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The Effects of Balanced Diets with Soy Bean Extract or Meat and Bone meal on Muscle and Liver Tissue Protein and Glycogen Levels of the Nile Tilapia (*Oreochromis niloticus* L.) Infected with *Vibrio anguillarum*

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Abstract
This study investigated the effects of balanced diets with solvent extracted soy bean and meat and bone meal on the levels of protein and glycogen in muscle and liver tissues of the Nile tilapia (*Oreochromis niloticus L.*) infected with *Vibrio anguillarum*. Compared to the control diet (fishmeal only), diets with solvent extracted soy bean (SBM) and meat and bone meal (MBM) significantly lowered ($P<0.05$) the protein levels in liver tissues. However, glycogen levels in muscle and liver tissues were also lower in fish fed with SBM and MBM diets than fish fed with control diet. When fish infected or not infected with *V. anguillarum* compared within the same diet groups, the liver protein levels were measured significantly higher ($P<0.05$) on day1, day3 and day5 of following injection. Glycogen levels both in muscle and liver tissues were also found to be significantly higher ($P<0.05$) in infected fish compared to control fish during the experiment. Considering the level of increase of metabolic reserves in muscle and liver tissues, it might be concluded from this experiment that the Nile tilapia has utilized SBM diet better than MBM diet under stress. Further research concentrating on higher amounts of solvent extracted soy bean and meat and bone meal, different environmental conditions and doses of infection with *V. anguillarum* and immune response parameters including physiological and cellular stress responses is needed in order to identify the effects of dietary plant and animal protein sources on overall mobilization of metabolic reserves in the Nile tilapia infected with *V. anguillarum*.
Key Words: *Oreochromis niloticus*, *Vibrio anguillarum*, protein, glycogen, soy bean, meat and bone meal

INTRODUCTION

Sustainable aquaculture is only possible using cost-effective aqua feeds since the feed cost comprises of up to 60% of operational costs in aqua farms. Fish meal is one of the most important and widely used protein source in aqua feeds and its production levels vary tremendously from one year to another resulting almost equal variability in its per unit price. Therefore, increasing amount of research is being conducted to identify cheaper and abundant alternative plant and animal protein sources that could be used partially or totally in aqua feeds [1, 2]. Since supplying the body with minimum required nutrients that are well balanced to each other is extremely important for a healthy growth [3], alternative proteins should be investigated first for their quality and nutritional value for fish species [4]. Having higher crude protein level, amino acid profile similar to the fish meal and higher nutrient digestibility increases the chance of alternative proteins being used for fish meal replacement in aqua feeds.

Previous studies showed that there was a linear relationship between the protein source in diets and the resistance against stress depending on these protein sources being plant or animal origin [5, 6]. Most of the infectious diseases occur mainly after exposing to sub-optimal environmental conditions and the severity of the infection is even worse when eating foods with lower nutritional quality. Nutrition could be an important tool for the prevention and cure of many diseases [7] and this is mainly valid for farmed animals. In this respect, formulating balanced diets according to the nutritional requirements of fish species is the most important aspects of fish nutrition and feeding under captivity [8].

Dietary protein quality and its amino acid profile and digestibility value are important defining factors of nutritional value of fish feeds. Proteins and other nitrogenous compounds and enzymes are also important elements in cell structure and cellular functions of hormones. Protein synthesis, for example, is strongly affected by the quality of dietary protein sources. Fish meal is considered one of the best protein source for fish feeding due mainly to its excellent protein quality judged by the well balanced amino acid profile for fish growth. Research is vigorously being conducted to investigate the effects of fish meal replacement by alternative plant and animal protein sources in fish feeds on growth performance and general well being including organ histology. Solvent extracted soy bean and meat and bone meal was also tested for some important farmed aquaculture species [9]. It is reported that feed containing either plant or animal protein sources can affect the prevalence of infections in fish differently [10]. Neji and de la Noue [10] demonstrated that the sensitivity in feeding behaviors was higher among infected fish fed with mainly plant proteins than fish fed with animal proteins.

Glycogen is the form of glucose stored in muscle and liver of living organisms. Although muscle tissue spend glycogen only for its need, liver glycogen is the precursor for plasma glucose [11]. In case of a need, glycogen stored in both muscle and liver tissues is catabolized through a process called glycogenolysis and the level depletes. In

addition, excretion of catecholamine, a stress indicator, could induce a dramatic change in the level of stored glycogen [12].

