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Effects of herbal supplements on growth performance of sea bass (*Dicentrarchus labrax*): Change in body composition and some blood parameters

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ABSTRACT

This study was conducted to investigate the effects of dietary thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*) and fenugreek (*Trigonella foenum graecum*) as feed additives on growth performance, proximate composition and ammonia excretion of European sea bass *Dicentrarchus labrax*. Four isonitrogenous (48% crude protein) and isocaloric (21 kJ/g) diets were formulated to contain 0% (control) or 1% of thyme, rosemary or fenugreek. The thyme supplementation significantly increased protein efficiency ratio, fillet protein levels, protein and energy retentions ($P<0.05$). The medicinal herb additives did not change serum urea, uric acid, creatinine and ammonia excretion rate ($P>0.05$). The results indicate that dietary thyme improved the protein and energy retentions of sea bass.

Key words: medicinal herbs, growth, proximate composition, ammonia excretion, seabass

Introduction

Herbs or spices have been reported to promote various functions like growth (Shalaby et al., 2006), appetite stimulation, antistress (Citarasu, 2010), immune functions (Dügenci et al., 2003; Dorucu et al., 2009; Ergün et al., 2011), skin coloration (Yılmaz & Ergün, 2011), egg hatching rates (Yılmaz & Ergün, 2012a), hematological and biochemical status (Yılmaz & Ergün, 2012b) and also increase disease resistance (Yılmaz et al., 2012; Yılmaz et al., 2013) in fish culture due to different active components.

Thyme has strong antimicrobial and antioxidant activity due to its very high contents of thymol, p-cymene, carvacrol, eugenol and 4-allylphenol (Lee et al., 2005; Rota et al., 2008). Carnosic acid and rosmarinic acid are the main chemical constituents of rosemary, and they are particularly high antioxidants (Erkan et al., 2008). Fenugreek is rich in flavonoids (such as apigenin, kaempferol and quercetin) and saponins (such as diosgenin and yamogenin). Their characteristic functions are to protect the oxidative damage

(Kaviarasan et al., 2004) and immunostimulatory properties (Bin-Hafeez et al., 2003).

Several studies have reported that oral administration of fenugreek in *Labeo rohita* and *Oreochromis mossambicus* (Paul et al., 2004; Mostafa et al., 2009), rosemary in *O. niloticus* (Abutbul et al., 2004; Zilberg et al., 2010), thyme, rosemary and fenugreek in *O. mossambicus* (Ergün et al., 2011) improved growth performance, disease resistance and immunity.

The objective of this study was to determine the effects of thyme, rosemary and fenugreek on the growth performance, fillet composition, ammonia excretion, and some serum biochemical parameters in the European sea bass *Dicentrarchus labrax*.

Materials and Methods

Fish and experimental conditions

Healthy cultured sea bass, *Dicentrarchus labrax* (20.43±0.03 g) were obtained from a local fish farm (Ida

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Gıda) in Çanakkale, Turkey. During the experimental period sea water quality remained as follows: temperature 25.10±0.26°C, pH 8.47±0.01, dissolved oxygen 6.37±0.06 mg/L, salinity 28.26±0.08 ppt and conductivity 44.25±0.20 mS.

Experimental herbs and diets

Thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*) and fenugreek (*Trigonella foenum graecum*) were obtained from a local market (Harman Business). The herbs were added to the feed at 1% thyme, 1% rosemary and 1% fenugreek. Additionally, a control group was fed a diet without herbal supplementation. The feed components of the diets are presented in Table 1.

Table 1. Percentage and proximate composition of the experimental diets.

