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Research Article

Growth, body composition, physiological responses and expression of immune-related and growth-related genes of Sobaity seabream (*Sparidentex hasta*) juvenile fed dietary bovine lactoferrin

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Abstract

Lactoferrin (LF) has been used as an immunostimulant in different fish species and have effects on growth and immunological parameters. The aim of this study was to evaluate the effects of dietary LF on growth and feeding performance, body composition, digestive enzymes activity, hematological factors, humoral immune response and expression of immune-related and growth-related genes in sobaity juvenile (*Sparidentex hasta*). One hundred and thirty five fish with an initial average weight of 35.64±0.30 g were fed for a period of 8 weeks. Basal diet was supplemented with 0 (control), 400 and 800 mg kg^{-1} LF in a completely randomized design trial in triplicate groups. The results showed no significant differences in growth and feeding parameters and body composition between control and treatment groups (p>0.05). Moreover, no significant difference was observed in digestive enzymes activity, non-specific immune response, hematological parameters and immune-related gene expression (IL-1β) between control and the treatment groups (p>0.05). Nonetheless, growth-related gene expression (IGF-I) of treatments and control group significantly varied (p<0.05). These results suggested that dietary LF used in this study is not appropriate for supplementation in the diet of sobaity juvenile as it did not influence the growth performance, feed utilization, body composition and the physiological parameters in this fish. This study encourages further research on different aspects of LF in sobaity culture with a clear emphasis on optimizing dosage levels.

Keywords: Immunostimulants, Hemato-immunological factors, Growth performance, Physiological response, Gene expression, Sobaity sea bream (*Sparidentex hasta*)

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Introduction

The antibiotics that are used as growth promoters appear to act by reducing pathogenic bacteria and modifying the microflora in the gut of the animal (Anderson, 1992; Bagni *et al*., 2000). However, dietary antibiotics lead to the presence of drug residues in edible animal products. Because the antibiotic supplementation has been shown to enhance antibiotic resistant strains of pathogens, antibiotic supplementation may result in reduced innate immune function (Anderson, 1992; Bagni *et al*., 2000). Thus, minimizing the use of antibiotics in controlling fish disease outbreaks, the safety of the consumer and the profitability for the farmer alternatives to antibiotics is needed (Anderson, 1992). Immunostimulants such as plant extracts, polysaccharides and lactoferrin (LF) are being promoted in aquaculture as a means that possess great value in the stimulation of the non-specific immune response and prevention of infectious diseases in fish (Anderson, 1992; Kumari *et al*., 2003). LF is an iron-binding glycoprotein present in milk, consisting of 2 lobes that possess essentially identical structural folds and iron-binding sites, which is generally considered to be a significant component of the host innate immune response (Brock, 2002). LF has a diverse range of physiological functions such as antimicrobial activities, initially attributed primarily to iron sequestration, antiviral activities, antioxidant activities, immunomodulation, and modulation of cell growth (Brock, 2002; Wakabayashi *et al*., 2006). This has extended the application potential of LF as a feed additive. A wide range of the effects of orally administered LF have been found in studies in mammals (Wakabayashi *et al*., 2006) and limited information is available regards LF in fish. Recently it has been recognized that oral administration of LF has improved immune response (Rahimnejad *et al.*, 2012; Moradian *et al.*, 2018), growth and feeding performance (Kakuta, 1996), physiological status (Welker *et al.*, 2007; Moradian *et al.*, 2018) and resistance to bacterial infections and lethal stress (Yokoyama *et al.*, 2006; Welker *et al.*, 2007) in different fish.