The aim of this study was to investigate the effects of balanced diets with solvent extracted soy bean or meat and bone meal on the mobilization of protein and glycogen levels in muscle and liver tissues of Nile tilapia (*Oreochromis niloticus* L.) on days 1, 3, 5, 7, 10 and 14 following infection with *Vibrio anguillarum*.

MATERIAL AND METHODS

Fish and the Maintenance

The Nile tilapia used in this experiment were supplied by Fish Culture Unit at Faculty of Fisheries and Aquaculture, Mersin University. Prior to experimentation all the fish was kept in 1000 L square tanks which had central drainage system and fed with commercial trout pellets (Çamlı Fish Feed Company, İzmir, Crude Protein:42%, 2-mm dry pellets). Experimentation was conducted using 9 glass aquaria measuring 40H×100L×40W cm. Two weeks before the experimentation, 20 fish (42.4 ± 2.3 g average initial wet weight) randomly selected from the stock tanks were housed in aquaria following individual weighing and acclimatized to experimental diets.

The experimental system was constructed in two sections. The fish not infected with *V. anguillarum* were investigated in the first 15-day period and designated as controls for the infected fish in the second experiment. Fish were fed at 4% and 3% BW/d in two equal meals from 09:00 to 10:00 h and 17:00 to 18:00 h during the 2-week acclimation period to experimental diets and in the actual trials respectively.

Fish were prevented from escaping using perforated plastic sheeting on top of each aquarium. Aeration to experimental system was supplied by central aeration system using air stones. The dissolved oxygen level in each aquarium was not allowed to fall below 5.5 mg/l during the experiments. Water temperature was kept at 26±2 ºC in the system using heaters with thermostatic control. Uneaten feed was collected from each aquarium following feeding during the experiments.

Diet Formulation, Preparation and Chemical Analysis

Two experimental diets were formulated to replace 30% of fish meal crude protein with SBM (solvent-extracted soybean meal, Toros Feed Company, Adana) and MBM (meat and bone meal, Toros Feed Company, Adana). Diets were formulated to contain similar crude protein and gross energy on a dry matter basis. Ingredients and chemical compositions of the diets are reported in Table 1. The amount of mineral and vitamin mixes incorporated into diets was decided in accordance with the dietary requirements of Nile tilapia (Table 1). L-lysine was added to SBM and MBM diets to bring the essential amino acid profiles in line with the control diet [13]. For further description of the diets see Engin and Özkan [14].

Diet samples were analysed for crude protein (Kjeldahl, selenium catalyst; %N×6.25). Gross energy in diets was also analysed using a bomb calorimeter (IKA® — WERKE, C2000 Auto bomb, basic model, Staufen, Germany, calibrated with benzoic acid). Crude fat analysis in diets was according to the soxhelet method where as dry matter (g per kg DM) and ash analysis in diets were according to standard methods [15].

Table 1. Formulation and chemical composition of the experimental diets.

DIETS			
Ingredients (g/kg diet)	CONT	SBM	MBM
Fish Meal	613.0	429.0	429.0
L-lysine	0.0	3.0	6.0
Soybean meal	0.0	302.8	0.0
Meat and Bone meal	0.0	0.0	434.5
Fish oil	83.0	95.0	45.0
Dextrose	50.0	50.0	47.0
Bentonite	175.5	41.7	0.0
Carboxymethylcellulose	50.0	50.0	10.0
Mineral mix*	12.5	12.5	12.5
Vitamin mix**	5.0	5.0	5.0
Stab-C***	1.0	1.0	1.0
Cr_2O_3	10.0	10.0	10.0
Chemical composition			
Dry matter (DM g/kg diet)	937.5±6.3	942.4±3.8	954.6±7.5
Crude protein (g/kg DM)	407.6±4.9	415.9±6.7	415.4±8.7
Crude fat (g.kg DM)	128.4±3.1	128.2±2.8	127.5±1.8
NFE+crude fiber**** (g/kg DM)	216.2	323.6	219.3
Ash (g/kg DM)	247.8	132.3	237.8
Gross Energy (MJ/kg DM)	16.8±0.01	18.6±0.01	16.5±0.8

*Mineral mixture (mg/kg food): Mn; 1000, Fe; 437.5, Zn; 625.0, Cu; 62.5, I; 25.0, Co; 5.0, Se; 1.875.

