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ORIGINAL ARTICLE

Effects of dietary butyric acid glycerides on growth performance, haemato-immunological and antioxidant status of yellowfin seabream (*Acanthopagrus latus*) fingerlings

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Abstract

Sixty-day research was conducted to investigate the effects of dietary butyric acid glyceride (BAG) on the performance of *Acanthopagrus latus* fingerlings (8.59 ± 0.33 g). Experimental feeds were prepared by supplementing a commercial feed (crude protein: 50%, crude lipid: 15%) with graded levels of BAG including 0 (control), 0.25% (BAG0.25), 0.5% (BAG0.5) and 1% (BAG 1). One hundred and twenty fish (10 fish in each tank) were stocked in twelve 300-L tanks then fed with the experimental feeds twice daily. Growth and feed utilization in fish fed BAG-supplemented diets were better than the control ($p < 0.05$). Fish fed BAG1 diet had more red and white blood cells counts than the other groups. Total immunoglobulin content and lysozyme activity in the skin mucus increased by increasing dietary BAG. Fish in BAG1 group had more skin mucosal alternative complement pathway activity than the other groups ($p < 0.05$). Liver catalase and glutathione S transferase activities increased in a dose–response manner to dietary BAG level. Fish in BAG1 group showed higher liver glutathione reductase activity than the other treatments. According to the findings of this study, supplementing diet with 1% of BAG can promote growth performance and health conditions in *A. latus* fingerlings.

KEYWORDS

Acanthopagrus latus, antioxidant capacity, butyric acid glycerides, feed utilization, mucosal immunity

1 | INTRODUCTION

The fast growth of the modern aquaculture systems (e.g. bioflock and recirculating aquaculture system) along with the application of the formulated feeds has facilitated the intensification of valuable farmed aquatic species and increased yield per unit of culture area. However, high stocking density, handling and reduction in water quality in such systems may cause chronic stress and subsequently compromise fish health (Dawood et al., 2018). Such stressful conditions in intensive aquaculture systems not only compromise welfare but also may suppress the immune system in farmed aquatic specimens and may cause infectious diseases outbreaks that eventually led to financial losses (Dawood & Koshio, 2016). On the other hand, using antibiotics and chemical therapeutics for controlling contagious diseases may have several drawbacks such as emergence of therapeutic-resistance pathogens, environmental pollution through bioaccumulation and biomagnification in wild aquatic animals, retention of hazardous drug residues in the final product and depression of immunocompetence in farmed aquatic species (Romero et al., 2012). Thus, finding and administrating safe and environmentally friendly agents such as feed additives (e.g. probiotics, prebiotics, herbal extracts and organic acids) have been considered

as a promising method to promote immunocompetence of aquatic species in aquaculture industry (Dawood et al., 2018; Wang et al., 2017).

Among different feed supplements, organic acids (OA) and their salts had positive effects on performance and health in different cultured aquatic species (Dawood et al., 2018; Ng & Koh, 2017). Organic acids prohibit the proliferation of pathogenic bacteria by penetrating through the cell envelope and disrupting the intracellular pH equilibrium that subsequently results in cell death (Defoirdt et al., 2009). Based on number of their carbons, OA are divided into short-chain fatty acids (SCFA) and medium-chain fatty acids, which SCFA group is mostly used in aquafeeds (Hoseinifar et al., 2017; Tran et al., 2020). Recent studies have indicated that SCFA can prevent diseases and improve growth, bioavailability of minerals, provoke digestive enzyme activity, induce proliferation of profitable microbiome in the gut (e.g. lactic acid bacteria) and enhance immunocompetance in farmed aquatic animals (Hoseinifar et al., 2017; Ng & Koh, 2017; Tran et al., 2020). These SCFA are rapidly assimilated by enterocytes and modulate host's gut-associated lymphoid tissue through different cellular mechanisms such as triggering immune-related genes transcription as well as provoking differentiation and proliferation of immune cells (Correa-Oliveira et al., 2016; Hoseinifar et al., 2017). Short-chain fatty acids attach to some receptors (GPR41, GPR43 and GPR109A) on the surface of immune cells in the gut-associated lymphoid tissue and affect transcription of immune-related genes that consequently can modify immune responses in the host (Hoseinifar et al., 2017; Tran et al., 2020). Among various SCFA, butyrate and its derivatives showed positive effects on physiological processes in different farmed aquatic animals such as growth (Jesus et al., 2019; Robles et al., 2013), feed utilization (Aalamifar et al., 2020), immune responses (Aalamifar et al., 2020; Tian et al., 2017), gut morphology and antioxidative status (Aalamifar et al., 2020; Tian et al., 2017). More than 90% of butyrate is metabolized by the colonic epithelial cells (Bedford & Gong, 2018). Short-chain fatty acids are used in aquafeeds in the forms of pure, salt and glyceride. However, when they are used in pure and salt forms, up to 100% of their quantities may be lost through leaching in water (da Silva et al., 2013). Due to its volatility, unfavourable odour and great absorbance in the foregut, butyrate cannot be used in free form (Bedford & Gong, 2018). Thus, it has been recommended to coat OA in glyceride form to reduce their leaching and elevate their efficiency (Jesus et al., 2019). Studies have shown that butyrate glycerides (BAG) are easily degraded by lipases in the digestive system and release butyrate into the gut (Bedford & Gong, 2018).

