INT. J. BIOL. BIOTECH., 14 (4): 669-676, 2017.

HAEMATOLOGICAL, IMMUNOLOGICAL CHANGES, SURVIVAL, GROWTH RATE, FOOD CONSUMPTION PROTEIN EFFICIENCY, AND BODY COMPOSITION OF HYBRID TILAPIA (*OREOCHROMIS MOSSAMBICUS* × *OREOCHROMIS NILOTICUS*): EFFECTS OF STOCKING DENSITY AND PROTEIN LEVELS IN SALINE ENVIRONMENT

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ABSTRACT

This trial was conducted for 42-days with 20, 40, 60 and 80 fish / 250L (stocking density: ST-20, ST-40, ST-60 and ST-80, respectively) with two dietary protein levels CP1 (25%) and CP2 (35%) on growth, feeding utilization and body composition. The results showed that growth performance in terms of final body weight, SGR, weight gain, survival rate, hepatosomatic index (HSI), total proteolytic enzyme activity and liver glycogen of hybrid tilapia fingerlings were significantly ($P < 0.05$) reduced at lower dietary protein level and higher ST. The feed intake, feed conversion ratio, protein efficiency rate (PER) and tissue protein levels were also significantly improved at CP2 and at lower stocking density (ST-20, ST-40). The haematological parameters such as plasma protein, cortisol, and glucose levels were significantly ($P < 0.05$) high at ST-60-80. The immunological parameters albumin (AL) and globulin (GL) were not significantly ($P > 0.05$) differed among these experimental groups. The haemoglobin and haematocrit (% Hct) were significantly varied among these groups also. The plasma protein, glucose and plasma cortisol levels were significantly increased at (ST-60 to ST-80) might be possible source of stress resulted in growth reduction in fish population. The results clearly demonstrated that fish growth was best at CP2 with lower stocking density (ST-20, ST-40). The physical parameters were at satisfactory level in all treatments.

Key words: Hybrid tilapia *(Oreochromis mossambicus* × *Oreochromis niloticus***)**, Stocking density, Dietary Protein level, Haematology

INTRODUCTION

Tilapia species have been frequently known as protein resource all over the World (Stickney, 1986). These species are commonly cluttered in a variety of environments due to its high salinity tolerance (Mian and Siddiqui 2015; Morgan and Iwama 1991). Beside, salinity the stocking density (ST) also effects on growth activity (Mian and Siddiqui 2014; Saoud *et al.,* 2008). In many fishes inverse correlation exist between growth rate and ST (Saoud *et al.,* 2008; Papoutsoglu *et al*, 1998) while other growth ameliorates with ST (Chua and Teng 1979). For example, the growth activity of channel catfish was severely affected by the interactions of its environmental conditions (e.g., salinity, temperature, DO, etc.) and ST (Dunham *et al.,* 1990). The quality feed is a main necessity for good health and growth in fish farming. In generally, fish need 40-50% dietary protein (Mian and Siddiqui 2014; Mian *et al*, 2015; Mian *et al.*, 2016; Omar 1994; Siddiqui *et al.,* 1982; Mazid *et al.,* 1979). Due to feed production cost, the enhancement of fish growth achieves incredible importance in aquaculture. Leal *et al.* (2009), Mian *et al.*, (2014) have investigated that shrimp protein hydrolysate (SPH) considered an exceptional amino acids sketch and low fiber content. Algal meal, yeast meal and shrimp head meal are the potential feed components for the fish sows as they are easily available and relatively cheap (Wassef *et al.*, 2001; Mian *et al.*, 2014).

The main aim of the trials was to evaluate the rearing capacity and protein requirements for fish growth and immunity under controlled laboratory conditions.

MATERIAL AND METHODS

These experiments conducted over the period of 42 days.