**Vitamin mixture (mg/kg food): Vitamin A; 114.000 IU, Vitamin D_3; 14.250 IU, Vitamin E; 1140.0, Vitamin K_3; 57.0, Vitamin B_1; 142.5, Vitamin B_2; 199.5, Vitamin B_6; 142.5, Vitamin B_{12}; 0.342, Biotin; 2.85, Folic acid; 57.0, Niacine; 1254.0, Pantothenic acid; 285.0, Vitamin C; 1425.0, Inositol; 1140.0, Antioxidants; 712.5. Vitamin mixture was supplied by Hoffman La Roche, Istanbul, Turkey. ***Stab-C (L-Ascorbyl-2-polyphosphate)

**** NFE=Nitrogen-free extractives. Calculated as the remainder of crude protein+crude fat+ash

The Experimental Infection Procedure of *V.anguillarum* to the Fish

V. anguillarum was injected to juvenile Nile tilapia intraperitoneally. A 24-h fresh culture of bacteria was used in the experimental infection procedure. Following the 24-h incubation period, bacteria were centrifuged for 10 min. at 3500 rpm. The supernatant was then re-centrifuged and washed twice with Phosphate Buffered Saline (PBS) solution. After being diluted with PBS, this bacterial suspension was prepared in four different doses [16]. The dilution consisting bacterial numbers as 1×10^6 cfu (colony forming units) was chosen and injected into fish using plastic syringes (1cc per fish).

Muscle and Liver Tissue Sampling and Determination of Protein and Glycogen Levels

Muscle and liver tissue samples were collected from a randomly selected fish in each aquarium of each treatment on days 1, 3, 5, 7, 10 and 14 (d1, d3, d5, d7, 10 and d14) by killing fish with a sudden blow to the head throughout the experiments. Following killing muscle and liver tissues were quickly excised from sample fish, weighed and frozen until analysis. Before determination of protein levels, tissue

samples were weighed and homogenized in 0.3 M sucrose solution at 24.000 rpm (Ultra-Turrax T-25 Homogenizer, Germany) for 5 min. then centrifuged at 3500 rpm for 10 min. Total protein concentration in homogenates were according to Lowry method [17]. Tissue samples analysed for glycogen reserves were first put into centrifuge tubes for protein and lipid extractions and then added with 3 ml KOH solution (30 %) and placed in hot water bath for 20 mins. Following hot water bath treatment, samples were added with 0.5 ml concentrated Na_2SO_4 and 3 ml 95% ethyl alcohol and boiled for 15 min. on a hot plate. Afterwards samples were centrifuged at 3500 rpm for 10 min. and supernatant were discarded.

Suspension in tubes were then dissolved in 2 ml distilled water and added with 2.5 ml 95% ethyl-alcohol and further centrifuged at 3500 rpm for 10 min. and supernatant were discarded. Suspension free from protein and lipid were then diluted to 50 ml and made ready for the glycogen analysis [17]. Glycogen concentrations in samples were according to Antron method [18].

Statistical Analysis

All data were subjected to one-way ANOVA using SPSS 10.0 and reported as mean ± standard error throughout the text. When a significant treatment effect was observed, a Tukey-Kramer HSD test was used to compare means. Significance was accepted at the probability of 0.05 or less. Student's t-test was also used to identify the significant differences between non-infected and infected groups for diets and individual sampling days.

RESULTS

There was no mortality throughout the experiment. The effects of balanced diets with solvent extracted soy bean or meat and bone meal on muscle tissue protein levels in the infected and non-infected fish are given in Figure 1. There were no significant differences (P>0.05) in muscle protein levels of fish fed with CONT, SBM and MBM diets (Figure 1). However, the protein levels of fish fed with MBM diet was significantly lower (P<0.05) after the d7 compared to the other treatments (Figure 1).

The liver protein levels of the infected and non-infected fish fed with CONT, SBM and MBM diets are given in Figure 2. The liver protein levels of fish fed with SBM and MBM diets were significantly lower (P<0.05) when compared to that of fish fed with CONT diets. The magnitude of decrements were even significantly higher (P<0.05) on d7, d10 and d14 in fish fed with MBM than that of fish fed with SBM diet (Figure 2). The same decrements in liver protein levels were also observed in the infected fish fed with SBM and MBM diets compared to that of fish in the non-infected group (P<0.05) (Figure 2).

Muscle glycogen levels of infected and non-infected fish fed with balanced diets containing SBM and MBM are given in Figure 3. There were no significant differences between muscle glycogen levels of fish fed with CONT and SBM diets during the first 15-day of the experimentation (non-infected fish or control group). However fish fed with MBM diet showed a significantly decreased (p< 0.05) muscle glycogen levels during this period (Figure 3). Fish experimentally infected with *V. anguillarum*, however, were demonstrated to have significantly lower (P<0.05) muscle glycogen levels when fed with SBM and MBM diets compared to that of fish fed with CONT (Fish meal only diet) diet.

