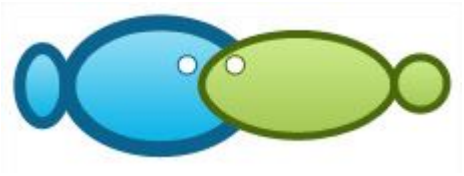

Assessment of stresful ambient water salinity on growth, feed utilization and hematological indices of European sea bass, *Dicentrarchus labrax*, juveniles

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Abstract. The present study was conducted to investigate the effect of two water salinity levels (10 and 15 ‰) on growth, feed utilization and hematological indices of European sea bass, *Dicentrarchus labrax* juveniles reared in net enclosures for 60 days. Fish were fed an experimental diet contained 45% crude protein and gross energy of 21.97 Mj kg^{-1} diet to apparent satiation for six days a week. A total of 120 *D. labrax* with an average initial body weight of 13.0±0.5 g $fish^{-1}$ were randomly distributed into six net enclosures measuring 3 x 8 x 1.25 m each representing two treatment groups (in triplicate) at a stocking density of 20 fish per net enclosure. Over the 60-days feeding period, growth, feed utilization efficiency and survival rate of *D. labrax* juveniles improved significantly ($p < 0.05$) with salinity 15 ‰ . The same trend was observed with feed conversion ratio (FCR). No significant difference ($p \geq 0.05$) was observed for the whole body proximate analysis of *D. labrax* juveniles with the tested salinities. Significant ($p < 0.05$) increase for mean red blood cells count (RBCs), mean white blood cells count (WBCs), hematocrit (Hct), hemoglobin (Hb), total plasma protein, total plasma globulin, plasma sodium chloride and glucose level of *D. labrax* juveniles was recorded with salinity level 15 ‰ . An opposite trend was observed for plasma cortisol, albumin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values. The result indicates that the tested salinity levels could be considered as stress factors. Thus, the present study highlights that the growth, feed utilization and hematological indices of *D. labrax* fingerlings improves at 15 ‰ salinity.

Key Words: *Dicentrarchus labrax*, salt water, physiological parameters, growth, blood parameters.

Introduction. In aquaculture, not only quality and quantity of feed are important, but also an inadequate environmental condition might be more critical. Growth performance of the fish is governed not only by its genetic potential, but also by its instant environmental conditions (Nguyen et al 2010). Salinity is one of the most important environmental factors that trigger fish growth. Changes in ambient salinity can directly influence fish metabolism (Cao et al 2015) and growth of some species (Lisboa et al 2015).

Salinity is a main abiotic factor in aquaculture (Ruscoe et al 2004), if its concentration is altered the internal stability could change causing fish to maintain many physiological responses to substantiate the prestressor condition (Enayati et al 2013). Tran-Ngoc et al (2017) reported that blood is the most sensitive media in fish for salinity changes. The first effect of salinity appears on fish through influencing osmoregulatory processes by gaining or losing ions in high or low salinities to conserve the ionic fluid concentration inside the body.

Stress due to salinity fluctuation has been reported to alter the standard hematological characteristic of teleosts, elevating plasma corticosteroids, reducing the levels of some blood parameters and also increasing the values of some blood components. These changes can affect oxygen transportation in blood and across the

gills. Both hematological and biochemical parameters have been frequently used as indicators for general conditions in various aquatic species (Tran-Ngoc et al 2017).

Fish response to any stressor could be achieved by many physiological changes to maintain homeostasis, osmolality and hematology (McDonald & Milligan 1997). Therefore, the aim of the present study is to evaluate the stressful effect of two different water salinity levels on growth performance, feed utilization, biochemical and hematological responses of European sea bass, *Dicentrarchus labrax* juveniles.

