PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 13 (6): 319-326, 2014
ISSN 1680-5194

Growth, Digestive Enzyme Activity and Health Status of Humpback Grouper (*Cromileptes altivelis*) Fed with Synbiotic

Rini Marlida[1], Muhammad Agus Suprayudi[2], Widanarni[2] and Enang Harris[2]
[1]Departement of Fisheries, Faculty of Agriculture, Achmad Yani University, Banjarmasin, South Kalimantan, Indonesia
[2]Department of Aquaculture, Faculty of Fishery and Marine Science, Bogor Agricultural University, Indonesia

Abstract: This research aimed to investigate biological responses of humpback grouper (*C. altivelis*) supplied by synbiotic supplement, which are growth, digestive enzyme activity and health status. Probiotic applied was isolated from humpback grouper digestive tract and screened for amilolytic, proteolytic and lipolytic activities; while prebiotic was obtained from the extracts of sweet potato (*Ipoemea batatas*). The experiments comprised of four combination of synbiotics containing 1% probiotic candidate in treatment RM3, RM4, RM5, RM7 + 2% prebiotic and one control (without symbiotic) supplementation. Each synbiotic treatment was added with 2% egg white to bind all ingredients together. Completely randomized design with three replicates was applied for the experiment during 40 days rearing period. The result indicated that treatment with synbiotics were significantly different ($p<0.05$) in protein and total digestibility as compared to control group, whereas treatment RM4 and RM7 had the best result in weight gain (ΔW), specific growth rate (SGR), protein retention, feed conversion ratio, RNA/DNA ratio, in comparison to treatment RM3, RM5 and without synbiotic. Application of synbiotic treatment combination of 1% probiotic RM4 + 2% prebiotic also showed enhancement of digestive enzyme activity (amylase, protease and lipase), biochemical plasma (glucose and triglyceride) and haematology parameter (haemoglobin, hematocrit and phagocytic activity).

Key words: Growth, humpback grouper, synbiotic

INTRODUCTION

Aquaculture has become the most important part in supplying the source of animal protein, in addition to husbandry for terrestrial animals. One marine species cultured with high economic value is humpback grouper (*C. altivelis*), which has been intensively applied in several places in Indonesia. However, intensive aquaculture practices were facing various obstacles related to growth rate, feed conversion and diseases, thus limiting their production. Humpback grouper nutrition has been widely researched (Laining *et al*., 2003; Shapawi *et al*., 2007; Williams, 2009), with emphasis on nutrient demand for fish growth. Nutrition for fish health management was primarily related to stress relief using combination of feed containing Fe (Setiawati, 2010) and Se (Hamzah, 2013). At present, fish nutrition research related to improving the status of fish health has become main priority in order to reduce the application of hazardous chemical substances. Pohlenz and Gatlin (2014) claimed a new term for such field, which is "immunonutrition", defined as a study to improve immunological functions through specific nutrients and other feed compositions with higher level than those required for optimal growth.

Nowadays, probiotic has become a part of aquaculture system functioning as production improved (Balcazar *et al*., 2006). Probiotic is organism living as a colony in digestive system. Exogenous induction as feeding supplement needs a property to differentiate into commensal microbes. Probiotic not only performs action mechanism in immune system, but also possess protective role, i.e. directly blocks pathogenic microbes and increases mucus integrity by epithelial cell stimulation (Gourbeyre *et al*., 2011). Nevertheless, the concept on probiotic remains limited as it is influenced by survival capability, colonization and nutrient competition. Extreme environmental changes can cause bacteria living in digestive system will be washed out. Survival of probiotic bacteria in digestive micro ecosystem is very much influenced by environmental factors, i.e. drugs and food (Gourbeyre *et al*., 2011). Alternative strategy for recovering the gut microbiota balance is using prebiotics (Gibson *et al*., 2004). Prebiotic is in digested feed yet it able to be fermented by bacteria, thus giving advantageous effect to the host organisms. Source of prebiotic can be obtained from tubers, such as raffinose, oligofructose and maltotriose as found in sweet potato *I. batatas* (Rini, 2008; Marlis, 2008). Sweet potato from Sukuh variety is locally

Corresponding Author: Muhammad Agus Suprayudi, Department of Aquaculture, Faculty of Fishery and Marine Science, Bogor Agricultural University, Indonesia

originated from Indonesia and potential as prebiotic source, containing oligosaccharide which is functional and indigestible by digestive enzymes.