During recent years, several studies have been carried out to evaluate the effects of various SCFA on growth and health indices of different marine fish species (Aalamifar et al., 2020; Katya et al., 2018; Lin & Cheng, 2017). Recently, yellowfin seabream, *Acanthopagrus latus*, has been introduced to marine cage culture on the southern coast of Iran. This species is a carnivorous marine fish with high economic value and great potential for mariculture in tropical and subtropical regions due to its suitable growth rate, ease of its propagation in captive conditions and high tolerance to various culture conditions (Tsui et al., 2016). Although, the nutritional requirements of *A. latus* are known (Mozanzadeh et al., 2017), the effects of dietary SCFA on this species have not been well understood. Previous studies demonstrated that supplementing diet with sodium acetate (Na-A) and sodium propionate(Na-P) (0.5% and 1% of diet) remarkably improved growth, humoral immune responses, antioxidative and digestive enzymes activities as well as haematological parameters in this species (Sangari et al., 2020; Sotoudeh et al., 2020). Therefore, in this research, it has been tried to investigate the influence of dietary BAG on growth rate and health status of *A. latus* fingerling.

2 | MATERIALS AND METHODS

2.1 | Experimental feeds

Experimental feeds were prepared by supplementing a commercial feed (Kimiaagaran, Shiraz, particle size: 2 mm, crude protein: 50%, crude lipid: 15%, ash: 12%, fibre: 1.5%, moisture: 8% and gross energy: 20 MJ kg^{-1}) with various levels of butyric acid glyceride (BAG) (1-monoglycerides of butyric acid, SILO Additives Ind. Co. Italy). Thus, graded levels of BAG were added to the basal diet to produce experimental feeds including 0 (control, without BAG supplementation), 0.25% (BAG0.25), 0.5% (BAG0.5) and 1% (BAG1). In this regard, the prescribed BAG levels were dissolved in a sufficient amount of chloroform: water solvent (80: 20, v/v) and sprayed over the commercial feed (Najdegerami et al., 2012). The experimental feeds were left exposed to air for chloroform evaporation then they were covered with gelatin (5%) (Guo et al., 2021). Finally, the experimental feeds were dried in an oven (40°C, 24 h) and then kept in a refrigerator (4°C) until used.

2.2 | Fish and culture conditions

This study was performed in the Marine Research Station of the Persian Gulf University, Bushehr, Iran. Yellowfin seabream (*Acanthopagrus latus*) fingerlings were transferred from a private hatchery centre to the laboratory, and then, they were adapted to the culture conditions for fifteen days. Fish were fed with the control diet during acclimation period. Next, 120 healthy fish (initial weight [BW_i] = 8.59 ± 0.33 g, mean ± SE) were randomly distributed into twelve 300-L cylindrical polyethylene tanks (10 fish $tank^{-1}$) in a flow-through system (1 L min^{-1}). Each diet fed to three groups of fish (three tanks). The tanks were supplied with disinfected and UV-treated seawater. Tanks were equipped with aquarium blubbers for aeration. The experimental feeds were given to fish two times a day (08.00 and 16.00 h) up to visual satiation for 60 days, making sure that no feed was left uneaten. During feeding trial, the physicochemical parameters of water were as follow:

temperature: 28.5 ± 1.5°C, salinity: 39.0 ± 1.0 ppt, dissolved oxygen: over 7 ppm, pH: 7.7 ± 0.3 and the photoperiod was 16L:8D (light: darkness).

2.3 | Sampling

At the end of feeding period, the samples were collected from fish after they being anesthetized with 2-phenoxyethanol (100 ppm). Body weight (BW_f) of all fish was individually measured. For evaluating haematological parameters, three fish per each tank (nine fish per treatment) were sedated with the same anaesthetic. Heparinized syringes were used for bleeding from caudal vein of fish and blood transferred into a vial and kept close to ice (4°C). The same fish were sacrificed using 500 ppm 2-phenoxyethanol, and their livers were dissected on an ice cube, transferred into tubes then kept in a −80°C freezer.