Material and Method

Experimental fish, diet and culture technique. A total of 120 *D. labrax* with an average initial body weight of 13.0±0.5 g fish^{-1} were obtained from a private commercial fish farm (El-Shrief farm, Wady Marriout, Alexandria), Egypt. The present experiment was conducted in the aquatic laboratory of the former private farm (El-Shrief farm, Wady Marriout, Alexandria) from September throughout December. Prior to the start of experiment, the fish were acclimated to the experimental conditions for one week in two indoor circular fiberglass tanks (1 m^3 each) by gradual decrease of water salinity at an approximate rate of 4 ‰ per day until reaching 10 and 15 ‰. Water salinity was declined slowly from 37 ‰ salinity to the target levels (either 10 or 15 ‰) by diluting with freshwater. After acclimation, the fish were randomly distributed into six net enclosures (3 x 8 x 1.25 m each) representing two treatments (in triplicates) at a stocking density of 20 fish per net enclosure in order not to cause any density stress on fish. Water temperature, dissolved oxygen (DO), pH, and ammonia were monitored weekly during the trial, to maintain water quality at optimum range for *D. labrax* juveniles.

DO levels were kept near saturation levels, continuous aeration with aeriation tubes was maintained in each net enclosure using an electric air pumping. Water temperature ranged from 18.0 to 19.0°C, DO from 6.00 to 6.59 mg L^{-1}, pH from 7.0 to 7.5, ammonia (NH_3) from 0.23 to 0.29 mg L^{-1} and a photoperiod regime of 12:12 h light: dark and supplemented with two led lamps (2000-2200 lux) to maintain the required photoperiod of *D. labrax*.

Fish were fed an experimental diet contained 45% crude protein and gross energy of 21.97 Mj kg^{-1} diet (Table 1) to apparent satiation for six days a week. Gross energy (GE) contents of diets were calculated according to gross caloric values of Brett (1973) using the values of 23.6, 39.5, and 17.2 kJ g^{-1} for crude protein, crude fat, and total carbohydrate, respectively. The daily ration was divided into three equal amounts and offered three times a day (09.00, 12.00 and 15.00 h).

Growth and feed utilization indices. The mean final body weight (FBW) was determined by dividing the total fish weight in each net enclosure by the available number of fish. Weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), protein productive value (PPV), energy retention (ER) and condition factor (K) were calculated using the following equations, according to Castell & Tiewes (1980), and Cho (1990):

$$WG = \text{final body weight (g)} - \text{initial body weight (g)};$$

$$FCR = \text{feed intake (g)}/\text{weight gain (g)};$$

$$SGR = 100 \times [(\ln \text{final body weight (g)} - \ln \text{initial body weight (g)})/ \text{duration of feeding (day)}];$$

$$PER\ (g) = \text{weight gain (g)}/\text{protein intake (g)};$$

$$PPV\ (g, \%) = (\text{protein gain (g)}/\text{protein intake (g)}) \times 100;$$

$$ER\ (kJ) = (\text{energy gain (kJ)}/\text{energy intake (kJ)}) \times 100;$$

$$\text{Survival}\ (\%) = 100 \times (\text{initial number of the fish}/\text{final number of fish});$$

$$\text{Fulton condition factor}\ (K) = W / L^3 \times 100.$$

where: W = fish weight (wet weight in g); L = total fish length (in cm).

Table 1

Shows the feed ingredients (%) and chemical composition (%) of the diet

Ingredient	*Experimental diet (g)*
Fish meal (68% CP)	317
Soy bean meal (47% CP)	375.4
Corn gluten (60% CP)	90
Wheat middling (13% CP)	50
Soybean oil	55
Fish oil	50
Salt	45
Dicalcium phosphate	10
Premix[1]	2
Methionine	2
Choline chloride	1
Lysine	1
Vitamin C	0.6
Antitoxic	1
Chemical composition (%, dry matter basis)	
Dry matter (DM)	90.51
Crude protein (CP)	46.13
Ether extract (EE)	17.41
Nitrogen free extract (NFE)[2]	24.59
Crude fiber (CF)	2.24
Ash	9.63
Gross energy (GE; Mj kg^{-1} DM)[3]	21.94