Combination of mannan oligosaccharides and *Bacillus* spp. in European lobster larvae (*Hommarus gammarus* L.) (Daniels *et al*., 2013), combination of mannan oligosaccharides and *Enterococus faecalis* in rainbow trout (*Oncorhyncchus mykiss*) (Rodriguez-Estrada *et al*., 2009) and combination of *Bacillus subtilis* and fructo-oligosaccharides in yellow croaker, *Larimichtys crocea* (Ai *et al*., 2011) supplemented in the feed have been successfully nurture growth, immune response and resistance towards disease. Less research had been conducted on growth response, digestive enzymes activity and haematology of humpback grouper (*C. altivelis*) feed on synbiotic food so far. Humpback grouper is one of the most valuable species of grouper. Commanding high prices in the live reef fish trade, the expansion in grouper aquaculture has seen in increasing need for more sustainable and environmentally responsible culture practices and especially the development of manufactured feeds that had better meet the nutritional requirements of the fish (Williams, 2009). Supplementation of synbiotic in fish feeding was expected to meet nutritional demand of humpback grouper, by improving digestive capability, nutrition absorption and feed efficiency, as well as rehabilitating the health status in humpback grouper aquaculture.

MATERIALS AND METHODS

Preparation of feed: This research was using commercial feed (*Otohime Marine Weaning Diet* EP 1 Japan) sized 1,5 mm of size. Prior to use, feed was weighed daily 3% from biomass weight and was added with 1% probiotic candidate that had been isolated and screened from humpback grouper digestive tracts, i.e., isolates RM3, RM4, RM5 and RM7 with 2% prebiotic extracted from sweet potato Sukuh variety (*I. batatas*) and control feed without synbiotic addition. All treatment was coated by 2% egg whites. Feed was wind-dried for approximately 15 min prior to fish feeding. Synbiotic feed production was carried out daily. Proximate analysis of feed was performed according to Association of Official Analytical Chemists (AOAC) method (1990) (Table 1).

Prebiotic extraction: Oligosaccharides extraction was referring to method by Muchtadi (1989), where 500 grams of mixed sweet potato flour (*I. batatas*) and water with ratio of 1:1 (w/v) was steamed for 30 min in 100°C. Mixed dough was dried in the oven for 18 h in 55°C, prior to milled and filtered with sieve until the steamed sweet potato flour was collected. During extraction process, 10 g of flour was suspended into 100 mL of ethanol 70% and stirred for 15 h using magnetic stirrer under room temperature. Filtrates were concentrated by using vacuum evaporator at 40°C. Concentrates were then centrifuged at 5000 rpm for 10 min to precipitate the impurities and solids, thus its extract will easily be sterilized. The required total dissolved solid content of oligosaccharides is 5%. Composition of oligosaccharides in sweet potato was estimated by using HPLC method (Table 2).

Table 1: Proximate composition of fish diets

	Treatments				
Proximate composition (%)	RM3	RM4	RM5	RM7	Without synbiotic
Moisture	11.45	10.17	10.92	10.94	10.61
Protein	37.99	37.07	38.71	39.66	39.46
Lipid	15.48	15.53	15.35	15.68	13.49
Ash	13.80	13.95	13.72	13.83	14.29
Crude fiber	0.65	0.68	0.28	0.36	0.17
NFE*	20.63	22.59	21.02	19.52	22.02

*NFE, Nitrogen free extract

Table 2: Composition of oligosaccharides in sweet potato extracts (*I. batatas*)

Parameter	Unit (g/100 g)
Fructo-oligosaccharides (FOS)	1.015
Galacto-oligosaccharides (GOS)	1.488
Inulin	1.115

Experimental animals and design: One hundred twenty fingerlings of humpback grouper (*C. altivelis*) were obtained from Research Agency for Marine Aquaculture (BPBL), Situbondo, Indonesia with average initial weight 4.75±0.02 g. Prior to the experiment, fish were acclimatized for one week in one-ton fiber glass tank with aeration system. Experiment was carried out based on complete randomized design with 4 synbiotic treatments and 1 control (without synbiotic). All treatments were did in three replications.

Fish were kept for 40 days and fed twice daily at 08.00 and 16.00 with 3%/BW. All fish were placed in 15 aquariums with size of 60 x 30 x 30 cm with 36 L of water. Aeration was applied in each aquarium where eight fishes were placed. During acclimatization, fish were kept without feed for 24 h prior to maintenance, in order to remove any residues in their digestive tracts. Siphoning was conducted after feeding to discard residual feed and feces. A proportion of 10% of water volume was exchanged daily and every 10 days for total water replacement, together with measuring fish weight and body size.

Data collection and statistical analysis: Growth performance parameters for analysis were body weight gain (WG); fish of all replicates were weighed individually and body weight gain determined every 10 days for fed adjustment 3 %/BW, specific growth rate (SGR), protein retention (PR) and lipid retention (LR) (Takeuchi, 1988), fed conversion ratio (FCR), RNA/DNA ratio (Glemet and Rodriguez, 2007), liver and muscle glycogen (Wedemeyer and Yasutake, 1977).