Effect of protein requirements and stocking density (ST) on growth

In this trial fish were stocked at 20, 40, 60 and 80 fish/ 250L (ST-20, ST-40 and ST-80/ 250L, respectively). For this trial, SPH (shrimp protein hydrolysate) was produced according to (Mian *et al.*, 2014; Leal *et al.*, 2009) and adjusted the feeds protein levels 25% and 35% crude protein (Table 1).

Statistical analysis

Statistical analysis of the results was conducted according to Snedecor and Cochran (1967).

Table 1. % Composition of the diets.

	% Composition	
Feed ingredients	25% (CP1)	35 % (CP2)
Fish meal	25.3	32.4
Shrimp head protein (SPH) *	2.5	2.5
wheat flour	15.6	12.3
Fish oil	0.5	0.5
wheat bran	19.23	17.4
vegetable oil	2	2
Soybean	21.2	21.2
Calcium carbonate	1	1
Vitamin C	1	1
Rice flour	11.4	10.2
¶Vitamins / minerals – premix	1	1
Analytical Composition		
Crude Protein	26.2	31.5
Lipids	6.2	5.3
Ash	13.3	12.1
Fiber	5.9	5.7
Moisture	10.4	9.4
NFE	32.2	27.2
Aminoacids		
Arginine	4.3	4.6
Lysine	5.5	5.3
Histidine	1.6	1.72
Threonine	4.2	4.1
Valine	3.1	2.95
Leucine	4.9	5.2
Isoleucine	2.85	3.01
Methionine	2.6	2.71
Cystine	2.2	2.3
Phenylalanine	4.4	4.3
Tyrosine	1.9	2.03
Tryptophan	1.1	1.22

‡Nitrogen- free extract = 100 – (% protein + % fat + % ash + % fiber); Fish meal (CP = 60.3 %): *Shrimp protein hydrolysate (SPH , CP =57%) Soybean (CP = 37.3): Wheat flour (CP = 12.4): Rice flour (CP =15.3): Wheat bran (CP = 16.6): Kidney bean (CP = 17.2)
¶Vitamins / minerals premix contained as (g kg^{-1}) described by Mian and Siddiqui, 2014.

Chemical analysis of formulated diets

The proximate analysis of the feeding ingredients and body tissues, salinity, DO were analyzed by standard methods AOAC (Helrich 1990) and Main and Siddiqui, 2015; Mian *et al*., 2014). Lipid was estimated using standard method (Folch *et al.,* 1957). The amino acids profile of feed ingredients and body tissue were analyzed (Model: 056-3019, Hitachi (Ltd), Tokyo, Japan: Wang *et al.,* 2006). Haematocrit (Hct %: Papoutsoglou and Voutsions, 1988) and RBC and WBC counts (Blaxhall and Daisley, 1973) were determined. Total plasma protein was estimated by means of commercial kit (Bio System, Barcelona, Spain). Serum albumin and globulin (Kenari *et al.,* 2010), proteolytic enzyme activity (Sawhney and Singh, 2000) and plasma cortisol, glucose and lactate (Mian and Siddiqui, 2014) were also estimated.

Final body weight (WG) weight gain, (%WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and feed intake were determined according to Steffens (1989). The hepatosomatic index HSI: was estimated following Du *et al.* (2006).

RESULTS

The Increasing ST from 20-80 fish/250L in tank and decreasing protein level from 35% (CP2) to 25% (CP1) indicated significant ($P<0.05$) decrease in growth activity. The increased protein level (CP2: 35%) presented best growth activity. The dietary feed intake (FI) was high at low (ST-20 and ST-40) (Table 2) The SGR were high at low (ST-10 and ST-20) at 35% (CP2) protein level. The lowest FCR were observed high up to ST-20. The lower survival rates were observed at ST10 and ST80/250L. PER, HSI, total proteolytic enzyme activity and liver glycogen were improved significantly ($P < 0.05$) at ST10-ST20 with 35% (CP2) protein level (Table 3). The haemoglobin (Hb) and % haematocrit were significantly ($P < 0.05$) different. Immunoglobin (albumin, AL and globulin, GL) were not significantly differed among the population which were also mentioned Table 4. The blood glucose, plasma cortisol and plasma protein level were significantly ($P < 0.05$) higher at (ST-60 and ST-80) Table 4.The body compositions were significantly ($P < 0.05$) varied with ST. As the ST increased the protein level of body tissue was significantly decreased Table5. The water parameters were at normal range Table 6.

