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Effect of Stocking Density and Protein level on Behaviour, Survival, Growth rate, Crowding Status, Stress Response, Food Consumption Protein efficiency, and Body Composition of Hybrid (*Oreochromis mossambicus* × *Oreochromis niloticus*) in Saline Environment

Jahangeer Mian* and Peer Zada Jamal Siddiqui

ABSTRACT
The present study was carried out at the Center of Excellence in Marine Biology University of Karachi over the period of 92 days to assess optimal salinity, effect of stocking density and two dietary protein level 30% (P1) and 40%(P2) crude protein on growth, survival rate feed conversion, body composition and production of Hybrid (*Oreochromis mossambicus* × *Oreochromis niloticus*). In present study, a 12 week trial conducted, 5, 10, 20, 40 (D1, D2, D3, D4/ P1 and D1, D2, D3, D4 /P2 respectively) fish/ 40L aquarium were stocked with two dietary protein level 30% (P1) and 40% (P2)CP on growth and feed utilization. The results were presented that growth of hybrid tilapia was significantly (P<0.05) condensed at P2 and high stocking densities (D3 and D4). Blood hemoglobin, RBC, WBC and %Hct were significantly different. Significantly (P<0.05) higher hematocrit concentration was observed at lower stocking densities indicating better physiological adaptation The resultant higher cortisol, blood sugar lactate and plasma protein levels at high stocking density were produced stress full condition. The physical parameters were at satisfactory level in all treatments.

Keywords: Aquaculture, Fish, Hybrid, Stocking density, Fish physiology.

1. Introduction

In agriculture, soil may be too saline to support profitable crop husbandry yet such soil may be used alternative for productive aquaculture of fish like tilapia species, which are more tolerable, grow faster under sub- nutritional conditions and high fecundity rate [1]. It has been also investigated that fish stocked at higher stocking density reduced growth performance due some extrinsic and intrinsic factors. Therefore to improve the fish growth, a tilapia seawater culture system was set up to assess the growth potential of hybrid tilapia (*Oreochromis mossambicus* × *O. niloticus*) under the combined effects stocking density and dietary protein level.

Stocking density also effects on growth [16, 17]. Aquaculturist should be know, how many juveniles can be reared in specific area since it might be positive or negative effect on growth performance [2]. Stocking density is a key factor to achieve the optimum production because it is directly associated with physiological and physical parameters like, water quality, capacity, nutrition, and type of culturing system, biochemical stages [17]. However, social behavior, fish genotype and ethology must be measured as key factors that will be directly effect on growth, survival rate and growth-stocking association [17]. Beside these factors the quality feed is a primary requirement for good growth and production of most fish in aquaculture. The majority of fish species require 40-50% protein in the diet. [14] found optimum growth of *Tilapia zilli* when fed a diet containing 35% protein. *Oreochromis niloticus* improved growth with dietary protein 30% CP [16]. *Oreochromis mossambicus* enhanced growth with30 to 40% dietary protein [10]. The dietary protein requirement of tilapia under Pakistan conditions has not been reported so far. Yet no information is available about survival rate, stocking density, feeding behavior, protein requirements, and growth rate of the hybrid (Oreochromis mossambicus × *O. niloticus*). Therefore, the objective of this study was to evaluate the optimal salinity, selective stocking density and protein requirements of tilapia hybrid (*Oreochromis mossambicus*× *O. niloticus*) under saline environment.

2. Material and methods

Experiments were carried out at Center of Excellence in Marine Biology (CEMB), University of Karachi over the period of 92 days. The fingerlings were collected from the Sindh Hatchery Thatta, Pakistan. Initially fish were acclimatized to achieve optimal salinity [7]. Fish were stocked initially with weight (2.2 to 2.4 g) at 5 (D1), 10 (D2), 30 (D3) and 40 (D4) fish/ 40L with 3 replicates. In this study two experimental diets containing 30% (P1) and 40% crude protein (P2) were formulated (Table 1). At the end of the trial representative fish from each aquarium were sacrificed and frozen for subsequent proximate analyses of the fish body composition and blood sections were taken by the cardiac prick of few fish per aquarium and sent to HEJ research institute (Institute for Chemical and Molecular Biology, University of Karachi) for the physiological assessments. Results were measured up to by means of TWO WAY ANOVA and Duncan's multiple range tests described by [24].