Table 3: Protein digestibility (ADprot) and total digestibility (ADCtn) of humpback grouper (*C. altivelis*) fed with different synbiotic

	Treatments				
Parameter	RM3	RM4	RM5	RM7	Without synbiotic
ADprot (%)	88.89±0.75^{a}	90.12±0.26^{a}	88.85±0.04^{a}	91.17±1.75^{a}	86.02±0.91^{b}
ADCtn(%)	64.32±1.44^{a}	63.73±1.61^{a}	65.90±0.16^{a}	66.92±2.08^{a}	51.16±0.97^{b}

Data expressed as mean±SD (n = 3)
Mean values in same row with different superscripts vary significantly ($p<0.05$)

Table 4: Blood glucose (mg/100 mL) and triglyceride (mg/100 mL) of humpback grouper (*C. altivelis*) fed with different synbiotic

	Treatments				
Parameter	RM3	RM4	RM5	RM7	Without synbiotic
Glucose	81.97±9.86^{b}	61.54±7.48^{c}	55.29±2.72^{c}	68.51±9.18^{bc}	104.81±6.12^{a}
Triglyceride	285.23±7.05^{bc}	330.26±23.59^{b}	243.39±17.72^{cd}	214.20±32.30^{d}	395.68±4.36^{a}

Data expressed as mean±SD (n = 3)
Mean values in same row with different superscripts vary significantly ($p<0.05$)

Body proximate analysis: Two fish from all replicates were taken for proximate analysis including protein content estimated by Kjeldahl method (Takeuchi, 1988), Folch method for lipid, ash content by tenure heating at 600°C temperature, water content by oven heating at 105-110°C temperature, crude fiber by sample dissolution with strong acid and base and heating as well. Determination of body proximate was conducted at the beginning and the end of maintenance.

Digestibility: The apparent digestibility coefficient (ADC) of the diets were determined by indirect method using 0,6% chromic oxide (Cr_2O_3) as digestibility indicator based on proximate analysis and indicator Cr_2O_3 on feces and feed (Takeuchi, 1988). Apparent digestibility coefficient of total nutrient (ADC tn) was calculated using formula; ADCtn = 100-(%marker in feed/%marker in feces x 100). Apparent digestibility of protein (ADprot) = 100 - [(%marker in feed/ % marker in feces) x (% nutrient in feces/% nutrient in feed) x 100].

Digestive enzymes activity, digestive tract of 3 fish from each replicate were collected and weighed. Extract filtrate of crude enzyme was taken to perform a test for amylase enzyme activity according to Worthington (1993) by using starch as substrate; protease enzyme activity according to Bergmeyer and Grassi (1983) with casein as substrate; and lipase enzyme activity according to Borlongan (1990) by using olive oil emulsion as substrate.

Haematological parameters, blood samples were collected from the caudal vein of 3 fish from each replicate using sterile syringe with anticoagulant. Blood samples for haematological analysis were directly processed according to the procedure, while samples for glucose and triglyceride analysis were centrifuged firstly for 5 min at 6000 rpm. Supernatants were then separated and stored at -20°C until analytical evaluation. Haemoglobin concentration was measured by Sahli method by using salinometer (Wedemeyer and Yasutake, 1977); haematocrit content and phagocytic index were measured by method from Anderson and Siwicki (1993). Concentration of blood glucose was analyzed by enzymatic colorimetric method using glucose liquicolor kit GOD-PAP. Concentration of blood triglycerides was analyzed according to the method of enzymatic colorimetric test for triglycerides with lipid clearing factor using GPO-PAP kit. GOD-PAP and GPO-PAP kit were obtained from Human Gesselischaft, Germany.

Survival was estimated starting from the beginning of feeding experiment until the end of 40-day rearing period.

Comparison among all treatments was carried out by one-way ANOVA followed by Duncan multiple range test method. Comparison was made at the 5% probability levels. The data were statistically analyzed by statistical package program Microsoft Excel 2007 and SPSS version 22 software.

RESULTS

Growth performance and survival: These results indicated that parameters of digestibility such as protein and total digestibility for the combination of prebiotics with probiotics in all candidates were significantly higher ($p<0.05$) than the control (Table 3). The synbiotic combination on treatment RM4 (1% probiotic RM4+2% prebiotic) and RM7 (1% probiotic RM7+2% prebiotic) is the best combination and significantly ($p<0.05$) increase weight gain (54.51±1.73 and 55.95±3.96 g, respectively), daily growth rate, protein retention, feed conversion, ratio of RNA/DNA (Table 5). Survival rates was 100 % for all treatment in 40 days rearing period. Probiotic candidates in this research were isolation from humpback grouper digestive tract and identification used kit API 20E for RM3 significantly 99.3% with *Ewingella americana*, RM4 significantly 86.0 % with *Vibrio alginolyticus*, RM7 significantly 96.9 % with *Pseudomonas flourescens* and RM5 identification used kit API 20NE, significantly 99.4% with *Sphingomonas paucimobilis*.