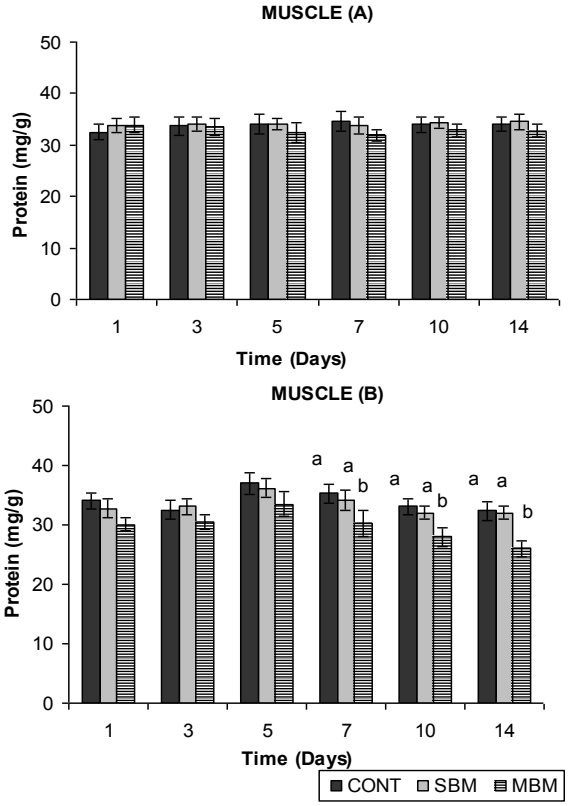

Figure 1. Muscle tissue protein levels of *O. niloticus* **(A)** non-infected and **(B)** infected with *V. anguillarum* when fed CONT, SBM and MBM diets. Each value is the mean±SE (n=3). Different letters indicate a significant difference (P<0.05) between means recorded for the same day in each group.

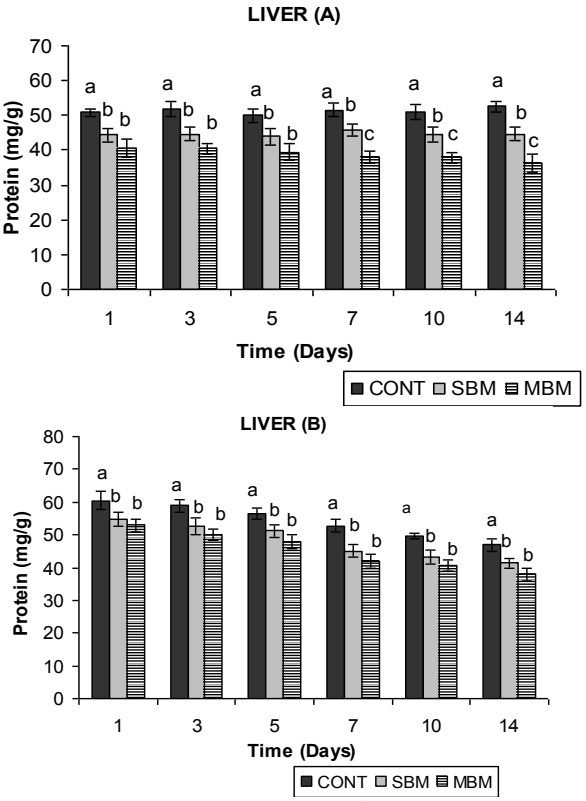

Figure 2. Liver tissue protein levels of *O. niloticus* **(A)** non-infected and **(B)** infected with *V. anguillarum* when fed CONT, SBM and MBM diets. Each value is the mean±SE (n=3). Different letters indicate a significant difference (P<0.05) between means recorded for the same day in each group.

