

Effect of Dietary supplementation of Green Tea (*Camellia Sinensis*) on Growth, Body Composition and Serum Biochemistry of the Asian Seabass, *Lates calcarifer* Fingerlings

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Abstract

The current study aimed to evaluate the effect of dietary supplementation of different levels of green tea (*Camellia sinensis*) on growth performance, body composition and serum biochemistry of Asian Sea bass, *Lates Calcarifer*. Therefore, Asian sea bass fingerlings with an initial body weight of 43.20 ± 0.11 grams were randomly assigned into 3 groups as triplicates. Fish were tested with three experimental diets of similar protein content (514.8 ± 0.06 g/kg⁻¹) and energy (48.4 ± 0.02 kcal/g) for 90 days. The first diet was treated as control without any inclusion of additives. The other two diets were mixed with green tea of 10 g/kg⁻¹ and 20 g/kg⁻¹ respectively. The results clearly shown a significant ($P < 0.05$) improvement of growth performance in all fish fed with green tea as dietary additive when compared with control, whereas the maximum performance is attained from the first treatment with lower dose (10 g/kg⁻¹) of green tea additive. the current study can concluded that, the dietary inclusion of low levels of green tea as feed additives has relatively improved the growth performance, feed utilization and general health without any adverse effects on biochemical profile of Asian sea bass (*Lates calcarifer*).

Keywords: Green tea; Asian Seabass; Dietary additive; Growth performance; Serum; Biochemistry

Introduction

Over the years, the total world fishery production decreased slightly and the human consumption for aquatic products increased as reported by FAO in 2012 [1]. The reduction in capture fisheries was partly compensated for the fast growth of aquaculture industry worldwide [2]. Research suggests that, aquaculture production should double by 2030 to meet the world's growing demand and needs as reported by FAO in 2014 [3]. On the other hand, high cost of fish feeds and diseases in the fish culture systems are major problems impeding aquaculture development globally [4]. Feed is one of the major inputs in aquaculture fish production and fish feed production technology is one of the least developed sectors in the aquaculture industry particularly in Saudi Arabia. The need for enhanced disease resistance, feed efficiency, and growth performance of cultured organisms is substantial for various sectors of this industry [5].

World Health Organization encourages the use of medicinal herbs as alternate to minimize the use of chemicals through the global trend to go back in to the nature. Recently there has been an increased interest for the application of immune stimulating function of medicinal herbs in aquaculture [6]. Various attempts has proven the positive effects of dietary medicinal plants/herbs as feed additives on growth and feed utilization in the aquaculture production of fishes and crustaceans [7-13].

Green tea (*Camellia sinensis*) is one of the world's most highly consumed beverages, attracted much attention in recent years due to its rich health benefits and biological activities [14,15]. Green tea has been shown to contain various types of catechins, including more than 70% polyphenols, vitamins, nitrogenous compounds, caffeine, inorganic elements, lipids and carbohydrates [16], and interest is continuously growing with regard to green tea's various beneficial health effects. Despite its excellent potential, little studies have thus far evaluated its potential use in the aquaculture practices [17-24]. Therefore we attempted to study the effect of dietary addition of green tea (*Camellia*

sinensis) on growth, body composition and serum biochemistry of a little studied commercially important species Asian seabass (*Lates calcarifer*) commonly called the giant sea perch or barramundi. Asian seabass is a euryhaline species belongs to the Centropomidae (snook) family widely distributed in the Indo-West pacific region and shown considerable potential as a suitable aquaculture candidate worldwide because of its delicately flavored flesh and high market demand. Although it has proven as one of the best candidate for fresh and marine aquaculture practices worldwide few studies have been published concerning its growth/nutritional aspects [17-24]. To the best of our knowledge, it is very hard to find a publication about the usage of green tea as dietary additive on the growth performance and serum biochemistry of Asian seabass fingerlings which is the main objective of the current study.