Haematology and blood biochemistry: Haematologic response can be seen from the number of haemoglobin, haematocrit and phagocytic activity (Table 6). While the biochemical response o f blood can be seen on glucose and triglycerides plasma (Table 4).

Table 5: Feed intake (FI.), body weight gain (BWG), specific growth rate (SGR), feed conversion ratio (FCR), protein retention (PR), lipid retention (LR), liver glycogen (LG), muscle glycogen (MG) and survival rate (SR) of *C. altivelis* for different synbiotic treatment

	Treatments				
Parameter	RM3	RM4	RM5	RM7	Without synbiotic
FI (g)	54.58±5.28^{ab}	53.73±1.82^{ab}	55.18±5.52^{a}	55.78±1.02^{a}	46.59±0.91^{b}
BWG (g)	44.55±4.18^{c}	54.51±1.73^{ab}	49.12±5.06^{bc}	55.95±3.96^{a}	36.78±2.36^{d}
SGR (%)	1.97±0.18^{bc}	2.22±0.03^{a}	2.01±0.14^{b}	2.32±0.02^{a}	1.79±0.10^{c}
FCR (%)	1.28±0.24^{a}	0.99±0.01^{b}	1.13±0.08^{ab}	0.99±0.04^{b}	1.27±0.07^{a}
PR (%)	40.56±6.13^{bc}	55.76±3.13^{a}	43.38±1.84^{bc}	44.83±4.94^{b}	36.81±3.04^{c}
LR (%)	50.27±4.95^{a}	49.91±2.88^{a}	55.43±4.41^{a}	51.43±3.20^{a}	53.57±2.33^{a}
RNA/DNA	1.24±0.03^{bc}	1.45±0.12^{a}	1.26±0.06^{bc}	1.39±0.13^{ab}	1.17±0.08^{c}
LG	1.44±0.11^{a}	0.77±0.45^{a}	0.17±0.12^{a}	1.29±0.04^{a}	0.55±0.11^{a}
MG	0.16±0.06^{a}	0.06±0.02^{b}	0.04±0.02^{b}	0.02±0.00^{b}	0.06±0.03^{b}
SR (%)	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}

Data expressed as mean±SD (n = 3)
Mean values in same row with different superscripts vary significantly (p<0.05)

Table 6: Haemoglobin (Hb), hematocrit (Ht) and phagocytic activity (PA) of *C. altivelis* for different synbiotic treatment

	Treatments				
Parameter	RM3	RM4	RM5	RM7	Without synbiotic
Hb (g%)	6.60±0.57^{a}	6.10±0.99^{a}	5.80±0.57^{a}	5.60±0.28^{ab}	4.22±0.31^{b}
Ht (%)	27.26±0.47^{ab}	30.78±0.67^{a}	25.72±4.03^{ab}	34.75±3.39^{a}	16.11±8.64^{b}
PA (%)	29.29±1.01^{a}	27.27±0.00^{a}	18.82±1.66^{b}	31.79±4.55^{a}	16.25±5.30^{b}

Data expressed as mean±SD (n = 3)
Mean values in same row with different superscripts vary significantly (p<0.05)

Digestive enzyme activity: The amylase activity in treatment B was significantly higher (p<0.05), than in other groups. Similar observation were also recorded for lipase and protease activity. Digestive enzyme activities for all treatments are showed in Fig. 1.

DISCUSSION

Growth and digestive enzyme activity: Feeding activity is closely related to appetite, which in turn determines the amount of food eaten (food intake). In this study the amount of feed consumed by the fish in the synbiotic treatment was higher (p<0.05) than the control (Table 5). Digestion is a complex, but closely orchestrated process, involving enzyme and fluid secretions and motility, culminating in absorption and evacuation (Ronnestad *et al*., 2007). Feed consumed by the fish will experience the process of food simplification, either through physical or chemical mechanisms, so that, food will become material that is easily absorbed and circulated throughout the body via the circulatory system. According to Ronnestad *et al*. (2007) the major function of the digestive system is to digest/degrade macronutrients from feedstuff into a form that can be easily absorbed, in order to supply dietary nutrients to the body tissues. Mechanical digestion occurs in the stomach and intestines happen effectively due to the activity of digestive enzymes.

