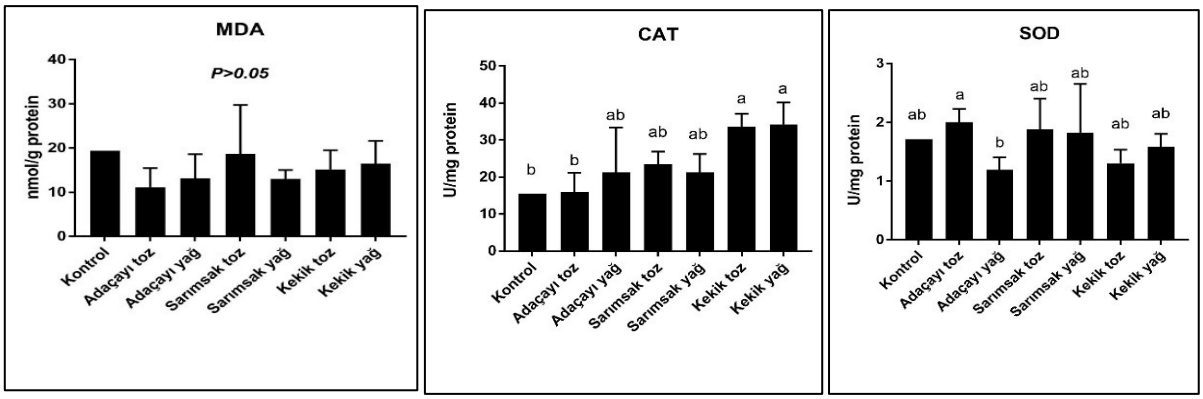

Fig 2. Graphical distribution of MDA, CAT and SOD data of the trial groups*

Where; Kontrol: Control group, Adaçayı toz: Sage powder, Adaçayı yağ: Sage oil, Sarımsak toz: Garlic powder, Sarımsak yağ: Garlic oil, Kekik toz: Thme powder Kekik yağ: Thyme oil

In this experiment, the growth performance and antioxidant activity of various medicinal plants were added to the feed of trout in powder (5%) and oil (1%) form. Also the used of spring water phsico-chemical properties as temperature; 8.0ºC, dissolved oxygen 9.6 ppm, pH 6.5.

Sage powder and oil, garlic powder and oil did not create a significant difference in terms of catalase enzyme activity compared to the control group ($P>0.05$). However, thyme powder and oil showed statistically significantly higher catalase activity than both the control and sage powder group (Fig 2) ($P<0.05$).

None of the forms of sage, garlic and thyme added to the feed caused any difference in MDA compared to the control group ($P>0.05$).

All feed additives used throughout the experiment showed similar SOD values to the control group ($P>0.05$). However, a statistically significant difference was detected between sage forms; The powder form showed significantly higher SOD activity than the oil form (Fig 2) ($P<0.05$).

Discussion and Conclusion

Medicinal plants have been reported as having a broad spectrum of growth promotion, appetite stimulation, antimicrobial, immunostimulant, antinflammatory, antistress, anticancer properties and their use in traditional medicine has been known for thousands of years around the world. Nevertheless, other plants which are used all over the world for both curative and culinary purposes such as garlic (Allium sativum), garlic chives (*Allium tuberosum*), green tea (*Camellia sinensis*), cinnamom (*Cinnamomum verum*), turmeric (*Curcuma longa*), Sundial lupine (*Lupinus perennis*), mango (*Mangifera indica*), peppermint (Mentha piperita), nutmeg (*Myristica fragrans*), basil (*Ocimum basilicum*), oregano (*Origanum vulgare*), rhubarb (*Rheum officinale*), rosemary (*Rosmarinus officinalis*) and ginger (*Zingiber officinale*) have been also screened (Bulfon et al., 2015). The use of medicinal plants in today's aquaculture industry is a rapidly spreading and increasingly popular issue. Studies on medicinal plants need to be investigated in more detail. It is of great importance to research the form, active ingredients, appropriate dosage and application times of traditionally used medicinal plants and to determine

the correct proportions. In addition, it must be easy to obtain and affordable in order to be used in routine applications in the aquaculture industry. Industrial processing, storage and marketing are needed to meet the needs of the rapidly growing sector.

The specific effects of garlic, thyme, and sage can vary depending on factors such as the type and amount of herb used, the fish's age and health status, and the overall diet composition. A better growth performance was observed in the garlic and garlic oil groups compared to the control group. In this study, thyme and thyme oil did not appear to make a significant difference on growth compared to the control group, but Zargar et al. (2022) reported that the addition of thyme essential oil to the rainbow trout diet had positive effects on growth performance and feed efficiency. Zheng et al. (2009); In their study, they added carvacrol (0.05), thymol (0.05), car (0.048)+thym (0.048), and OS (*Origanum heracleoticum*) (0.05) oil to *I.punctatus* fish. Accordingly, carvacrol, thyme, carvacrol+thyme and *Orego Steam* (OS) achieved a significant positive increase in terms of WG, FCR, SR, which are among the growth parameters of the experiment, compared to the negative control group ($p < 0.05$). Also the addition of Oregano Stim® also effectively enhanced hepatosomatic index (HSI), viscerosomatic index (VSI) and condition factor (CF) compared to the control diet ($P<0.05$). OS also remarkably enhanced the antioxidant activity of channel catfish. Both the combination of carvacrol and thymol and OS reduced fish mortality.