Table 2. Growth parameters of hybrid (*Oreochromis mossambicus* × *Oreochromis niloticus*).

		Stocking density fish per 250L		
+ SPH protein level (%)	ST-20	ST-40	ST-60	ST-80
		Initial weight (g)		
CP1	2.1	2.3	2.4	2
CP2	2.2	2.24	2.3	2.03
Average*	2.15^{a}	2.27^{b}	2.35^{c}	2.01^{d}
		Final body weight (g)		
CP1	9.2	10.4	8.03	6.2
CP2	10.12	12.91	8.56	6.5
Average*	9.66^{a}	11.65^{b}	8.3^{c}	6.35^{d}
		Survival rate (%)		
CP1	100	100	95	87
CP2	100	100	96	89
Average*	81^{a}	100^{b}	95.5^{b}	88^{a}
		Weight gain (g)		
CP1	7.1	8.1	5.63	4.2
CP2	7.92	10.67	6.33	4.47
Average	7.51^{a}	9.385^{b}	5.98^{c}	4.3^{d}
		SGR		
CP1	3.67	3.6	2.88	1.39
CP2	3.8	4.1	3.1	1.42
Average*	3.735^{a}	3.85^{b}	2.99^{c}	1.4^{d}

Means that have same superscript letter within a column are not significantly different (*P*> 0.05). (Mean ±SE, n= 3)
Weight gain= Terminal weight- initial weight
SGR (% day^{-1}), specific growth rate: 100 × [ln terminal weight (g) – ln initial weight (g)/ Duration (days)]
Sr, survival rate (%) = 100× [final number of fish /initial number of fish]

Table 3. Physiological parameters of juveniles (*Oreochromis mossambicus* × *Oreochromis niloticus* at various stocking densities (fish /250L)

	Stocking density			
+ SPH protein level (%)	ST-20	ST-40	ST-60	ST-80
		Feed intake		
CP1	35.3	37.5	31.34	28.5
CP2	37.1	42.6	33.12	31
Average *	36.2 [a]	40.05[b]	32.76[c]	29.75[d]
		‡ FCR		
CP1	1.5	1.1	1.52	1.65
CP2	1.23	1.3	1.4	1.62
Average *	1.365 [a]	1.2 [b]	1.46[c]	1.63[d]
		¶ PER		
CP1	1.72	1.2	0.67	0.5
CP2	2.26	1.82	0.86	0.71
Average *	1.99 [a]	1.51 [b]	0.765[c]	0.605[d]
		* HSI		
CP1	2.02	2.12	1.62	1.3
CP2	2.1	2.3	1.7	1.6
Average *	2.1[a]	2.2[b]	1.67[c]	1.45[d]
		Liver glycogen mg g^{-1}		
CP1	2.25	2.3	1.9	1.32
CP2	2.2	2.45	2.03	1.45
Average *	2.22[a]	2.4[b]	1.96[c]	1.4[d]
		Total proteolytic enzyme activity mg $g^{-1}h^{-1}$		
CP1	1.87	1.91	1.72	1.64
CP2	1.9	1.93	1.82	1.71
Average *	1.9[a]	1.92[a]	1.77[b]	1.67[c]

Means that have same superscript letter within a column are not significantly different ($P > 0.05$). (Mean ±SE, $n = 3$)
‡FCR = Feed conversion ratio, HSI = Hepatosomatic index, ¶PER = Protein efficiency ratio

DISCUSSION

In present trial, the effect of the interaction between dietary protein levels and ST on the growth performances of tilapia was recorded. Few authors have reported that the substitution of fish meal by plant protein-based diets decrease the growth activity of fishes drastically Plascentia-Jatomea *et al.*, (2001), Mian et *al.*, (2014). This may be due to lacking of various important amino acids, such as, lysine and methionine.