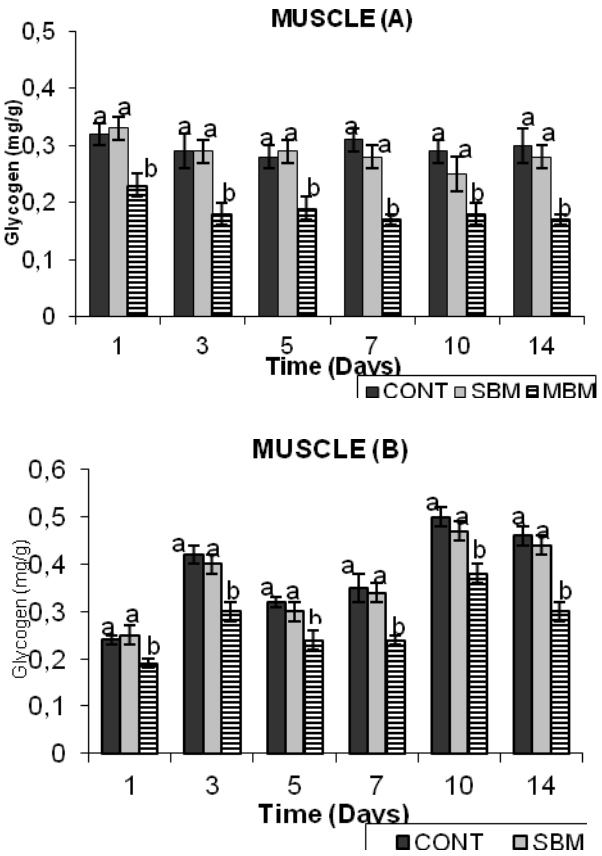

Figure 3. Muscle tissue glycogen levels of *O. niloticus* **(A)** non-infected and **(B)** infected with *V. anguillarum* when fed CONT, SBM and MBM diets. Each value is the mean±SE (n=3). Different letters indicate a significant difference (P<0.05) between means recorded for the same day in each group.

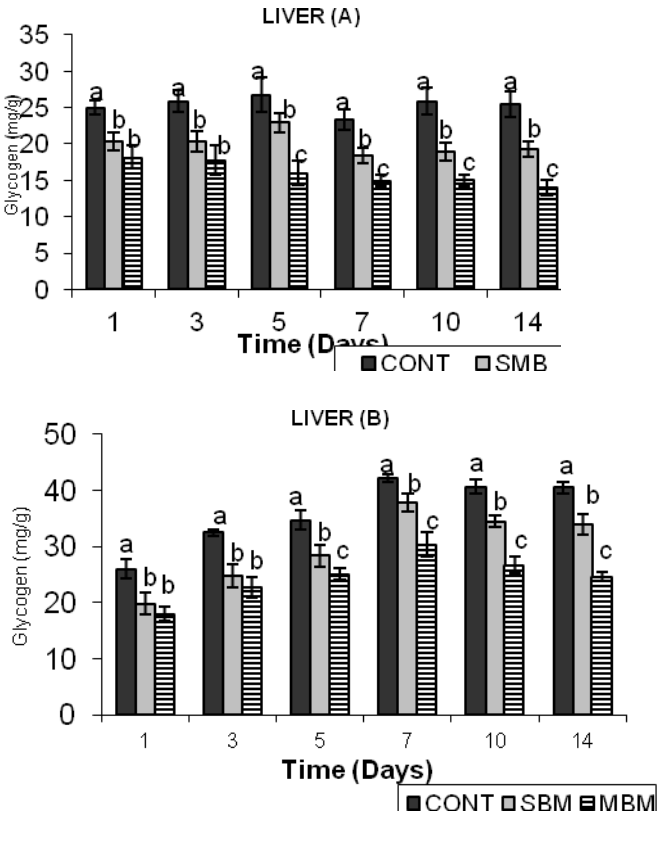

Figure 4. Liver tissue glycogen levels of *O. niloticus* **(A)** non-infected and **(B)** infected with *V. anguillarum* when fed CONT, SBM and MBM diets. Each value is the mean±SE (n=3). Different letters indicate a significant difference (P<0.05) between means recorded for the same day in each group.

Liver glycogen levels of infected and non-infected fish fed with balanced diets consisting SBM and MBM are given in Figure 4. Liver glycogen levels were found to be significantly lower (P<0.05) in fish fed with SBM and MBM diets compared to that of fish fed with CONT diet. The decrements were also more evident beginning d5 onwards during the first 15-day of the experimentation (Figure 4). Fish infected with *V. anguillarum*, however, had significantly lower liver glycogen levels (P<0.05) throughout the second 15-day of the experimentation when fed with MBM diet compared to that of non-infected fish in the first 15-day of the experimentation (Figure 4).

DISCUSSION

Protein synthesis is a biochemical process through which all the necessary amino acids are sufficiently supplied and linked in a correct way. Therefore, protein sources that are deficient of certain essential amino acids could disrupt the protein synthesis in living organisms. In addition, fish immune system has previously been shown that it is directly affected by the type of dietary protein source whether it is a plant or animal origin [19]. Furthermore, it was demonstrated that tissue protein levels could substantially be affected by simply the alteration in protein synthesis and the hazards that infections might cause on tissues [7].