	Control	Thyme	Rosemary	Fenugreek
Ingredients (%)				
Fish meal	63	63	63	63
Corn gluten meal	7	7	7	7
Soybean meal	7	7	7	7
Wheat flour	7	7	7	7
Fish oil	10	10	10	10
Mineral mix ^a	0.5	0.5	0.5	0.5
Vitamin mix ^b	1	1	1	1
Vitamin C	0.06	0.06	0.06	0.06
Salt+Ethoxyquin	0.75	0.75	0.75	0.75
Starch	3.69	2.69	2.69	2.69
Thyme		1		
Rosemary			1	
Fenugreek				1
Chemical analyses (%)				
Protein	48.35	48.38	48.33	48.58
Fat	16.28	16.19	16.12	16.32
Ash	12.17	12.80	12.89	12.97
NFE ^c	23.20	22.63	22.66	22.13
Energy (kJ/g) ^d	21.79	21.66	21.63	21.67
P:E (mg protein kJ ⁻¹ energy)	22.19	22.34	22.35	22.41

^aVitamin Mix: Vit. A, 18000 IU; Vit. D3, 2500 IU ; Vit. E, 250 mg/kg; Vit. K3, 12 mg/kg; Vit. B1, 25 mg; Vit. B2, 50 mg; Vit. B3, 270 mg; Vit. B6, 20 mg; Vit. B12, 0.06 mg; Vit. C, 200 mg; Folic acid, 10 mg; Calcium d-pantothenate, 50 mg; Biotin, 1 mg; Inositol, 120 mg; Choline chloride, 2 g.

^bMineral Mix: Fe, 75.3 mg; Cu, 12.2 mg; Mn, 206 mg; Zn, 85 mg; I, 3 mg; Se, 0.35 mg; Co, 1 mg.

^cNitrogen-free extracts (NFE) = 100 - (crude lipid+crude ash+crude protein)

^dEnergy calculated according to 23.6 kJ g⁻¹ protein, 39.5 kJ g⁻¹ lipid, and 17.0 kJ g⁻¹ NFE.

The ingredients were mixed in a blender and the mixture was then pressed through a 2-mm die in a pelleting machine. The

pellets were dried in a drying cabinet (40°C) until moisture dropped well around 10% and stored in bags in a deep freeze at -20°C until used.

Experimental design and feeding trial

The experiment was performed with 204 specimens of sea bass. Fish were divided into 140 L fiberglass tanks (17 fish / 3 tanks per dietary group). Before the initiation of the experiment fish were given a diet that contained 48% protein and 12% fat. The recirculating system used in the experiment consisted of filters, protein skimmer and biofilter containing bioballs. Water was exchanged daily corresponding to about 10% of the total volume. Fish were fed with an experimental diet three times a day at 08:00 a.m., 12:00 a.m. and 16:00 p.m. at a rate of 2% of their body weight. Fish experiments were performed in accordance to the guidelines for fish research from the animal ethic committees at Çanakkale University.

Growth performance and proximate composition

Growth performance and feed utilization were calculated according to the formulae given below. Proximate analyses of the diets and fish fillets were performed using standard methods (AOAC, 1998). Dry matter was analyzed by drying at 105°C in an oven to a constant weight, crude fat by methanol: chloroform extraction, crude protein by the Kjeldahl method, and crude ash by incineration at 525°C in a muffle furnace for 12 h.

$$\text{Weight gain (\%)} = \frac{\text{final fish weight} - \text{initial fish weight}}{\text{initial fish weight}} \times 100$$

$$\text{CF (Condition factor)} = \frac{\text{body weight (g)}}{\text{standard length}^3} \times 100$$

$$\text{FCR (Feed conversion ratio)} = \frac{\text{feed intake (g)}}{\text{weight gain (g)}}$$

$$\text{PER (Protein efficiency ratio)} = \frac{\text{weight gain (g)}}{\text{protein intake (g)}}$$