For evaluating serum total protein and albumin, two fish of each tank (six fish per treatment) bled with unheparinized syringes, and then, blood was allowed to clot in the refrigerator (2 h at 4°C). The clotted blood was centrifuged (6000 *g*, 15 min), serum was separated then kept in a freezer.

For skin mucus extraction, three fish of each tank (nine fish per treatment) was anesthetized and individually transferred to a polyethylene bag contained 2 ml of 50mM NaCl and gently rubbed (1 min), and then, the collected mucus transferred into tubes. The collected mucus was centrifuged (1500 *g*, 10 min, 4°C), and the supernatant stored at −80°C until their analyses (Ross et al., 2000). Growth parameters were calculated according to the standard formulae:

$$\text{Weight gain(WG, \%)} = \left(\frac{[BW_f(g) - BW_i(g)]}{BW_i(g)}\right) \times 100$$

$$\text{Specific growth rate}\left(\text{SGR, \% BW}_i\text{day}^{-1}\right) = \left(\frac{[\ln(BW_f) - \ln(BW_i)]}{\text{days of the feeding trial}}\right) \times 100$$

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

$$\text{Protein efficiency ratio (PER, \%)} = \left[\frac{\text{wet weight gain (g)}}{\text{total protein intake (g)}}\right] \times 100$$

$$\text{Survival rate (\%)} = \left(\frac{\text{number of fish at the end of the experiment}}{\text{initial number of fish}}\right) \times 100$$

2.4 | Haematological parameters

The complete blood count parameters including red blood cell (RBC), white blood cell (WBC), haemoglobin (Hb), haematocrit (Hct) and leucocytes differentiation were evaluated according to the methods described by Houston (1990). Haematological indices were determined using the following formulae (Dacie & Lewis, 2001).

$$\text{Mean corpuscular haemoglobin (MCH, pg)} = \left[\frac{\text{Hb}\left(\text{g dl}^{-1}\right)}{\text{RBC}\left(\times 10^6 \mu l\right)}\right] \times 10$$

$$\text{Mean corpuscular volume (MCV, fl)} = \left[\frac{\text{Hct (\%)}}{\text{RBC}\left(\times 10^6 \mu l\right)}\right] \times 10$$

$$\text{Mean corpuscular haemoglobin concentration (MCHC, g/dl)} = \left[\frac{\text{Hb}\left(\text{g dl}^{-1}\right)}{\text{Hct (\%)}}\right] \times 100$$

2.5 | Immunological parameters

Immunoglobulin content in the skin mucus was measured using the protein precipitation method using polyethylene glycol (PEG) described by Siwicki et al. (1994). One hundred µl of skin mucus was mixed with the same volume of PEG (12%) and incubated (25°C, 2 h) with continuous agitation. After incubation, the mixture was centrifuged (400 *g*, 10 min, 25°C) and total protein content in the supernatant measured using the Bradford (1976) method.

Total Ig = total protein content in the skin mucus − protein content in the supernatant.

The lysozyme activity was determined using the modified turbidimetric method using *Micrococcus lysodeikticus* (Sigma, St Louis, MO, USA) as described by Esteban et al. (2001). Twenty-five µl of mucus was added to 175 µl of substrate solution (0.2 mg *M. lysodeikticus* per ml (w/v) of 0.02 M sodium citrate buffer, pH 5.8) and absorbance read at 450 nm from 0 to 15 min at 25°C. One unit of lysozyme activity was defined as a reduction in absorbance of 0.001 min^{-1}.

The rabbit red blood cells (RaRBC) were used for determination of ACH50 activity according to Yano et al. (1991). In summary, 25 µl of RaRBC suspension was added to serially diluted mucus (100 µl) in MgEGTA gelatin veronal buffer (GVB). The mixture was incubated at 25°C for 2 h with permanent agitation. Following incubation time, cold EDTA-GVB buffer (1 ml) was added to the mixture, then it was centrifuged (400 g, 10 min, 25°C). The amount of haemolysis was spectrophotometry measured at 414 nm by considering 50% haemolysis of RaRBC by skin mucosal samples as ACH50 unit.

Serum total protein and albumin contents were measured using commercial diagnostic kits (Pars Azmoon, Tehran, Iran) and an autoanalyzer tool (Technicon RA-1000, Technicon Instruments, USA).

