

# Effects of GroBiotic®-A supplementation on growth performance, body composition and liver and intestine histological changes in European Seabass (*Dicentrarchus labrax*) juveniles

## Grobiyotik A ilavesinin levrek (*Dicentrarchus labrax*) juvenillerinde büyüme performansı, vücut kompozisyonu, karaciğer ve bağırsak histolojik değişimleri üzerine etkileri

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**Abstract:** The effects of GroBiotic®-A supplementation on growth performance, body composition, liver and intestine histology in European seabass (*Dicentrarchus labrax*) juveniles were evaluated. The commercial GroBiotic®-A was added to diets at four different levels (0, 1, 2 and 3%), three replicates and fed 4 times a day (9:00, 11:30, 14:00, 16: 30 hours) for 60 days as ad libitum. Total 480 European seabass juveniles with a starting weight of 1.40±0.08 g were randomly stocked into 12 tanks with a volume of 1 m<sup>3</sup>. At the end of the study, the changes observed in weight, feed conversion ratios (FCR) and survival rates were calculated as 6.69 ± 5.35-7.40 ± 5.47, 0.80 ± 0.18-0.88 ± 0.20 and 96.6 ± 1.51-100 ± 0.0, respectively. When the body composition of the control and treatment groups were compared, no statistically significant differences were observed between the protein and lipid values (p>0.05), except ash (p <0.05). Histological sections of intestinal tissue; the number of goblet cells was higher than that of the control group. The highest values were determined in the group supplemented 2% GroBiotic®-A. The highest microvillus length was found in the group added 1% GroBiotic®-A. It was detected an inverse relationship between microvillus length and contribution rates as the the GroBiotic®-A additive levels increased. Also, degeneration and necrosis was detected in hepatocyte cells of seabass juvenile fed with diets supplemented 2% and 3% GroBiotic®-A as well as increase of the number of fatty vacuoles in liver tissue due to the increase in the amount of GroBiotic®-A. In conclusion, when the growth parameters, body composition and histological data were evaluated together, the feeding group supplemented 1% GroBiotic®-A performed the best.

**Keywords:** GroBiotic®-A, European seabass, growth, histology, aquaculture

**Öz:** Ticari bir prebiyotik olan Grobiyotik-A ilavesinin levrek (*Dicentrarchus labrax*) juvenillerinde büyüme performansı, vücut kompozisyonu, karaciğer ve bağırsak histolojik değişimleri üzerine etkileri değerlendirilmiştir. Grobiyotik-A, dört farklı seviyede (%0, 1, 2 ve 3) yemlere eklenmiştir ve günde 4 kere (9:00, 11:30, 14:00, 16: 30 saatlerinde) doyana kadar 60 gün boyunca besleme yapılmıştır. Çalışma 3 tekrarlı olarak yürütüldü. Başlangıç ağırlıkları 1,40±0,08 g olan kırk levrek rastgele 1 m<sup>3</sup> hacimli 12 tanka stoklandı. Çalışma sonunda ağırlıkta gözlenen değişiklikler, yem dönüşüm oranları (FCR) ve yaşama oranları sırasıyla 6,69 ± 5,35-7,40 ± 5,47, 0,80 ± 0,18-0,88 ± 0,20 ve 96,6 ± 1,51-100 ± 0,0 olarak ölçülmüştür. Kontrol ve deneme gruplarının vücut kompozisyonları karşılaştırıldığında protein ve lipid değerleri arasında istatistiksel olarak anlamlı bir fark gözlenmezken, kül değerlerinde gözlenmiştir (p <0,05). Bağırsak dokusunun histolojik kesitlerinde; goblet hücrelerinin sayısı kontrol grubuna göre daha yüksek bulunmuştur. En yüksek değerler %2 Grobiyotik-A eklenen grupta tespit edilmiştir. Mikrovillus uzunluğu, en yüksek %1 Grobiyotik-A eklenmiş grupta bulunmuştur. Grobiyotik-A katkı miktarı arttıkça, mikrovillus uzunluğu ile katkı oranları arasında ters bir ilişki olduğu tespit edilmiştir. İlave olarak, %2 ve %3 yemle beslenen balıkların hepatosit hücrelerinde dejenerasyon ve nekrozun yanı sıra, Grobiyotik-A miktarındaki artış nedeniyle karaciğer dokusunda yağ vakuol sayısında artış tespit edilmiştir. Sonuç olarak, büyüme parametreleri, vücut kompozisyonu ve histolojik veriler birlikte değerlendirildiğinde, %1 Grobiyotik-A takviyesi deneme sonunda en iyi büyüme performansını göstermiştir.