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$$\text{SGR (Specific growth rate)} = \frac{\ln \text{ final fish weight (g)} - \ln \text{ initial fish weight (g)}}{\text{experimental days}} \times 100$$

$$\text{PR (Protein retention)} = \frac{(\text{final protein concentrations} \times \text{final fish weight (g)}) - (\text{initial protein concentrations} \times \text{initial fish weight (g)})}{\text{protein content of the diet} \times \text{FCR} \times (\text{final fish weight (g)} - \text{initial fish weight (g)})} \times 100$$

$$\text{FR (Fat retention)} = \frac{(\text{final fat concentrations} \times \text{final fish weight (g)}) - (\text{initial fat concentrations} \times \text{initial fish weight (g)})}{\text{fat content of the diet} \times \text{FCR} \times (\text{final fish weight (g)} - \text{initial fish weight (g)})} \times 100$$

Ammonia excretion experiment

At the end of the growth trial (45 days), each group of fish was kept in a tank (70 L) at 23°C in order to measure ammonia excretion rates. After feeding fish at a rate of 2% of their body weight within 25 minutes, the water inflow was stopped. Water samples were taken every hour for eight hours and total ammonia concentrations (NH₄⁺ and NH₃) were analyzed by the Nessler method with a HANNA C 200 (HI 83200) photometer (Hanna Instruments, Co., Italy) (Ergün *et al.*, 2008) according to the following formulae:

$A = [(N_2 - N_1) \times V_2] / B / T_{2-1}$, where A = ammonia excretion rate (mg total NH₃-N / kg wet fish weight / hour), N₁ = ammonia concentration at time 1 (mg total NH₃-N/L), N₂ = ammonia concentration at time 2 (mg NH₃-N/L), V₂ = volume of the medium at time 2 (ml), B = wet weight of the fish (g) and T₂₋₁ = time interval between samplings 1 and 2 (h).

Blood sampling and biochemical analysis

For blood sampling, fish were anaesthetized with 20 mg/L clove oil. Six fish on day 0 and six fish per dietary group on day 45 were used for blood analysis. Fish blood samples were collected randomly from the caudal vein using a vacutainer (Kima-vacutest®, Italy) fitted 5 ml. Blood serum was separated by centrifugation (4000g, 10 min) in vacutainer and stored at -20°C until used for biochemical analysis. Biochemical indices for the serum included urea, uric acid and creatinine which were determined using bioanalytic test kits (Bioanalytic Diagnostic Industry, Co) by a Shimadzu spectrophotometer (PG Instruments, UK).

Statistics

Each value was expressed as mean ± standard error of mean (SEMs) for each parameter measured. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by a TUKEY multi comparison test with SPSS 17.0 package software. Statistical significance was established at P<0.05.

Results

The four diets were equally accepted by the fish and there was no mortality or disease in any treatment. There were no significant differences (P>0.05) in average weight gain, feed conversion ratio (FCR), specific growth rate (SGR) and fat retention (FR) (Table 2). However, thyme supplementation increased protein efficiency ratio (PER), protein retention (PR) and energy retention (ER) (Table 2), and fillet protein composition (P<0.05) (Table 3). The total ammonia excretion by fish in an 8 h period is shown in Figure 1. Peak values developed at 6 h after feed distribution. The total amount of ammonia excretion in the water was slightly lower for the thyme (215.45 mg/kg fish / 8 h), rosemary (225.65 mg/kg fish / 8 h) and fenugreek (225.63 mg/kg fish / 8 h) groups than the control (232.93 mg/kg fish / 8 h) group (P>0.05) (Figure 1).

Serum biochemical results are shown in Table 4. Results have shown that T, R and F diets did not change significantly the serum urea, uric acid and creatinine levels (P>0.05).

Discussion

In this study, rosemary and fenugreek did not change growth performance in sea bass (Table 2) and fillet proximate composition (Table 3). However, the ER and PR were increased in fish fed diets containing the thyme (Table 2). It appears that the thyme improves the nutrient utilization of sea bass turning out a better growth of the fish. Most probably fat was used for energy, and protein was used for growth in thyme diet. It has been shown that herbs stimulate the secretion of pancreatic enzymes, important factors in nutrient digestion and assimilation (Frankic *et al.*, 2009). This trend can be related with the higher, though not significant, weight gain, PER, SGR and muscle protein, and better FCR obtained with thyme supplementation.

