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Effect of salinity and protein levels on haematological, and physiological changes and growth of hybrid tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*)

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Abstract

The experiment was conducted to determine the effect of salinity and dietary protein levels on physiological and haematological changes as well as growth of the juveniles of hybrid tilapia (*Oreochromis mossambicus*×*Oreochromis niloticus*). Fish were stocked at 20 fish $50L^{-1}$. The initial average weight of the juveniles was 2.1±0.02 g and they were kept in salinities 0, 10, 20 and 35 ppt (S1, S2, S3 and S4, respectively) and fed with 30% (P1) and 40% (P2) dietary protein levels for 45 days. The harvesting weight and SGR were not significantly (p>0.05) different between S2 and S3 treatments for 30% to 40% protein levels varied in connecting S2-S3 with P1diets or in between S2-S3 with P2 diets. The food consumption significantly (p<0.05) increased with decreasing protein levels (40% to 30%) in diets at 10-20 ppt salinities. The survival rates were not significantly (p>0.05) different between 10-20 ppt salinities with P1 and P2 diets. The lowest FCR and highest muscular hydration were achieved at intermediate salinities with low protein diets. No obvious changes occurred in Hct%, Hb, plasma protein, muscles and liver protein levels in all treatments. Furthermore, the plasma cortisol and glucose levels tended to increase at higher salinities but were not significantly (p>0.05) different among all treatments. The physico-chemical parameters of water (i.e. dissolved oxygen: DO, temperature, ammonia etc.) were at a normal range throughout the study. Results showed that environmental salinity (0-35 ppt) had no adverse effects on growth and biochemical changes and also no required high protein levels in diets at any salinity.

Keywords: Hybrid tilapia, Haematology, Physiology, Cortisol, Muscular hydration

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Introduction

In agriculture, soil may be too saline to support profitable crop husbandry. So, such soil may be used alternatively for productive aquaculture of fish like tilapia species, which are more tolerant, and grow faster under sub- nutritional conditions (Ahmed, 1962; Kuweye *et al*., 1993; Asad *et al.*, 2010; Khan *et al*., 2014). Tilapia species have been generally known as the protein resources throughout the world (Mazid *et al*., 1979; Siddiqui *et al*., 1997; Stickney, 1986; Mian and Siddiqui, 2014; Mian and Siddiqui, 2015). Some of the tilapia species are commonly cultured in brackish water, estuaries and coastal lagoons due to high salinity tolerance. Among these species, the hybrid tilapia *Oreochromis mossambicus* × *Oreochromis niloticus* which is euryhaline can also tolerate a relatively wide range of salinities. It is extremely tolerant in a variety of water conditions (Mian and Siddiqui, 2015; Gracia-Ulloa *et al*., 2001). Many authors have worked on tilapia species and its salinity tolerance (Likongwe *et al*., 1996; Wang *et al*., 1997; Nolan *et al*., 1999; Romana-Eguia and Eguia, 1999; Boeuf and Payan, 2001; Gracia-Ulloa *et al*., 2001; Jameel *et al*., 2004; Kamal and Mair, 2005; Asad *et al*., 2010; Khan *et al*., 2014; Mian and Siddiqui, 2014; Mian and Siddiqui, 2015). Early investigation showed fast growth rates in isotonic salinity because of reduced cost of osmoregulation Febry and Lutz (1987). However, few researches have shown that tilapia grew remarkably in high salinities (Kuweye *et al*., 1993). Poor growth and mortality rate of tilapia in brackish water was reported by Doudet (1986). Consequently, the euryhaline fish species need more protein due to higher energy budget, metabolism and osmoregulation (Larumbe-Morán *et al*., 2010). Beside, food quality feed is a primary requirement for good growth and production of cultured fish. The majority of fish species require 40-50% protein in the diet (Mian and Siddiqui, 2015; Mazid *et al*., 1979). Efficient management of diet protein contents of fish species leads to increasing tolerance against the adverse environment as a main strategy. Also, the study of hematological parameters helps to know the health status, feeding, growth of the fish species and flexibility of the species to environmental conditions (Satheeshkumar *et al*., 2011; Mian and Siddiqui, 2015). The dietary protein requirement of tilapia under conditions in Pakistan has not been reported so far. The objective of this study is to evaluate the effect of salinity and protein requirements on growth, physiological and haematological aspects.

