Effect of Sangrovit® on the Growth and Performance of Sea Bass (*Dicentrarchus labrax* L., 1758)

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ABSTRACT

In this experiment, 540 juvenile sea bass (*Dicentrarchus labrax* L., 1758) (average initial weight = 17.03±0,43 g) were fed diets containing the active ingredients of Sangrovit® – the Benzo[c]phenanthridine and Protopin alkaloids (QBA/PA) – which have been shown to improve feed intake, increase the secretion of digestive enzymes, enhance feed digestibility and availability, improve feed conversion and liver function, and provide antibacterial and anti-inflammatory benefits. In this study, two levels of Sangrovit® were added to a commercial sea bass fry feed, which also served as the control diet (Group A: 0 ppm Sangrovit®). Group B received 500 g Sangrovit® premix (1:10 dilution) per ton of commercial feed, which was equivalent to 50 ppm Sangrovit®. Group C received 1000 g Sangrovit® premix (1:10 dilution) per ton of commercial feed, which provided 100 ppm of Sangrovit®. Nine (9) cylindrical-conical polyester tanks (300 litre capacity) were used in this study. Sixty five (65) fish were placed in each tank, and there were three (3) replicates per treatment. The trial was conducted over ninety (90) days, and all fish were fed at the same rate for the duration of the experiment. Growth performance, feed conversion ratio (FCR), specific growth rate (SGR), condition factor (CF), hepatosomatic index (HSI), and vicserosomatic index (VSI) were all recorded. ANOVA was used to assess variance within and among treatment groups and repetitions, and differences between initial and final measured values were assessed using the t-test. Due to a lack of homogeneity among groups, data were analyzed using the Kruskal-Willis test. Final average body weights for Groups A, B and C were 49.907±1.28 g, 55.243±1.03 g, and 62.217±1.35 g, respectively. Group A and Group B final average body weights were not significantly different ($p>0.05$), but the final average body weight of the fish in Group C was significantly greater ($p<0.05$) than was the final average body weight of fish in Group A.

Key Words: Sea bass, *Dicentrarchus labrax*, Sangrovit®, feeding, growth performance

1. INTRODUCTION

In recent years, aquaculture has gained in importance as a renewable source of dietary protein and as a viable commerical activity. To maintain this position in the future and to continue to provide a good investment opportunity, the problems that the sector currently faces must be addressed. One of the more important of these concerns is the cost of feed, which is estimated to be 50-60% of the total cost of production. Numerous studies on the use of different feed formulations, feed ingredients and feeding techniques have been conducted (Kaushik et al.,2004; Thiessen et al.,2003; Martinez et al., 2004; Enes et al.,2006; Izquierdo et al.,2003). These studies have included assessments of various alternative raw materials, vitamins and minerals, monitoring the amount of feed provided to the fish, and the addition of pigments and other feed additives to the diet. Especially, various feed additives with growth promoting properties came into prominence in these

studies (Francis et al., 2005; Haroun et al., 2006; Abdel-Tawwab et al., 2008; Lara-Flores et al.,2003; Li and Gatlin, 2004). Growth promoting feed additives may contain different ingredients as plant extracts, organic acids, probiotics, hormones etc. The benzo[*c*]phenanthridine and protopin alkaloids (QBA/PA) extracted from plants are known to have antimicrobial, anti-inflammatory, and immune-modulatory effects (Vieira et al., 2008; Rawling et al., 2009). These alkaloids include sanguinarine, chelerythrine, allocryptopine and Protopin.
The commercial product Sangrovit®, an organic and plant-based material containing benzo[*c*]phenanthridine and protopin alkaloids (QBA/PA), increases feed intake in various animal categories such as swine and poultry and may stimulate digestive enzyme secretion, which would improve feed digestibility, nutrient availability and thereby feed conversion.
In the present study, the effect of Sangrovit® on growth, feed utilization and liver and visceral fat reduction of sea bass, was investigated.