**Anahtar kelimeler:** Grobiyotik A, levrek, büyüme, histoloji, prebiyotik

## INTRODUCTION

Aquaculture is the fastest growing animal farming sector in the last 30 years, providing food to the world and contributing increasingly to sustainable economic growth

(Bjørndal et al., 2019). In commercial facilities, the need to produce more in the culture system leads to undesirable consequences for the fish, which weakens the immune

system of the fish and eventually leads to disease outbreaks (Kurt et al., 2019). In commercial aquaculture, different antibiotics were used together with feeds for the prevention and treatment of bacterial diseases of aquatic animals (Vechklang et al., 2012). The use of uncontrolled and excessive antibiotics in aquaculture to prevent or treat bacterial diseases can lead to the development of bacterial resistant strains that may be a threat to the environment and human (Mancuso, 2019). The use of antibiotics extensively in animal production as growth promoters is banned in EU countries. Subsequently, various measures have been taken to reduce or even stop antibiotic use in aquaculture (Yazici, 2017; Mancuso, 2019).

In this regard, meeting the requirements of environmentally friendly aquaculture according to consumer demand and food safety several functional feed additives such as prebiotics, probiotics, plant extracts, immunostimulants etc. as alternative to antibiotics have been used to improve growth performance and animal health (Suzer et al., 2008; Dimitroglou et al., 2009; Vechklang et al., 2012; Yu et al., 2019). The main aims of commercial aquaculture are to increase the growth of culture organisms and to control the diseases that may occur (Adel et al., 2016). Proper nutrition has long been recognized as a vital factor in promoting normal growth and maintaining health of fish. Prepared diets provide essential nutrients necessary for normal physiological functionality, as well as other components that may protect their health (Li & Gatlin, 2004; Adel et al., 2016).

Prebiotics are defined as indigestible food components that beneficially affect the host by stimulating growth or activity of a limited number of health-promoting bacteria in the intestine while potentially limiting pathogenic bacteria (Ringø et al., 2010). Torrecillas et al., (2011) showed that prebiotics can improve feed utilization and growth positively in many different fish species. The researches of prebiotics in finfish and crustacean have mainly focused on: the effects of growth performance, feed conversion, gut microbiota, gut and liver histology, resistance against pathogenic bacteria and innate immune parameters (Ringø et al., 2010; Yu et al., 2019).

Mannan oligosaccharide (MOS) as a prebiotic has been shown to increase nutrient absorption by increasing villus height and number in the intestine, and some benefits in improving health by maintaining intestinal integrity (Dimitroglou et al., 2009). Another prebiotic used in aquaculture is also commercial GroBiotic®-A that contains a combination of partially autolyzed brewer's yeast, dairy components, and dried fermentation products (Li & Gatlin, 2004; Adel et al., 2016). The benefits of this GroBiotic®-A prebiotic have been reported in many fish to promote growth, food intake, survival, improve the immune system, and disease resistance (Li & Gatlin, 2005; Burr et al., 2009; Buentello et al., 2010; Zheng et al., 2011; Adel et al., 2016).

In recent years, European seabass (*Dicentrarchus labrax*) has become one of the most cultivated and valuable commercial fish in Mediterranean aquaculture (Carbone and

Faggio, 2016). European seabass market size was valued at \$1082 million in the world 2016. In the last decade, Turkey has a 43% share of world production in 2016 European seabass. This is followed by Greece (23%), Egypt (13%), Spain (12%) and Italy (4%) (Bjørndal et al., 2019). In addition, 37% of total cultured fish production in Turkey was provided from European seabass. Seabass is a species with high tolerance and high growth potential against water quality parameters. However, it is very sensitive to some stress factors caused great losses under aquaculture conditions (Carbone and Faggio, 2016). Studies on the effects of prebiotics have been limited to Mannan Oligosaccharides (MOS), Fructo Oligosaccharides (FOS), Short Chain Fructo Oligosaccharides (ScFOS) and Xylo Oligosaccharides (XOS) prebiotics (Guerreiro et al., 2015; Guerreiro et al., 2017; Yazici, 2017). Although many studies have investigated the documented benefits of GroBiotic®-A on different fish species by adding various ratios, there was no study investigating the effect of GroBiotic®-A on economically important European seabass. Hence, the aim of present study was to reveal the effects of GroBiotic®-A on growth parameters, body composition, intestine, and liver histology in European seabass.

## MATERIALS AND METHODS

In a total of 600 (0.2-0.3± 0.08g) seabass fry were obtained from a commercial fish farm (Kılıç Seafood Corporation) at Muğla, Turkey. Before starting the study, they were kept in two circular tanks with a volume of 1 m<sup>3</sup> and fish at Marine Science and Technology Faculty, Aquaculture Research Facilities at Iskenderun Technical University were fed a commercial feed (Kılıç Seafood Corporation) with 63.78 % crude protein and 9.78% crude lipid for 4 weeks.