Probiotic candidates isolated from the digestive tract of humpback grouper and supplemented into feed could serve as providers of exogenous enzymes and help the simplification process of feed macromolecules into micromolecules that are easily absorbed so it can be used as sources of energy or as precursors in the synthesis of cells components. Enzyme activities of amylase, lipase and protease in treatment RM4 were significantly higher (p<0.05) was 8.52±0.39, 0.07±0.00 and 0.07±0.00 U/mL/min, respectively, compared with other treatments and control (Fig. 1). Yang *et al*. (2005) explained that the increase in digestive enzymes activity may be caused by the synbiotic that stimulates a healthier digestive tract microbial ecology and modifies the selection of bacterial enzymes.

Some researchers explain that the action and beneficial effects of probiotics in fish farming are in their ability to improve the nutrition of host species through the production of supplemental digestive enzymes, growth and high feed efficiency, protection against intestinal disorders and antinutritional factors' absorption contained in the feed material (Verschuere *et al*., 2000; Ziaei-Nejad *et al*., 2006; Mohapatra *et al*., 2011). Bacillus species isolated from the digestive tract of *Cyprinus carpio* fish turn out to have a large number of activities amylolytic, proteolytic and lipolytic extracellular (Bairagi *et al*., 2002). The existence of probiotics can stimulate the production of endogenous enzymes (Wang, 2007).

Among the four synbiotic compositions studied: 2% prebiotic blended with each candidate probiotic RM 4 and RM 7 was a synergy that best increased weight gain, specific growth rate, feed conversion rate, retention ratio of protein and RNA/DNA (Table 5). Synergistic action was also demonstrated by a combination of mannan oligosaccharides and *Bacillus* spp. in European lobster larvae (*Hommarus gammarus* L.) (Daniels *et al*., 2013), as well as the combination of mannan oligosaccharides and *Enterococus faecalis* in rainbow trout (*Oncorhyncchus mykiss*) (Rodriguez-Estrada *et al*., 2009).

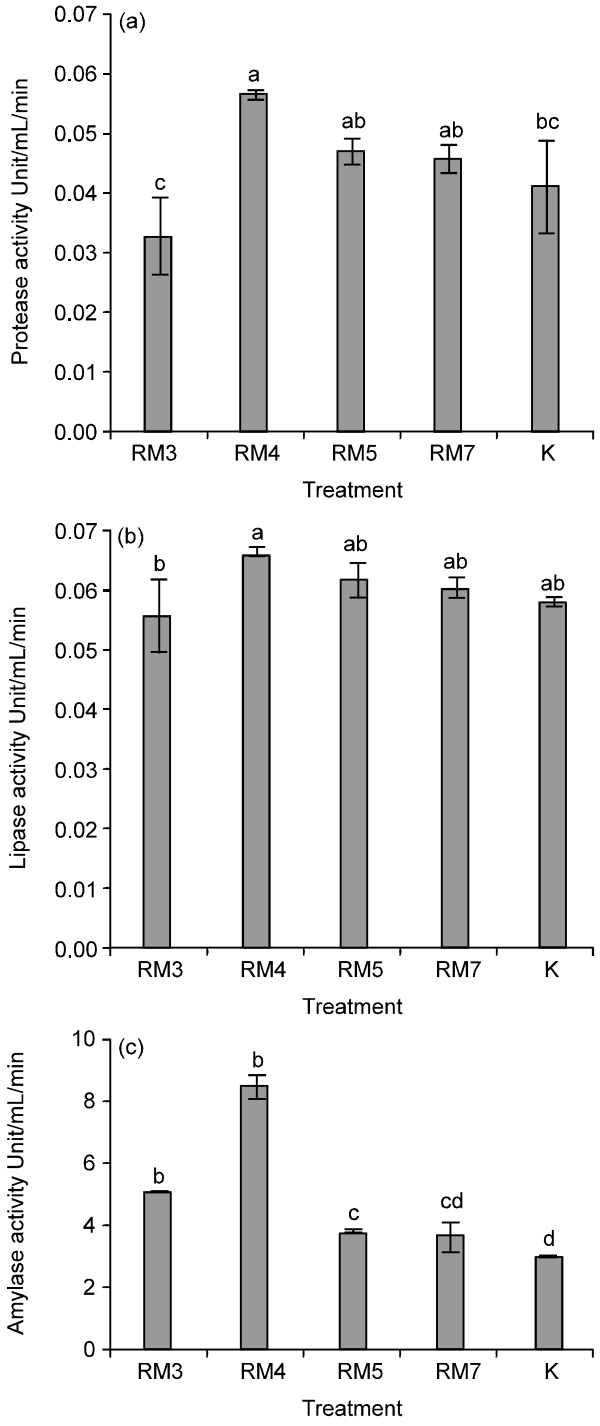

Fig. 1: Protease activity (a), lipase activity (b) and amylase activity (c) in *C. altivelis* digestive tract at different synbiotic treatment. K (without synbiotic)

Prebiotic extracted from sweet potato variety Sukuh (*I. batatas*) was suitable for the treatment of RM4 and RM7, but for the growth parameter: the treatment RM3 and RM5 gave no different results from the control. Synbiotic experiment on yellow croaker, *Larimichtys crocea*, also shows the same thing: there is no significant interaction between *Bacillus subtilis* and fructo-oligosaccharide observed under the conditions of the study (Ai *et al*., 2011). Further, it explains also that prebiotics can be selectively fermented by specific intestinal bacteria, thus, modulate the growth and activity of those bacteria. The synbiotic effect is also potentially affected by the species and the environment.