2. MATERIALS AND METHODS

This work was conducted at the Aegean University Faculty of Fisheries hatchery facilities in Urla-Iskele in Turkey. Sea bass fry (average live weight = 17.03 ± 0.43 g) were placed in nine (9) 300 litre cylindrical-conical polyester tanks (Figure 1). In this study, 585 sea bass in total were used. Sixty five (65) fish were placed in each tank, and there were three replications per treatment. The experiment was conducted during the month of March, April and May 2010, for a total of 90 days. The hatchery water was obtained directly from the sea by passing it through sand filters in an open system, without the use of any heating apparatus. Water temperature was between 14.3 ±0.18 and 16.49 ±0.170C, dissolved oxygen was between 7.43±0.02 and 6.37±0.05 mg l-1. The fish were fed three times a day at a rate of 0.7% - 1.1% of total live weight, depending on the water temperature during the experiment.

Figure 1: Experimental tanks

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Three experimental diets were formulated (44% crude protein, 16% fat, 12% ash, and 3470 Cal/kg diet) (Table 1) to contain different levels of Sangrovit® premix (1:10 dilution) (ANC Animal Nutrition Center, Phytobiotics Futterzusatzstoffe GmbH), which was supplemented at 0.0 (control Group A), 50 ppm (Group B) and 100 ppm (Group C) in the diet. The control and treatment group diets were formulated as 2 mm extruded pellets by Agromarin Feed Factory in Turkey. The nutrient content of this pellet is described at below:

Table 1: Formulation of experimental diets

Raw Materials	Group A	Group B	Group C
Herring meal*	260	260	260
Anchovy meal**	180	180	180
Fish oil*	127	127	127
Soybean meal***	217.97	217.47	216.97
Corn gluten 60% CP	30	30	30
Wheat gluten	10	10	10
Wheat meal	165	165	165
Vitamin/mineral Premix	10	10	10
Methionine and Lysine	0.03	0.03	0.03
Sangrovit® (ppm)	0	50	100
Moisture max	12	12	12
Crude ash max	12	12	12
Crude protein min	44	44	44
Crude fat min	16	16	16
Starch max	10	10	10
Metabolic energy Kcal/kg	3470	3470	3470
Crude fibre max	2.5	2.5	2.5

*65,5% CP, Peru
**71% CP, North of Turkey
***44% CP, ASA, USA
aProvided per kg of diet: 15 mg of vitamin A (500,000 IU/g); 15 mg of vitamin D3 (100,000 IU/g); 60 mg of vitamin E (500 IU/g); 2.5 mg of vitamin K; 7.5 mg of thiamin; 15 mg of riboflavin; 7.5 mg of pyridoxine; 87.5 mg of nicotinic acid; 2.5 mg of folic acid; 25 mg of vitamin B12 (1,000 mg/kg); .5 g of inositol; 62.5 mg of biotin (2%); 25 mg of calcium pantothenate; 2 g of choline (50%).

The diets were prepared under special conditions, and imported Sangrovit® was added to the experimental feeds after being dissolved in fish oil. Biometric measurements for growth performance (body weight, total length, fork length) were obtained at the beginning of the study, and this process was repeated every 30 days. Fish body weight was determined using a 0.001 g precision scale, and body length was measured using a 30 cm ruler. Growth performance and feed utilization were assessed by net weight gain (NWG), feed conversion ratio (FCR), spezific growth rate (SGR) and condition factor (CF). Calculations of this formulations were made as follows:

FCR = feed intake/ weight gain (Barrias and Oliva-Teles, 2000)
SGR = (ln Final Weight/ ln İnitial Weight)/ days (Barrias and Oliva-Teles, 2000)
CF = Final Weight/(Final Length)

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3 Fish were anesthetized with a phenoxy-phenolic compound. Also, at the beginning and at the end of the experiment, five (5) fish from each tank were dissected to obtain their internal organs, liver weights were recorded, and the viscerosomatic (VSI) and 4 hepatosomatic (HSI) indexes were then calculated. Calculations were made using the following formulae (Metailler, 1986; Kaushik, 1998; Martinez and Vasquez, 2001; Hoşsu et al., 2003; Cheng et al., 2005; Korkut et al., 2007):

HSI= Liver Weight/Body Weight x 100
VSI= Viscera Weight/ Body Weight x100

ANOVA was used to assess variance within and among treatment groups and repetitions, and differences between initial and final measured values were assessed using the t-test. Due to a lack of homogeneity among groups, data were analyzed using the Kruskal-Willis test. Statistical analysis was conducted using SPSS 09.01 for Windows.