Experiments were conducted in 1m<sup>3</sup> cylindrical fiberglass tanks (n =12). A 20% water exchanged of the each tank was performed daily using filtered seawater. Forty European sea bass (*Dicentrarchus labrax*) juvenile (mean±standard deviation) body weight 1.43±0.08 g per fish were randomly stocked into 12, 1m<sup>3</sup> cylindrical fiberglass tanks filled with 0.8 m<sup>3</sup> of filtered seawater (40 fish/tank). Each treatment tank was supplied with aeration by using a 0.55-Greenco blower (Greenco, Model 7RB 310-7AA01, Zeguo Wenling Zhejiang, China) and air stones. Siphoning was carried out daily in the tanks with its own water inlet and outlet. Photoperiod application was set to 12 hours light and 12 hours dark. Abiotic measurements such as dissolved oxygen (DO, mg/L), temperature (°C), salinity (g/L) and pH were measured daily with a multifunction oxygen meter (YSI, Model Y85). DO, water temperature, salinity and pH were determined as 4.45±0.55mg/L, 25.75±1.25°C, 35.65±0.34g/L and 7.85±0.15, respectively.

### Experimental diets and feeding

The prebiotic used in the study is commercially known as GroBiotic®-A (International Ingredient Corporation, St Louis, MO, USA) consisting of partially autolyzed brewer's yeast, dairy ingredient components and dried fermentation products (Table 1).

**Table 1.** GroBiotic®-A product analysis (International Ingredient Corporation, St Louis, MO, USA)

Proximate composition	Percent (%) value
Crude Protein	30.0-32%
Crude Fat	0.1-2%
Crude Fiber	2-3.0%
Carbohydrate	53.0%
Ash	6.0%
Moisture	5.0%
ME (calculated)	3,580 kcal/kg

Experimental design was arranged in triplicate by 4\*3 factorial. All diets were prepared at the same time and kept in sterile plastic bags at 4°C until used. The commercial Grobiotic-A was added to diets at four different levels as a control 0, 1 (GBA1), 2 (GBA2) and 3 (GBA3) %, and fed 4 times a day (9:00, 11:30, 14:00, 16: 30 hours) for 60 days as *ad libitum*. The size and amount of diets offered the fish according to growth performance of experimental groups was readjusted every 15 days (Table 2).

Commercial feeds were placed into the mixer chamber of Alphie1 (Hexagon Product Development Pvt. Ltd. India) with GroBiotic®-A 3-D mixing feature and 25 min (1000 µ), 20 min (1200µ), 15 min. min (1500 µ) at 80 rpm with stirring. Feed sizes were adjusted according to fish measurements in 20-day periods. Alphie1 used in the study, mixing at low speed, the integrity of the feed was not disturbed, and because of the multi-dimensional mixing feature, it was ensured that GroBiotic®-A was added to the feeds homogeneously. Prepared feeds were stored at +4°C until used in plastic containers.

### Growth parameters and proximate composition

#### Sampling strategies

Fish were weighed at the beginning and end of the trial, and survival was monitored daily. No feed was given 24 hr prior to weighing and sampling the fish. Fish were anesthetized with clove oil (5mg/L). The growth performance parameters of the fish were carried out on day 0<sup>th</sup>, 20<sup>th</sup>, 40<sup>th</sup> and 60<sup>th</sup>. The following formulas were used to calculate the growth parameters and feed consumption of fish: final weight (FW, g), weight gain (WG, g) = (final weight – initial weight), specific growth rate (SGR, % day<sup>-1</sup>) = (ln final weight – ln initial weight)/times (days) × 100, weight gain (WG, %) =

[(final weight – initial weight)/initial weight] × 100, feed conversion ratio (FCR) = weight gain/feed intake and survival (%) = (final animal × 100)/ initial animal (González-Félix et al., 2018).

#### Proximate analysis of experimental fish and feed

At the end of the experiment, standard AOAC (1997) procedures were used for the crude protein content of fish carcass samples and experimental feeds from each treatment group, Bligh and Dyer (1959) method for crude lipid content, and Vollenweider et al., (2011) method for raw ash content. Proximate analysis of fish and experimental feeds were performed in triplicate.