In this trial, the compositions of formulated diets shrimp head protein hydrolysate (+SPH) had complete sequences of aminoacids reported Mian *et al.*, (2014). In present trial, decreasing protein level from CP2 to CP1 diets and increasing stocking density from ST-20 to ST-80 fish/ 250L resulted in a significant ($P < 0.05$) reduction in growth performances. The final body weights, %WG, SGR were improved in (ST-20 and ST-40) groups with CP2 diet. Similar findings were also noted Islam and Hossain (1994), Mian and Siddiqui (2014). Santiago *et al.*, (1982) examined that the 35 to 40% dietary CP in diet for *O. niloticus* fry showed excellent results.

Table 4. Haematological and Immunological parameters of (*Oreochromis mossambicus* × *Oreochromis niloticus*) kept at various stocking density (20, 40, 60 and 80/ 250L).

	Stocking density			
+ SPH protein level (%)	ST-20	ST-40	ST-60	ST-80
		(Haematocrit) Hct (%)		
CP1	37.27	39.75	36	35.5
CP2	36.5	38	36.3	35
Average*	36.8[a]	38.7[b]	36.15[c]	35.25[d]
		Haemoglobin (mg/ dl)		
CP1	9.16	9.53	9.3	9.02
CP2	9.1	9.21	9.14	9
Average*	9.13[a]	9.37[b]	9.22[c]	9.01[d]
		Glucose (mg/ dl)		
CP1	58.98	57.59	61.76	64.9
CP2	56.52	56.5	60.2	64.78
Average*	57.75[a]	57.045[a]	61[c]	64.84[d]
		Plasma cortisol (ng/ ml)		
CP1	3.5	3.7	17.2	25.3
CP2	3.6	3.83	18	24
Average*	3.55[a]	3.76[b]	17.6[c]	24.65[d]
		Plasma protein mg/ 100 ml		
CP1	1.9	1.96	2.3	2.42
CP2	1.86	1.92	2.34	2.4
Average*	1.88[a]	1.94[a]	2.32[b]	2.41[c]
		Albumin g/dl		
CP1	1.38	1.41	1.42	1.51
CP2	1.4	1.39	1.44	1.45
Average*	1.39[a]	1.4[a]	1.43[a]	1.48[a]
		Globulin g/ dl		
CP1	1.8	1.81	1.82	1.84
CP2	1.82	1.79	1.83	1.81
Average*	1.81[a]	1.8[a]	1.825[a]	1.82[a]

Means that have same superscript letter within a column are not significantly different ($P > 0.05$). (Mean ±SE, n= 3)

The results of this trial indicate that, the increasing rearing capacity above ST-40, the tissue lipid, protein and feed intake were significantly decreased, and this phenomenon was vice versa, when dietary protein increased from CP1 to CP2. In present findings, the observed dietary feed intake (FI) was highest in fish reared at low ST. Similar findings also reported Clark *et al.* (1990), Zonneveld and Fadholi (1991), Mian and Siddiqui (2014) for red tilapia. The best SGR, WG, final body weight and FCR were observed in fish reared at the (ST-20 to ST-40) with fed on CP2 diet. These results are similar to those reported Holm *et al.* (1990), Rowlend *et al.* (1995) and Papoutsoglu *et al.*, (1998). The improved feed utilization and protein efficiency rate (PER) were also observed at low ST with CP2 diet. Similar studies have reported previously Zonneveld and Fadholi (1991).In our present study also observed the high survival rate showed at to ST20 to ST40 with both protein levels. Similar type of the study have been also reported (Coulibaly *et al.*, 2007) but many studies also have been investigated not get significant effect of stocking density and survival rates (Hengsawat *et al.*, 1997). Some studies have reported that the inversely correlation existed between the stocking density and survival rates.