The diversification of the aquaculture industry, which is based on social, economic and ecological considerations, is a main tool for the sustainability of this fast-growing industry. In this regard, silvery-black porgy or sobaity (*Sparidentex hasta*, Valenciennes, 1830) is considered as a potential candidate for coastal aquaculture diversification in the Persian Gulf and Oman Sea regions due to its readiness to spawn in captivity, rapid growth, and tolerance to a relatively wide range of culture conditions (Pavlidis and Mylonas, 2011). In recent years, this species has been regularly propagated in Iran and juvenile fish are transferred to sea cages for marine aquaculture extension projects (Mozanzadeh *et al.*, 2015). Thus, this species has received a considerable attention from the scientific community in order to

develop its intensive culture and improve diet formulation. Hence, recommending LF as a feed additive in aquaculture relies on the prior determination of its biological effects on different aquatic species under specific conditions. Thus, based on previous studies we decided to evaluate potential effects of different levels of dietary LF on sobaity juvenile.

Material and methods

Experimental design and fish maintenance

This study was carried out at the Mariculture Research Station of the Aquaculture Research Center- Southra of Inian (SIARC), Sarbandar, Iran. One hundred and thirty five juveniles of silvery-black porgy were randomly distributed into 9 cylindrical polyethylene tanks and each tank stocked with 15 fish (initial body weight 35.64±0.30 g, mean±standard error). Fish were acclimated for 2 weeks before the onset of the nutritional trial. Tanks were supplied with filtered running seawater (1 L min^{-1}), salinity ranged between 47 and 49‰ (48.0±0.5‰) and temperature between 25 and 31°C (28.9±1.5ºC) during the experimental period (56 days). Average values for dissolved oxygen and pH were 6.8±0.4 mg L^{-1} and 7.7±0.2, respectively. The photoperiod was held under natural condition (30°32′N, 49°20′E; 14 h). Bovine lactoferrin (LF) (97.2% purity, Japan) used as a supplement in this study was kindly provided by the Morinaga Milk Industry Company. The commercial diets (Beyza, Iran, containing 47% crude protein, 17% crude fat, crude fiber 2% and 14% ash) were supplemented with different levels of LF (0 [control], 400, 800 mg kg^{-1}) according to previously published studies in other marine fish species (Esteban *et al*., 2005; Ren *et al*., 2007). To prepare LF-supplemented feeds 400 and 800 mg of LF was dissolved in 100 mL distilled water and sprayed on 1000 g of commercial dry feed. The feeds were then oven-dried with a continuous 35ºC air current for 60 min. The dried feeds with supplements and the control feed (commercial dry feed only) were sprayed with 50 mL gelatin and stored at 4 ºC until used (Chang and Liu, 2002; Chitsaz *et al*., 2016). The fish were hand-fed *ad libitum*, two times a day at 90:00, and 15:00.

Sample collection

At the end of the trial, fish were fasted for 24 h before being anaesthetized (2-phenoxyethanol at 0.5 mL l^{-1}; Merck, Schuchardt, Germany). At the end of the experiment all fish were weighed individually to the nearest 0.1 g. Three specimens from each replicate were anaesthetized and then sacrificed with an overdose of 2-phenoxyethanol to evaluate whole body proximate composition, digestive enzymes activities and gene expression. Blood was collected from the caudal vein in 3 fish (n = 9 fish per diet treatment, n = 3 fish per replicate) with heparinized syringes, while the collected blood from

the other 3 fish was added into non-heparinized tubes and centrifuged (4000 g, 10 min, 4ºC) in order to be prepared for further analysis of serum (Azodi *et al.*, 2015a; Eslamloo *et al.*, 2017).

Growth measurement

Growth performance was evaluated by specific growth rate (SGR), weight gain (WG), feed conversion ratio (FCR) and protein efficiency ratio (PER) as follows (Azodi *et al.*, 2015b, 2016):

SGR (% day^{-1}) = 100 × [Ln (Mean final body weight) - Ln (Mean initial body weight)] / time (days)

WG (%) = 100 × [(Mean final body weight - Mean initial body weight) / Mean initial body weight]

FCR = dry feed intake (g) / wet weight gain (g)

PER (%) = wet weight gain (g) / protein intake (g)

Chemical analysis

The analyses of proximate composition of fish were performed using the standard methods of AOAC (AOAC, 1995). Briefly, dry matter was measured gravimetrically after oven drying of homogenized samples for 24 h at 105ºC. Crude protein (N×6.25) was determined by the Kjeldahl procedure using an automatic Kjeldahl system. Crude lipid was determined by ether extraction using Soxhlet.