#### Histological analysis

At the end of the study, five fish randomly selected from each experimental group were autopsied and tissue samples taken from the digestive tract and liver were fixed in 10% phosphate buffered formaldehyde. After fixation, the manually processed tissue samples were coated with embedding material and embedded in paraffin blocks. 4-5 µm thick tissue samples were stained with hematoxylen-eosin (HE) staining method and examined under light microscope (Bullock, 1978).

#### Statistical analysis

SPSS package program was used in statistical calculations. The homogeneity of the variances was tested before comparisons between treatment groups were made. One-way ANOVA was used for statistical comparisons among the treatment groups and then the mean and standart deviation (±SD) of initial weight, weight gain, SGR, FCR, and survival of different levels of GroBiotic-A on growth performance of European seabass was compared with Duncan's multiple comparison tests to compute the 95% confidence interval.

## RESULTS

### Growth performance

At the end of the study, it was observed that weight gain, feed rate, specific growth rate and survival rates were statistically similar and there were no significant differences among the treatments groups ( $p > 0.05$ ) (Table 2).

**Table 2.** Mean and standart deviation (±SD) of initial weight, weight gain, SGR, FCR, and survival of different levels of GroBiotic®-A on growth performance of European seabass fry (*Dicentrarchus labrax*)

Parameter	Treatment Groups			
	Control	GBA1	GBA2	GBA3
Initial weight (g)	1.40±0.07	1.46±0.08	1.41±0.09	1.45±0.12
Final weight (g)	14.75±0.69	15.12±0.59	14.08±1.16	14.08±0.73
Weight gain (g)	13.35±0.76	13.66±0.60	12.67±1.09	12.64±0.66
Weight gain (%)	952.48	933.19	896.52	873.56
Feed Conversion Ratio (FCR %)	0.80±0.18	0.82±0.18	0.86±0.26	0.88±0.20
Specific Growth Rate (SGR %)	3.92±0.77	3.89±0.60	3.83±1.09	3.79±0.67
Survival Rate (SR %)	97.5	100	96.6	98.5

### Biochemical composition of fish

At the end of the study, ten fish randomly sampled and pooled for biochemical composition. When the body composition of the control and treatment groups were compared, the differences between protein and lipid values were not statistically significant ( $p > 0.05$ ), except ash ( $p < 0.05$ ) (Table 3). The highest protein, lipid and ash values were found as  $24.28 \pm 0.29$  (GBA3),  $2.12 \pm 0.53$  (GBA1) and  $3.23 \pm 0.13$  (GBA2), respectively.

**Table 3.** Mean and standard deviation ( $\pm$ SD) of protein, lipid, and ash body composition of European seabass fry (*Dicentrarchus labrax*) fed on diets containing (Control 0), 1, 2, and 3% GroBiotic®-A for 60 days. (%)

Treatments	Protein	Lipid	Ash
Control	$22.07 \pm 0.75^a$	$1.73 \pm 0.87^a$	$2.33 \pm 0.16^a$
GBA1	$22.84 \pm 0.62^a$	$2.12 \pm 0.53^a$	$2.95 \pm 0.37^b$
GBA2	$24.01 \pm 1.63^a$	$1.81 \pm 0.86^a$	$3.23 \pm 0.13^b$
GBA3	$24.28 \pm 0.29^a$	$1.31 \pm 0.35^a$	$3.20 \pm 0.03^b$

### Biochemical composition of feeds

The differences between lipid and ash values of feeds used in the current study were not statistically significant ( $p >$