Materials and methods

The experiments were carried out at Center of Excellence in Marine Biology (CEMB), University of Karachi for a period of 45 days. Juveniles of hybrid tilapia (*O. mossambicus* × *O. niloticus*) were collected from the Gharo Creek, Sindh, Pakistan. The 2.1±0.1 g juveniles were stocked initially at 20 fish $50L^{-1}$ at 0, 10, 20 and 35 ppt salinities (S1, S2, S3 and S4,

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respectively) in 0.071 m^3 with three replicates. Adjustment of salinity was done following the methods of Jameel *et al*. (2004). In the present trail, two protein levels 30% (P1) and 40% (P2) were also formulated (Table 1) according to (Mian and Siddiqui, 2014; Mian and Siddiqi, 2015). The physico-chemical parameters such as dissolved oxygen, temperature, pH and ammonia were maintained regularly. The photoperiod was set at 14h light and 10h dark. Formulated diets were offered twice a day (Jobling, 1994; Mian *et al*., 2014) at 08:00-09:00 am and16:00-17:00 pm. 10 fish were sampled randomly from each aquarium. Fish were weekly weighed, counted and measured to achieve growth parameters. At the end of the experiment the body tissues and blood samples of fish were collected for haematological and physiological assessments.

Chemical analysis

The approximate analysis of feed and body compositions were analyzed by using the standard methodology of AOAC (Helrich, 1990; Mian *et al*., 2014). Haematocrit (Hct %) was determined as proposed by Papoutsoglou and Voutsions (1988). Total plasma protein was estimated by means of commercial kits (Bio System, Barcelona, Spain). Haemoglobin was measured using the method of Beckman Coulter (HMX, U.S.A.).

Growth parameters

Harvesting weight, FCR, SGR and food consumption rates were calculated (Steffens 1989).

Sr (%)=[100×Nf/Ni], (Ni=initial number and Nf = final number of fish)

FCR (Feed conversion rate)=[I/Wt– Wo +Wd], (I=Total supplied feed, Wt= final weight, Wo=initial weight, Wd= number of dead fish

SGR=100×[In (Wt)-In(Wo)/t, (t=total days)

The feed consumption rate=[(food ingested) ×100/ (wet body weight)]

Statistical analysis

SPSS software was used for the statistical analysis of survival and growth performance. One-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to compare the means. The significance of results was considered at 95% confidence level.

Results

The composition and amino acid profiles of feeds are mentioned in Table 1. The harvesting weight and % Sr were significantly (p<0.05) higher at salinities S1-S3 with 30% protein level (P1) than with S1-S3 with 40% protein level (P2) diets (Table 2). The significantly (p<0.05) higher feed consumption ratio was also achieved at S1-S3 with P1 diet than with S1-S3 with P2 diet. The SGR was not significantly (p>0.05) different between S2-S3 with P1 diet and S1-S4 with P2 diet. The lower survival rates were observed at S4 with P1 diet and S4 with P2 diet (Table 2). The total plasma

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protein levels increased with increasing salinity but were not significantly different in S1-S4 with P1 diet or S1-S4 with P2 diet. The %Hct, Hb, tissue protein level and liver protein level were not significantly (p>0.05) varied among all diets and salinities (Table 3). The physico-chemical parameters such as DO, temperature, ammonia and pH were at normal ranges in all treatments (Table 4). The plasma glucose and cortisol levels were high at S4 with P1 diet or at S4 with P2 diet but were not significantly (p> 0.05) different among all treatments (Fig. 1). The muscular hydration tended to decrease in high salinities but did not significantly differ among all the treatments (Fig. 2).

Table 1: % composition of formulated diets.