2.1 Chemical analysis of formulated diets

The approximate analysis of the feeding ingredients and body composition are shown in (Table 1 and Table 4 respectively) were analyzed by using the standard methodology AOAC [8]. Moisture was determined by oven (Memmert GmbH 2880, Schwanbach , Germany) at 105 °C for 24h, ash was analyzed by muffle furnace (model: MV-2 / CV1434 Electrodruzstvo, Parha, Czechoslovakia), crude protein estimated by Kajedhal process with routine Kajedhal schemeBuchi 433 / 324, Flawil (Switzerland), lipid was approximated by [6]. The amino acids profile of ingredients was estimated (described by [25]. Total protein determined by means of commercial kits (Bio System, Barcelona, Spain).Dissolved oxygen measured by (970.DO.2 meter / 02668, Mar 09 U.K) and hand salino-meter was using for salinities monitored (Mod: 9111,STAGO, S/Mill, salinity, Japan), Haematocrit (Hct %) and total lipid were analyzed, proposed by [18]. The RBC and WBC counts calculated as described by [15]. Total protein, cholesterol and urea were determined by means of commercial kits (Bio System, Barcelona, Spain). Plasma glucose estimated by (Hitachi; 902, S.NO. 1811-002, automatic analyzer, Roche, Ibaraki, Japan), lactate estimated by lactate dehydrogenase [9] and hemoglobin measured by (Beck man coulter, HMX, U.S.A). Plasma cortisol was determined by radioimmunoassay as described by [19].

3. Results

Total amino acids of formulated diet contenting essential aminoacid (EAA) and non- essential aminoacid mainly tyrosine, cystine, leucine, lysine, tyrosine and arginine showed (Table 1) amino acids requirements of tilapia species described by [22]. Increasing stocking density from D1 toD4/aquarium and increasing protein level from P1to P2% resulted in a significant (P<0.05) reduction in growth performance. P1 protein diet produced the best growth performance. The dietary feed intake was higher at low stocking density D1/P1 and D2/ P1 fish/ aquarium (Table 2). SGR were high at D1/P1 andD2/P2. The FCR were observed high at D4/P1and D4/P2. PER were also observed high at low stocking densityD1/P1, D2/P1, andD1/P2 and D2/P2. The blood glucose, cortisol level, hemoglobin, plasma protein serum lactate were significantly (P <0.05) higher at D3/P1, D4/P1, D3/P2 and D4/P2 (Figure 1). The Hct% was satisfactory level at lower stocking densityD1/P1, D2/P1, D1/P2 and D2/P2 (Figure 1). The body compositions were varied among all treatments but not significantly different except protein, this components increased with decreasing of stocking density (Table 4). During the beginning of the trial no significantly change in all groups and were not found any change in between the replicates. The physical parameters were at satisfactory levels among all treatments. A positive correlation was also observed between plasma cortisol and serum glucose levels (r^2 = 0.92, P < .05). A strong correlation was also observed in between stocking density and, cortisol level (r^2 =0.96, P <0.05).

Table 1: Composition and chemical analysis of the experimental diets

Feed ingredients*	% Composition 25% Protein (P1)	35% Protein (P2)
Fish meal	27.3	33.4
Rice flour	9.4	8.6
wheat flour	15.6	10.3
Fish oil	0.5	0.5
wheat bran	14.23	12.4
vegetable oil	2	2
Soybean	22.3	22.3
Calcium carbonate	1	1
Vitamin C	1	1
Shrimp head protein	2.5	2.5
Vitamins / minerals – premix	1	1
Analytical Composition		
Crude Protein	30.7	40.3

Lipids	6.3	5.4
Ash	13.7	12.2
Fiber	6.2	5.6
Moisture	10.5	9.3
NFE	33.2	28.2
Aminoacids		
Lysine	5.54	5.5
Arginine	4.4	4.7
Histidine	1.65	1.7
Threonine	4.2	4.2
Valine	2.9	3
Leucine	4.92	5.1
Isoleucine	2.9	3.02
Methionine	2.68	2.7
Cystine	2.3	2.24
Phenylalanine	4.43	4.4
Tyrosine	1.9	2.02
Tryptophan	1.13	1.2

3.1 Stress Indicators
The plasma cortisol level, blood sugar, serum lactate, hemoglobin and plasma protein levels significantly higher at highest stocking density.