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Table 2. Fish performance and feed utilization for sea bass fed diets containing different herb supplements for 45 days.

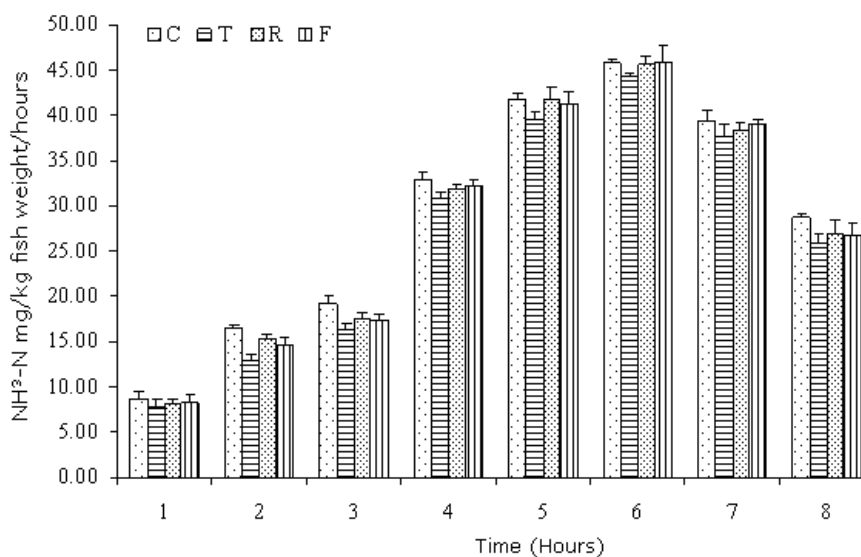
	Control	Thyme	Rosemary	Fenugreek
Initial fish weight (g)	20.43±0.18	20.44±0.22	20.45±0.13	20.39±0.29
Final fish weight (g)	39.67±0.92	40.72±0.82	38.94±0.50	39.20±0.70
Weight gain (%)	90.42±2,24	92.26±1,12	99.18±1,89	94.12±2,90
Initial CF	1.14±0.03	1.14±0.04	1.14±0.03	1.14±0.05
Final CF	1.27±0.06	1.27±0.04	1.26±0.03	1.27±0.02
FCR	1.08±0.02 ^{ab}	1.02±0.01 ^a	1.13±0.02 ^b	1.11±0.01 ^b
SGR (%/day)	1.47±0.03	1.53±0.02	1.43±0.03	1.45±0.01
PER	1.93±0.03 ^b	2.04±0.01 ^a	1.85±0.03 ^b	1.89±0.02 ^b
PR (%)	43.69±0.14 ^b	54.17±0.79 ^a	44.65±1.09 ^b	45.56±1.01 ^b
FR (%)	21.19±0.64	18.11±1.15	16.21±2.14	16.68±1.88
ER (%)	32.52±0.83 ^b	37.63±0.68 ^a	30.89±0.27 ^b	30.67±0.96 ^b
Survival (%)	100	100	100	100

Legend: Different letters in the same line indicate significant differences among groups ($P < 0.05$). Values are mean \pm SEMs ($n=3$).

Table 3. Proximate composition of the fillet for sea bass fed diets containing different herb supplements for 45 days.

