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Effects of stock density on texture-colour quality and chemical composition of rainbow trout (*Oncorhynchus mykiss*)

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Abstract

This study describes the effects of different stocking densities on texture/colour characteristics, protein content /amino acid and lipid content/fatty acid composition of rainbow trout fillet. Stocking density was selected 5 (Group A), 15 (Group B), 25 (Group C) kg fish m^{-3}. Tukey's Multiple Comparison Test showed insignificant differences between measured size/weight measurement and condition factors. No significant differences were found between A and C groups for colour analysis (L* and a* value) and texture profile analysis (hardness, adhesiveness, cohesiveness and gumminess values) of rainbow trout fillets. The proximate composition analysis showed rainbow trout fillets from the A and C groups to exhibit higher values of moisture than the B group. Fish from the A and B groups had a lower of ash and protein in comparison to C group samples. The highest fat values of rainbow trout were measured in B group samples. The content essential amino acid and non-essential amino acid was lower in A and B groups than in C groups. Fish from the C group had higher content of polyunsaturated fatty acids, especially n-3, docosahexanoic acid (DHA) and eicosapentaenoic acid (EPA) in comparison to A and C group. As a result, 25 kg fish m^{-3} is recommended stock density in terms of product texture/color and composition quality.

Keywords: Rainbow trout, *Oncorhynchus mykiss*, Stock density, Texture-Colour quality, Chemical composition

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Introduction

The rainbow trout is a fast-growing carnivorous fish, highly popular as a food fish and a valuable species in world aquaculture. This fish is intensively cultured by commercial fish farms in Turkey, Norway, USA, Chile, Denmark and France. The fish, popularly known as "Alabalik" was introduced in Turkey from Germany during the beginning of 1970s and has been popular among fish farmers and consumers. Immediately after introduction, the fish proved to be a popular fish in aquaculture, and at present, rainbow trout is the most important fish species used for commercial aquaculture in Turkey. Rainbow trout production in Turkey was about 111.335 metric tons in 2012 (TurkStat, 2013). However, culture of this fish has been largely restricted to concrete pond aquaculture systems (Çelikkale *et al*., 1999; Alpaz, 2005).

Stocking density is a key factor in determining the productivity and profitability of commercial fish farms (North *et al*., 2006a).

Papoutsoglou *et al*. (1998) have reported that the stocking density is important in achieving the aims of the controlled production of several fish species. Lambert and Dutil (2001) noted that increasing stocking density has a negative effect on feeding and growth of fish.

According to M'balaka *et al*. (2012), the effects of stocking density on the growth andsurvival rate for the aquaculture a diversity of fish species are well documented and seem to impact the final production differently. Some researchers have been working in lower stocking densities on growth performance and survival rate for species (Hecht *et al*., 1996; Cuvin-Aralar *et al*., 2007; Mazlum, 2007; Sorphea *et al*., 2010; Pouey *et al*., 2011; Zhu *et al*., 2011; Khatune-Jannat *et al*., 2012).

There have been discussions at European Union level to introduce an upper limit of stocking density in fresh water at 25–30 kg fish m^{-3} though no such regulations has yet been established (Hosfeld *et al*., 2009). Some people state that the average fish density in Norway is estimated to be between 40 and 50 kg fish m^{-3}; and more than 80% of the fish farmers have used a density in excess of 25 kg fish m^{-3}. In Europe, North America and Australia, 60kg fish m^{-3} is generally viewed as the maximum density. However, an increase in stocking density will in general cause the deterioration of water quality due to a reduction in dissolved oxygen, and build up of fish metabolites and carbon dioxide followed by a reduction of pH level.

Most of the studies on the impact of stocking density on fish performance have been carried out with freshwater species, mainly salmonids, and little information is available for marine species (Turnbull *et al*., 2005).

High stocking densities can have a detrimental impact on the health and welfare of aquaculture fish as well (Sirakov and Ivancheva, 2008). In particular, high densities can lead to: increased stress, increased susceptibility to disease, increased incidence of physical injuries (North *et al*., 2006a), poor body condition, and reduction in growth, feed

intake and feed conversation efficiency (Ellis *et al*., 2002).

In this study, we have investigated the effect of different stocking densities on the growth performance, texture/colour quality and amino acid/fatty acid composition of rainbow trout.

Materials and methods

Samples

All the rainbow trout specimens (with a mean length of 15 cm and a mean weight 50g) used for this research were obtained from the Istanbul University, Sapanca Aquaculture Research Center, (Sakarya, Turkey). The experiment was carried out in square tanks (each with 2×2×0.60 meter dimentions). We had three different treatments, each with two replicate tanks. The fishes were stocked in duplicate tanks at densities of 5 kg fish m^{-3} (Group A), 15 kg fish m^{-3} (Group B), and 25 kg fish m^{-3} (Group C) (n=1.760, 3.520, 5.280 fish per tank, respectively). Tanks were supplied with untreated water directly from a reservoir at ambient temperature with a flow through rate of one liter per minute. Water quality characteristics of inflow were typically within the following ranges: dissolved oxygen min. 7,5 mg/l; pH 7,66 – 8,00; total water hardness 120 mg/l. The experiment was carried out for 45 days (April to May 2010). Fish were hand-fed with a ration in accordance with the manufacturer's tables (BIOAQUA Standard Extruder Trout Grower Feed), and feeding was done twice between 09:00 and 16:00 under ambient lighting.