4. Discussion
The present study interacts between stocking density (D1-D4) and dietary protein levels (P1- P2) on the growth and behaviour of hybrid tilapia (Table 2). In present research article the resultant data showed that increasing protein level from P1to P2 and increasing stocking density from D1 to D4 fish were affected in a significant (*P*< 0.05) diminution in growth and behavior. This study showed that the P1 diet produced the best growth performance. This study agreed with [10, 13]. [15] reported that 40% CP was best for growth of *Oreochromis niloticus* fry, 30% crude protein presented the better growth of *Diplodus sargus* reported by [21] and 30% CP was better for growth of *Oreochromis niloticus* suggested by [16]. The results showed that increasing the stocking density above D2/P1 or D2/P2 produced in a significant (*P* <0.05) lower lipid and protein at ease. Furthermore, an increase in crude protein level from P1 to P2 resulted in significantly decreased (*P*<0.05) body protein content. Those fish stocked at lowest stocking density (D1and D2, had highest feed intake (Table 2). These results are being in agreement with those reported by [16, 26] for hybrid tilapia. The fish reared at (D4) had also reduced growth performance credited to their lower fed intake. The fish fed with P1 and reared at D1 and D2 had also lowest FCR. These findings agreed with [18, 20] likewise, PER were also found to be affected by dietary crude protein level, stocking density of fish fed the P1 and reared at the D1 and D2 displaying the best protein efficiency ratio (PER). Similar studies reported by [16, 26]. The tendency of higher Cv at high stocking density was expected to increase population interaction. The coefficient variation were also significantly higher at high stocking density than fish at low stocking density and fed 30% protein suggesting the lower stocking density suitable for rearing system in order to lowest coefficient variation achieved in this experiment. Significantly higher (*P*<0.05) hematocrit concentration was observed at lower stocking densities indicating better physiological adaptation. In this present study, it was clearly demonstrated that increasing stocking density responsible the fish –fish interaction to compete and reduce to assess the feed energy distribution that suppress the fish growth [20]. The other main physiological factor that co-related to high stocking density, the cortisol, plasma glucose levels are considered to be a good indicator of stress levels in fish [2]. Results from this study indicated that fish held at high stocking density had higher levels of plasma cortisol, lactate, plasma protein, hemoglobin and glucose level than fish held at lower stocking densities. Its primary function is to increase blood sugar through gluconeogenesis and thus increase energy availability. Activities of cortisol also include down regulation of the Interleukin-2 receptor (IL-2R), enhancement of glycogenolysis, and proteolysis [2, 4]. Cortisol has been linked directly with growth reduction, immunosuppression and declined reproductive capabilities [3]. Evidences were showed that higher stocking density produced high stress full situation and energy demand rather than low stocking density (Fig.1). This key factor was also explained by [2, 3, 4]. The main aim of this study is to compare the optimal stocking density and also assist the protein level according to the stocking density. The important object of this study is to enhance the socio-economic values of poor communities and to develop the inland culturing system.