[1]Vitamin and mineral mixture (supplements per kg of the mixed feed): vitamin A - 4,500 IU; vitamin D3 - 4,500 IU; vitamin E - 400 mg; vitamin B1 - 30 mg; vitamin B2 - 40 mg; vitamin B6 - 40 mg; vitamin B12 - 0.08 mg; vitamin K3 - 15 mg; ascorbic acid - 750 mg; nicotinic acid - 300 mg; Ca-pantothenate - 100 mg; folic acid - 10 mg; biotin - 3 mg; inositol - 500 mg; p-amino benzoic acid - 200 mg; Ca - 2.1 g; Fe - 250 mg; Mn - 40 mg; Zn - 60 mg; I - 4 mg; Cu - 12 mg; Se - 0.3 mg; Co - 2 mg; [2] NFE: calculated using the following equation: NFE = 100 (crude protein + ether extract + crude fiber + ash); [3] Gross energy (GE) contents of diets were calculated according to gross caloric values of Brett (1973) using the values of 23.6, 39.5, and 17.2 kJ g^{-1} for crude protein, crude fat, and total carbohydrate, respectively.

Carcass composition. At day (0) of the trial, a random pooled sample of 20 fish from each treatment group were collected, anaesthetized with t-amyl alcohol and sacrificed for determination of initial whole-body proximate composition analysis. At the end of the feeding trial, five fish were randomly selected from each replicate and anaesthetized with t-amyl alcohol, sacrificed and homogenized in a blender to determine the final whole-body proximate composition. The fish were pooled for each treatment, oven-dried at 105°C overnight, grounded and stored at -20°C for subsequent analysis. The chemical composition of fish and diet samples was assessed according to procedures of AOAC (2000). Dry matter was determined after drying the samples in an oven (105°C) for 24 h. Ash was measured following incineration at 550°C for 12 h. Crude protein was determined by the micro-Kjeldahl method, with N%×6.25 (using a Kjeltec Auto Analyzer, Model VELP Scientifica, UDK 127, Usmate, Italy), and crude fat was assessed by Soxhlet extraction (Model VELP Scientifica, SER148) with diethyl ether (40-60°C).

Blood sampling. Blood samples were collected at the end of the experiment. Each of the experimental treatment was sampled once, with five fish net^{-1} enclosure for hematological indices analysis and five fish net^{-1} enclosure bled for plasma content analysis. The fish were anesthetized with t-amyl alcohol and the blood samples were taken by puncturing the caudal vein. The collected blood was divided into two tubes, one containing heparin as anticoagulant agent for hematological assessment and the other was anticoagulant free for biochemical estimation. The hematological parameters are expressed in international units (IU).

The red blood cell counts (RBCs) were determined by using a Bürker counting chamber and Hayem solution. The findings and instructions published by Blaxhall & Daisley (1973) and Hrubec et al (2000) were followed in the RBCs determineation. Hematocrit (Hct) was determined by using microhematocrit-heparinized capillary tubes

and a microhematocrite centrifuge (10,000 x g for 5 min). The values of Hct were determined within 30 min after bleeding. Hemoglobin (Hb) concentrations were determined by the cyanhemoglobin method, at 540 nm. The total white blood cell (WBC) count was determined according to the methods of Stoskopf (1993) and Hrubec et al (2000). WBCs and Hb values were determined within 6 h after blood sampling.

Total plasma protein (g dL^{-1}) was determined using biuret method according to Doumas et al (1981). Albumin (g dL^{-1}) was determined by the bromocresol green method according to Reinhold (1953) and globulin (g dL^{-1}) was calculated as the difference between total protein and albumin. Glucose (mg dL^{-1}), sodium chloride (mq L^{-1}) and cortisol (P mol^{-1}) were determined according to (Brown & Taylor 1985; Morineau et al 1997).

Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were assayed using the method of Gella et al (1985). The principle reaction of the colorimetric determination of AST or ALT activity was based on the reaction of aspartate or alanine with ɑ-ketoglutaric acid to form oxaloacetate or pyruvate respectively. The oxaloacetate or pyruvate was measured by monitoring the concentration of oxaloacetate or pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine. The absorbance is read at a wavelength of 505 nm, then interpolated in the calibration curve.

Statistical analysis. One-way ANOVA and Duncan's (1955) multiple range tests were calculated, effects with a probability of ($p < 0.05$) were considered significant. The data of the experiments were statistically analyzed using general linear model (GLM) procedure according to Statistical Analysis System (SAS® 2004).

Results and Discussion. *D. labrax* is widespread and a very important commercial marine fish species in the Mediterranean Sea and Egypt. It is a euryhaline marine teleost species that tolerates a wide range of salinities (Kousoulaki et al 2015). The lack of conditions for offshore aquaculture and pollution of sea water in certain parts of the Egyptian lakes has forced aquaculturists to consider estuaries, or even freshwater resources to maintain their crops. Thus the effect of decreased water salinity to 15 and 10 ‰ on growth, feed utilization and hematological indices of *D. labrax* juveniles were investigated over a period of 60-days.

The findings of the overall feeding period, showed no apparent evidence of infected fish and subsequently preventive veterinary measures were not required. No mortality was recorded for the *D. labrax* juveniles throughout the 60-days of the experimental period duration (Table 2).

Table 2

Growth performance and nutrient utilization of European sea bass, *Dicentrarchus labrax* after 60 experimental days

Measured variable	*Experimental treatments*	
Salinity levels	10 ‰	15 ‰
IBW(g $fish^{-1}$)	13.38±0.06	13.38±0.08
FBW (g $fish^{-1}$)	22.40±0.25^{b}	23.97±0.30^{a}
WG (g $fish^{-1}$)	9.02±0.27^{b}	9.58±0.34^{a}
Feed intake (g $fish^{-1}$)	16.48±0.01	17.24±0.01
FCR	1.75±0.04^{a}	1.51±0.05^{b}
SGR (% days)	0.86±0.02^{b}	0.90±0.02^{a}
PER	1.21±0.02	1.23±0.05
PPV (%)	18.03±0.01	18.12±0.02
ER (%)	17.74±0.06^{b}	18.53±0.04^{a}
K	1.22±0.04	1.12±0.03
S (%)	100	100

Values are means±SD of triplicate analyses. Means in the same row bearing different superscript differ significantly ($p < 0.05$); IBW - initial body weight; FBW - final body weight; WG - body weight gain; FCR - feed conversion ratio; SGR - specific growth rate; PER - protein efficiency ratio; PPV - protein productive value; ER - energy retention; K - condition factor; S - survival.

The data revealed that all fish FBW, WG and SGR increased significantly ($p < 0.05$) with water salinity 15 ‰. The same trend was observed for the best FCR and the highest ER values. No significant influences of increased water salinity was reported on PER, PPV and K values. Although Saillant et al (2003) reported that growth and survival of sea bass was improved by 15 ‰ salinity at the beginning of larval rearing (14 days) and at the end of pre-growing (234-458 days). But Hamed et al (2016) reported that each fish species seems to have its own optimum salinity for growth, however, osmoregulatory imbalance is often associated with whole animal-level stress changes, including decreased growth performance (Imsland et al 2008). Boeuf & Payan (2001) reported that numerous studies have shown that 20 to > 50% of the total fish energy budget are dedicated to osmoregulation. However, food intake and stimulation of food conversion are both reported to be environmental salinity-dependent. Each species has its own optimum salinity for growth, under a certain water temperature and ontogenetic phase, although existing data are sometimes contradictory. For instance, the best salinity conditions for growth in gilthead sea bream, *Sparus aurata* have been reported at 12 ‰ (Laiz-Carrión et al 2005) or 28 ‰ (Klaoudatos & Conides 1996), in turbot, *Scopthalmus maximus* at 15 ‰ (Imsland et al 2008) and in *D. labrax* at 15 ‰ (Saillant et al 2003) or 28-30 ‰ (Dendrinos & Thorpe 1985; Conides & Glamuzina 2006).