Blood sampling and serum analysis

White blood cell (WBC) and red blood cell (RBC) counts were assessed according to methods described by Houston (1990). Hemoglobin concentration (Hb) and hematocrit value (HCT) were estimated by photometric assay of cyanomethemoglobin and microhematocrit method, respectively (Houston, 1990; Morshedi *et al.*, 2011). Differential WBC counts (neutrophils and monocytes) were conducted on Giemsa stained blood smears.

Serum lysozyme activity was determined by a turbidimetric assay according to the method described by Ellis (1990) based on the lysis of the lysozyme sensitive Gram positive bacteria, *Micrococcus lysodeikticus* (Sigma, USA). Hen egg white (in 0.1 M phosphate citrate buffer, pH=5.8) was used for the preparation of the standard curve. The optical density was measured after 15 and 180 s, using a spectrophotometer (Hitachi 220A, Japan) at 670 nm. The results of lysozyme activity are given as mg mL^{-1}. Complement activity was assayed via the alternative pathway (ACH50) using rabbit red blood cells according to the procedure of Tort *et al.* (1996). The concentration of serum total immunoglobulin (Total Ig) was performed as described by Siwicki *et al.* (1994).

Digestive enzyme assays

Samples of the fish intestines (3 per replicate) were homogenized immediately in 100 mM Tris–HCl

buffer with 0.1 mM EDTA and 0.1% Triton X-100, pH 7.8, followed by centrifugation (30000×*g*; 12 min at 4°C). After centrifugation, the supernatant was collected and frozen at −80°C (Furne *et al.*, 2008). Total protease, lipase and amylase were assayed according to the methods described below. The specific activity of lipase was performed by the enzymatic photometric method using lipase kit (Bionik, Canada). It was based on 1, 2-o-Dilauryl-rac-3glutaric acid (6-methyresorufin) ester as a substrate that was broken down into 6-methyresorufin and Glutaric acid 6-ethylresorufin-ester by lipase. Specific activity of amylase was measured using the enzymatic photometric method by amylase kit (Bionik, Canada). It was based on 4, 6-ethylidene-(G7)-p-nitrophenyl-(G1)- alpha-D-maltoheptaoside (EPS-G7) as a substrate. Total protease activity quantification followed according to the method published by Anson (1938). In this method, azocasein was used as substrate. The reaction mixture contained 1 mL of 1.5% casein solution, pH 7.0, was placed at 37°C and then, 1 mL of a supernatant sample was added. The reaction was incubated for 10 min before adding 2 mL of 0.4 M trichloroacetic acid. The solution was filtered, and 2.5 mL of 0.4 M Na_2CO_3 and 0.5 mL of Folin reagent were added. Finally, the color density was determined at 660 nm. Total soluble protein was measured by the Bradford (1976) method using bovine serum albumin as a standard. Enzyme activity is expressed as specific activity/mg protein.

Gene expression analysis

Gene cloning: Primers used in the identification of the *Sparidentex hasta* interleukin-1β (IL-1β) and insulin-like growth factor I (IGF-I) sequences were designed with Primer 3. Primers for IL-1β and IGF-I genes were designed based on nucleotide sequences with the gene bank numbers AY669059.1, AJ277166.2, JQ973887.1, and AJ269472.1: AY608674.1, AF030573.1, AY996779.2, and AB902571. Primer sequences are shown in Table 1. Total RNAs were extracted from liver and kidney tissue samples using the RNA extraction kit (Roche). Total RNA concentration was measured by spectrophotometry and the quality was assessed by 1% agarose electrophoresis. The first strand cDNA were synthesized using M-MLV Reverse Transcriptase (Ferments). cDNA of liver and kidney were used as template. PCR was performed with the following setting: denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 60 s, 58°C for 45 s, and 72°C for 1 min, with a final extension step of 72°C for 10 min. The PCR products were separated on a 1% agarose gel and then with PCR purification kit (Qiagene). Purified fragment was sequenced by a commercial company (Tops gene).