2.6 | Liver antioxidant enzymes

To measure antioxidant enzyme, frozen livers were defrosted, homogenized in nine volume (w/v) of 0.05 M phosphate buffer (pH = 6.6) contained 1% Triton X-100. Then, the homogenized samples were centrifuged (10 min, 30,000 *g*, 4°C) and supernatants were separated. The total protein content of the samples was measured using the Bradford (1976) method. Catalase (CAT; EC 1.11.1.6) activity was determined at 25°C by determining the reduction in

absorbance at 240 nm (ε = 40 mM cm^{-1}) using 13.2 mM H_2O_2 in 50 mM phosphate buffer (pH 7.0) and 100 µl of homogenized sample (Aebi, 1984). A blend of 100 µl of liver homogenate and the same buffer was used as a control.

Glutathione reductase (GR; EC 1.6.4.2) activity was assayed as described by (Carlberg & Mannervik, 1975). The NADPH oxidation was measured at 340 nm (ε = 6.22 m M^{-1} cm^{-1}) using 20 mM glutathione disulphide and 2 mM NADPH as substrates.

Glutathione S-transferase (GST: EC 2.5.1.18) was measured using glutathione (GSH) and 1-chloro-2,4 dinitrobenzene (CDNB) as a substrate in a phosphate buffer (110 mM phosphate buffer, pH 6.5, 30 mM CDNB, 100 mM GSH, distilled water and 50 µl tissue homogenate) (Habig et al., 1974) The GST-catalysed by formation of GS-DNB produces a dinitrophenyl thioether that spectrophotometrically measured at 340 nm (ε 9.6 mM^{-1} cm^{-1}).

2.7 | Statistics

Data were analysed using SPSS 18 software. The normality and homogeneity of data tested by Kolmogorov–Smirnov and Leven's tests respectively. A one-way ANOVA test was used to find possible differences among treatments at 95% confidential level. For multiple comparisons, a Duncan's post hoc test was used. All the data are presented as means ± standard error of the mean calculated from three replicates.

3 | RESULTS

3.1 | Growth performance

The survival rate of fish did not affect by supplementing BAG in diet (Table 1). Growth performance factors including BW_f and SGR in fish fed diets contained BAG were higher than the control ($p < 0.05$). In this regard, WG in fish fed with BAG-supplemented feeds about 34% on average was higher than the control. Feed utilization parameters including FCR and PER were improved in BAG-supplemented groups compared with the control.

3.2 | Haematological parameters

Fish fed BAG1 diet had superior number of RBC than the other treatments (Table 2, $p < 0.05$). The values of blood Hb and Hct in BAG1 group were higher than the control and BAG0.5 groups ($p < 0.05$). Fish in the BAG0.25 treatment had a higher MCV value than the control and BAG0.5 groups, whereas the BAG1 group demonstrated an intermediate value. The percentage of lymphocytes gradually decreased with increasing BAG level in diet, whereas the percentage of neutrophils increased with increasing dietary BAG ($p < 0.05$). The values of MCH and MCHC as well as the percentages of monocytes and eosinophils were not affected by diets.

3.3 | Immune and antioxidative responses

Fish in BAG1 and the control groups had the highest and lowest total Ig (Figure 1a) levels and lysozyme (Figure 1b) activities in the skin mucus, respectively, and the other groups demonstrated intermediate values ($p < 0.05$). Fish in BAG1 group had higher skin mucosal ACH50 (Figure 1c) activity compared with the other groups.

The number of blood WBC (Figure 2a) and serum albumin (Figure 2b) levels was highest and lowest in BAG1 and the control groups respectively ($p < 0.05$). Serum total protein content (Figure 2c) increased with increasing the dietary BAG level.

The activities of CAT (Figure 3a) and GST (Figure 3b) in the liver of fish gradually enhanced with increasing BAG level in diet ($p < 0.05$). Fish fed BAG1 diet had higher GR (Figure 3c) activity in the liver than the other treatments ($p < 0.05$).

