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Influence of Black Cumin Seeds (*Nigella sativa*) and Turmeric (*Curcuma longa* Linn.) Mixture on Performance and Serum Biochemistry of Asian Sea Bass, *Lates calcarifer*

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Abstract: A total of 270 fingerlings of Asian sea bass (*Lates calcarifer*) were allotted into three equal treatments with three equal replicates for each to investigate the effect of dietary supplementation of two levels of Black cumin seeds (*Nigella sativa*) and Turmeric (*Curcuma longa*) mixture (BTM) on growth performance and blood chemistry throughout the experimental period (98 days). Fish of the first treatment fed on basal diet and served as a control while fish of the second and third treatments kept on BTM (1:1 w w^{-1}) constitute either 5g kg^{-1} or 10g kg^{-1} basal diet. The examined doses of BTM were weak growth promoters. Carcass analysis revealed unchanged composition in all treatments. Hematological and examined parameters of protein, lipid and mineral metabolism were not altered in all treated fish. Furthermore, the examined BTM doses were safe to fish as both liver and kidney functions were not disturbed. The present study can conclude that, inclusion of Black cumin seed and Turmeric combination in Asian sea bass (*Lates calcarifer*) diets relatively improved the growth performance and did not affect blood chemistry. Both medicinal plants were safe to liver and kidney at the examined dose as reflected on their biomarkers.

Key words: Herbs % Barramundi % Nutrition % Biochemistry % Fish

INTRODUCTION

Different studies were conducted to minimizing fish feed costs by using medicinal plants "back to nature" as feed additives instead of using synthetic drugs of serious side effects [1]. Previous studies [2, 3] focused on the effects of herbs on growth performance and biochemistry. Asian seabass *(Lates calcarifer*) is commercially cultivated in Asia, in both brackish water and freshwater ponds, as well as in marine cages in coastal water. Because of its relatively high marketing value, it has become an attractive commodity to both large to small-scale aquaculture enterprises [4].

The focus of the current study includes uses of plant mixture of Black cumin seed (*Nigella sativa L.*) and turmeric (*Curcuma longa*) in the diet of Asian sea bass fingerlings. We named this plant mixture as BTM. Black cumin seed is herbaceous plant which is a member of the Ranunculacea Family. Previous studies demonstrated the positive effect of black cumin seed on performance and blood chemistry of *Mugil cephalus* fish [3], Catfish [5], Nile tilapia [2], Pekin ducklings [6] and Japanese quails [7]. Turmeric *(Curcuma longa)* is a perennial herb that grows to a height of three to five feet and is cultivated extensively in Asia and other countries with a tropical climate. *Curcumin*, the active ingredient from the spice turmeric is a potent antioxidant [8, 9] and hepatoprotective properties [10]. The herbal synergistic effect has been reported in fish including, Japanese flounder [11], Nile tilapia [12] and *Mugil cephalus* [3]. The current study aimed to investigate the effect of the mixture of two famous traditional medicinal plants (BTM) searching for their synergistic effect concerning performance and blood chemistry in Asian sea bass fingerlings.

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MATERIALS AND METHODS

Fish and Aquaria: In the present study, Asian seabass (*Lates calcarifer*) fingerlings were obtained from Fish Farming Center, Jeddah, Saudi Arabia and transferred to fish culture wet laboratory of the Collage of Agricultural and Food Sciences, King Faisal University, Saudi Arabia. Fish were kept for 2 weeks in two 3000 liters capacity tanks for acclimation to the experimental conditions. Tanks were cleaned biweekly throughout the study.

Fish were fed a mixture of the all tested experimental diets in order to habituate them to locally formulated feed. After the acclimatization period, healthy Asian seabass fingerlings (n=270) with an average of initial fresh body weight of 10.52±0.34g fish^{-1} were randomly distributed into three equal treatments with three replicates (30 fingerlings for each) in 1 m^3 circular fiberglass tank throughout the experimental period (98 days). All the tanks were supplied with freshwater from a recirculatory closed system and were continuously aerated by electric air pump. The system was subjected to a photoperiod of 12 h light: 12 h darkness and temperature of 25.8±1.8°C.