Gene expression

Real-time PCR primers were designed based on *S. hasta* IL-1β and IGF-I sequences, and to the beta actin gene (based on alignments from AY491380.1, KY388508.1, and AY510710.2; primer sequences are shown in Table 1 using Primer 3 online software. Head kidney and liver tissues were homogenized in liquid nitrogen, and total RNA was extracted using RNA extraction kit (Sinagen) according to the manual of manufacturer. RNA samples quality were checked using a NanoDrop ND-1000 spectrophotometer. cDNA was synthesized from 1 µg of total RNA using a RevertAidTM First Strand cDNA Synthesis kit (Fermentas, K1622). The genes determined in this study included IGF-I and IL-1β. The cDNAs were used to perform real-time PCR with specific primers which previously reported as shown in Table 1. β-actin gene was used as a housekeeping gene in all experiments. The real-time PCR was performed with the SYBR Green Real-time PCR Master Mix (Sinagene, Iran) in a Rotor-Gen3000 real-time PCR detection system (Corbett research). Each 20-µL amplification contained 10 uL SYBR Green Real-time PCR Master Mix (Sinagene), 10 pmol of each primer and 1 µl cDNA. The mixtures were run with the following thermal cycling program: an initial activation step at 95°C for 3 min and then 40 cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 30s. All real-time PCRs were performed at least three times. Melt curve analysis was performed on the PCR products at the end of each run to ensure that a single product was amplified. Relative targeted gene expression was calculated for each reaction by the ΔΔCt method.

Statistical method

All data were analyzed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Normality and homogeneity of variances were tested initially using the Kolmogorov–Smirnov and Levene tests, respectively. Differences between the dietary groups were tested using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Data are presented as means ± standard error (n = 3) and differences were considered to be significant at $p<0.05$.

Results

There was no mortality in the trial. The growth performance of juvenile sobaity seabream fed the diets containing LF over a period of 8 weeks is reported in Table 2. No significant differences were observed for final weight, percent weight gain, specific growth rate, and protein efficiency ratio of juvenile sobaity seabream between LF-treatments and control treatment ($p>0.05$). In the present study, whole body dry matter, protein and lipid contents did not change among dietary treatments ($p>0.05$; Table 3).

Table 1: Nucleotide sequences of the primers used to cloning and assay gene expression.

Primer Name	Nucleotide sequences (5'– 3')
IGF-I Forward	ATGTCTAGCGCTCTTTCCTTTC
IGF-I Reverse	CTACATTCGGTAATTTCTGCCCC
IL-1β Forward	TGGAATCCGAGATGACATGCAAC
IL-1β Reverse	CACTCGCCATCCCCCACTG
IGF-I Forward(Real-Time PCR)	TGTAGCCACACCCTCTCACT
IGF-I Reverse (Real-Time PCR)	AGCCTCTCTCTCCACACACA
IL-1β (Real-Time PCR)	CTGGACTTGGAGATTGCCCA
IL-1β Reverse (Real-Time PCR)	CTTCCACTGCGCTCTCCAG
Beta actine F (Real-Time PCR)	AGGGAAATCGTGCGTGACAT
Beta actine R (Real-Time PCR)	CGAGGAAGGATGGCTGGAAG

Interleukin-1β (IL-1β), Insulin-like growth factor I (IGF-I)

Table 2: Growth and feeding performance of sobaity juvenile fed different levels of lactoferrin (LF) for 8 weeks.