3. RESULTS AND DISCUSSIONS

The water parameters reflected the natural water conditions in the location where the study was conducted. They were characteristic spring semester conditions, and this environment had no negative impact on fish development or behavior, their feeding pattern, and or on the level of stress that they were subjected to.
During the study period, fish average live weight and live weight gain for all treatment groups increased incrementally (Table 2). Final average body weights for Groups A, B and C were 49.907±1.28 g, 55.243±1.03 g, and 62.217±1.35 g, respectively. Group A and Group B final average body weights were not significantly different ($p>0.05$), but the final average body weight of the fish in Group C was significantly greater ($p<0.05$) than was the final average body weight of fish in Group A. Mortality during the experiment was 20%, 19.4% and 20% for Groups A, B and C, respectively (Table 2). The values for FCR, SGR, VSI, HSI and CF are listed in Table 2, and although incremental trends are evident for each parameter based on Sangrovit® content, there were no significant differences ($p>0.05$) among treatment groups, except for the SGR in Group C (SGR 0.74), which was elevated relative to Group A (control) (SGR 0.67) ($p<0.05$).

Table 2: Growth Performance Parameters for Experimental Groups

Parameters	Group A (Control)	Group B (50 ppm)	Group C (100 ppm)
Initial number of fish	180	180	180
Initial Average Live Weight (g)	17.027±0,36	17.037±0,44	17.027±0,49
Final Average Live Weight (g)	49.907±1.28^{a}	55.243±1.03^{ab}	62.217±1.35^{b}
Live Weight Gain (g)	32.88±0.41^{a}	38.21±0.83^{ab}	45.19±1.18^{b}
Mortality (number of dead fish)	36	35	36
FCR	1.29	1.27	1.26
SGR	0.67^{a}	0.71^{b}	0.74^{b}
VSI	6.84	6.81	6.69
HSI	1.79	1.73	1.67
CF	0.92	1.01	1.14

Values expressed as means ± standard deviation
^{ab}Significant differences between groups are indicated by difference in superscript letters.

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There have been few Sangrovit® studies conducted using aquatic species, but Rawling et al., 2009 reported on the effect of Sangrovit® in red tilapia (*O. niloticus*). Fish were fed equal amounts of diets containing various proportions of Sangrovit® for 60 days: 25 mg/kg (Diet 25S), 50 mg/kg (Diet 50S) 75 mg/kg (Diet 75S) and 100 mg/kg (Diet 100S), and growth, performance and health status were subsequently monitored.
The Sangrovit®-fed fish gained significantly more weight (71.85±8.98, 67.85±3.32, 66.80±1.98, 67.70±8.06 respectively) than control fish (51.00±1.84). SGR was significantly improved in Sangrovit®-fed fish (4.05±0.20, 3.98±0.08, 3.94±0.05, 3.96±0.18 respectively) versus control fish (3.54±0.06).
Similarly, we have shown here that sea bass, when fed 100 ppm Sangrovit® for 90 days, exhibit a significant improvement in body weight gain over fish that receive no Sangrovit® in the diet. The values for FCR for all groups were similar, but fish growth, body weight gain and SGR for fish in Group C (100 ppm) were significantly different from the control group. These data suggest that the application of Sangrovit® to the diet of sea bass from the fry stage through to harvest can contribute to low mortality, a good FCR, and improved growth and performance relative to fish that do not consume Sangrovit®. However, studies on commercial farms (soil pools, net cages, etc.) may provide different results, due to the varying environmental and feeding conditions that would be encountered.

In conclusion, recent increases in raw material prices have made it necessary to find alternative feed ingredients and feed additives that will help to reduce the overall cost of the rations. Sangrovit® has been shown here to have a positive impact on the growth and performance of sea bass, warranting its inclusion in the feeding program of this economically important species.

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