4 | DISCUSSION

According to the presented results, supplementing diet with BAG pronouncedly increased growth rate in *A. latus* fingerlings that mainly associated with improvements in FCR and PER. It has been confirmed butyrate or its derivatives can boost up growth in fish by modifying gut morphology (Tian et al., 2017; Zhou et al., 2019) and strengthening antioxidative status (Aalamifar et al., 2020; Liu

TABLE 1 Growth performance and feed utilization of *Acanthopagrus latus* fingerling fed different levels of butyric acid glyceride for 60 days (mean ± SE, $n = 3$)

Treatments	Control	BAG0.25	BAG0.5	BAG1
BW_i (g)	8.97 ± 0.33	8.23 ± 0.43	8.46 ± 0.16	8.7 ± 0.017
BW_f (g)	14.33 ± 0.63^a	15.70 ± 0.11^b	17.13 ± 0.31^b	16.66 ± 0.12^b
WG %	59.6 ± 1.28^a	92.0 ± 10.69^b	96.6 ± 11.09^b	91.6 ± 4.97^b
SGR (% BW_i day^{-1})	0.77 ± 0.01^a	1.08 ± 0.09^b	1.18 ± 0.04^b	1.08 ± 0.04^b
FCR	2.29 ± 0.18^a	1.77 ± 0.07^b	1.53 ± 0.20^b	1.83 ± 0.03^b
PER	1.52 ± 0.04^a	2.41 ± 0.04^b	2.54 ± 0.04^b	2.74 ± 0.04^b
Survival (%)	100 ± 0.0	100 ± 0.0	96.6 ± 1.8	100 ± 0.0

Note: A different superscript in the same row denotes statistically significant differences ($p < 0.05$).
Abbreviations: BW_f, final body weight; BW_i, initial body weight; FCR, feed conversion ratio; PER, protein efficiency ratio; SGR, specific growth rate; WG, weight gain.

TABLE 2 Effects of different levels of butyric acid glyceride on haematological parameters of *Acanthopagrus latus* fingerling (Mean ± SE, *n* = 3)

Treatments	Control	BAG0.25	BAG0.5	BAG1
RBC (×10^6 µl)	1.50 ± 1.45^a	1.58 ± 2.51^a	1.54 ± 1.52^a	1.77 ± 8.95^b
Hb (g dl^{-1})	6.2 ± 0.05^a	6.7 ± 0.15^{ab}	6.3 ± 0.05^a	7.36 ± 0.43^b
HCT (%)	31.0 ± 0.57^a	34.3 ± 0.66^{ab}	32.0 ± 0.57^a	37.3 ± 1.76^b
MCV (fl)	206.0 ± 1.73^a	213.3 ± 0.88^b	207.6 ± 1.85^a	209.6 ± 1.85^{ab}
MCH (pg)	41.23 ± 0.03	41.6 ± 0.15	40.9 ± 0.15	41.4 ± 0.40
MCHC (g dl^{-1})	19.96 ± 0.2	19.5 ± 0.1	19.7 ± 0.17	19.6 ± 0.31
Lymphocyte (%)	77.0 ± 1.52^c	75.3 ± 0.33^{bc}	71.6 ± 1.2^b	67.0 ± 1.52^a
Neutrophil (%)	17.6 ± 0.88^a	19.6 ± 1.2^a	22.6 ± 0.33^b	26.3 ± 0.88^c
Monocyte (%)	4.8 ± 0.88	4.1 ± 0.57	5.2 ± 0.57	6.3 ± 0.88
Eosinophil (%)	0.6 ± 0.33	1.0 ± 0.57	0.6 ± 0.33	0.4 ± 0.33

Note: A different superscript in the same row denotes statistically significant differences ($p < 0.05$).
Abbreviations: Hb, haemoglobin; Hct, haematocrit; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; MCV, mean cell volume; RBC, red blood cell.