Parameters	Levels of LF (mg kg^{-1})		
	Control	400	800
Initial weight (g)	35.98±0.03	36.06±0.23	35.95±0.20
Final weight (g)	76.09±1.75	76.84±0.26	73.26±0.39
SGR (% day^{-1})	1.34±0.04	1.35±0.00	1.27±0.02
WG (%)	111.46±4.67	113.08±1.61	103.83±2.23
FCR	1.57±0.03	1.64±0.02	1.70±0.06
PER	1.23±0.03	1.18±0.01	1.13±0.04

SGR=100×[Ln (Mean final body weight) - Ln (Mean initial body weight)] /time (days), WG (%)=100[(Mean final body weight - Mean initial body weight)/ Mean initial body weight], FCR=dry feed intake (g)/wet weight gain (g), PER=wet weight gain (g)/ protein intake (g), standard error (SE).
No significant changes were observed (mean±SE, $p<0.05$).

Table 3: Body composition of sobaity juvenile fed the different levels of lactoferrin (LF) for 8 weeks

Parameters (% wet weight)	Levels of LF (mg kg^{-1})		
	Control	400	800
Protein (%)	23.20±0.66	21.30±0.46	22.01±0.22
Lipid (%)	7.91±0.47	7.77±0.47	8.64±0.49
Dry mater (%)	39.96±1.21	36.86±0.93	37.89±0.92

No significant changes were observed (mean±SE, $p<0.05$).

The changes in some hemato-immunological parameters of sobaity seabream fed different levels of dietary LF are shown in Table 4. At the end of the experiment, dietary LF had no significant effects on RBC, HCT content, Hb concentration and differential WBC counts ($p>0.05$). The amount of total Ig, ACH50 and lysozyme activity in sobity seabream did not markedly vary between the control and the supplemented diet with LF ($p>0.05$; Table 5).

Table 4: Hematological factors of sobaity juvenile fed the different levels of lactoferrin (LF) for 8 weeks

Parameters	Levels of LF (mg kg^{-1})		
	Control	400	800
HB (g/dl)	8.32±0.18	7.88±0.55	8.18±0.34
HCT (%)	59.00±1.58	55.25±3.90	57.25±2.21
RBC ($\times 10^6$/mm^3)	2.54±0.06	2.39±0.01	2.45±0.01
WBC (mm^3)	4950±306.8	5000±227.3	4925±768.5
Monocytes (%)	2.75±0.47	4.00±0.40	3.50±0.95
Neutrophilis (%)	24.25±0.25	24.50±0.28	25.00±2.67
Lymphoctes (%)	72.75±0.47	70.75±0.62	70.50±3.37

Hemoglobin (Hb), Hematocrit (HCT), Red blood cell (WBC), White blood cell (RBC)
No significant changes were observed (mean ± SE, $p<0.05$).

Table 5: Immune responses of sobaity juvenile fed the different levels of lactoferrin (LF) for 8 weeks.

Parameters	Levels of LF (mg kg^{-1})		
	Control	400	800
Total Ig (mg/dl)	18.00±0.31	22.81±5.30	12.72±2.30
Lysozyme (mg/ml)	2.98±0.20	2.98±0.77	4.90±0.64
ACH50 (mU/ml)	0.068±0.00	0.067±0.00	0.072±0.00

No significant changes were observed (mean±SE, $p<0.05$).

As are presented in Table 6, the supplementation of diet with LF did not coincide with the activities of total protease, lipase and amylase ($p>0.05$). Expression of immune-related and growth-related genes of sobaity fed diets with different levels of LF is presented in Table 7. There were significant differences in IGF-I gene expression of treatments and control group ($p<0.05$). Nonetheless, IL1β gene expression did not affect by different experimental groups ($p>0.05$).

Table 6: Digestive enzymes activity of sobaity juvenile fed the different levels of lactoferrin (LF) for 8 weeks (mean ± SE).

Parameters	Levels of LF (mg kg^{-1})		
	Control	400	800
Total protease (U mg protein)	0.15±0.01	0.11±0.01	0.07±0.00
Amylase (µmole/mg protein)	4.82±0.12	4.04±0.04	4.35±0.09
Lipase (µmole/g/h)	0.33±0.02	0.33±0.01	0.23±0.01

No significant changes were observed (mean±SE, $p<0.05$).