Material and Methods

Formulation of experimental diets

In order to meet the nutritional requirement of Asian seabass fingerlings [25] three isonitrogenous (514.8 ± 0.06 g/kg⁻¹) and isocaloric

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(48.4 ± 0.02 kcal/g) experimental diets were formulated containing 0.0 g/kg⁻¹, 10 g/kg⁻¹ and 20 g/kg⁻¹ of green tea respectively. The constituents of each diet were thoroughly mixed and blended using a Hobart blender machine to make a paste of each diet. Pelleting of each diet was carried out by passing the blended mixture through a laboratory pellet machine equipped with 2 mm-diameter die. Further the pellets were dried in a hot air oven for 24 h at 60°C and after drying the diets were broken up, sieved and stored in air-tight transparent plastic containers, labeled and stored in refrigerator (40°C) until feeding. The diets were analyzed for the proximate composition (Table 1) as per the standard and official methods by AOAC 1995 [26].

Experimental fish and feeding trial

Fingerlings of the Asian seabass (*Lates calcarifer*) were obtained from the hatchery unit of the of National aquaculture group (NAQUA), Saudi Arabia and transported to the fish culture wet laboratory of the College of Agricultural and Food Sciences of King Faisal University. All fish are stocked in a 3000 liters capacity tanks in order to acclimatize them to the experimental conditions. The fish were fed with a mixture of all tested diets in order to familiarize them to locally formulated feed. Prior to the experiment healthy Asian seabass fingerlings (n=270) with an average initial body weight of 43.20 ± 0.11gm were randomly allocated into 3 treatments groups with replicates (3 × 3 × 30) in a 628 liters circular fiberglass tank. The treatment tanks were supplied with freshwater from a closed circulatory system and aerated continuously using 2 HP air blowers. All the systems are exposed to a photoperiod of 12 h light: 12 h darkness with temperature of 26°C. During the experimental period, all treatment groups were fed with their respective diets at a rate of 3% from their fresh body weight/day in two equal portions, given at 8.00 a.m. and 2.00 p.m. Fish weighed and counted fortnightly and the feeding rate was adjusted according to the new biomass. The fish were not fed on the weighing day.

Treatments			
Ingredients	Control (0g)	10g	20g
Fish meal	481.4	481.4	481.4
Soybean meal	296.6	292.9	289.1
Yellow corn	127.0	120.7	114.5
Fish oil	70	70	70
Green tea powder	0.0	10	20
Premix ²	10	10	10
Dicalcium phosphate	0.5	0.5	0.5
Limestone	0.5	0.5	0.5
Sodium chloride	0.5	0.5	0.5
Total	1000	1000	1000
Proximate analysis			
Dry matter	945.0	928.6	943.2
Crude protein	521.1	519.9	503.4
Ether extract	114.5	113.4	120.1
Crude Fiber	33.7	30.6	35.3
Crude Ash	129.5	129.5	130.9
NFE ⁴	201.3	206.6	210.3
Gross energy ³ (kcal g ⁻¹)	48.5	48.5	48.4

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, 1 and 20 g kg⁻¹) of Green tea powder

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

3. NFE (nitrogen free-extract)

4. GROSS Energy values were according to (NRC, 2011).

Table 1: Composition of the experimental diet (All values are g/ kg⁻¹ unless otherwise specified) containing dietary green tea powder for Asian seabass fingerlings.

Physico-chemical water parameters

Dissolved oxygen, pH, Total ammonia and water temperature were measured by using YSI professional plus multiparameter water quality meter. Water quality parameters throughout the experimental period were maintained as per the optimum range suggested earlier [27].