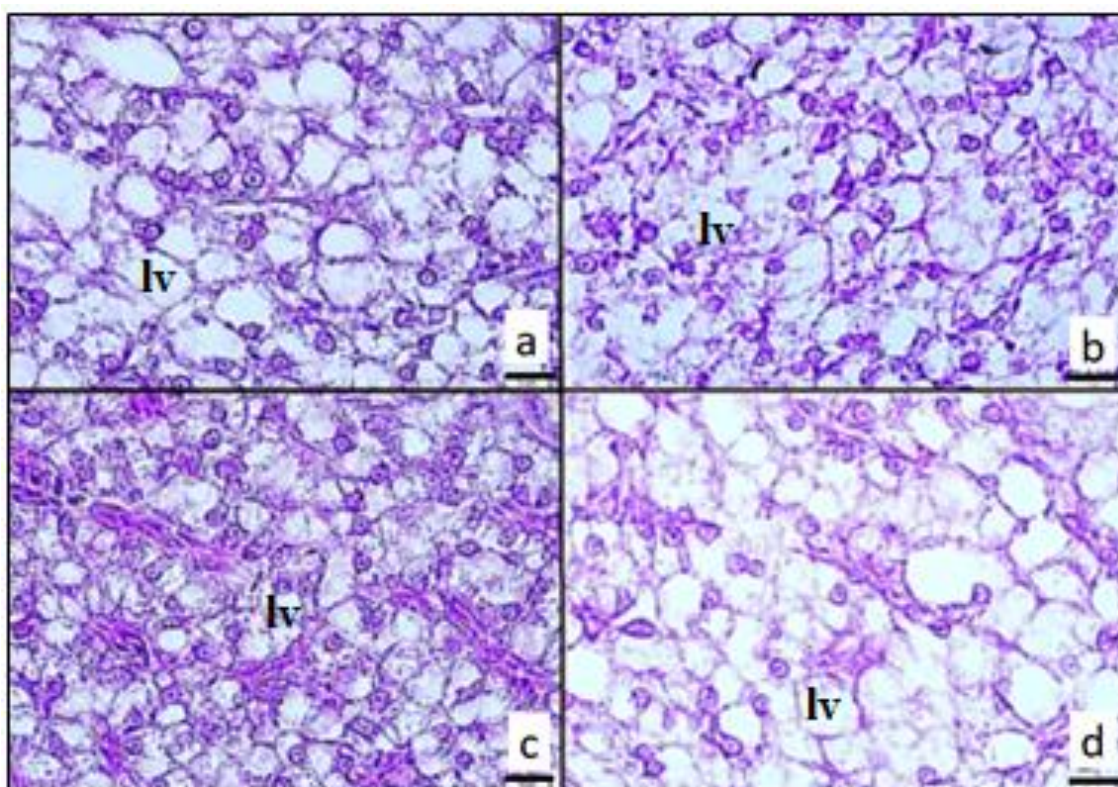
$0.05$ ) except protein ( $p < 0.05$ ) (Table 4). The highest protein, lipid and ash values were determined as  $63.78 \pm 0.20$  (Control),  $10.99 \pm 0.46$  (GBA3) and  $10.63 \pm 0.18$  (Control), respectively.

**Table 4.** Mean and standard deviation ( $\pm$ SD) of protein, lipid, and ash belong to commercial feed diets of European seabass fry (*Dicentrarchus labrax*) fed on diets containing (Control 0), 1, 2, and 3% GroBiotic®-A for 60 days. (%)

Treatments	Protein	Lipid	Ash
Control	$63.78 \pm 0.20^a$	$9.78 \pm 0.92^a$	$10.63 \pm 0.18^a$
GBA1	$63.50 \pm 0.51^a$	$10.22 \pm 1.02^a$	$10.46 \pm 0.07^a$
GBA2	$62.03 \pm 0.60^b$	$9.90 \pm 0.37^a$	$10.36 \pm 0.03^a$
GBA3	$61.77 \pm 0.62^b$	$10.99 \pm 0.46^a$	$10.51 \pm 0.03^a$

### Histological results

In liver tissue sections, the mean number of fat vacuoles observed in the liver tissue of the control (Figure 1a) and experimental group of 1% GBA was found to be moderate (Figure 1b). In other groups (2% GBA and 3% GBA), there was a significant increase in the number of fat vacuoles due to the increase in the additive. In addition, degeneration and necrosis of hepatocyte cells of the liver were observed in the experimental group of 2% GBA and 3% GBA (Figure 1c, d).