Immanuel et al. (2009) in their study of 3 different medicinal plant extracts on tilapia (*Oreochromis niloticus*) fish, they obtained higher values in terms of SGR in the groups treated with medical plant extracts than the control group. Similarly, in our study, the SGR value was found to be significantly higher in the groups administered oil and powder forms of medicinal plants compared to the control group ($p<0.05$). Giannenas et al. (2012); In the growth results of rainbow trout (*Onchorhynchus mykiss*) to which 1 g/kg carvacrol and thymol were added to their feed, a significant difference was obtained in terms of FCR in the pyhtogenic supplemented groups compared to the control group, similar to our study, but no significant difference was obtained in terms of WG, unlike our study. ($p>0.05$). In the same study, unlike our study, the herbal groups had lower results than the control group in terms of MDA, while a significant positive difference compared to the control group was obtained in terms of CAT values, similar to our study results ($P<0.05$).

Ahmedifar et al. (2011) added thyme thymol+carvacrol) (*Origanum vulgare*) powder at different rates (1.0, 2.0, 3.0 g/kg) to rainbow trout (average 8 g.) feed. As a result of this study, a significant positive difference was observed in weight gain, specific growth rate, feed conversion ratio, feed efficiency rate values in the thyme group compared to the control group, similar to our study ($p<0.05$). Aly & Mohamad (2010) added echinacea (1.00ppt) and garlic (3% powder form) to tilapia fish feed in summer and winter periods. While other groups performed better than the control group in the growth parameters weight gain, specific growth rate and survival rate data for the summer period ($p<0.05$), these parameters were found to be insignificant for the winter period ($p>0.05$).

Diler et al. (2017) studied growth performance and antioxidant enzyme activities in their study where they added thymol (*Origanum onites*) oil at different rates (0.125, 1.5, 2.5 and 3.0 ml/kg) to rainbow trout fish (avr. 26 g.). In the results of working; Thyme groups showed a positive effect compared to the control group in terms of WG ($p<0.05$), and likewise, in terms of FCR, it was found to be positively significant in the 1.5 and 3.0 mL/kg groups compared to the control group. The results obtained in this study, which were similar to our study in terms of fish used and weight, were also parallel to our study in terms of weight gain, specific growth rate, feed

conversion ratio, feed efficiency rate and SR (Table.1; Fig.1) ($p<0$ .05). In the same study, levels of plasma superoxide dismutase (SOD) and plasma catalase (CAT) activities, which are antioxidant enzymes, were found to be positively significant in the group to which 3.0 mg/kg thyme was added, similar to our study ($p<0.05$). In our study, CAT values were positive in the herbal groups compared to the control group ($p<0.05$), and a significant difference was observed in SOD values in the AT and ST groups (Fig.2).

Volpati et al. (2014), avg. 65.0 g. In the study where they added 0.025% and 0.05% carvacrol to the feed of sea bass fish (*Dicentrarchus labrax*), both weight gain, specific growth rate and feed conversion ratio and VSI (Viserosomatic Index), HSI (Hepatosomatic Index) and MFI (Mesenteric Fat Index). The difference between groups was not found to be significant ($p>0.05$). In the same study, a positive and significant difference was found in the lysozyme activity 0.05% group compared to the other groups ($P<0.05$). Abdel Rahman et al. (2022), as a result of the study in which they added 2, 4, 8 g/kg garlic powder to the feed of carp fish, control in terms of growth performance (weight gain, specific growth rate, feed conversion ratio, feed efficiency rate) was achieved in groups of 4 and 8 g/kg. They had a significant difference compared to the group ($p<0.05$). Additionally, 4 and 8 g/kg groups were found to be significant in terms of superoxide dismutase (SOD), one of the antioxidant enzyme activities ($p<0.05$). In our study, similar data were obtained, although the fish species were different.

In the CAT analysis of antioxidant activities, a significant difference was observed in plasma only in the OS group compared to the other groups ($p<0.05$), while there was no significant difference between the groups in terms of SOD ($P>0.05$). Superoxide dismutase (SOD) catalyzes the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide. Subsequently, catalase (CAT) activity decomposes this hydrogen peroxide into oxygen and water and this constitutes a crucial part of the cellular antioxidant defense mechanism. In this study, the activities of superoxide dismutase and catalase were assessed in the plasma of canal catfish and these values were markedly enhanced in fish fed the OS diet compared to the control diet and other treatments (Zheng et al., 2009). Similarly, in our study, a significant increase was observed in SOD and CAT between the groups ($p<0.05$), while no difference was observed in MDA ($p>0.05$). Hussein et al. (2023) in their study where they added garlic powder to the feed of European sea bass (*Dicentrarchus labrax*) at rates of 2.4, 6.8 g/kg, and found the best growth performance values (weight gain, specific growth rate, feed conversion ratio, feed efficiency rate and survival). rate) 2 and 4 g/kg. was obtained in proportional groups ($p<0.05$). In our study, similar data were obtained, although the fish species were different.

As a result the practical implications of phytogenic inclusion in aquafeeds include the potential for increasing growth performance and feed utilisation of cultured fish. This would allow for faster fish growth leading to improved production time; however, research is required with different important aquaculture species and longer time scales to fully evaluate the value of including phytogenics at industrial farming levels. Phytogenic products could be also further tested in experimentally infected fish in order to evaluate their health implications under stress or health challenge conditions (Giannenas, 2012).

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Contribution of authors

Author 1: Conceptualization, Data curation, Formal analysis, Writing original draft
Author 2: Conceptualization, Funding acquisition, Investigation, Methodology, Writing original draft, Software

All authors have read and agreed to the published version of the manuscript.

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