	% Composition	
Feed ingredients	**(P1) 30%**	**(P2) 40%**
Fish meal protein	18.3	23.4
Shrimp head protein (SHPH) *	3.3	4.2
Wheat flour	25.3	18.6
Fish oil	0.5	0.5
Wheat bran	19.23	17.4
vegetable oil	2	2
Rice flour	19.8	21.2
Calcium carbonate	1	1
Vitamin C	1	1
Tapioca flour	11.4	10.2
¶Vitamins / minerals – premix	1	1
Analytical Composition		
Crude Protein	29.7	40.3
Lipids	6.2	5.3
Ash	13.3	12.1
Fiber	5.9	5.7
Moisture	10.4	9.4
§NFE	33.6	27.2
Gross energy (MJ $100g^{-1}$)	19.3	18.1
Amino acids (%)		
Arginine	4.2	4.1
Lysine	5.4	5.5
Histidine	1.56	1.7
Threonine	4.1	4.12
Valine	3.0	3.1
Leucine	4.8	5.3
Isoleucine	2.8	3.03
Methionine	2.54	2.7
Cysteine	2.22	2.8
Phenylalanine	4.3	4.4
Tyrosiı	1.92	2.02
Tryptopl	1.12	1.17

§Nitrogen- free extract = 100 - (% protein + % fat + % ash + % fiber)

¶ Vitamins/minerals premix contained as (g) Riboflavin (0.85g): Folic acid (0.75g): Vitamin B6: (0.02): Vitamin B12 (0.04g): Vitamin E (0.2g), Vitamin D3 (0.3): Niacin (0.18g). Minerals premix: Iodine (0.01g), Iron (0.01g), Selenium (0.005g), Chromium (0.05g), □ SHPH (CP = 39.8), Fish meal protein, FMP: (CP= 57.6%): Tapioca flour: *Metroxylon sago* (CP=3.12%); wheat flour: *Triticum aestivum* (CP = 16.4), Rice flour: (14.7, CP).Vitamins/minerals premix were used as described by (Mian *et al*., 2014)

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Table 2: The harvesting body weight, % survival rate, feed consumption rate, FCR and SGR of hybrid tilapia *Oreochromis mossambicus* × *Oreochromis niloticus* cultured in different treatments.

Salinity/% Protein	Harvesting weight (g)	Sr (%)	Feed consumption rate	FCR	SGR
S1/P1	16.1 ± 0.01^{a}	86	2.9 ± 0.02^{a}	2.2 ± 0.02^{a}	4.57 ± 0.02^{a}
S2/P1	17.8 ± 0.03^{b}	100	3.5 ± 0.01^{b}	1.4 ± 0.02^{b}	4.73 ± 0.02^{b}
S3/P1	18.03 ± 0.02^{b}	100	3.57 ± 0.01^{b}	1.42 ± 0.03^{b}	4.78 ± 0.03^{b}
S4/P1	15.3 ± 0.02^{c}	84	2.4 ± 0.01^{d}	2.8 ± 0.3^{c}	4.33 ± 0.001^{c}
S1/P2	15.7 ± 0.01^{ab}	85	2.7 ± 0.02^{ab}	1.6 ± 0.02^{d}	4.6 ± 0.02^{a}
S2/P2	16.4 ± 0.02^{bc}	100	3.02 ± 0.04^{ac}	1.5 ± 0.03^{ab}	4.64 ± 0.03^{d}
S3/P2	16.51 ± 0.02^{ac}	100	3.1 ± 0.01^{bc}	1.62 ± 0.03^{d}	4.67 ± 0.01^{d}
S4/P2	14.2 ± 0.02^{bc}	80	2.3 ± 0.01^{ad}	2.9 ± 0.3^{bc}	4.2 ± 0.04^{ab}

Mean (n = 3, mean ± S.E) each n consists 10 fish replicate $^{-1}$ in the same column followed by the different letters are significantly different (p<0.05).

Table 3: Haematocrit, haemoglobin, plasma protein, muscle protein and liver protein at different salinities and protein levels.