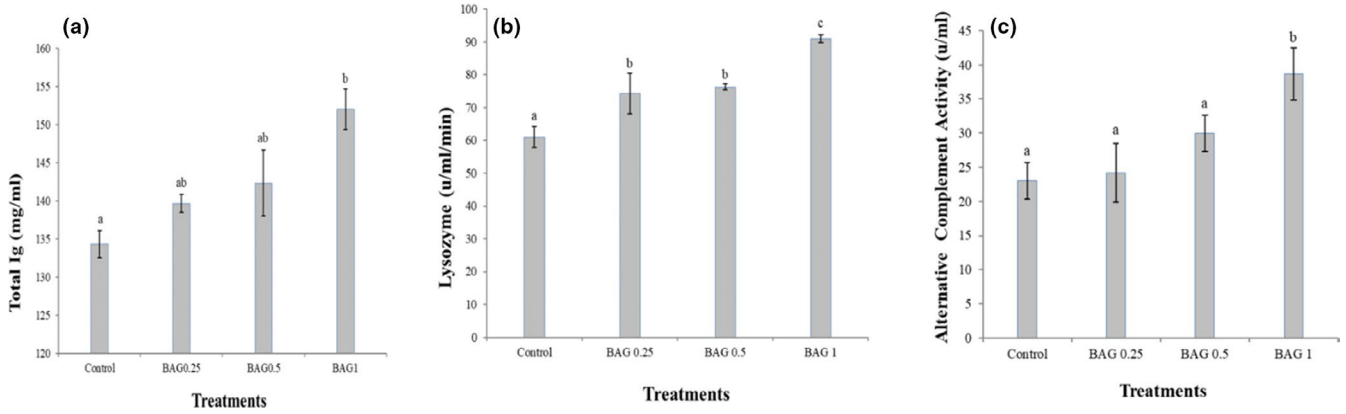

FIGURE 1 Total immunoglobulin (a), lysozyme (b) and ACH50 (c) levels in the skin mucus of *Acanthopagrus latus* fingerling fed with different dietary supplement of butyric acid glyceride for 60 days. Data are expressed as mean ± SE. Bars of different letters indicate significant differences ($p < 0.05$)

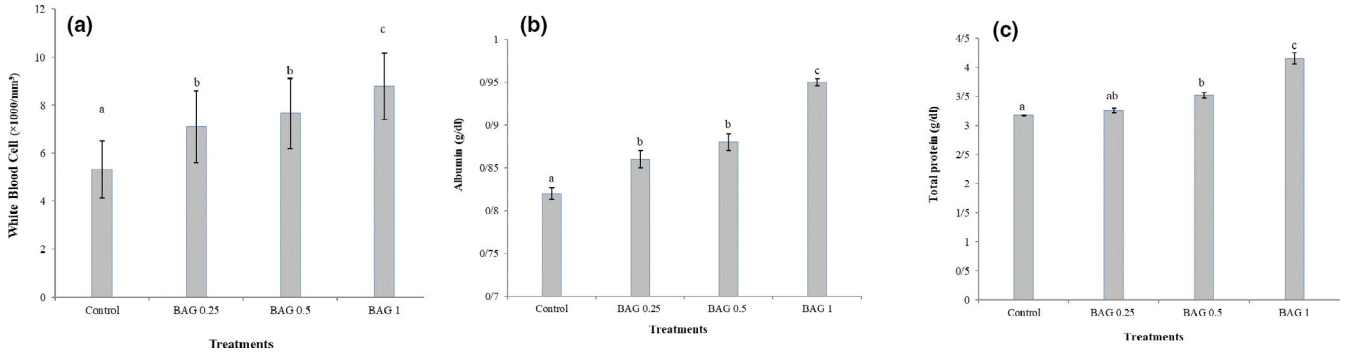

FIGURE 2 White blood cell (a), serum total protein (b) and Albumin (c) levels in *Acanthopagrus latus* fingerling fed with different dietary supplement of butyric acid glyceride for 60 days. Data are expressed as mean ± SE. Bars of different letters indicate significant differences ($p < 0.05$)

et al., 2017; Wu et al., 2018), increasing digestive enzyme activities (Aalamifar et al., 2020; Reyshari et al., 2019; Silva et al., 2016; Tian et al., 2017), stimulating propagation of beneficial microbiota as well as decreasing harmful bacteria in the host's gut (Reyshari et al., 2019; Tian et al., 2017). In addition, OA by reducing the pH of digesta in the gastrointestinal tract provoke the activity and / or synthesis of acid proteases especially pepsin (Castillo et al., 2014). Furthermore, OA by decreasing pH of digesta can stimulate release

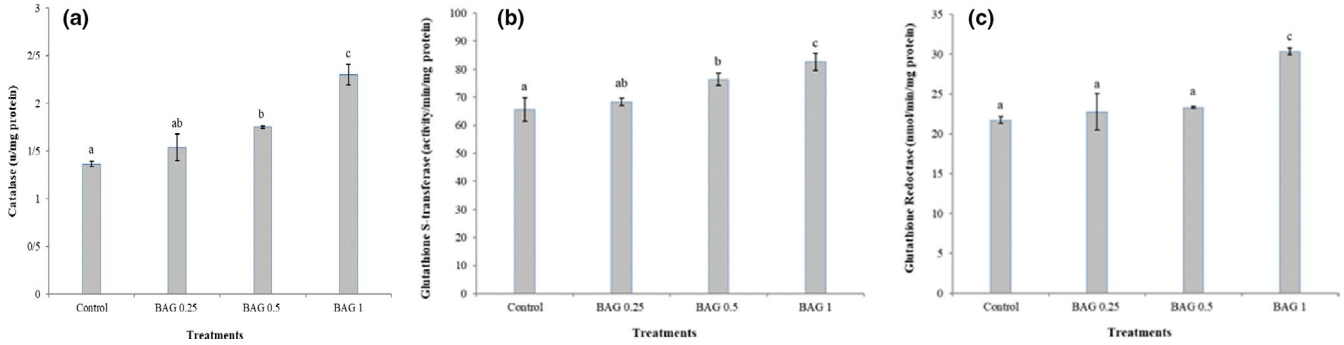

FIGURE 3 Liver antioxidant enzymes including catalase (a), glutathione-s-transferase (b) and glutathione reductase (c) in *Acanthopagrus latus* fingerling fed with different dietary supplement of butyric acid glyceride for 60-days. Data are expressed as mean ± SE. Bars of different letters indicate significant differences ($p < 0.05$)