Calculation of growth and feed utilization indices

Prior to the start and at the end of the experiment, fish of the each treatment tank (n=30) were anesthetized by using 0.1 g/L tricainemethane sulfonate (MS-222; Argent Chemical Laboratories, Redmond, WA, USA) and weighed individually. Following growth and feed utilization, indices were calculated after the experimental period as per the standard methods: Weight gain% = (final weight - initial weight) × 100/initial weight. Daily gain = weight gain/period (days). Specific growth rate (SGR)% = {[ln (final weight) - ln (initial weight)]/ days} × 100 (ln is the natural log). Survival rate % = (number at end - number at start)/number at start × 100. Daily feed intake (DFI) = feed intake × 100/[(initial weight + final weight)/2] × days. Feed efficiency (FE) % = wet weight gain × 100/wet feed intake. Feed conversion ratio (FCR) = feed intake (g)/weight gain (g). Protein efficiency ratio (PER) = weight gain (g)/protein intake (g). Protein productive value (PPV) % = {(retained protein (g)/protein intake (g)) × 100}. Energy efficiency ratio (EER) = weight gain (g)/energy intake (kcal). Energy productive value (EPV) % = {(retained energy (kcal)/energy intake (kcal)) × 100}.

Analytical methods followed

Ingredients of formulated diets, pre-and post-experiment carcass samples were analyzed in triplicate by using standard methods by AOAC in 1995 [26]. Concerning carcass sampling, fish were dried at 70°C for 48-72 h subsequently passed through a meat grinder into one composite homogenate per treatment. All experimental 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 were analyzed for the same parameters except for CF%. The nitrogen free-extract (NFE %) are calculated by difference [100 - (% CP + % EE + % Crude ash + % CF)] g kg⁻¹. CP content (total nitrogen × 6.25) was determined using a BUCHI digestion unit K-435 subsequently distillation and titration was performed using Foss analyze Unit kjeltec 8400 automatic system, while the crude lipid concentrations were estimated by petroleum ether extraction by using a Foss soxtec TM 2043 system. In addition, ash contents were obtained by incinerating samples in a muffle furnace (VULCANTM) at 550°C for 12 h whereas, the dry matter was identified by drying the sample in an oven (Mettler) 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 Scientifica unit.

Serum Biochemical analysis

At the end of the feeding experiments, fish were given anesthesia (0.1 g/L⁻¹ Tricainemethane sulfonate), and the blood samples were collected from the heart using disposable tuberculin syringe [28] without anticoagulant for serum separation as described by Lee et al. [29]. The obtained sera were used for the spectrophotometric determination of the activities of Aspartate Transaminase (AST) and Alanine Transaminase (ALT) [30]. In addition, serum glucose [31], total protein [32], albumin [33], globulin [34], Blood urea nitrogen [35], Uric acid and creatinine [36] were also determined. Furthermore, the collected sera was used for the spectrophotometric analysis of serum triacylglycerol (TAG), total cholesterol by using the enzymatic method of spin react kits [37,38], respectively. 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

This experiment was designed with a completely randomized design (CRD) to test for significant differences in the mean of treatments. The data were expressed as mean \pm standard deviation (SD). The obtained data subjected to one-way ANOVA analysis of variance [39]. Differences among treatment means were determined by Duncan's multiple range tests at a ($P < 0.05$) level of significance.

Results

Growth and feed utilization performance

The results of the growth and feed utilization performance of the Asian Seabass fingerlings (*Lates calcarifer*) fed with various levels of Green tea are demonstrated in the Table 2. The results revealed that, fish group fed with diet containing 10 g/kg⁻¹ of green tea powder exhibits a significant ($P < 0.05$) higher final body weight and daily weight gain as compared with control (0.0 g/kg⁻¹) and 20 g/kg⁻¹ diet. There was no significant difference noticed in the survival rate (%) among the treated fish groups. Regarding the feed utilization efficiency significantly ($P < 0.05$) better DFI, PPV and EPV values were observed in the 0.0 g/kg⁻¹ treatment group as compared with other two treatments. FCR values obtained were not differing much between the treated fishes.