The present results indicate that salinity affects FCR and growth in *D. labrax* juveniles adapted to low salinity. The metabolic and energetic cost of osmoregulation should, at least partly, reflect the effect of salinity on growth, feeding behavior, appetite, or stimulation/inactivation of other metabolic and endocrine pathways (Boeuf & Payan 2001; McCormick 2001). The same trend for growth and FCR data were observed in the present study. In the present study, the FCR for 15 ‰ salinity group was better compared to 10 ‰ salinity group, indicating that stressful condition lowers the fish ability to utilize the offered feed.

No statistical difference ($p \geq 0.05$) was detected for the influence of water salinity levels on whole body proximate analysis of *D. labrax* juveniles herein (Table 3). The results agree with the findings of Dendrinos & Thorpe (1985) who stated that salinity has no significant effects on the protein and lipid contents in the meat of sea bass. Alliot et al (1983) reported that the body composition of sea bass was influenced by temperature rather than salinity, which was almost constant (18-19^{o}C) throughout the present experimental duration.

Table 3

Whole-body proximate composition of European sea bass, *Dicentrarchus labrax* after 60 experimental days

Proximate composition	*Experimental treatments (salinity levels)*	
	10 ‰	*15 ‰*
Dry matter (DM, %)	27.90±0.34	28.57±0.52
Crude protein (CP, %)	53.25±0.56	51.46±0.79
Ether extract (EE, %)	27.83±0.40	29.17±1.78
Ash (%)	18.93±0.00	19.37±0.42
Gross energy (Mj/100 g)	5.64±0.11	5.66±0.09

Values are means±SD of triplicate analyses. Means in the same row bearing different superscript differ significantly ($p \leq 0.05$).

Meanwhile, the hematological parameters presented by mean RBCs count, mean WBCs count, hematocrit (Hct), Hb (Figure 1), total plasma protein, total plasma globulin (Figure 2) and plasma sodium chloride and glucose (Figure 1) of *D. labrax* fingerlings significantly ($p \leq 0.05$) increased with water salinity 15‰. An opposite trend was observed for plasma cortisol (Figure 1), albumin (Figure 2), AST and ALT values (Figure 3). The blood parameters in fish reflected its health status and give the physiological responses to stressors as a result of the effect of environmental factors (Schutt et al 1997). Salinity considered as a stressor factor, any change in blood criteria has been considered as part of preliminary response for stress (McCormick 2001). Thus, the change in blood

parameters refers to the way the fish trying to keep balanced form and considered as physiological endeavor to get back to the internal stability after exposing to different salt concentrations (Schreck 1990). This might have led to occurring changes in oxygen consumption rate and energy consumed and changes in ions transfer across gills via increasing the blood factors that works as an intermediate for achieving the increase in oxygen and transferring ions across blood (Akinrotimi et al 2007).