of secretin and cholecystokinin that consequently enhance both of endocrine and exocrine pancreatic secretions and increase digestibility of the dietary nutrients (Castillo et al., 2014; Ng & Koh, 2017). Moreover, OA by reducing acidity of the gut environment can elevate minerals bioavailability in chyme by chelating them especially in plant protein sources (e.g. phosphorous) (Hoseinifar et al., 2017). Short-chain fatty acids can assimilate through passive diffusion in the gut (Freitag, 2007) and provide energy for the rehabilitation and proliferation of the gut epithelial cells that consequently enhance efficiency of nutrients absorption in this tissue (Baruah et al., 2007; Diebold & Eidelsburger, 2006). Also, OA promote the palatability and physical characteristics of aquafeeds, which result in better FCR in farmed aquatic species (Da Silva et al., 2013; Morken et al., 2012). Several studies also showed growth-promoting effects of OA by improving feed utilization in marine fish species, such as calcium lactate, citric acid (CA) and potassium diformate (KDF) in red drum (*Sciaenops ocellatus*, Castillo et al., 2014), CA in large yellow croaker (*Larimichthys crocea*, Zhang et al., 2016), butyrate and lactate in giant grouper (*Epinephelus lanceolatus*, Lin & Cheng, 2017), sodium diformate (NaDF) and butyrate in Asian seabass (*Lates calcarifer*, Reyshari et al., 2019; Alamifar et al., 2020) and sodium propionate and sodium acetate in *A. latus* (Sotoudeh et al., 2020).

Results of the current research revealed that supplementing diet with 1% BAG increased RBC, WBC, Hb and Hct in *A. latus* suggesting oxygen-carrying capacity of blood and immunocompetence could be boosted up in this treatment. It has been confirmed that OA could increase the liberation of iron, phosphorous, calcium and copper in feedstuffs and by reducing the pH of the gut the solubility of these minerals enhance that eventually increase their absorption (Baruah et al., 2007; Lückstädt, 2008). Yong et al. (2017) reported that commercial butyrate (Butipearl™) at 0.05% of diet increased Hct and Hb in hybrid catfish (*Clarias microcephalus* × *C. gariepinus*). Furthermore, Benedito-Palos et al. (2016) reported that using a PP-based diet induced anemia in gilthead seabream (*Sparus aurata*), but inclusion of butyrate (0.4%) in this diet restored blood Hb content in normal range in this species. Sangari et al. (2020) also reported that inclusion of sodium propionate and sodium acetate in a plant protein rich diet (0.5%–1.0%) enhanced Hb in *A. latus* juveniles. Similar to our results, Reda et al. (2016) showed that the combination of calcium propionate, propionic acid and formic acid (0.1%–0.2%) elevated the values of RBC, WBC, neutrophil and lymphocyte in Nile tilapia (*Oreochromis niloticus*). Dawood et al. (2020) also reported haematopoietic inducing effects of sodium butyrate (0.1%) in elevation of Hb content, RBC and WBC counts in Nile tilapia.

The skin mucus is the vital part of the skin associated with lymphoid tissues and contains specific (e.g. immunoglobulins and B lymphocytes) and non-specific (e.g. complement and lysozyme) immune factors with bactericidal, anti-viral and anti-fungal characteristics (Lazado & Caipang, 2014). It has been confirmed that butyrate can strengthen the immunocompetence and disease resistance in fish by improving gut immune function through inducing specific (e.g. immunoglobulin M) and non-specific (e.g. lysozyme and alternative complement pathway) immune responses as well as downregulation of the pro-inflammatory cytokines along with upregulation of anti-inflammatory cytokines in different segments of the gut (Tian et al., 2017). In the current research, skin mucosal immune parameters including total Ig, lysozyme and ACH50 remarkably elevated in BAG-supplemented treatments and it was associated with increasing blood WBC and serum total protein and albumin contents. These results indicating the supplementing diet with BAG can induce skin mucosal-related immune factors in *A. latus*. Although research about the effects of dietary OA on skin mucosal immunity of fish are scarce, several studies demonstrated the immune-stimulating effects of OA on serum/plasma lysozyme, total Ig and ACH50 in marine fish species such as butyrate in European (*Dicentrarchus labrax*, Abdel-Mohsen et al., 2018) and Asian seabass (Aalamifar et al., 2020), NaDF in European (Wassef et al., 2017) and Asian seabass (Reyshari et al., 2019) and sodium propionate and sodium acetate in *A. latus* (Sotoudeh et al., 2020).