	Experimental period (days)	Control	Thyme	Rosemary	Fenugreek
Moisture (%)	0	75.53±0.92	75.53±0.92	75.53±0.92	75.53±0.92
	45	73.34±0.46	71.58±0.39	73.11±0.55	73.50±0.39
Protein (%)	0	19.78±0.84	19.78±0.84	19.78±0.84	19.78±0.84
	45	21.31±0.22 ^b	23.41±0.32 ^a	21.78±0.33 ^b	21.59±0.25 ^b
Fat (%)	0	2.35±0.16	2.35±0.16	2.35±0.16	2.35±0.16
	45	2.99±0.15	2.66±0.16	2.62±0.28	2.64±0.24
Ash (%)	0	1.29±0.17	1.29±0.17	1.29±0.17	1.29±0.17
	45	1.37±0.05	1.44±0.15	1.56±0.05	1.56±0.06

Legend: Data at the same exposure time with different lower case is significantly different among treatments ($P < 0.05$). Values are mean \pm SEM ($n=6$).

**Figure 1.** Ammonia excretion rate of sea bass fed with control and herbal supplementation diets during the experiment. C: Control, T: Thyme, R: Rosemary and F: Fenugreek. Values are mean \pm SEM ($n=3$).

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Table 4. Some serum biochemical parameters for sea bass fed diets containing different herb supplements for 45 days.

	Experimental period (days)	Control	Thyme	Rosemary	Fenugreek
Urea (mg/dL)	0	0.15±0.01	0.15±0.01	0.15±0.01	0.15±0.01
	45	0.15±0.01	0.15±0.02	0.15±0.01	0.14±0.03
Uric acid (mg/dL)	0	1.23±0.05	1.23±0.05	1.23±0.05	1.23±0.05
	45	1.14±0.03	1.18±0.04	1.20±0.04	1.20±0.04
Creatinine (mg/dL)	0	0.27±0.02	0.27±0.02	0.27±0.02	0.27±0.02
	45	0.26±0.01	0.23±0.03	0.23±0.02	0.26±0.02

Legend: Values are mean ± SEM (n=6).

Mostafa et al. (2009) conducted an experiment with Nile tilapia fingerlings, fed with a basal diet containing 0, 0.5, 1 and 1.5 g/100g fenugreek seed meal for 12 weeks, and they found that the use of 1 g/100g fenugreek seed meal improved the fish performance. However, in the present study, fenugreek did not significantly affect growth performance. These differences could be explained by the different application time of feeding and fish species, or the level of fenugreek in the diets.

The other important factor which can minimize the nitrogenous waste is output of the fish (Ergün et al., 2008). In this study, the thyme, rosemary and fenugreek slightly decreased the total ammonia excretion at 17.48, 7.28 and 7.30 mg/kg fish/8 h respectively, compared to control group (Figure 1). It is known that the main internal source of ammonia in fish is through catabolism of proteins (Ip et al., 2001). The less protein that is catabolised, the more nitrogen is accumulated in the fish body, indicating that the dietary protein is used for growth than as source of energy (Yigit et al., 2003). In accordance with this, the increase in growth performance, PR, PER and muscle protein value found in the thyme group corroborate the above findings concerning ammonia excretion. Similar results were also obtained with *Yucca schidigera* as feed additive in Nile tilapia diets resulting in decreased ammonia excretion rate (El-Saidy & Gaber, 2004; Reyes & Chien, 2009).

The physiological role of urea, uric acid and creatinine is not clearly understood. Nevertheless, they are useful indices for overall health of gill and kidney (Campbell, 2004), feed utilization (Tulli et al., 2007) and amino acid (arginine) requirement (Tibaldi et al., 1995) in fish. The creatine represents more than 50% of the nitrogenous waste that is excreted through the fish kidney (Campbell, 2004). Herb additives did not change serum urea, uric acid and creatinine levels in the present study (Table 4). Similar results were obtained when the fenugreek was included at 1% in tilapia

diet (Mostafa et al., 2009). On the other hand, the rosemary decreased serum urea and uric acid in chicken (Ghazalah & Ali, 2008). Another study has shown that the stinging nettle decreased serum urea and creatinine in rainbow trout (Awad, 2010).

In conclusion, the results indicate that a dietary thyme level of 1.0 % could improve the protein and energy retentions of the sea bass.

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