This study showed that SBM diet had a decreasing effect on the liver protein levels of the Nile tilapia throughout the study indicating probably the alteration in protein synthesis due to anti-nutritional factors that soy bean meal has. Soy bean is considered one of the most suitable plant protein alternatives for fish meal in aqua feeds due to its reasonably high crude protein content and as nearly equally balanced essential amino acid profile as fish meal [1]. However, the deficiency of some sulfur containing amino acids and protease inhibitor as an anti-nutritional factor in soy bean meal could mainly prevent its use in higher amounts in aqua feeds. It was previously demonstrated that supplementation of sulfur deficient amino acids to feeds and mechanical treatment of soy bean meal have both increased its use in tilapia feeds [20]. Feeding trout with diets containing plant proteins has substantially altered the liver protein levels compared to that of fish fed with fish meal only diets [21]. They also concluded that this alteration was mainly due to the limitation in protein use or the availability of essential amino acids caused by anti-nutritional factors present in plant proteins [21]. Liver is the main organ where proteins and other nitrogenous compounds are synthesized. Any disruptions to this process could not only severely affect the protein utilization efficiency but also the capacity of organisms to fight disease infections in vertebrates. Previously it has been reported that net protein utilization of tilapia (*Oreochromis niloticus* x *O. aureus*) substantially affected by inclusion of soy bean meal into their diets [22].

Fish fed with MBM diet had decreased muscle and liver protein levels compared to that of fish fed with CONT and SBM diets in this study. The levels were even lower in the infected group fed with MBM diet probably indicating the faster mobilization of energetic reserves due to stress caused by the combined effect of dietary protein source and *V. anguillarum* infection [19]. MBM is not a primary protein source that would be used for fish meal replacement in aqua feeds due mainly to its unbalanced amino acid profile and lower nutritional quality to aquatic organisms [23, 24]. In addition, high amount of crude ash in meat and bone meal was demonstrated to be the main reason of lower dry matter and protein digestibility in gilt head sea bream (*Sparus aurata*) [4, 25], fresh water shrimp (*Macrobrachium nipponense*) [24] and the Nile tilapia [14].

The decrements observed in muscle and liver glycogen levels of fish fed with SBM and MBM diets in this study might primarily be attributed to alterations in the metabolic pathways due to the anti-nutritional factors present in soy bean and the relatively lower apparent nutrient digestibility of meat and bone meal in the Nile tilapia [14]. The variation between blood glucose and liver glycogen levels is directly affected by metabolic pathways that are mainly determined by the oxidative status and conditions of organisms [11, 13, 26]. Amino acids are the primary substrates for gluconeogenesis in fish [27]. Von Der Decken and Lied [28] demonstrated that increasing amount of soy bean protein concentrates in balanced diets caused lower muscle protein and glycogen levels in cod (*Gadus morhua*). They also concluded that lower energetic reserves found in muscle tissue were probably due to altered metabolic functions and decreased enzyme activities as a result of plant protein inclusion in diets [10].

The liver protein levels significantly increased in the infected fish compared to that of fish in the non-infected group in each dietary treatment during the study. Liver is an indicator organ for any dietary and physiological alterations occurring in the body [10]. It also plays an important role on the biosynthesis of immunoglobulin in the immune system [7]. Therefore, higher liver protein levels in the infected group might be an outcome of increased biosynthesis of immunoglobulin against bacterial infection in the Nile tilapia as a defense mechanism [10].

The muscle and liver glycogen levels of infected fish were found to be higher than that of non-infected fish fed with CONT, SBM and MBM diets in this study indicating probably the higher rates of gluconeogenesis and increased glycogen deposition as a result of cortisol excretion triggered by stress due to bacterial infection. Circulation levels of stress hormones are greatly affected by the easily occurring stressful conditions in aqua farming units [30, 31]. Therefore glucose production is expected to increase substantially as a result of increments in the excretion of stress hormones like cortisol and catecholamine in fish under captivity. Glucose, the most important metabolic substrate for tissues, is catabolized from glycogen in liver in order to provide increased energy needs due to stress in animals [32]. These hormones specifically play roles in the mobilization of energetic or metabolic reserves and increase the gluconeogenetic potential of cells [33, 34]. Pathogen organisms are considered as natural stressors and causes substantial changes in animal physiology [31]. It was previously demonstrated that plasma cortisol levels increased in the rainbow trout due to *V. anguillarum* infection [35]. Similarly, increments of plasma cortisol levels caused by disease infections were also reported in several other important aquaculture species [36, 37].