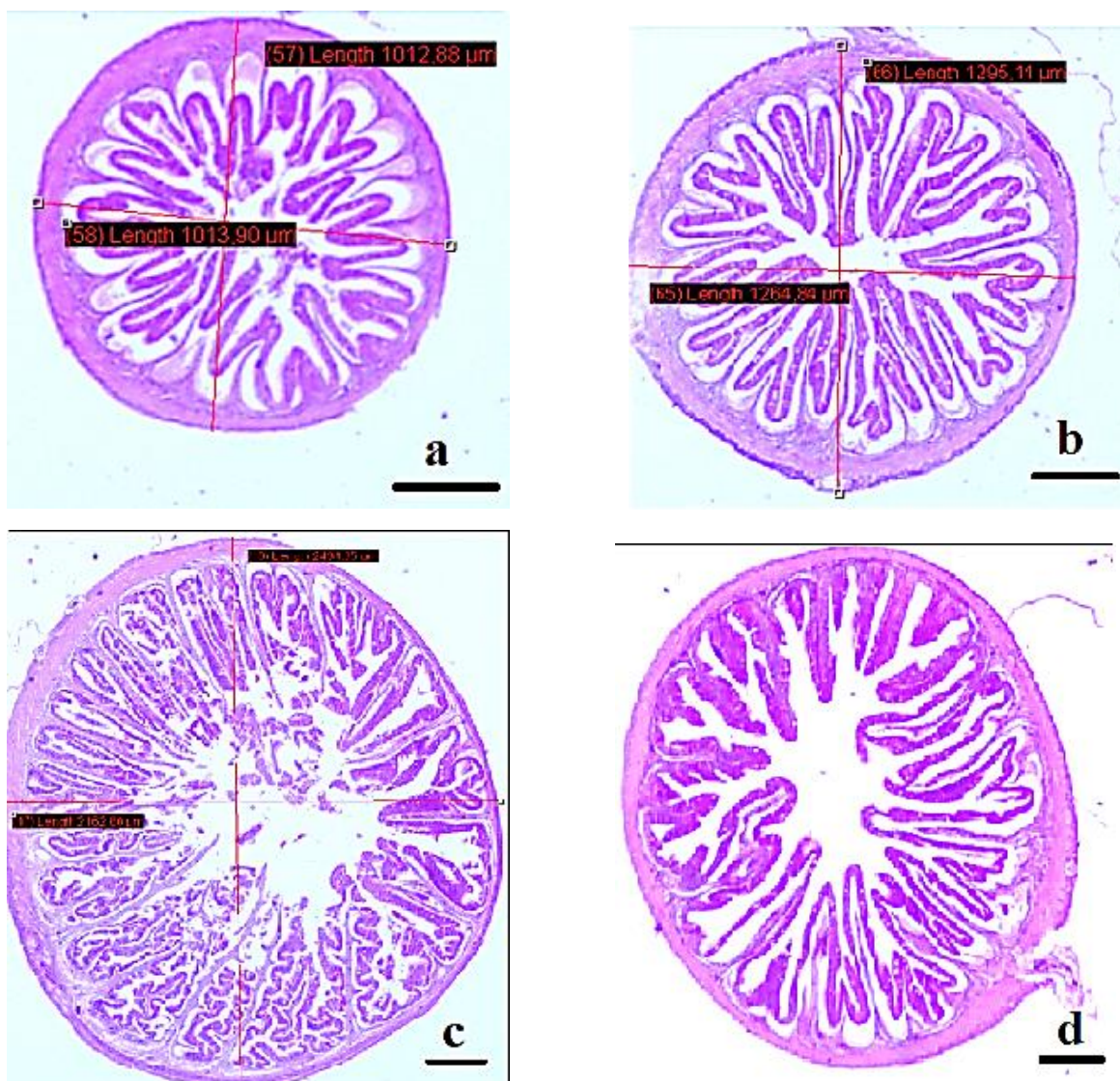
**Figure 1.** Light photomicrograph of liver of European seabass showing increase diffuse macro-vesicular lipid accumulation in liver tissue (a: control group; b: 1% group; c: 2% group; d: 3% group) a,b: Moderate lipid vacuoles (lv) c,d: Excessive increase in lipid vacuoles (lv), necrosis in hepatocytes (bar: 200  $\mu$ m, H&E)

In intestinal tissue sections, of midgut intestinal diameter, villus length and villus width of all fish were measured (Table 5). Light photomicrograph of intestine sections of European seabass villus structure in intestine in all treatments group (Figure 2; a,b,c,d).

While the increase in the GBA2 feeding group was remarkable compared to the control group, it was found that there was an inverse relationship between villus length and contribution rates in the GBA3 feeding group (Figure 2c,d).

**Table 5.** The villus lengths and gut diameters measured in the intestine of European seabass fry (n=5) treatment groups