Humpback grouper is a carnivorous fish and has limited ability to utilize carbohydrates as energy sources for growth. Hidalgo *et al*. (1999), with respect to the enzyme activity in liver and digestive tract stated that the carnivorous species have stomach with low amylase activity, while the stomach less omnivorous species have higher amylase activity. In this study, the high activity of amylase enzyme on treatment RM4 compared to other treatments, can be utilized by the humpback grouper fish to improve their protein retention (55.76±3.13), which showed significantly higher ($p<0.05$) compare to other treatments and control (Table 5).

Fish may also utilize glucose as energy source, which showed by low blood glucose and liver glycogen levels. Low blood glucose levels in fish consuming symbiotic was associated to faster turnover rate of glucose, as the consequence on increasing glucose absorbed by cells and the presence of insulin. According to Fu and Xie (2004), carnivorous fish utilizes gluconeogenesis as main process to produce glucose and remains active at time of high glucose condition. Blood glucose value in the control treatment was significantly higher ($p<0.05$), reaching to 104.808±6.12 mg/100 mL compared to other synbiotic treatment (Table 4). Hyperglycemia can cause excessive production of free radicals lead to oxidative stress and indirectly affect the fish metabolism.

Excess blood glucose, after the metabolic energy needs are met, immediately are converted into triglycerides and then stored in adipose tissue. High levels of triglycerides at the time of fasting are due to the endogenous synthesis of triglycerides derived from the glucose mobilization resulted from the liver glycogen and free fatty acids transported from adipose tissue to the liver (Groff and Gropper, 1999). Moreover, glucose levels in fish were lower than the control treatment after 15 h of feeding, showed a pattern of glucose utilization as the energy source. Glucose that has entered into cell will be metabolized to supply the energy, thus, avoiding the use of some amino acids as sources of metabolic energy. This situation will eventually improve the growth and deposition of materials, such as protein and fat. Glycogen is a form of carbohydrate stored in liver and muscles. Because the ability of liver and muscles to store glycogen is limited, then, the excess carbohydrates are stored in the form of fat (lipogenesis). The high activity of lipogenesis in all the treated fish can be seen from the high retention value of fat, which was not significantly different ($p>0.05$) from the control.

Fish growth can be estimated using the ratio of RNA/DNA (Glemet and Rodriguez, 2007). The ratios of RNA/DNA in treatment RM4 and RM7 were 1.45±0.12 and 1.39±0.13, respectively, significantly higher ($p<0.05$) than in other treatments and control (Table 5). This was in line with the increase of fish weight at the end of study: 54.51±1.73 and 55.95±3.96 g for the treatment of RM4 and RM7, respectively.

In an effort to increase the production of humpback grouper fish, feed is a factor that is very important since it becomes the largest cost in production inputs, ranged from 25-88% (Suprayudi, 2010). This ability would be advantageous for the diet as carbohydrates could be added at greater proportion than for strictly carnivorous fish and thereby save on costs protein (Furne *et al*., 2005). According to Williams *et al*. (2004) commercial feed for humpback grouper, *C. altivelis* in particular, requires high protein content (50%). The increase in amylolytic ability of humpback grouper through synbiotic supplementation opens opportunities for further research, with regard especially to the carbohydrate-protein ratio in feed composition.

Fish health status: Fish health management is very important in the aquaculture industry, because it deals with the purpose of obtaining profit value. Improving the health status of fish is a preventive measure against the spread of disease in aquatic environment. Aquatic environment is the living medium not only for fish, but also for a wide variety of organisms, including pathogens. Disturbance to fish that could potentially cause disease is the result of environmental conditions, the presence of pathogenic organisms and the decline of fish health status. According to Verschuere *et al*. (2000), in the aquatic environment: host and other microorganisms share the ecosystem. In such environment, microbes have the option to live in association with potential hosts in their digestive tract, gills, skin, or live solitarily.

The health status of the humpback groupers treated with feed containing synbiotic can be seen from the haematology parameter. The highest phagocytic activity value obtained from the treatment RM7, followed by RM3 and RM4 ($p<0.05$), in contrast to the RM5 treatment and control (Table 6). Phagocytes are the most powerful and the most important of the body's defense system that can operate immediately (without delay) in the fight against the invasion of microorganisms after crossing the surface of the body and into the body (Mims *et al*., 2001).