Salinity/ % Protein	Haematocrit %	Haemoglobin g 100 mL^{-1}	Plasma protein	Muscles protein mg g^{-1}	Liver protein mg g^{-1}
S1/P1	37.4 ± 3^{a}	9.8 ± 0.1^{a}	9.03 ± 0.1^{a}	162.15 ± 1.3^{a}	205.5 ± 2^{a}
S2/P1	40.2 ± 1^{a}	10.02 ± 0.2^{a}	9.1 ± 0.02^{a}	156.5 ± 1.4^{a}	193.3 ± 1.4^{a}
S3/P1	41.3 ± 1^{a}	10.1 ± 0.1^{a}	9.17 ± 0.2^{a}	153.4 ± 2^{a}	196.5 ± 2^{a}
S4/P1	32.1 ± 2^{a}	9.2 ± 0.3^{a}	9.23 ± 0.01^{a}	15.6 ± 3^{a}	186.4 ± 1^{a}
S1/P2	36.4 ± 3^{a}	9.4 ± 0.1^{a}	9.07 ± 0.2^{a}	162.15 ± 2^{a}	208.5 ± 2^{a}
S2/P2	38.2 ± 1^{a}	9.82 ± 0.2^{a}	9.2 ± 0.2^{a}	156.5 ± 1.3^{a}	189.3 ± 1.4^{a}
S3/P2	40.2 ± 1^{a}	10.03 ± 0.4^{a}	9.22 ± 0.01^{a}	153.4 ± 1.5^{a}	187.5 ± 2^{a}
S4/P2	30.6 ± 2^{a}	9.03 ± 0.1^{a}	9.23 ± 0.2^{a}	148.6 ± 3^{a}	181.4 ± 1^{a}

Mean (n = 3, mean ± S.E) each n consists 10 fish replicate^{-1}, in the same column followed by the different letters are significantly different (p<0.05).

Table 4: The physical parameters of water.

Salinity % Protein^{-1}	Dissolved oxygen (mg L^{-1})	Temperature °C	pH	NH3-Nitrogen (mg L^{-1})
S1/P1	5.22 ± 0.1	26.9 ± 0.1	7.1 ± 0.1	0.31 ± 0.2
S2/P1	5.3 ± 0.1	27.1 ± 0.2	7.1 ± 0.04	0.32 ± 0.2
S3/P1	5.15 ± 0.1	27.2 ± 0.4	7.3 ± 0.02	0.31 ± 0.01
S4/P1	5.23 ± 0.1	26.8 ± 0.3	7.2 ± 0.01	0.3 ± 0.1
S1/P2	5.22 ± 0.1	27.2 ± 0.1	7.1 ± 0.01	0.32 ± 0.01
S2/P2	5.2 ± 0.1	26.9 ± 0.1	7.2 ± 0.03	0.34 ± 0.01
S3/P2	5.27 ± 0.1	27.2 ± 0.2	7.3 ± 0.02	0.32 ± 0.1
S4/P2	5.22 ± 0.1	27 ± 0.1	7.1 ± 0.02	0.35 ± 0.2

(Mean ± S.E)

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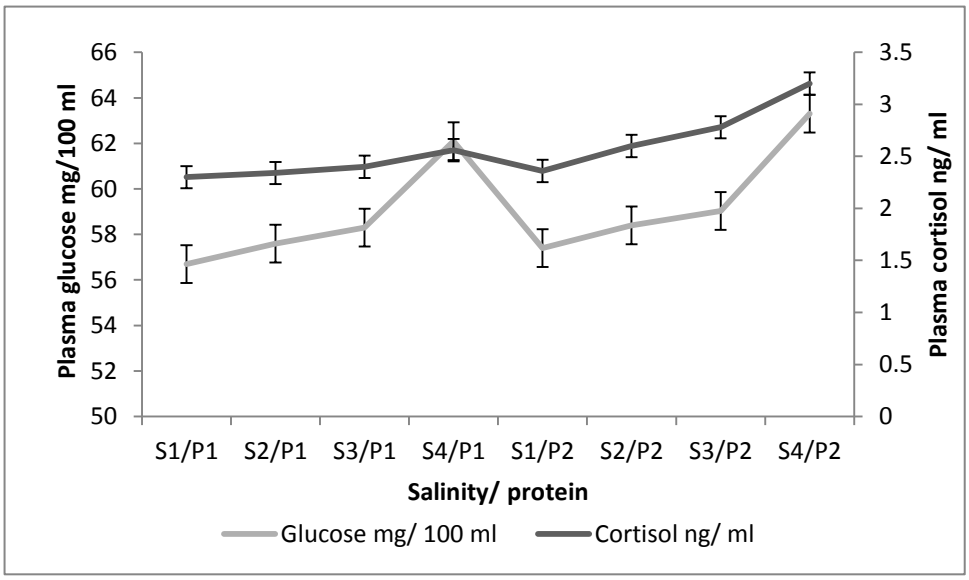


Figure 1: Effect of salinity and protein levels on plasma glucose and cortisol levels (error bars shows standard deviation).