In conclusion, muscle protein levels did not change considerably with dietary treatments both in the infected and non-infected Nile tilapia in this study. However, muscle glycogen levels were found to be significantly lower in fish fed with MBM diet in each experimental group suggesting SBM is a better alternative protein source in the Juvenile Nile tilapia. The lowest liver protein and glycogen levels were obtained on fish fed with MBM diets also indicated that SBM was a preferred choice of the two alternative sources in terms of utilization and mobilization of energetic reserves in liver during stress management. Further research specifically investigating the activities of key enzymes playing role in the mobilization of energetic reserves is needed to draw a clear conclusion about the utilization of alternative proteins under stress.

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REFERENCES

[1] El Sayed A-FM 1998. Total replacement of fishmeal with animal protein sources in nile tilapia *Oreochromis niloticus* (L.) feeds. Aquacult. Res., 29(4):275-280.

[2] Xue M, Cui Y. 2001. Effect of several feeding stimulants on diet preference by juvenile gibel carp (*Carassius auratus gibelio*), fed diets with or without partial replacement of fish meal by meat and bone meal. Aquaculture, 198 (3-4):281-292.

[3] Peres H, Lim C, Klesius PH. 2004. Growth, chemical composition and resistance to *Streptococcus iniae* challenge of juvenile nile tilapia (*Oreochromis niloticus*) fed graded levels of dietary inositol. Aquaculture, 235:423-432.

[4] Robaina L, Moyana FJ, Izquierdo MS, Socorro J, Vergara JM. Montero D. 1997. Corn gluten meal and meat and bone meals as protein sources in the diets for gilthead sea bream *Sparus aurata* nutritional and histological implications. Aquaculture, 157:347-359.

[5] Pouliot T, de la Noue J. 1992. Apperent digestibility in rainbow trout (*Salmo gairdneri*) influence of hypoxia. Can. J. Fish Aquat. Sci. 45:2003-2009.

[6] Thomas PT, Woo PTK. 1990. Dietary modulation of humoral immune response and anemia in rainbow trout (*Oncorhynchus mykiss* Walbaum) infected with *Cryptobia salmositica* Katz. J. Fish Dis., 13:435-446.

[7] Post, G. 1987. Text book of fish health, Second Edition, T.F.H. Puplication, Inc. 288.

[8] Barros MM, Lim C, Klesius PH. 2002. Effect of soybean meal replacement by cotton seed meal and iron supplementation on growth, immune response and resistance of channel catfish (*Ictalurus puctatus*) to *Edwardsiella ictaluri* challenge. Aquaculture, 207:263-279.

[9] El Sayed A-FM 1999. Alternative dietary protein sources for farmed tilapia, *Oreochromis spp*. Aquaculture, 179:149-168.

[10] Neji H, de la Noue J. 1998. Effect of animal and vegetal protein diets on feed intake and apparent digestibility of nutrients in rainbow trout (*Oncorhynchus mykiss*) infectec by *Aeromonas salmonicida*, with and without chronic hypoxia stress. Can. J. Fish. Aquat. Sci., 55: 2019-2027.

[11] Hemre GI. 2002. Carbohydrates in fish nutrition: Effects on growth, glucose metabolism and hepatic enzymes. Aquacult. Nutr., 8: 175-194.

[12] Heath AG. 1995. Water pollution and fish physiology, CRC Pres, Lewis Publis. 360.

[13] National Research Council (NRC), 1993. Nutrient requirements of warmwater fishes and shellfishes. National Academy of Science, Washington, DC, USA.

[14] Engin K, Özkan F. 2008. Apparent nutrient digestibility of balanced diets with soy bean extract or meat and bone meal in the Nile tilapia (*Oreochromis niloticus* L.) infected with *Vibrio anguillarium*. Turk. J. Vet. Anim. Sci., 32 (3):173-181.

[15] Association of official analytical chemist. 1995. Official methods of analysis, 16th edn. AOAC, Arlington, VA.

[16] Eldar A, Bejerano Y, Livoff A, Horovitcz A, Bercovier H. 1995. Experimental Streptococcal meningo-encephalitis in cultured fish. Vet. Microbiol., 43:33-40.

[17] Wedemeyer GA, Yasutake WT. 1977. Clinical methods for the assessment of the effects of environmental stress on fish health. U.S. Tech. Pap. U.S. Fish Wildl. Ser., 89:1-18.