Haemoglobin and hematocrit values in fish supplemented with synbiotic were also better than in control (Table 6). Satheeshkumar *et al*. (2010) have reported the possibility of using haematocrit as a tool in the aquaculture and fisheries management for checking the anaemic condition. Fish hematocrit values are usually between 20 and 35% and rarely attain greater than 50%.

ACKNOWLEDGEMENTS

The Directorate General of Higher Education, Ministry of National Education of Indonesia is gratefully acknowledged for their support through the Doctoral Program Grants over the fiscal year 2014.

REFERENCES

Ai, Q., H. Xu, K. Mai, W. Xu, J. Wang and W. Zhang, 2011. Effects of dietary supplementation of *Bacillus subtilis* and fructooligosaccharide on growth performance, survival, non-specific immune response and disease resistance of juvenile large yellow croaker, *Larimichthys crocea*. Aquacult., 317: 155-161.

AOAC, 1990. Official Methods of Analysis of the Association of Official Analytical Chemists, Arlington, VA, USA.

Anderson, D.P. and A.K. Siwicki, 1993. Basic hematology and serology for fish health programs. Paper presented in second symposium on diseases in Asian aquaculture "Aquatic Animal Health and Environment". Phuket, Thailand 25-29th October, 1993.

Bairagi, A., Sarkar K. Ghosh, S.K. Sen and A.K. Ray, 2002. Duckweed (*Lemna polyrhiza*) leaf meal as source of feed stuff in formulated diets for rohu (*Labeo rohita* Ham.) fingerlings after fermentation with a fish intestinal bacterium. Bioresour. Technol., 85: 17-24.

Balcazar, J.L., de I. Blas, I. Ruiz-Zarzuela, D. Cunningham, D. Vendrell and J.L. Muzquiz, 2006. The role of probiotics in aquaculture. Vet. Microbiol., 114: 173-186.

Bergmeyer, H.U. and M. Grassi, 1983. Methods of Enzymatic Analysis. Volume 2. Weinheim: Verlag Chemie.

Borlongan, T.G., 1990. Studies on the lipases of milkfish *Chanos chanos*. Aquacult., 89: 315-325.

Daniels, C.L., D.L. Merrifield, E. Ringo and S.J. Davies, 2013. Probiotic, prebiotic, synbiotic applications for the improvement of larval European lobster (*Homarus gammarus*) culture. Aquacult., 416-417: 396-406.

Furne, M., M.C. Hidalgo, A. Lopez, M. Garcia-Gallego, A.E. Morales, A. Domezain, J. Domezaine and A. Sanz, 2005. Digestive enzyme activities in Adriatic sturgeon *Acipenser naccarii* and rainbow trout *Oncorhynchus mykiss*. A comparative study. Aquacult., 250: 391-398.

Fu, S.J. and X.J. Xie, 2004. Nutritional homeostasis in carnivorous southern catfish (*Silurus meridionalis*): is there a mechanism for increased energy expenditure during carbohydrate overfeeding? Comp. Biochem. Physiol. Part A 130: 359-363.

Gibson, G.R., H.M. Probert, J.V. Loo, R.A. Rastall and M.B. Roberfroid, 2004. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr. Res. Rev., 17: 259-275.

Glemet, H. and M.A. Rodriguez, 2007. Short-term growth (RNA/DNA ratio) of yellow perch (*Perca flavescens*) in relation to environmental influences and spatio-temporal variation in a shallow fluvial lake. Can. J. Fish Aquat. Sci., 64: 1646-1655.

Gourbeyre, P., S. Denery and M. Bodinier, 2011. Probiotics, prebiotics and synbiotics: impact on the gut immune system and allergic reactions. J. Leukocyte Biol., 89: 685-695.

Groff, J.L. and S.S. Gropper, 1999. Advanced Nutrition and Human Metabolism. Stamford; Wadsword.

Hamzah, M., 2013. Increasing growth performance and stamina of humpback grouper (*Cromileptes altivelis*) by selenium-inserted feed. [Doctoral Thesis]. School of Postgraduate Studies, Bogor Agricultural University.

Hidalgo, M.C., E. Urea and A. Sanz, 1999. Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. Aquacult., 170: 267-283.

Laining, A., Rachmansyah, T. Ahmad and K. Williams, 2003. Apparent digestibility of selected feed ingredients for humback grouper, *Cromileptes altivelis*. Aquacult., 218: 529-538.

Marlis, A., 2008. Isolation of oligosaccharide from sweet potato (*Ipomoea batatas* L.) and processing influence for prebiotic potential. [Magister Thesis]. School of Postgraduate Studies, Bogor Agricultural University.