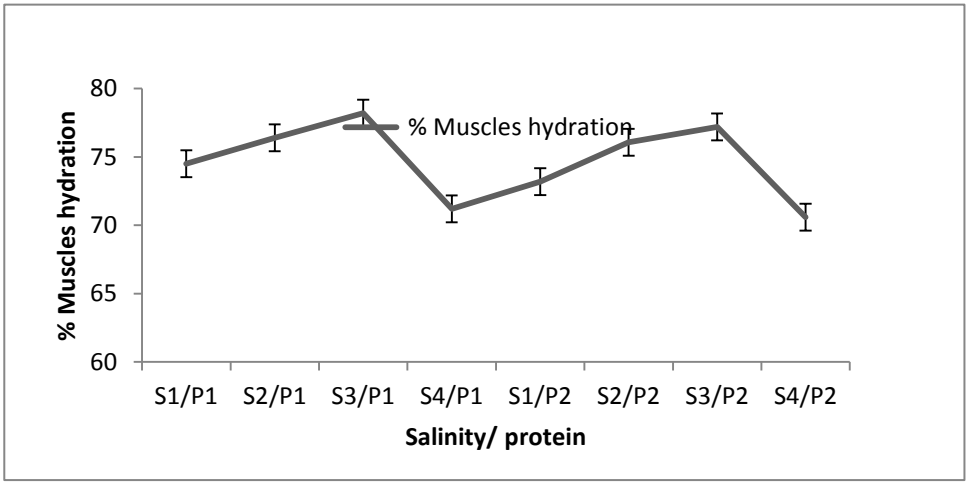


Figure 2: Effect of protein and salinity on % muscles hydration (error bars shows standard deviation).

Discussion

Feed quality is a primary requirement for good growth and production of fish in aquaculture. Mazid *et al*. (1979) found optimum growth of *Tilapia zilli* when fed a diet containing 35% protein. *O. niloticus* showed improved growth with 30% dietary protein (CP) (Siddiqui *et al*., 1997; Omar and Al-Sagheer, 1994). *O. mossambicus* showed best growth with 30 to 40% dietary protein (Islam and Hossain, 1991). Evidence suggests that the tilapia species also showed remarkable growth at salinity ranging from 10-20 ppt (Suresh and Lin, 1992; Mian and Siddiqui, 2015). Present findings showed that increasing protein level from 30% to 40% and salinity from 0 to 10, 20 and 35ppt resulted in significant (p<0.05) diminution in fish growth and behavior. Previous researches showed that the hybrid tilapia can be easily introduced to fluctuating saline waters (Graci-Ulloa *et al*., 2001). According to Bril *et al*. (2003) fish growth was depressed at