In present trial, the hepatosomatic index significantly reduced at high (ST-60 and ST-80) and decreasing of this biological index of fishes may be pointed their lower growth performance (Mian *et al*., 2014). In addition, this lower biological index may be due to higher utilization of lipids in liver to achieve the energy requirements, conservation and growth (Du *et al*. 2006; Mian *et al.*, 2014). The liver glycogen and total proteolytic enzyme activity (TPEA) was progressed at ST to (20-ST-40) than (ST-60 and ST-80) indicate the better growth performance (Mian *et al*. 2014).

Table 5. The composition of body protein, lipids, ash, moisture at various stocking density (20, 40 and 80 fish/ 250L).

		Stocking density	(fish / 250L)	
+ SPH protein level (%)	ST-20	ST-40	ST-60	ST-80
		Crude Protein (%)		
CP1	16.67	16.43	16.21	16.09
CP2	16.8	16.61	16.35	16.2
Average*	16.735[a]	16.52[b]	16.28[c]	16.145[d]
		Lipids (%)		
CP1	12.74	12.56	12.44	12.31
CP2	12.63	12.6	12.41	12.22
Average*	12.68	12.58	12.425	12.26
		Ash (%)		
CP1	4.6	4.32	4.43	4.62
CP2	4.5	4.28	4.52	4.5
Average*	4.55	4.3	4.47	4.56
		Moisture (%)		
CP1	67.2	66.54	66.1	66.45
CP2	66.3	67	66.3	66.74
Average*	66.75	66.77	66.64	66.6

Means that have same superscript letter within a column are not significantly different ($P > 0.05$). (Mean ±SE, n= 3)
ST: Stocking density

Table 6. Physico-chemical parameters

	Dissolved Oxygen (mg L^{-1})	Temperature °C	pH	NH_3 - Nitrogen (mgL^{-1})
Min.	4.7	27.85	6.2	0.35
Max.	4.9	28.2	6.93	0.36

The haematological studies are further supported the health status, growth and resistant to toxicant (Satheesh *et al*., 2011; Mian *et al*., 2014). The immunological parameters, such as, albumin (AL) and globulin (GL) were not significantly ($P < 0.05$) differed among this trial. The haemoglobin and % Hct were also significantly varied, this therefore, due to oxygen consumption rate among fish in different stocking densities. The high ST had high levels of serum glucose, plasma cortisol; this may be produce due to high stress and energy demand than the low ST. This study is agreement with Barton and Iwama (1991) and Costas *et al.*, (2008), Mian and Siddiqui (2014). The overall out outcomes in present trials also suggest that the low (ST20- ST40/250L) with 35% dietary protein level had showed excellent growth behavior and physiological characteristics in hybrid tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*). However, the increasing in stocking density and decrease the dietary protein level may interrupt the fish growth performances.

ACKNOWLEDGEMENTS

The first author is thankful to Dr. Imink Anton, Stirling University (Department of Aquaculture Management, U.K), Dr. Shouqi XIE, Institute of Hydrobiology, Chines Academy of Science (China), and Dr. Ali Turkur, Mugla Sitki Kocman University, Faculty of Fisheries (Turkey) and appreciate, acknowledge the critical review and guidance in technical and aquaculture management. The financial assistance for this research was entirely covered by DelPHE project (Development of Partnership for the Enhancement of Biodiversity Research, Aquaculture and Fisheries Technologies) and also funded by DFID (UK) and HEC Pakistan. Our thanks are also to Director, Center of Excellence in Marine Biology, University of Karachi, Karachi, Pakistan..

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(*Accepted for publication September 2017*)