At the beginning, during and at the end of the experiment, fish in each tank (n=30) were anesthetized with 0.1g L^{-1} tricainemethane sulfonate (Argent Chemical Laboratories, Redmond, WA, USA) and weighed collectively. Fish were fed the tested diets at a rate of 3% from their fresh body weight per day in two equal portions; each portion was given at 8.00 AM and 2.00 PM. During the experimental period, fish were weighed collectively biweekly to adjust the feeding level for the subsequent period according to the new biomass. The fish were not fed on the weighing day. Dissolved oxygen and water temperature were measured by Oxygen Meter (YSI Model 57) whereas pH was estimated by using Jenway 370 Meter. Total ammonia and Nitrite were determined by using Spectrophotometric analysis method [13].

Experimental Design: Acclimated Asian seabass fingerlings were allotted into 3 equal treatments with three replicates (30 fingerlings for each). All treatments were assigned randomly in the aquaria and each aquarium was represented one observation. Fish of the first treatment fed on basal diet and served as a control whereas fish of the second and third treatments kept on BTM (1:1 w w^{-1}) constitute either 5g kg^{-1} (0.5%) or 10g kg^{-1} (1.0%) basal diet (Table 1) [3].

Table 1: Formula and proximate analysis of the experimental diets supplemented with various levels of Black cumin seed and Turmeric mixture. (All values are g kg^{-1} unless otherwise indicated)

	Treatment[1]		
	T_1	T_2	T_3
Ingredients			
Fish meal (65%)	487.7	487.7	487.7
Soybean meal (48%)	165.0	165.0	162.0
Corn gluten meal (60%)	130.0	130.0	132.0
Yellow corn	127.3	122.3	118.3
Fish oil	70.0	70.0	70.0
BTM[2]	0.0	5.0	10.0
Dicalcium phosphate	5.0	5.0	5.0
Premix[3]	10.0	10.0	10.0
Limestone	5.0	5.0	5.0
Total	1000	1000	1000
Proximate analysis			
Dry matter	983.60	977.50	986.60
	DM basis		
Crude protein	525.72	523.58	525.34
Ether extract	155.35	155.91	154.37
Fiber	38.94	31.61	39.43
NFE	184.53	192.84	183.76
Ash	95.47	96.06	97.10
Gross energy (KJ g^{-1})[4]	21.57	21.68	21.50
CP-GE Ratio (g KJ^{-1})[5]	24.37	24.15	24.43

Values in parentheses are percentage protein of ingredients. Proximate analysis values are mean values of three replicates for each treatment.

1 Diets with various levels (0, 5 and 10g kg^{-1}) of BTM.

2 BTM: Black cumin seed and Turmeric mixture.

3 Premix of vitamins and minerals according to NRC (1983) recommendations for fish.

4 Energy values were calculated using the factors 23.4 KJ g^{-1}, 39.2 KJ g^{-1} and 17.2 KJ g^{-1}, for protein, lipid and carbohydrates, respectively (Goddard 1996).

5 CP-GE: crude protein to gross energy ratio.

DM (Dry matter), NFE (nitrogen free-extract)

Medicinal Plants and Preparation of Diets: Three isocaloric and isonitrogenous diets treatment 1 (T_1), treatment 2 (T_2) and treatment 3 (T_3) were formulated, to meet the nutrient requirements of Asian seabass (*Lates calcarifer*) fingerlings [14]. All the experimental diets (T_1, T_2 and T_3) were formulated to have almost similar crude protein, CP (524.88g kg^{-1} ± 0.66) and gross energy, GE (21.48 kJ g^{-1} ± 0.05) and were arranged to contain BTM (1:1 w w^{-1}) at levels of 0, 5 and 10g kg^{-1} represented (0, 5 and 1%) basal diet, respectively [3]. Diets composition and chemical analysis are shown in Table 1. Black cumin seeds (*Nigella sativa*) and Turmeric roots (*Curcuma longa*) were supplied from a local market of Al-Ahsa city, Saudi Arabia and identified by botanists of

College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa, Saudi Arabia. The whole seeds and roots were crushed in a blender and mixed well with the treatments before their administration.