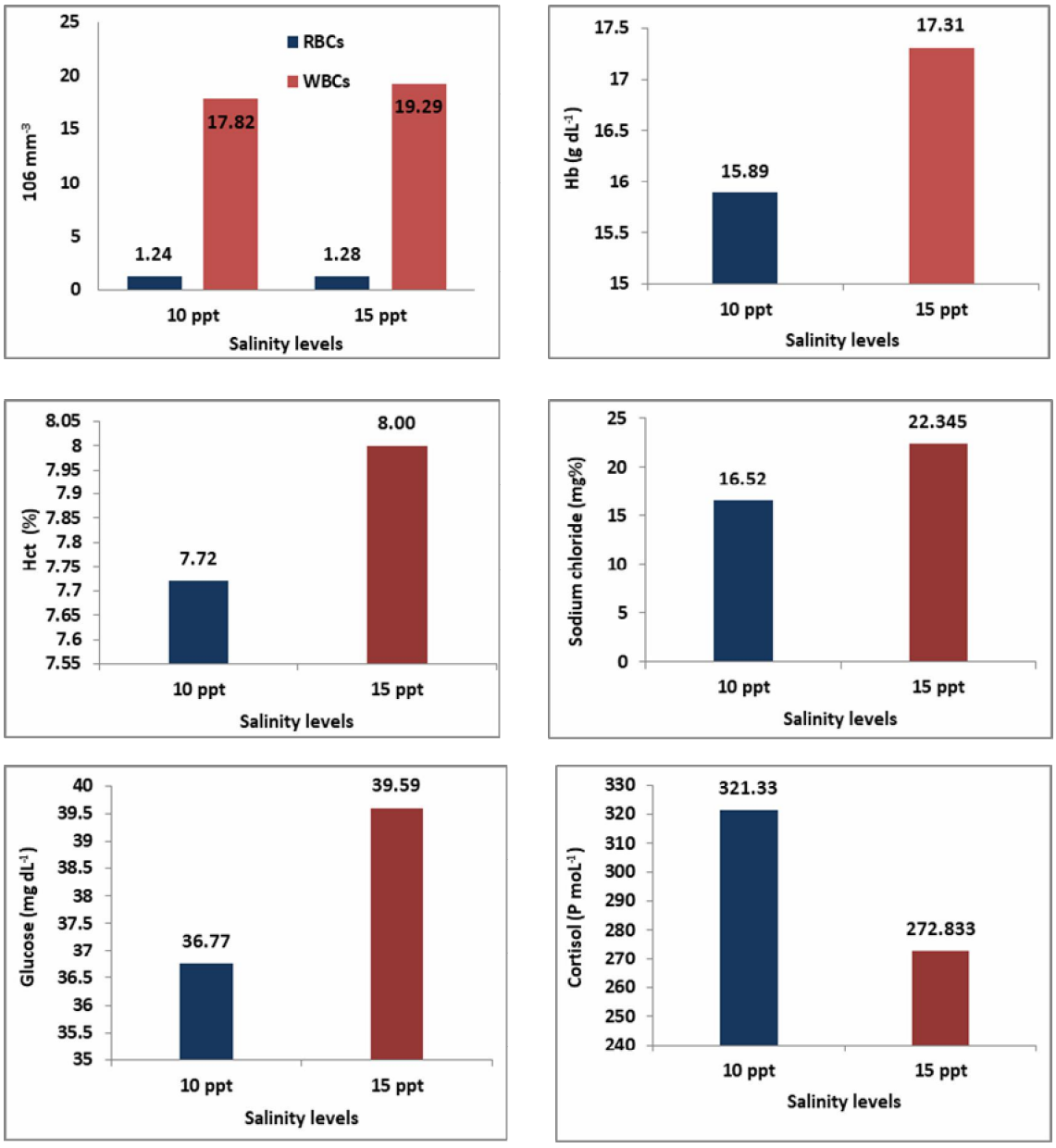

Figure 1. Effect of different salinity levels on hematological characteristics of European sea bass, *Dicentrarchus labrax* after 60 experimental days.