Whole-body chemical composition

The whole fish body composition (Table 3) of Asian seabass fed with experimental diets relatively shown significant improvement in 0.0 g/kg⁻¹ and 10 g/kg⁻¹ treatment whereas the least results were noticed in the fish provided with diets containing 20 g/kg⁻¹ green tea.

Parameters	Treatments		
	Control (0g)	10g	20g
Growth Performance			
Initial weight (g fish ⁻¹)	42.98 \pm 0.50	43.58 \pm 0.59	43.04 \pm 0.50
Final weight (g fish ⁻¹)	^b 100.60 \pm 0.47	^a 111.28 \pm 3.88	^b 100.37 \pm 1.44
weight gain (g fish ⁻¹)	^b 57.63 \pm 0.35	^a 67.70 \pm 3.95	^b 57.69 \pm 1.45
Daily gain (g fish ⁻¹)	^b 0.69 \pm 0.03	^a 0.80 \pm 0.05	^b 0.69 \pm 0.02
SGR (%)	1.01 \pm 0.01	1.11 \pm 0.05	1.01 \pm 0.02
Survival (%)	98.98 \pm 1.11	97.78 \pm 2.22	93.33 \pm 5.09
Feed Utilization			
DFI (g fish ⁻¹)	^{ab} 131.11 \pm 3.42	^a 137.23 \pm 1.20	^b 128.65 \pm 1.93
FCR	2.28 \pm 0.07	2.03 \pm 0.11	2.23 \pm 0.02
FE (%)	41.60 \pm 1.31	45.79 \pm 2.51	42.28 \pm 0.44
Protein Utilization			
PER	0.89 \pm 0.03	1.02 \pm 0.06	0.94 \pm 0.01
PPV(%)	^{ab} 21.10 \pm 0.32	^a 22.71 \pm 0.69	^b 20.01 \pm 0.31
Energy utilization			
EER	9.68 \pm 0.31	10.97 \pm 0.60	9.83 \pm 0.10
EPV(%)	^{ab} 21.30 \pm 0.32	^a 23.91 \pm 1.62	^b 19.59 \pm 0.82

* values are mean \pm standard error (SE) of three replicates for each treatment.
*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 2: Growth performance and feed utilization of Asian seabass fed with diet containing various levels of green tea powder.

Parameters	Treatments		
	Control (0g)	10g	20g
Dry matter	^a 306.74 \pm 6.40	^a 303.85 \pm 3.56	^b 284.83 \pm 3.86
Crude protein	^a 190.38 \pm 2.08	^{ab} 185.91 \pm 4.08	^b 176.86 \pm 1.05
Ether extract	66.72 \pm 4.10	72.30 \pm 8.30	62.00 \pm 4.17
Crude Ash	^a 49.64 \pm 0.59	^b 45.64 \pm 1.37	^b 45.97 \pm 0.93
Gross energy (kcal/g) ¹	17.04 \pm 0.49	17.31 \pm 0.57	15.83 \pm 0.43

Values are mean \pm standard error (SE) of three replicates for each treatment. Gross Energy values were calculated for protein, lipid and carbohydrates according to (NRC, 2011).

Table 3: Whole-body chemical composition of Asian seabass fish fed the experimental diets containing various levels of green tea powder as feed additives.