Serum/plasma total protein is a reliable health index in fish because it contains a wide range of immune-related proteins and peptides such as lysozyme, anti-proteases, Ig, lectins and complement factors (Uribe et al., 2011). Generally, using immunostimulant in aquafeeds can stimulate the immune system and increase the biochemical indices such as serum albumin, protein and globulin in fish (Andrews et al., 2009). In the present experiment serum, total

protein and albumin values gradually increased with increasing BAG level in diet suggesting BAG can act as an immunostimulant and can promote health status in *A. latus*. In this regard, inclusion of dietary OA elevated serum total protein and albumin in different cultured fish species such as calcium propionate and calcium lactate (1%–1.5%; Hassaan et al., 2014) or malic acid (0.5%–1%; Hassaan et al., 2018) in Nile tilapia, NaDF (0.3%–0.5%) in European seabass (Wassef et al., 2017) and sodium propionate and sodium acetate (0.5%–1%) in *A. latus* (Sangari et al., 2020).

Oxidative stress has been correlated with a spectrum of processes such as diseases, mutagenesis and contributor to damage to various body cells fish (Martínez-Álvarez et al., 2005). Antioxidant enzymes and non-enzymatic compounds (e.g. glutathione) are the main scavengers of reactive oxygen species in fish. Various biotic and abiotic factors such as nutrition, fish age and diseases can change the antioxidant defences of fish (Martínez-Álvarez et al., 2005). Our finding revealed the antioxidant capacity of fish fed BAG-supplemented diets enhanced that manifested by the increased activities of antioxidant enzymes including CAT, GST and GR in the liver especially in BAG1 group. Organic acids can strengthen the activity of antioxidant enzymes such as Cu/Zn-SOD by increasing the bioavailability and concentration of dietary minerals (e.g. Cu, Zn and Mn) in the gut that serve as cofactors for these enzymes (Robles et al., 2013; Zhang et al., 2016). In addition, it has been proved that sodium butyrate can enhance the antioxidant capacity in fish by up-regulating the expression of NF-E_2-related factor 2 and downregulation of kelch-like-ECH-associated protein which elevate the mRNA expression of Cu/Zn-SOD, Mn-SOD, CAT, glutathione peroxidase, GST, GR mRNA levels and consequently elevate the activities of corresponding antioxidant enzymes (Mirghaed et al., 2019; Wu et al., 2018). Similar to the results of the current study, previous research also demonstrated the positive effects of dietary SCFA on antioxidant capacity of different fish species, such as CA (0.8%–1.6%) in large yellow croaker (Zhang et al., 2016), butyrate or lactate (1%) in giant grouper (Lin & Cheng, 2017), fulvic acid (1%–2%) in juvenile loach (*Paramisgurnus dabryanus*, Gao et al., 2017), sodium butyrate (0.05%–0.1%) in rice field eel (*Monopterus albus*, Zhang et al., 2020), butyrate (0.25%–0.5%) in Asian seabass (Aalamifar et al., 2020) and sodium propionate and acetate (0.5%–1%) in *A. latus*.

In conclusion, from the results of this research it can be suggested that dietary BAG at 1% of supplementation level can increase the growth rate of *A. latus* that mainly attributed to the improvement of feed efficiency (FCR and PER). In addition, health conditions of fish fed with BAF-supplemented diets was promoted that manifested by higher skin mucosal (e.g. lysozyme, ACH50 and total Ig) and humoral (e.g. WBC counts and total protein) immune responses as well as liver antioxidant capacity in this species.

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CONFLICT OF INTEREST

There is no conflict of interest in this paper.

AUTHOR CONTRIBUTIONS

Zarei. S. carried out the experiment. Badzohreh. G developed the original idea and the protocol, abstracted and analysed data, wrote the manuscript. Davoodi R. contributed to the critical revision of the manuscript for important intellectual content. M. Nafisi Bahabadi and F Salehi supervised the work. All authors provided critical feedback and helped shape the research, analysis and manuscript.

ETHICAL APPROVAL

All applicable national and institutional guidelines for the care and use of fish were according to the Iran Veterinary Organization.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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