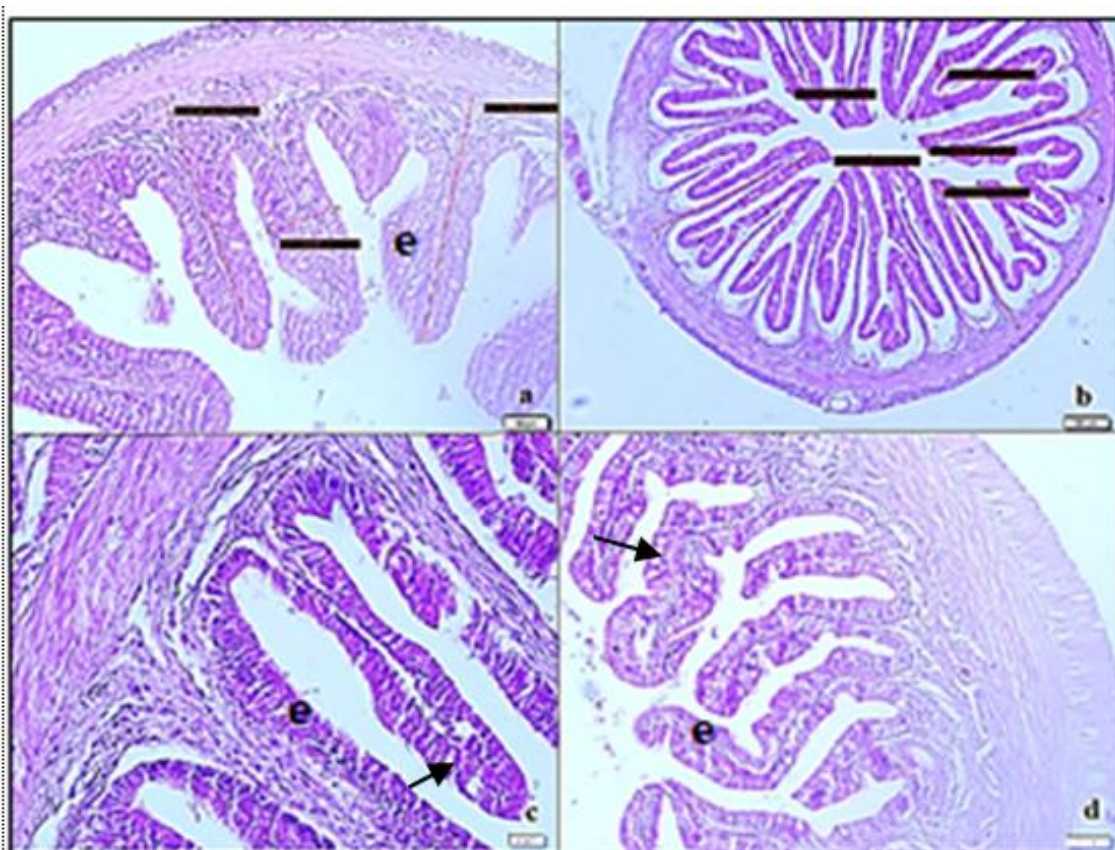
Parameters	Treatments			
	Control	GBA1	GBA2	GBA3
Intestinal diameter ( $\mu\text{m}$ )	1253	1790	1863	1662
Villus length ( $\mu\text{m}$ )	420	550	480	380
Villus width ( $\mu\text{m}$ )	101	115	111	99



**Figure 2.** Light photomicrograph of intestine sections of seabass showing normal villus structure in intestine in all treatments; a: control group; b: 1% group; c: 2% group; d: 3% group (bar: 200  $\mu\text{m}$ , H&E)

In the detailed microscopic examination of the intestine sections, it was observed that the structure of enterocyte cells and the number of goblet cells were normal in the control and 1% GBA group, but there was an excessive increase in the number of goblet cells (Figure 3b). However, high villi length

was observed in the GBA2 group (Figure 3c) and enlargement of the lamina propria in the GBA3 group. Among the enterocyte cells, goblet cells increased compared to the control group (Figure 3d). In addition, no pathological picture was observed in all intestinal preparations examined.



**Figure 3.** High magnification of histological sections for treatment groups and structure of enterocyte (e) and goblet cells (arrowed); a: control; b: GBA1; c: GBA2 ; d: GBA3 (bar: 200  $\mu$ m, H&E)

## DISCUSSION

In the current study, it was investigated that the addition of GroBiotic®-A affect on the growth performance, body composition, gut and liver histology of European seabass. There were no significant effects of supplementing the basal diet with GroBiotic®-A (GBA) within the range of 1–3 % on Weight gain, FCR and SGR of the European seabass ( $P > 0.05$ ). Hoseinifar et al., (2016) indicated that effects of prebiotic on growth performance on fish are inconsistent. Li & Gatlin, (2004) indicated that the addition of GBA to the juvenile Hybrid striped bass (*Morone chrysops* x *Morone saxatilis*) diet improved growth performance compared to fish fed a basal diet, while Burr et al. (2010), working on the same species in adult size noted that no change was observed in growth performance.

The lack of growth enhancement in European seabass with the addition of prebiotics was consistent with previous studies on trout (*Oncorhynchus mykiss*) (Sealey et al., 2007), Westslope cutthroat trout (*Oncorhynchus clarkii lewisii*) (Sealey et al., 2015), red drum (*Sciaenops ocellatus*) (Burr et al., 2009), Caspian kutum, (*Rutilus frisii kutum*) (Yousefian et al., 2012) and Nile tilapia (*Oreochromis niloticus*) (Vechklang et al., 2012; Peredo et al., 2015). In contrast to these studies

such as tilapia (Zheng et al., 2011), rainbow trout fry (*Oncorhynchus mykiss*), (Azari et al., 2013), juvenile starry flounder (*Platichthys stellatus*), (Wang et al., 2014), rainbow trout (Staykov et al., 2007; Yilmaz et al., 2007), largemouth bass (*Micropterus salmoides*) (Yu et al., 2019) and beluga sturgeon juvenile (*Huso huso*) (Adel et al., 2016; 2017) indicated differences in growth performance. Peredo et al., (2015) showed that differences in the size or age of the fish used in prebiotic studies may have different effects depending on the microbiota. In addition, such intraspecific, as well as interspecific differences are quite common in prebiotic studies and may be likely attributed to initial differences in the composition of intestinal microbiota, although this has not been studied in most studies.