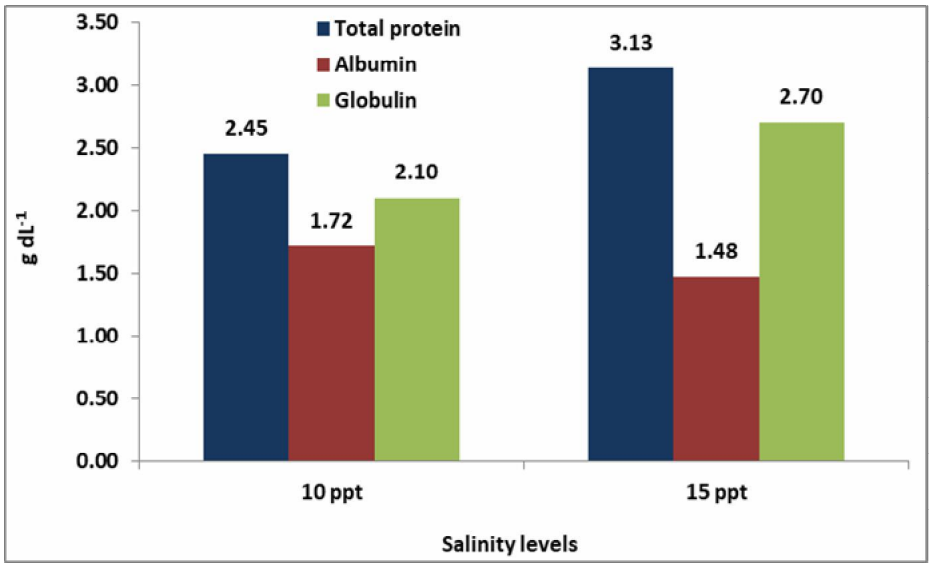

Figure 2. Effect of different salinity levels on total plasma protein, albumin and globulin of European sea bass, *Dicentrarchus labrax* after 60 experimental days.

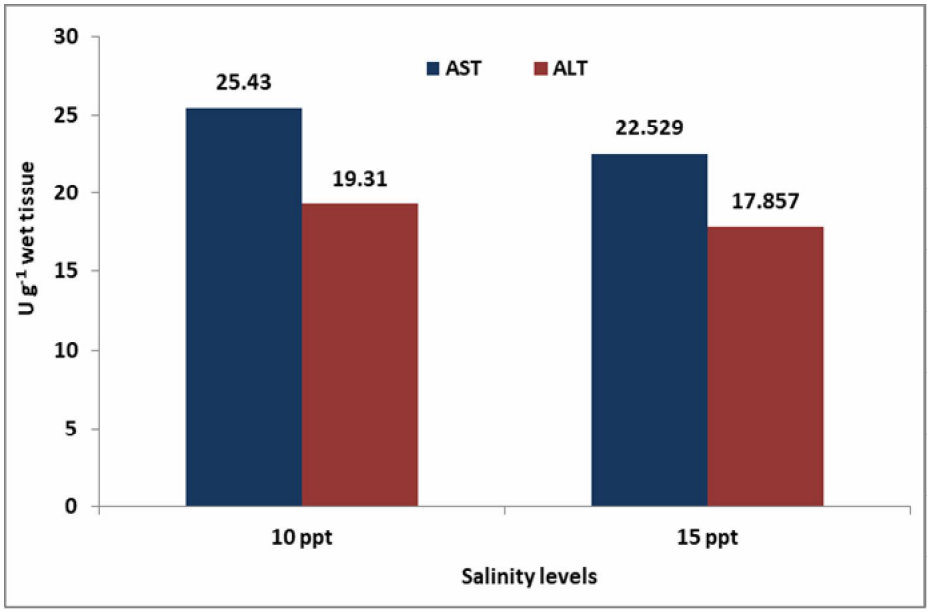

Figure 3. Effect of different salinity levels on plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of European sea bass, *Dicentrarchus labrax* after 60 experimental days.