Parameters	Treatments		
	Control (0g)	10g	20g
Glucose (mg/dl)	13.1 \pm 0.8	14.0 \pm 1.9	13.3 \pm 1.9
Total Protein (g/l)	4.3 \pm 0.1	4.5 \pm 0.1	4.8 \pm 0.1
Albumin (g/l)	1.6 \pm 0.1	1.7 \pm 0.1	1.5 \pm 0.1
Globulin (g/l)	2.7 \pm 0.3	2.8 \pm 0.1	3.3 \pm 0.3
A/G ratio ¹	0.6 \pm 0.1	0.6 \pm 0.1	0.5 \pm 0.1
Total cholesterol (m g/dl)	230.3 \pm 5.3	236.2 \pm 5.2	231.7 \pm 5.0
ALT (U/l) ²	12.5 \pm 0.3	12.0 \pm 0.2	10.0 \pm 2.2
AST (U/l) ³	29.2 \pm 1.1	30.8 \pm 1.1	30.2 \pm 1.2
ALP (U/l) ⁴	18.0 \pm 2.2	16.2 \pm 2.4	17.0 \pm 2.4
Bilirubin (mg/dl)	0.1 \pm 0.02	0.1 \pm 0.02	0.1 \pm 0.02
BUN (mg/dl) ⁵	1.7 \pm 0.1	1.8 \pm 0.2	1.7 \pm 0.1
Uric acid (mg/dl)	1.6 \pm 0.4	1.5 \pm 0.1	1.9 \pm 0.1
Calcium (mg/dl)	11.9 \pm 1.2	12.5 \pm 1.0	12.8 \pm 1.0
Phosphorus (mg/dl)	10.8 \pm 3.0	9.8 \pm 2.2	13.8 \pm 3.2
Magnesium (mg/dl)	1.2 \pm 0.3	1.0 \pm 0.2	1.0 \pm 0.2
Chloride (mEq/l)	173.6 \pm 5.2	171.7 \pm 2.9	183.8 \pm 6.9

Values are mean \pm standard error (SE) of three replicates for each treatment. A/G ratio: Albumin/globulin ratio; AST: Aspartate Transaminase; ALT: Alanine Transaminase; ALP: Alkaline phosphatase; BUN: Blood urea nitrogen

Table 4: Effect of different levels of green tea powder on Serum biochemical parameters of in Asian seabass.

The highest protein content value was obtained at 0.0 g/kg⁻¹ (190.38 \pm 2.08) and lipid value at 10 g/kg⁻¹ (72.30 \pm 8.30) group, meanwhile fish fed the 20 g/kg⁻¹ diet exhibited low crude protein and fat contents (176.86 \pm 1.05 and 62.00 \pm 4.17), respectively. Ash content found to be significantly ($P > 0.05$) lower in fish fed with green tea diets of 10 g/kg⁻¹ and 20 g/kg⁻¹ as compared with control diet (0.0 g/kg⁻¹).

Serum biochemistry

The results of the biochemical analysis of the fish serum samples by spectrophotometric methods are presented in the (Table 4). The overall results revealed that, throughout the experimental period the values of biochemical parameters namely glucose, total protein, albumin, globulin and their ratio have not varied significantly between the treatments. Also, liver enzyme (ALT and AST) activities, blood urea nitrogen, uric acid and creatinine levels were not changed among the treatments. Furthermore, the TAG and total cholesterol levels were normal and not differ significantly ($P > 0.05$) in all treated groups during the experimental period. In addition, the values of calcium, phosphorus and magnesium levels were not altered significantly ($P > 0.05$) between the treated groups.

Discussion

The use of herbs/medicinal plants as alternative solution to substitute chemicals and antibiotics used in aquaculture has been a topic of discussion and active researches worldwide. The result