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

		Growth Parameters			
Stocking density/ Protein (%)	**Initial weight**	**Final weight**	**SGR**	**Weight gain**	**Survival rate**
D1/P1	2.1± 0.3^{a}	34.23± 0.4^{b}	3.3± 0.01^{b}	32.12± 0.2^{b}	95± 1.2^{b}
D2/P1	2.3± 0.1 ^{b}	41.7± 0.2^{a}	3.4± 0.03^{a}	39.4± 0.2^{a}	100± 1.4^{a}
D3/P1	2.2± 0.3 ^{c}	30.2± 0.5^{c}	2.9± 0.1 ^{c}	27.8± 0.1 ^{c}	85 ± 0.7^{c}
D4/P1	2.2± 0.1 ^{c}	23.2± 0.1^{d}	2.7± 0.02 ^{d}	22± 0.3 ^{d}	70 ± 1^{d}
D1/P2	2.2± 0.2 ^{c}	31.7± 0.9^{be}	3.1± 0.02^{e}	29± 0.1^{e}	80± 1.2 ^{ab}
D2/P2	2.3± 0.4 ^{b}	37.2± 0.7^{f}	3.2± 0.01^{f}	35.1 ± 0.5^{f}	100± 1.3 ^{b}
D3/P2	2.3± 0.1 ^{b}	26.2± 0.8^{ac}	2.8± 0.01^{ac}	24± 0.4^{ac}	80± 0.9^{ab}
D4/P2	2.31± 0.1 ^{b}	20.21± 1^{cd}	2.6± 0.01^{bc}	18± 0.3^{bc}	65± 1.4^{cd}
		Physiological parameter			
Stocking density/ P	**Feed intake**	**Final Cv**	**PER**	**HSI**	**FCR**
D1/P1	42± 0.2^{b}	1.2± 0.1^{b}	0.72 ± 0.02^{c}	2.4 ± 0.01^{a}	1± 0.1^{c}
D2/P1	47 ± 0.1^{a}	0.48± 0.1^{a}	1.4 ± 0.1^{b}	2.3 ± 0.2 ^{b}	0.2± 0.1^{a}
D3/P1	34.9± 0.1^{c}	1.65± 0.3^{c}	0.5 ± 0.1^{bc}	2.1 ± 0.1^{c}	1.8 ± 0.1^{e}
D4/P1	30.1± 0.3 ^{d}	4.1± 0.2 ^{d}	0.38± 0.02^{ab}	2.2 ± 0.01 ^{d}	2± 0.02^{ab}
D1/P2	34± 0.3^{e}	2.8± 0.4^{e}	0.84 ± 0.01^{d}	2.1 ± 0.03^{c}	1.6 ± 0.1^{d}
D2/P2	38± 0.5^{f}	2 ± 0.2^{f}	2.2± 0.03^{a}	2.0± 0.01 ^{bc}	0.7 ± 0.2^{b}
D3/P2^{cd}	28.7± 0.6^{ad}	3 ± 0.1^{ab}	0.6 ± 0.1^{cd}	1.8 ± 0.2^{d}	2± 0.03^{ab}
D4/P2 ^{ac}	24.5± 0.3^{ac}	5 ± 0.3^{ae}	0.4 ± 0.01^{f}	1.7 ± 0.01^{e}	2.4± 0.01^{ac}

*Mean in the same column with different superscripts are significantly (P <0.05) different
*Each value was the mean of three replicates (Mean ± S.E)
SGR = 100 [In (Wt) - In Wo) / t]. FCR = I / Wt – W$_{o}$ + Wd, PER = weight gain (g)/ protein intake (g), Cv = S. E / Mean weight x 100, Sr (%) = 100 Nt / Ni, Feed intake (% d^{-1}) = 100 × I/ [Wt – W$_{o}$ / 2×t], HSI = Mean liver weight/ Wet weight of fish× 100, [Nt = final number of fish, Ni = Initial number of fish, Wt = final weight of fish, Wo = Initial number of fish, I = total supplied feed to fish.

Table 3: Biochemical and physical parameters of hybrid (*Oreochromis mossambicus* × *O. niloticus*)

		Biochemical Parameters			
Stocking density/ (Protein %)	**Cholesterol (mg 100ml^{-1})**	**Urea (mg 100ml^{-1})**	**RBC (× 10^{6}/mm^{3})**	**WBC (× 10^{3}/mm^{3})**	**Total plasma lipids mg100ml^{-1}**
D1/P1	162 ± 1.04^{a}	3 ±0.1^{b}	4.4 ± 0.2 ^{b}	3.9 ± 0.2 ^{b}	1756 ± 15.1 ^{b}
D2/P1	163± 1.2 ^{a}	3.1 ±0.1^{c}	4.4 ± 0.3^{a}	4 ± 0.1^{a}	1834 ± 9.2^{a}
D3/P1	167± 1 ^{a}	3.33±0.1 ^{a}	4.32 ± 0.1^{c}	3.9 ± 0.1^{b}	1678 ± 11.2^{c}
D4/P1	170± 2 ^{a}	3.34 ±0.1 ^{a}	3.9± 0.2 ^{d}	3.78 ± 0.2 ^{c}	1456 ± 7 ^{d}
D1/P2	163± 1 ^{a}	3.1 ±0.1 ^{c}	3.91 ± 0.1^{d}	3.8± 0.01 ^{c}	1745 ± 9 ^{b}
D2/P2					