In the present study, a decrease in Hb content, RBCs and Hct in 10‰ treated groups, are in agreement with other researcher who found a significant effect of salinity on RBCs, Hct and Hb in different species, this result may be associated with osmoregulatory disfunction induced by high salinity levels (Usha 2011; Fazio et al 2013; Soltanian et al 2016). Low Hct percentages in stressed fish could be explained by reduced volume of RBCs due to osmotic changes caused by ion leakage from plasma (Alwan et al 2009; Elarabany et al 2017). However, the increase of RBCs may be triggered by the increase of oxygen consumption owing to the increase of energy requirements. Besides, RBCs have an important role to transfer the oxygen, the increase in WBCs may be related to immunity reaction because of raising the cortisol hormone that is responsible for organizing the osmosis in salt water or might be the transferring of WBCs throughout the body as existing of factor leading to facilitate the infection of fish with diseases. Moreover, the increase in Hb is leading to increase in RBCs as Hb regarded as protein carried by RBCs and having a role in respiration (Al Hilali & Al-Khshali 2016). Concurrently, the increase in concentration of Hb might be due to the increase in salinity

which was detected for *D. labrax* in this study and corresponds with the findings of Yavuzcan-Yildiz & Kirkavgac-Uzbilek (2001).

Moreover, the significant ($p < 0.05$) effect of different salinity levels on hematological parameters was found in the present study. Total plasma protein level recorded the highest values for fish in salinity 15 ‰ groups. Helmy et al (1974) reported that the increase in serum protein would result when anabolic processes exceeded catabolic ones, and reserved protein is being produced in greater quantity to meet increased metabolic requirements of the fish. They added that an increased catabolic rate would explain the decreases in serum protein level. Moreover, the cyclic nature of the total serum protein is an indicator of the changes taking place in the serum globulin fraction. This contradicts with other studies reporting either no changes or decreased protein levels in parallel with increased salinity (Herrera et al 2012). Actually, the data herein are in covenant with the findings of Soltanian et al (2016) whom showed the decrease of total protein when salinity reached 17 ‰ .

The possible importance of increased serum protein as a fuel for tissues during osmotic acclimation has not been addressed yet, but may be related to a metabolic reallocation of energy resources once carbohydrate stores have been mobilized. Amino acids seem to play an important role in allowing fish to adjust to the different environmental salinities, either as energy sources or as important osmolytes for cell volume regulation (Aragão et al 2010).

In the study herein, cortisol level has the tendency to increase by lowering salinity, and it was associated with their low activity during low salinity rearing as the impact of stress increase. Cortisol is well known to suppress fish immune functions in relation to various stresses (Harris & Bird 2000). In this present study, low salinity exposure as the stressor triggered the hypothalamus pituitary-interrenal activity. This condition resulted in cortisol secretion. The increase of plasma cortisol value is considered to be a primary indicator of stress response (Cataldi et al 1998). As a result, the circulating level of cortisol is commonly used as a stress indicator of fish (Wendelaar Bonga 1997). The physiological response of fish may be compromised or influenced their health and well-being, or may become maladaptive by long-term environmental stressors exposure (Barton & Iwama 1991).

The AST and ALT, found mainly in hepatocytes and cardiomyocytes of fish, respectively, play important roles in protein metabolism. When liver and myocardial cells are damaged or their permeability increases, AST and ALT will be released into the blood, resulting in elevated blood transaminase activity. The activities of serum AST and ALT can, therefore, be used to monitor the health status of fish (Wang et al 2005). The current findings showed that the AST and ALT where significantly higher in 10 ‰ salinity group than that of 15 ‰ salinity group, indicating that AST and ALT increase due to stress affected liver and myocardial cells. In the present study, significantly elevated values of AST and ALT, correlated with the low salinity, indicate increased permeability of the hepatocytes and cellular leakage.

Conclusions. The results of this study suggest that salinity could be considered as a stress factor. The water salinity level influenced growth, feed utilization and hematological indices of European sea bass, *D. labrax* juveniles. Any change in blood criteria as effect by water salinity or dietary nutrients had been considered as part of preliminary response for stress. Further research is needed to detect the effect of different dietary protein under different water salinity levels on European sea bass, *D. labrax* juveniles performance.

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