Ingredients per each diet were grinded and mixed with vitamins and minerals mixture. Fish oil was sprayed on the mixture and the control diet. Distilled water was added to each diet until stiff dough resulted and this was pressed through meat grinder machine and the resulting pellets were dried in the oven at 40°C over night. All diets were stored at -4°C to avoid oxidation and subsequent rancidity. The ingredients of each diet are illustrated in Table 1.

Sampling and the Analytical Methods: Ingredients, formulated diets, pre-and post experiment carcass samples were analyzed in triplicate using standard methods [15]. Concerning carcass sampling, fish were dried at 70°C for 48-72 h and were passed through a meat grinder into one composite homogenate per treatment. The diets were analyzed for crude protein (CP), ether extract (EE), crude fiber (CF), ash and moisture while whole body composition of Asian sea bass fingerlings samples was also analyzed for the same parameters except for CF. The nitrogen free-extract (NFE %) was calculated as 1000 - (crude protein + crude fat + fiber + ash) g kgG1.

CP content (total nitrogen×6.25) was determined using a BUCHI digestion unit K-435 and Distillation unit B-324 and B-324 nitrogen analyzer while crude lipid concentrations were determined by petroleum ether extraction using a BUCHI extraction B-811 Automatic system. In addition, ash content was obtained by incinerating samples in a muffle furnace (VULCAN™) at 550°C for 12 h whereas dry matter was determined by drying the sample in an oven (MEMMERT) at 105°C for 16 h and weighing to the nearest 0.1 mg. Ingredients and diets samples were analyzed for fiber by using VELP SCLENTIFICA unit.

At the end of the experiment and after anesthesia (0.1 g LG^{1} tricainemethane sulfonate), blood samples were collected from the heart using disposable tuberculin syringe [16] for estimation of total erythrocytic count (TEC), total leucocytic count (TLC), packed cell volume (PCV) [17] and hemoglobin (Hb g LG^{1}) [18]. The Mean corpuscular volume (MCV) was calculated as ([hematocrite value×10]/ erythrocyte count). Mean corpuscular hemoglobin (MCH) was calculated as ([hemoglobin g LG^{1}×10]/ erythrocyte counts), whereas Mean corpuscular hemoglobin concentration (MCHC) was calculated as ([hemoglobin g LG^{1} ×100]/ hematocrite value).

Similarly, blood samples were collected without anticoagulant for serum separation [19]. The obtained sera were used for spectrophotometric determination of the activities of Aspartate Transaminase (AST) and Alanine Transaminase (ALT) [20]. In addition, serum glucose [21], total protein [22], albumin [23] and globulin [24] blood urea nitrogen [25], uric acid, creatinine [26], triacylglycerol (TAG) [27] and total cholesterol [28] were also determined. Very low density lipoprotein cholesterol (VLDL-c) was calculated by division of TAG by 5 [29]. Calcium, phosphorus and magnesium were determined by using Commercial diagnostic kits (United Diagnostic Industry, UDI, Dammam, Saudi Arabia) on ELIPSE full automated chemistry analyzer (Rome, Italy). Concentration of the biochemical constituents was calculated according to the manufacture instruction.

Statistical Analysis: Data of the current study were analyzed by SAS software [30], analysis of variance was conducted using general liner model procedure, (Proc GLM; SAS 6.12). Duncan's multiple range tests was used to compare dietary treatments means. Differences were considered significant at $P<0.05$.