Table 7: Expression of immune-related and growth-related genes of *S. hasta* juveniles fed experimental diets supplemented with lactoferrin (LF) for 8 weeks.

Parameters	Levels of LF (mg kg^{-1})		
	Control	400	800
IGF-I	0.99±0.01^{a}	1.48 ± 0.08^{b}	1.39 ± 0.13^{ab}
IL1ß	1.00±0.10^{a}	1.34 ± 0.09^{a}	1.53 ± 0.14^{a}

A different superscript in the same row denotes statistically significant differences (mean±SE, p<0.05).

Discussion

The results of our study showed that supplementation of diet with different levels of LF did not significantly affect on the growth and feeding performance including FW, WG, SGR, PER and FCR in sobaity. This suggests that supplementation of LF at range of 400 to 800 mg kg^{-1} was not optimal for the growth of sobaity fingerling. It has been already established that LF effectively improves immune response, growth, nutritional or physiological status and resistance to bacterial infections of different animals (Brock, 2002; Wakabayashi *et al.*, 2006). However, limited information is available regarding the LF in fish especially marine fish. Similar results have been reported following administration of LF in diets of gilthead seabream (*Sparus auratus*), rainbow trout (*Oncorhynchus mykiss*) and African cichlid (*Sciaenochromis fryeri*) (Esteban *et al.*, 2005; Rahimnejad *et al.*, 2012; Moradian *et al.*, 2018). Moreover, Yokoyama *et al*. (2006), Welker *et al*. (2007) and Eslamloo *et al*. (2012) showed that administration of 400 to 1200 mg kg^{-1} in the diet of orange spotted grouper (*Epinephelus coioides*) and 100 to 1600 mg kg^{-1} Lf in Nile tilapia (*Oreochromis niloticus*) and Siberian sturgeon (*Acipenser baeri*) had no significant effect on growth performance and feed utilization. In disagreement with the present results, Badawy *et al*. (2013) found elevated growth performance and feed utilization of Nile tilapia fed 200 to 600 mg kg^{-1} and administration of 0.6 to 60 mg kg^{-1} LF in the diet of juvenile goldfish (*Carassius auratus*) significantly improved growth performance (Kakuta, 1996). From previous studies, it has been suggested that the ability of different species to absorb LF, dosage, different duration of LF administration, different fish species are the main factors that determine the efficacy of dietary LF on fish growth and feed utilization (Eslamloo *et al.*, 2012; Moradian *et al.*, 2018). According to Yokoyama *et al*. (2006), the interaction between LF and some unknown ingredients observed in their study may explain the conventional controversy about LF effects on fish growth. To our knowledge, the interaction between diet ingredients and LF is not clear and merit future research.

Fish body composition is influenced by several factors such as morphological, physiological and

environmental factors (Salam and Davies, 1994). In the present study, whole body protein, lipid and dry matter did not change among dietary treatments. There is no available data on the body composition of fish fed with LF. However, from previous studies, it has been suggested that changes in body composition especially protein and lipid likely to be attributed to changes in deposition rate in muscle and different growth rate (Abdel-Tawwab *et al.*, 2006; Abdel-Tawwab *et al.*, 2008). The results of the present study showed that the effects of LF on body composition were not efficient.

The hematological parameters of fish can be influenced by many biotic and abiotic factors such as age, sex, water temperature, seasonal pattern, and feeding status (Řehulka *et al.*, 2004). In the present study, hematological parameters of sobaity juvenile were not affected by dietary LF. Similar to our results; Kauta *et al*. (1996) and Eslamloo *et al*. (2012) observed a non significant improvement in hematological parameters (WBC, RBC, and hematocrit) in red sea bream (*Pagrus major*) and Siberian sturgeon fed on dietary LF after 8 weeks feeding. Moreover, Welker *et al*. (2007), Rahimnejad *et al*. (2012) and Moradian *et al*. (2018) reported that hematological parameters of Nile tilapia, rainbow trout and African cichlid were not affected by dietary LF. Kawakami *et al*. (1988) reported that the supplementation of diet with LF in iron-deficient anemic rats significantly increased RBC, hemoglobin and hematocrit values along with a subsequent improvement of anemia. It seems that the physiological conditions of the animal and the availability of iron in the diet have contributions to improving hematological factors by LF (Eslamloo *et al.*, 2012).