Table 4: The body composition of hybrid (*Oreochromis mossambicus* × *O. niloticus*)

		Body composition		
Stocking density/ Protein (%)	**Crude Protein (%)**	**Lipids (%)**	**Ash (%)**	**Moisture (%)**
D1/P1	15.6± 0.1[a]	12.74± 0.2	4.6± 0.1	67.2± 2
D2/P1	16.8± 0.1[b]	12.56± 0.1	4.32± 0.2	66.54± 2
D3/P1	14.3± 0.1[c]	12.44± 1	4.43± 0.1	66.6± 0.1
D4/P1	15.02± 1[d]	12.31± 1	4.62± 0.1	66.45± 0.1
D1/P2	15.5± 2[ab]	12.63± 0.2	4.5± 0.1	66.3± 1
D2/P2	16.6± 0.2[bc]	12.6± 0.1	4.28± 0.3	67± 0.5
D3/P2	14.4± 0.4[ca]	12.41± 0.1	4.52± 0.1	66.7± 0.4
D4/P2	14.2± 0.1[cd]	12.22± 0.3	4.5± 0.2	66.74± 0.1

*Mean in the same column with different superscripts are significantly (P <0.05) different
*Each value was the mean of three replicates (Mean ± S.E).

4. Conclusions

The overall out outcomes in the present study suggested that hybrid tilapia fed on 30% (P1) diet and reared at stocking density (10 fish/ 40L) produced the selective growth behavior. Nevertheless, the increase in stocking density and dietary protein level would be delay the fish growth performance. However, the higher rearing capacity might be create the stress full situations.

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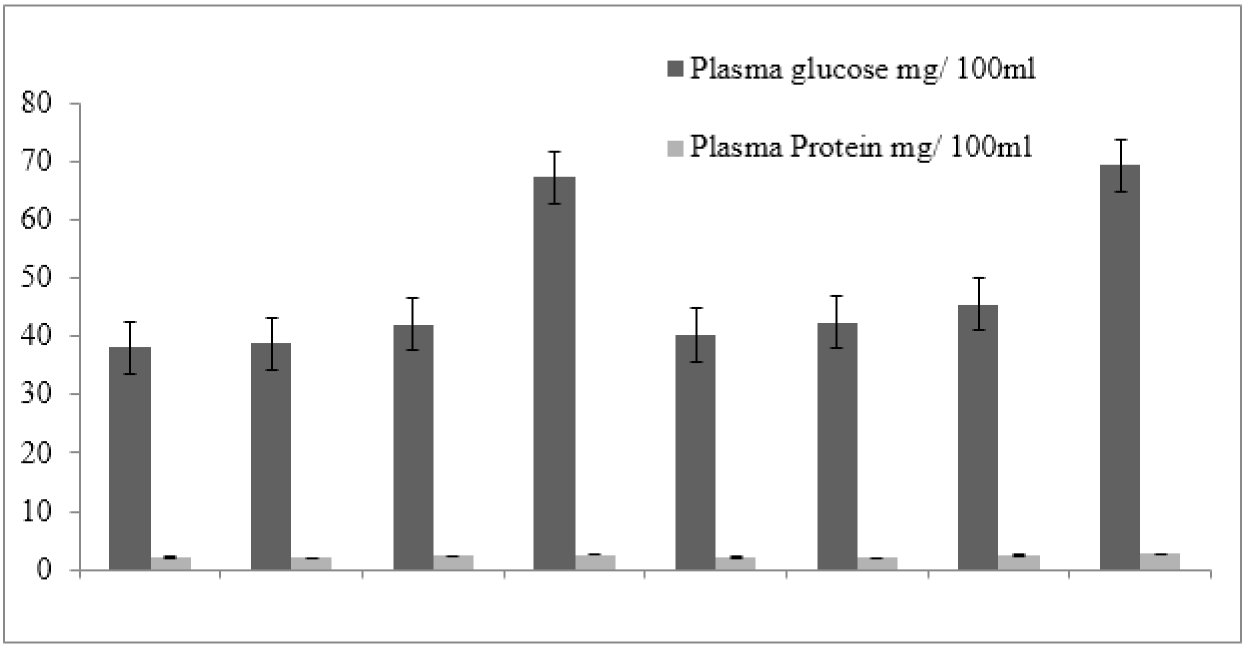