of the present study revealed that, the dietary inclusion of green tea has relatively improved the growth performance and feed utilization however significantly ($P>0.05$) better growth performance (final body weight and daily gain) and feed utilization levels (DFI, PPV% and EPV%) observed in the diet comprise of lower dose of green tea powder (10 g/kg^{-1}). The results agree with the findings of Abdel-Tawwab et al. [21]. They [21] have observed a growth-promoting influence of green tea on Nile Tilapia and reported that the optimum growth and feed utilization were attained at inclusion of lower dose of 0.5 g/kg^{-1} green tea diet. They [21] explained that, the improved fish growth and feed utilization may be because of the palatability or attractiveness of the diets, which in turn cause increased feed intake and enhanced fish performance. In addition, they [21] specified that, green tea may play an inhibiting role with the potential pathogens in the digestive tract, may enhance the population of beneficial microorganisms, and/or may enhance the microbial enzyme activity that consequently improves the feed digestibility and nutrient absorption. Similar observations [28] reported that, green tea extract relatively improved the growth performance in juvenile black rockfish (*Sebastes schlegeli*) and they [28] concluded that, the dietary inclusion of green tea extract could have enhanced the nutrients digestibility in fish leads to improved nutrient utilization. In addition, dietary inclusion of green tea extract at lower levels results improved growth performance and feed utilization in freshwater prawn (*Macrobrachium rosenbergii*) [24]. On the contrarily, few studies [18,40] reported no significant effects of green tea as dietary additives in fish growth performance. This may be due to the variation in the inclusion level of green tea dose or difference in the fish species or experimental conditions.

The survival rate (%) was not significantly affected among the dietary treatments during the experimental period. This is probably due to the extreme cannibalistic nature of Asian sea bass in the fingerling stage. Similar observation [41] reported that dietary supplementation of two levels (5 and 10 g/kg^{-1} diet) of black seed turmeric mixture (BTM) has not affected the mortality rate of Asian sea bass in the fingerlings. Result also agrees with the earlier findings [21] which stated that, survival rate of juvenile Nile tilapia were not significantly affected by dietary inclusion of green tea leaves. Furthermore, previous results [42] explained that, the dietary inclusion of black cumin seeds, green tea and propolis extracts were not significantly affected the survival rate of the Nile tilapia.

The whole body composition particularly protein and fat level were significantly higher in 10 g/kg^{-1} green diet which is close to the control than other treatment, which in turn suggested that, the addition of dietary green tea in lower dose play a role in enhancing feed intake with a subsequent improvement of the nutrient deposition in fish body. The dietary effect of green tea in Nile tilapia induced similar changes in protein and lipid contents in fish body which might be linked with changes in their synthesis, deposition rate in muscle and/or different growth rate [21]. Similar observation made [42] has been detected in Nile tilapia. They [42] found higher protein and fat levels at the inclusion of lower dose of 0.5% of green tea and subsequent inclusion results in the decline of its levels. It indicates that, the higher level of dietary inclusion of green tea may effective in lowering the fat content of the fish. The current findings disagree with previous findings [41] reported that, there is no significant changes in the whole body composition of seabass fingerlings while treated with the 10 g/kg^{-1} of black cumin seed and turmeric mixture (BTM).

Biochemical parameters in serum show the nutritional health status of the fish [43]. The current findings indicated that, there were

no significant changes observed in the serum biochemical parameters of total protein, glucose, albumin, globulin, total cholesterol and TAG among the treated fish. This might indicated that, the inclusion level of dietary green tea did not affect the health condition of the fish. This finding agree with previous findings [19,41] and disagree with other findings [21] which reported that, fish fed green enriched diets had higher levels of biochemical parameters as compared to control fish. Likewise other report [44] reported that plasma total protein level in rainbow trout increased significantly after feeding the fish with various herbal extracts. Liver and Kidney function found to be normal in the present study as reflected in the unchanged values of ALT, AST albumin (in case of liver) and blood urea nitrogen, urea and creatinine (in the Kidney instance). Similar observation [21] has been reported in Nile tilapia during studying the effect of green tea as dietary additive. In addition, previous results [41] illustrated in their report that, the inclusion of black seed and turmeric mixture (BTM) in Asian sea bass (*Lates calcarifer*) diets relatively improved the growth performance and did not affect Liver and kidney functions. This is in agreement with the findings in the present study and other previous reports [45,46].

Conclusion

The results of the present study indicated that dietary inclusion of green tea powder especially in lower (10 g/kg^{-1}) dose have the potential to improve growth performance and feed utilization without any adverse effect in the serum biochemistry of seabass fingerlings.

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