The complement and lysozyme activity are important components of non-specific immune defense for protecting fish against potentially invasive organisms through lysis of their cellular membranes (Chipman and Sharon, 1969; Müller-Eberhard, 1988). Proteins such as immunoglobulin are the main serum components that a basic requirement for an efficient immune system (Kumar *et al.*, 2005). In the present study, non-specific immune response of sobaity including ACH_{50}, lysozyme activity and total immunoglobulin did not show significant variations induced by dietary LF. Similar results have been reported in the other studies on some fishes such as gilthead seabream (Esteban *et al.*, 2005), Nile tilapia, channel catfish (*Ictalurus punctatus*) (Welker *et al.*, 2007, 2010) and Siberian sturgeon (Eslamloo *et al.*, 2012). In contrast to this result, significant differences were observed in rainbow trout and African cichlid (Rahimnejad *et al.*, 2012; Moradian *et al.*, 2018). In our study, LF supplementation did not significantly affect hematological parameters. These results suggest that this supplementation could not play a role in enhancing the general health status of the fish with subsequent enhancement of immune response. It is clear that

optimizing LF levels requires further attention as there is often a fine line between achieving benefits and achieving negative effects.

It has been reported that LF possesses serine protease activity in a gram-negative bacteria, *Haemophilus influenza* (Brock, 2002). Teraguchi *et al*. (1994) reported that mice fed cows' milk containing 2% bLF or bLFhyd, the over growth of Enterobacteriaceae and *Streptococcus* was significantly suppressed; but, LF did not suppress the intestinal *Bifidobacterium*. In our study, there was no significant difference in amylase, lipase and protease activities between LF-treatment and the control groups. It has been demonstrated that LF had a diverse range of biological activities including antimicrobial activities, antiviral activities, intestinal bacterial flora and digestive tract-related infections (Wakabayashi *et al.*, 2006) that it could subsequently changes intestine digestive enzymes. However, it is not clear that whether these enzymes activities are related to endogenous (host) and/or exogenous microbial activities (potentially modulated by supplements). Unfortunately, no information regarding the digestive enzyme activities such as amylase, lipase and protease affected by LF is available. However, more research is needed to expand knowledge on the variation of digestive enzyme activities in sobaity when fish diet supplemented with LF.

It is well known that the expression of immune-related genes can be considered as a useful tool for evaluating immune response (Alejo and Tafalla, 2011). The results of this study showed that the growth-related gene such as IGF-I did not affect by administration of dietary LF, although fish fed LF_{400} showed higher expression than the control groups. Nonetheless, the immune-related gene such as IL1β did not indicate significant variations induced by diet supplementation.

Although earlier studies have widely documented the effects of LF administration on growth performance and immune responses in several fish species, but a limited number of studies have focused on the expression of immune-related and growth-related genes. Carnevali *et al*. (2006) reported an increase of IGF-I transcription in European sea bass (*Dicentrarchus labrax* L.) fed diet containing probiotic *Lactobacillus delbrueckii delbrueckii*. These authors suggested that at molecular level, the expression of genes involved in muscular growth was also positively affected by bacterial integrators confirming a beneficial role of probiotics on the whole metabolism. However, more research is needed to expand knowledge on the variation of gene expression in sobaity when fish diet supplemented with LF.

Overall, the results of the present study indicate that any effects on growth and feeding performance and body composition after 8 weeks feeding with LF supplemented diet were not observed for sobaity fish. There were no significant effects through dietary

LF on other measured parameters in the present study. Therefore, the present results do not support using dietary LF as an immunostimulant-supplemented feed in diet of sobaity. This preliminary study encourages further research on different aspects of LF administration in sobaity culture as well as cellular immunological responses and disease resistance.

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