The body composition, including protein and lipid content, is of vital importance as it affects the growth and survival of cultivated species as it generally reflects the state of nutrition and the health of aquatic species (Hoang, 2019). Dietary prebiotic inclusion affects the protein content in the tissues of culture animals, and may also vary depending on the species. (Burr et al., 2010; Genc et al., 2007; Yilmaz et al., 2007) In this study, prebiotic supplementation on the body composition of fish did not affect protein and lipid content compared to the control group.

This situation is similar to the studies of some researchers (Adel et al., 2017, 2016; Vechklang et al., 2012; Wang et al., 2014; Zheng et al., 2011), but it shows difference from some of the studies (Burr et al., 2010; Sealey et al., 2007). They were indicated that there is a significant increase in protein. Unlike other researchers who reported no change in ash content, it was significantly higher. Survival rate at the end of the trial was higher than 97.5% in all treatments, and no significant differences were observed among treatment groups ( $P > 0.05$ ). Results of Peredo et al. (2015) revealed that tilapia fed the diet containing 2% GBA had significantly higher survival than that of the other treatment groups. FCR of juvenile European seabass significantly tended to decrease with the supplemented GroBiotic®-A (1%), but no significant difference was observed among the treatment groups. The results belong to the basal group of Wang et al. (2014) supported the results of current study.

The studies have shown that some prebiotics supplementation in the diets may cause significant differences in gastrointestinal morphology in some fish (Anguiano et al., 2013). Changes to the morphology of the intestine may be attributed to the production of short-chain fatty acids through the microbial fermentation of prebiotic substances. Peredo et al. (2015) GBA an improvement in gut morphology ensure benefit feed utilization, but the maintenance of an intact, healthy mucosal epithelium may help to prevent opportunistic indigenous bacterial infections. Dimitroglou et al. (2009) reported that the results of the histological studies may help to explain the improved growth performance, feed utilization, and survival of fish. In the current study, histological examinations demonstrated that the villus length and the number of goblet cells of the fish in the GBA1 feeding group increased significantly compared to the other groups in the intestinal tissue. Studies on rainbow trout and sea bream (*Sparus aurata*) with different prebiotics are consistent with the present study (Dimitroglou et al., 2009; Yilmaz et al., 2007), gilthead sea bream (Eryalçin et al., 2017). Similar to the results of the current study, previous investigations with red drum (Zhou et al., 2010), hybrid seabass (Anguiano et al., 2013) showed that GBA supplementation improved gut

morphology. In this study, it was observed that prebiotic level had a positive effect on fish intestine and liver when 1% was added to the diet, but it had a negative effect due to the increase in GBA level. It was reported that the addition of 2% GBA in striped bass was effective on intestinal structures at week 4 but not at week 8 (Anguiano et al., 2013). In the present study villus lengths were found to be longer in the treatment groups supplemented with GroBiotic®-A than in the control group. The highest villus length has been determined as GBA2 (Table 5). Differences between the results of studies on the effects of prebiotics on the villus structure, using different dose levels, studying with different species, the presence of different intestinal microbiota in these species, has been reported to be caused by reasons such as the use of different culture conditions (Adel et al., 2016; Anguiano et al., 2013; Dimitroglou et al., 2009).

In conclusion, the findings of the present study indicated that weight gain, feed conversion ratio, survival, and whole body proximate composition of European seabass following 8 weeks of feeding were not significantly affected by dietary supplementation of 1% and 2% %3 GroBiotic®-A. Addition of 1% GBA to the feed showed a positive effect on the liver and intestine tissues of the seabass. However, an increase in the amount of Grobiotic-A (2% and 3%) was found to increase the number of fatty vacuoles in the liver tissue as well as degeneration and necrosis in the hepatocyte cells of the fish. Prebiotic Grobiotic®-A (1%) could be a potential dietary supplement for seabass juveniles. In particular, dietary content appears to improve the growth performance and Gastrointestinal tract GIT of juveniles. However, Further studies should be designed to investigate the effects of GroBiotic®-A on immune response and disease resistance applying challenge studies in European seabass.

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