[18] Plummer DI. 1971. Practical Biochemistry. Mc Graw Hill Book Company Ltd., England.

[19] Landolt LM. 1989. The relationship between diet and the immune response of fish. Aquaculture, 79:193-206.

[20] Davies SJ, Williamson J, Robinson M, Bateson RI. 1989. Practical inclusion levels of common animal by-products in complete diets for tilapia (*Oreochromis mossambicus*, Peters). In: Proc. 3rd. Intl. Symp. On Feeding and Nutr. Fish. Toba. Japan. 325-332.

[21] Vilhemsson TO, Martin SAM, Medale F, Kaushik SJ, Houlihan DF. 2004. Dietary plant-protein substitution affects hepatic metabolism in rainbow trout (*Oncorhynchus mykiss*). Br. J. Nutr., 92(1):71-80.

[22] Shiau Y, Chuang JL, Sun CL. 1987. Inclusion of soybean meal in tilapia (*Oreochromis niloticus* X *O. aureus*) diets at two protein levels. Aquaculture, 65:251-261.

[23] Watanabe T, Pongmaneerat J. 1991. Quality evaluation of some animal protein sources for rainbow trout *Oncorhynchus mykiss*. Nippon Suisan Gakkaishi, 57:459-501.

[24] Yang Y, Xie S, Lei W, Zhu X, Yang Y. 2004. Effect of replacement of fish meal by meat and bone meal and poultry by-product meal in diets on the growth and immune responce of *Macrobrachium nipponense*. Fish and Shellfish Immunol., 17:105-114.

[25] Alexis MN. 1997. Fish meal and fish oil replacers in Mediterranean marine fish diets. In: Feeding tomorrow's fish proceedings of the workshop of the CIHEAM Network on technology of aquaculture in the Mediterranean. Eds: A. Tacon; B. Barsureo, CIHEAM, Zaragoza Spain, 1989. 183-204.

[26] Rueda-Jaso R, Concescao LEC, Dias J, De Coen W, Comes E, Rees JF, Soares F, Dinis MJ, Sorgeloos P. 2004. Effect of dietary non-protein energy levels on condition and oxidative status of senegalese sole (*Solea senegalensis*) juveniles. Aquaculture, 231(1-4):417-433.

[27] Vijayan MM, Pereira C, Grau EG, Iwama GK. 1997. Metabolic responses associated with confinement stress in Tilapia: The role of cortisol. Comp. Biochem. Physiol., 116C:89–95.

[28] Von Der Decken A, Lied E. 1993. Metabolic effects on growth and muscle of soya bean protein feeding in cod (*Gadus morhua*). Br. J. Nutr., 69:689-697.

[29] Segner H, Juario JV. 1986. Histological observations on the rearing of milkfish, *Chanos chanos*, fry using different diets. J. Appl. Ichthyol., 2:162-173.

[30] Wendelaar Bonga SE. 1997. The stress response in fish. physiol. Rev., 77:591–625.

[31] Iwama GK, Vijayan MM, Forsyth RB, Ackerman PA. 1999. Heat shock proteins and physiological stress in fish. Am. Zoologist, 39:901-909.

[32] Ackerman PA, Forsyth RB, Mazur CF, Iwama GK. 2000. Stress hormones and cellular stress response in salmonids. Fish Physiol. Biochem. 23(4):327-336.

[33] Mommsen TP, Vijayan MM, Moon TW. 1999. Cortisol in teleosts: Dynamics, mechanisms of action, and metabolic regulation. Rev. Fish Biol. Fish., 9:211-268.

[34] Laiz-Carrion R, Martin Del Rio MP, Miguez JM, Mancera JM, Soengas JL. 2003. Influence of cortisol on osmoregulation and energy metabolism in gilthead sea bream *Sparus aurata*. J. Exp. Zool., 298A:105-118.

[35] Ackerman PA, Iwama GK. 2001. Physiological and cellular stress responses of juvenile rainbow trout to vibriosis. J. Aquat. Anim. Health. 13:173-180.

[36] Laidley CW, Woo PTK Leatherland JF. 1988. The stress response of rainbow trout to experimental infection with the blood parasite *Cryptobia salmositica* Katz, 1951. J. Fish Biol., 32:253-261.

[37] Rand TG, Cone DK. 1990. Effects of *Ichthyophonus hoferi* on condition indices and blood chemistry of experimentally infected rainbow trout (*Oncorhynchus mykiss*). J. Wildlife Dis., 26:323-328.