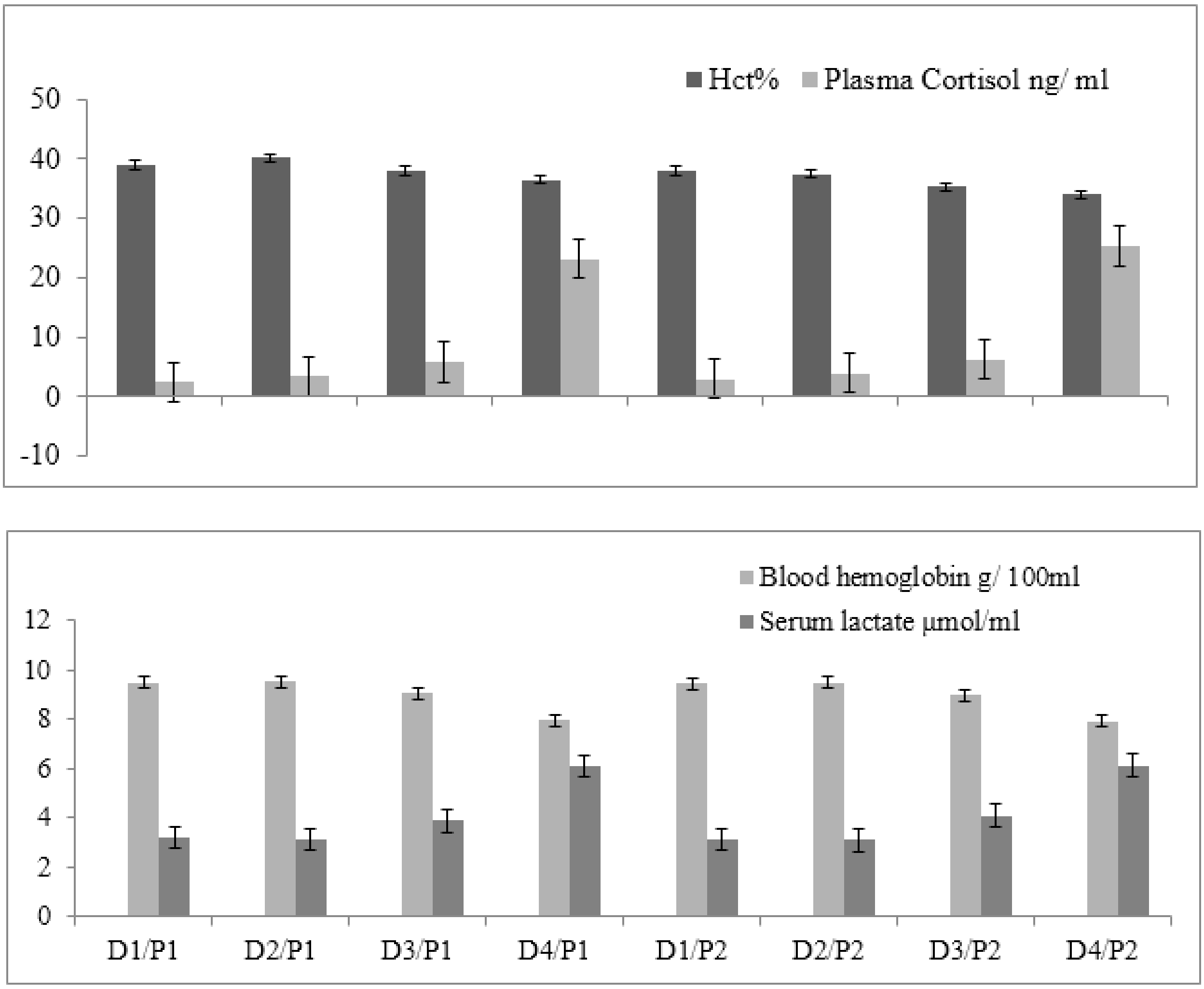

Fig 1: Analysis of blood hemoglobin, serum lactate, %Hct, plasma cortisol, total plasma protein, plasma glucose level are showing at four stocking densities D1-D4 (fish/ 40L) and with two protein levels (25% CP = P1, 35% CP = P2).Test dimension is 5 fish per collection. Statistics are resources ± SE.

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