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high salinities in case of osmoregulation in fish that consumed high proportion of available energy ranging from 25% to 50% total metabolic activity. Similarly, the cost of osmoregulation in Nile tilapia (*O. niloticus*) was 19% of the total metabolic cost in fresh water and 29% in 30 ppt sea water (Farmer and Beamish, 1969). Febry and Lutz (1987) proposed that cost of osmoregulation was 16% in fresh water and 12% in sea water in red hybrid tilapia which is more euryhaline (Iwama, 1989). By contrast, in the present study, the harvesting weight and SGR weights of fish were improved by increasing salinity from 0 to 20 ppt and decreasing dietary protein level (40% to 30%). Our present findings were also comparable to results reported by other researches (Gracia-Ulloa *et al*., 2001; Suresh and Lin, 2003; Jameel *et al*., 2004; Larumbe-Morán *et al*., 2010; Mian *et al*., 2014; Mian and Siddiquai, 2015). Thus this study indicated that salinity probably did not have adverse effects on growth, survival, haematological or physiological changes in tilapia juveniles because the biomass was favorable in all treatments and never exceeded the serious maxima as described (Legal, 1958; Larumbe-Morán *et al*., 2010). Indeed, growth increased in intermediate salinities suggesting that, they are competent at growing in brackish water (20 ppt) with similar protein levels in diets and, can even display superior capability in high salinities than in freshwater conditions. In support of the S3 with P1diets treatment (20 ppt: 30% protein) harvesting weight was higher than that of fresh water with similar protein levels. This was also higher in S3 with P2 diets (20 ppt: 40% protein). Similarly, the highest SGR of Nile tilapia has obtained at 0 ppt (Likongwe *et al*., 1996) and *Sparus sarba* at 15ppt (Woo and Kelly**,** 1995). It has been also reported that salinity affected food consumption (Wang *et al*., 1997). Consequently the growth rate was decreased with decreasing food consumption (Leod, 1977). It was also found that the better growth was achieved in low salinity due to an increase in the feed conversion efficiency and low energy budget (Lambert *et al*., 1994). In our present findings the highest feed consumption was achieved at S1-S3 with P1 diets and was significantly better than at S1-S3 with P2 diets. These findings therefore indicate that high salinity and protein levels may cause reduction in food consumption. Similar findings were also reported by Likongwe *et al*. (1996) and Mian and Siddiqui (2015). Estimates of FCR were low at intermediate salinity with low protein diets, which reflect the improved efficiency in metabolism (Kamal and Mair, 2005). Similar findings were also reported by (Watanabe *et al*., 1988). Evidence also proved that high salinity caused stressful condition. This may lead to increased glycogenolysis and proteolysis through increasing plasma level of cortisol during the down regulation of the interleukin-2 receptor (IL-2R) (Mian *et al*., 2014; Mian and Siddiqui, 2015). In our present investigations the plasma glucose and

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cortisol levels tended to increase in high strength (S4 with P1dites and S4: P2 diets) but were not significantly different among treatments with the same dietary protein levels. Earlier works reported consistency of hydration, which may contribute to great adaptability of *O. mossambicus* × *O. niloticus* in heterosmotic environments. It was also demonstrated that the water content of muscle tissue decreased with increasing environmental salinity (Venkatachari, 1974). In our present study, when the fish were transferred from fresh water to high saline water, muscular hydration was not significantly decreased with the same dietary protein levels (S1-S4/P1 and S1-S4/P2: Fig.2). By contrast, few authors suggested salinity influence on variation of hydration in muscles. For example, when sturgeon *Acipenser naccarii* was transferred from fresh water to sea water, the muscular hydration was significantly decreased (Martínez-'Alvarez *et al*., 2002). Furthermore, they also reported that the protein content of the body tissues fluctuated due to variation of environmental salinity. In our present findings the protein levels of liver and muscle were not affected by any of the studied salinities. So the present study indicates that the salinity range 0-35 ppt may not have undesirable effects on physiological changes.

Furthermore, the haemoglobin level of fish species correlates with their metabolic activity, and ecological condition (Kumar *et al*., 2011; Mian *et al*., 2014; Satheesh Mian and Siddiqui, 2014 and 2015). In our present findings the haematocrit (Hct %) and haemoglobin (Hb) levels were not significantly affected by different salinities and dietary protein levels (Table 3). By contrast, Martínez-'Alvarez *et al.,* 2002 noted that the plasma Hb did not return to their initial position (haemoglobin: Hb level in fresh water) while gradually increasing salinity up to 20 ppt for sturgeon species. Evidence from the work on fish in saline environments indicates that the total plasma protein levels of some fish species of saline environments decreased with increasing salinity (Martínez-'Alvarez *et al*., 2002; Mian *et al*., 2014; Mian and Siddiqui, 2015). This may be due to higher osmoregulatory energy demand and reduced appetite of fish at higher salinity. According to Legal (1958) the electrophoretic pattern of serum protein of *Blennius pavo* was affected by salinity changes. In contrast, the present study showed that total plasma protein was not significantly (p>0.05) affected by different salinities (S1-S4) at similar protein levels.

The results indicate that culture of hybrid tilapia is feasible in environments with salinities at 10-20 ppt without affecting growth, survival rate, haematological and physiological changes, with no increase in required dietary protein if the standing biomass remained under critical maxima. Nevertheless, the tilapia do not require higher protein intake at higher salinities (10 to 30 ppt) to achieve growth rates corresponding to those observed in low saline context.

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