Mims, C.A., A. Nash and J. Stephen, 2001. Mim's Pathogenesis of Infectious Disease: Fifth Edition. Academic Press. London, 474 p.

Mohapatra, S., T. Chakraborty, A.K. Prusty, P. Das, K. Paniprasad and K.N. Mohanta, 2012. Use of different microbial probiotics in the diet of rohu (*Labeo rohita*) fingerlings: effect on growth, nutrient digestibility and retention, digestive enzyme activities and intestinal microflora. Aquacult. Nutr., 18: 1-11.

Muchtadi, D., 1989. Evaluation of Food Nutritional Value. Bogor: Centre for Inter-University, Bogor Agricultural University.

Pohlenz, C. and D.M. Gatlin, 2014. Interrelationships between fish nutrition and health, *Aquaculture*. http://dx.doi.org/10.1016/j.aquaculture.2014.02.008. (*Article in press*).

Ronnestad, I., Y. Kamisaka, L.E.C. Conceicao, S. Morais and S.K. Tonheim, 2007. Digestive physiology of marine fish larva: hormonal control and processing capacity for proteins, peptides and amino acids. Aquacult., 268: 802-97.

Satheeshkumar, P., G. Ananthan, D. Senthilkumar, A.B. Khan and K. Jevananthan, 2010. Comparative investigation on haematological and biochemical studies on wild marine toleost fishes from Vellar estuary, Southeast Coast of India. Comp. Clin. Pathol. DOI 10.1007/s00580-010-1091-5.

Rini, D.S., 2008. Bioassay of prebiotic potentials originated from fresh sweet potatoes and their produced *cookies* and *flakes*) [Magister Thesis]. School of Postgraduate Studies, Bogor Agricultural University.

Rodriguez-Estrada, U., S. Satoh, Y. Haga, H. Fushimi and J. Sweetman, 2009. Effect of single and combined supplementation of *Enterococcus faecalis*, mannan oligosaccharide and polyhydrobutyric acid on growth performance and immune response of rainbow trout *Oncorhynchus mykiss*. Aquacult. Sci., 57: 609-617.

Setiawati, M., 2010. Improving stamina of juvenile humpback grouper (*Cromileptes altivelis*) supplied with Fe and reared under different environments. [Doctoral Thesis]. School of Postgraduate Studies, Bogor Agricultural University.

Shapawi, R., W.K. Ng and S. Mustafa, 2007. Replacement of fish meal with poultry by product meal in diets formulated for the humpback grouper, *Cromileptesaltivelis*. Aquacult., 273: 118-126.

Suprayudi, M.A., 2010. Development of local raw materials for fish/shrimp feed: current status and future prospects. Paper presented on Workshop and Seminar of Fish/Shrimp Nutrition and Feeding Technology. Bogor, October 26, 2010. Agency for Marine And Fisheries Research and Development.

Takeuchi, T., 1988. Laboratory work-chemical evaluation of dietary nutrients. Di dalam: Watanabe T, editor.Fish Nutrition and Mariculture. Tokyo:JICA Textbook the General Aquaculture Course. hlm, 179-233.

Verschuere, L., G. Rombaut, P. Sorgeloos and W. Verstraete, 2000. Probiotic bacteria as biological control agents in aquaculture. Microbiol. Mol. Biol. Rev., 64: 655-671.

Wang, Y.B., 2007. Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. Aquacult., 269: 259-264.

Wedemeyer, G.A. and W.T. Yasutake, 1977. Clinical methods for the assessment of the effects of environmental stress on fish health. Technical paper of the U.S. fish and wildlife service. Vol. 89. U.S. Department of the Interior Fish and Wildlife Service, Washington DC.

Williams, K.C., S. Irvin and M. Barclay, 2004. Polka dot grouper *Cromileptes altivelis* fingerling require high protein and moderate lipid diets for optimal growth and nutrient retention. Aquac. Nutr., 10: 125-134.

Williams, K.C., 2009. A review of feeding practices and nutritional requirements of post larval groupers. Aquacult., 292: 141-152.

Worthington, V., 1993. Worthington Enzyme Manual. Enzymes and Related Biochemicals Worthingthon Chemical, New Jersey, US., 399 p.

Yang, S.C., J.Y. Chen, H.F. Shang, T.Y. Cheng, S.C. Tsou and J.R. Chen, 2005. Effect of synbiotics on intestinal microflora and digestive enzyme activities in rats. World J. Gastroenterol., 11: 7413-7417.

Ziaei-Nejad, S., M.H. Rezaei, G.A. Takami, D.L. Lovett, A.R. Mirvaghefi and M. Shakouri, 2006. The effect of Bacillus spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus*. Aquacult., 252: 516-524.