RESULTS

Growth Performance and Survival Rate: Results presented in Table 2 demonstrated that, initial body weight (g) of Asian seabass fingerlings fed the experimental diets did not differ, indicating that all treatments were homogenous. However, fish fed both levels of BTM showed similarly a significant ($P<0.05$) higher SGR and weight gain % when compared with the control. Moreover, survival rate was not changed among all treatments throughout the experimental period.

Feed Utilization Efficiency and Body Chemical Composition of Asian Sea Bass: Data summarized in the Table 2 revealed also that, feed utilization efficiency in terms of Feed intake, DFI, FE, FCR, PER, PPV, EER and EPV were not altered among all treatments. Regarding carcass DM, CP, EE, crude ash and GE contents of Asian seabass carcass, insignificant differences were observed among all treatments (Table 3).

Hematological and Biochemical Parameters: The obtained results demonstrated that, TEC, TLC, Hb, PCV, MCV, MCH and MCHC were not changed significantly ($P>0.05$) in all treatments throughout the experimental period when compared with the control (Table 4).

Table 2: Growth performance and feed utilization of Asian seabass fed diets with various levels of Black cumin seed and Turmeric mixture

	Treatment		
Parameter	T_1	T_2	T_3
Initial weight (g fish^{-1})	11.35±0.74	10.53±0.32	9.69±0.24
Final weight (g fish^{-1})	64.50±3.87	69.55±1.71	60.69±2.33
Weight gain (%)	468.93±11.89^{b}	561.04± 8.31^{a}	526.26±13.22^{a}
Daily gain (mg fish^{-1})	542.31±32.43	602.31±14.55	520.44±21.66
SGR (% day^{-1})	1.77±0.02^{b}	1.93± 0.01^{a}	1.87± 0.02^{a}
Relative SGR (%)	100	109	106
Survival rate (%)	97.78±1.11	95.56±1.11	97.78±1.11
Feed intake (g fish^{-1})	70.96±5.85	73.97±1.60	62.80±2.29
DFI (g fish^{-1})	2.42±0.06	2.40±0.01	2.32±0.05
FE (%)	76.50±2.44	81.63±0.27	82.32±1.66
FCR (feed gain^{-1})	1.33±0.04	1.26±0.00	1.23±0.03
Protein utilization			
PER	1.43±0.05	1.52±0.00	1.55±0.03
PPV (%)	27.58± 0.35^{b}	29.86± 0.43^{ab}	31.23± 0.97^{a}
Energy utilization			
EER	3.49± 0.11^{b}	3.68± 0.01^{ab}	3.78± 0.08^{a}
EPV (%)	23.96±0.55	25.66±0.51	26.49±1.04

Values are mean ± standard error (SE) of three replicates for each treatment. Within a row, values with different superscripts are significantly different among dietary treatments ($P < 0.05$). SGR (Specific growth rate), DFI (Daily feed intake), FE (Feed efficiency), FCR (Feed conversion ratio), PER (Protein efficiency ratio), PPV (Protein productive value), EER (Energy efficiency ratio), EPV (Energy productive value)

Table 3: Whole-body chemical composition of Asian seabass fed the experimental diets supplemented with various levels of Black cumin seed and turmeric mixture as feed additives.

		Treatment		
Parameter	At the Start	T_1	T_2	T_3
Dry matter (g kg^{-1})	243.10	283.20±3.16	288.20±3.66	293.53±10.45
Composition on DM basis				
Crude protein (g kg^{-1})	682.47	664.34±3.21	664.15±5.97	669.04±4.44
Ether extract (g kg^{-1})	178.19	202.16±4.50	202.02±4.13	190.29±5.71
Ash (g kg^{-1})	139.34	133.50±1.41	133.83±2.65	140.67±2.68
Gross energy (KJ g^{-1})	22.95	23.47±0.10	23.46±0.06	23.12±0.14

Values are mean ± standard error (SE) of three replicates for each treatment

Table 4: Effect of different levels of Black cumin seed and Turmeric on hematological indices in Asian seabass

	Treatment		
Parameter	T_1	T_2	T_3
TEC (10^{12} L^{-1})	3.10±0.20	3.10±0.10	3.06±0.30
TLC (10^{9} L^{-1})	75.20±0.50	76.00±0.60	77.00±0.30
PCV (Volume fraction)	0.25±0.02	0.25±0.03	0.25±0.01
Hb (g L^{-1})	81.00±5.00	76.00±2.00	79.00±1.00
MCV (fL)	80.60±0.80	80.60±0.60	81.60±0.10
MCH (pg cell^{-1})	26.10±1.00	24.50±1.80	25.80±1.20
MCHC (g L^{-1})	324.00±9.00	304.00±9.00	316.00±3.00

Values are mean ± standard error (SE) of three replication for each treatment.TEC (Total erythrocytic count), TLC (Total leucocytic count), PCV (packed cell volume), Hb (Hemoglobin), MCV (Mean corpuscular volume), MCH (Mean corpuscular hemoglobin), MCHC (Mean corpuscular hemoglobin concentration)

Table 5: Effect of different levels of Black cumin seed and Turmeric on biochemical parameters in Asian seabass

	Treatment		
Parameter	T_1	T_2	T_3
Glucose (mmol LG^{1})	10.44±0.12	10.41±0.23	10.66±0.11
Total Protein (g LG^{1})	30.80±0.02	30.80±0.03	30.80±0.05
Albumin (g LG^{1})	20.70±0.20	20.60±0.30	20.70±0.10
Globulin (g LG^{1})	10.10±0.10	10.20±0.20	10.30±0.30
A-G ratio	2.04±0.14	2.01±0.11	2.01±0.13
Total cholesterol (mmol LG^{1})	1.01±0.10	0.99±0.01	0.95±0.05
TAG (mmol LG^{1})	1.70±0.40	1.71±0.30	1.72±0.30
VLD-c (mmol LG^{1})	0.34±0.04	0.34±0.03	0.34±0.03
ALT (U LG^{1})	20.20±0.10	19.70±0.61	20.10±0.14
AST (U LG^{1})	100.10±0.91	101.20±1.04	102.20±0.89
BUN (mmol LG^{1})	3.78±0.61	3.89±0.40	3.92±0.54
Uric acid (µmol LG^{1})	77.32±0.73	77.32±0.73	78.27±0.54
Creatinine (µmol LG^{1})	18.68±0.42	17.52±0.54	17.89±0.28
Calcium (mmol LG^{1})	2.53±0.31	2.45±0.46	2.55±0.14
Phosphorus (mmol LG^{1})	0.72±0.05	0.71±0.06	0.71±0.06
Magnesium (mmol LG^{1})	0.33±0.05	0.33±0.05	0.34±0.08

Values are mean ± standard error (SE) of three replicates for each treatment. Albumin/globulin ratio (A/G ratio), AST (Aspartate Transaminase), ALT (Alanine Transaminase), TAG (triacylglycerol), VLDL-c (Very low density lipoprotein cholesterol)

Spectrophotometric analysis of serum samples indicated that, during the whole experimental period all biochemical parameters related to glucose and protein metabolism (Total protein, albumin, globulin and their ratio) remain unchanged in fish fed BTM (two doses) when all compared with the control group (Table 5). Uses of BTM with different levels did not disturbed liver and kidney functions as reflected on unchanged measured liver enzyme (ALT and AST) activities, blood urea nitrogen, uric acid and creatinine (kidney function, Table 5). The present findings also demonstrated that TAG, VLDL-c and total cholesterol were not changed significantly ($P > 0.05$) in all treated groups throughout the experimental period (Table 5). Moreover, the electrolytes balance was not altered in all treated groups as reflected in unchanged ($P>0.05$) values of calcium, phosphorus and magnesium (Table 5).

DISCUSSION

Growth promoters are commonly added to the animals feed for growth enhancement and efficient feed utilization. They are chemical products, antibiotics, enzymes and/or natural extractives. Since the use of chemical products antibiotics might have some unfavorable side effects, therefore researchers tended to use natural additives which meet the requirements of good growth promoting agents [1]. Medicinal plants have received increasing attention as spices for human and additive in diets for animals. However, only few studies have been done on the use of feed additives in fish nutrition and chemistry [3, 31]. Interestingly, dietary supplementation of BTM (5g kgG^{1} diet) for 6 weeks improved performance and blood chemistry of *Mugil Cephalus* with average body weight around 60g/fish [3].

Weak synergistic effect of BTM was observed in the present study reflected on the relative promotion of growth performance whereas feed utilization efficiency remained unchanged significantly. In consistence with our results, there was no significant difference in survival rate and feed conversion ratio in white shrimp (average weight of 0.25g) fed ethanolic extract of Turmeric for 9 weeks [32]. The same authors reported that ethanolic turmeric extract could improve body weight gain when supplemented in White shrimp diet at 15g kgG^{1} which exceeds the dose of turmeric used in our study. Moreover, increased specific growth rate and food conversion ratio in *Labeo rohita* fingerlings fed four different dosages of turmeric at 0.1, 0.5, 1.0 and 5g kgG^{1} feed for 60 days were reported [33]. We did not use the dose of turmeric described above (15g kgG^{1}) because we are interested with the whole seed not with the ethanolic extract. Moreover we gained success with the same dose of the present study in *mugil cephalus* fish [3].

Although, some dietary herbs changed some hematological and biochemical parameters of fish [34], differences in these parameters (TEC, TLC, Hb, PCV, MCV, MCH and MCHC) were not observed in the current study. However, values of the hematological parameters of the control group agree with previous study [35] in the same fish species. In consistence with our results, no significant difference were found in PCV values of Rainbow trout fed diet containing 1 and 2.5% black cumin seed [36]. Furthermore, some previous studies [37, 38] demonstrated that, curcumin never changed the hematological parameters in laboratory animals. In the contrary, the present hematological findings disagree with those demonstrated the positive effect of black cumin seed in Nile tilapia [39], catfish [5], *Mugil Cephalus* [3] and some laboratory animals [40-43] and disagree also with other findings in *Mugil Cephalus* [3], mice [44] and broiler chicken [45] kept on curcumin diet. The present findings disagree also with previous research demonstrating lower hemopiotic values of black cumin seed when its extract was used to inhibit snake venom *in vitro* [46] and when goat administered black seed orally [47]. This confliction might be attributed to different doses, species, age, period of administration and drug combination.

The unchanged values of biochemical parameters (serum total protein, glucose, albumin, globulin, total cholesterol, TAG, VLDL-c) observed in the present study disagree with those obtained previously in *Mugil cephalus* fish fed the same dose of BTM [3]. In addition, previous researches demonstrated the positive effect of black cumin seed on biochemical parameters of Nile tilapia [39], catfish [5] and birds [6, 7]. Improvement of such biochemical parameters was also observed after turmeric supplementation in *mugil cephalus* fish [3], Rohu [33] and rodents [9, 44].

Liver damage was observed after dietary supplementation of *Nigella sativa* extract to rodent [48, 49] and ducklings [6]. This liver damage was not observed in the current study as reflected on unchanged values of ALT, AST and albumin indicated that, the reported black cumin seed toxicity might be reduced by its combination with turmeric as plant mixture. The safety of BTM as fish food additives was extended to involve kidney functions which were not disturbed based on unchanged values of blood urea nitrogen, urea and creatinine recorded in the present study. In consistence with our results, no significant difference among all turmeric treated Rohu, *Labeo rohita* [33]. The present study can conclude that, inclusion of Black cumin seed and Turmeric combination in Asian sea bass (*Lates calcarifer*) diets relatively improved the growth performance and did not affect blood chemistry. Both medicinal plants were safe to liver and kidney at the examined dose as reflected on their biomarkers.

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