The condition factor

The condition factor was calculated according to the following formula $CF=W/L^3*100$ where W is the fish weight in grams; and L is its length in cm.

Colour analysis

The colour of the fish samples was determined with the help of a Konica Minolta chromo meter (model CR 400/410; Minolta, Osaka, Japan). L* (brightness), a* (+a, red; -a, green) and b* (+b, yellow; -b, blue) values were measured. The colorimeter was calibrated using white references (CR-A44**).**

Texture analysis

Textural analyses of the fillets were performed by CT3 Texture Analyser (Brookfield Texture Analyser, Guangzhou, China) equipped with a load cell of 1,5 kg. The conditions of the apparatus were as follows: test type: texture profile analysis (TPA), test target: distance, target value: 4,0 mm, trigger load: 0,020 N, test speed: 1,00 mm/second, probe type: TA 52, dimension of probe: 3x3x3 cm. Texture parameter: hardness, adhesiveness, resilience, cohesiveness, springiness, gumminess, chewiness.

Chemical analysis

Moisture determination

Moisture content was determined by drying the sample at 105°C±2ºC for 3 h (FN500, Nüve, Turkey) to constant weight. The weight difference before and after drying was multiplied by 100 and divided by the initial weight of the sample (Mattissek *et al.*, 1992).

Ash determination

Homogenized sample (5 g) was weighed in a well-dried porcelain basin and subjected to a low Bunsen flame. Samples were subjected to 550°C (3 hours) (MF100, Nüve, Turkey) and cooled in desiccators. The amount of ash was calculated considering the difference of weight after and before this procedure (AOAC, 1998a).

Protein analysis

Crude protein was determined by the Kjeldahl method. The sample was heated to 420°C for 20 min with 98% H_2SO_4 and catalyst using Buchi Scrubber B-414 Heating digester (Buchi Labortechnik, Switzerland), and then treated with 33% NaOH and 4% boric acid by Kjel Flex K-360 distillation unit (Buchi Labortechnik, Switzerland). The amount of nitrogen was estimated after titration with 0.2 N HCl. It was multiplied by the coefficient 6.25 (AOAC, 1998b).

Fat analysis

Fat was extracted with the Weilmeier and Regenstein (2004) given method after modification. Clean aluminium pans were weighed after drying at 105°C for about 2 h. Approximately 2–2.5g of ground or finely chopped fresh fish muscle were weighed into a 100-mL beaker and mashed with 2mL H_2O and 2mL concentrated HCl. Six millilitres of HCl were added, and the mixture was digested on a hot plate (about 80°C) for about 90min. The mixture was then transferred into a flask, followed by rinsing the beaker with 10mL water and 15 mL acetone. The tightly capped flask was shaken vigorously. An additional 25mL of petroleum ether was added to the flask and the flask was shaken again. The mixture was allowed to separate until the layers were visibly separated (about 20min), and the ether layer was poured into the aluminium pan. The pan was placed on a hot plate until the ether evaporated (Buchi Rotavapor R3000, Buchi Labortechnic, Switzerland). Two more extractions with 25 mL petroleum ether was performed, always adding the upper ether layer into the same pan. Once all the ether had been evaporated from the pans, the pans were dried for at least 20 min in an oven (Wiseven, Won 105, South Korean), allowed to cool, and re-weighed. Fat content was calculated from the following equation:

Fat (%) = (weight of A1 pan after oven – weight of A1 pan before oven)/weight of sample

Amino acid analysis

For amino acid analyses, The high performance liquid chromatograph (HPLC) system consisted of a system controller, auto injector, liquid chromatography pump A, liquid chromatography pump B, fluorescence detector and degasser, all from Shimadzu LC- 10 VP (Kyoto, Japan). The computer program used was Class-VP 6.14 (Shimadzu, Kyoto, Japan). All chemicals used were of analytical grade.

Muscle tissue of fishes was homogenised with 6% (v/v) perchloric acid in a 1:25 ratio (v/w) in an Ultraturrax homogenizer and prepared in accordance with the hydrolysis and derivatization technique described by Waters AccQ•Tag Chemistry Package Instruction Manual.[15] Amino acids were identified by

comparison of their retention time with those of authentic standards (Pierce, Amino Acid Standard Hydrolyzate, Product No: 20078 20088 20089 1800180 NCI0180, Rockford, IL 61105 USA) and their contents were calculated on a weight basis (Erkan *et al.,* 2010).

Fatty acids analysis

Fatty acid composition was determined after methylation (Ichihara *et al.*, 1996), by gas chromatography (Perkin Elmer Clarius 500 GC, Singapore) using a Perkin Elmer Elite WAX Capillary Column (30m-0.25mm ID-0.25µm film) (Cat. N9316403). The chromatographic conditions were as follows: injection volume: 0.5 µL; injection temperature: 240^{o}C; Air: 450mL/min; H_2:45ml/min; split flow: 50mL/min; split ratio: 49,0; detector temperature: FID-240^{o}C; column temperature program; 140^{o}C for 5 min, programmed at 4^{o}C/min up to 240^{o}C 15 min. Fatty acids were identified by comparison of their retention time with those of authentic standard (Supelco® 37 Component FAME Mix, Cat. 47885-U) and their contents were calculated on a weight percentage basis. All chemicals used were of analytical grade.

Statistical analysis

Data analyses were carried out using the Microsoft Excel 2010 (Seattle, USA) software; one-way analysis of variance (ANOVA) and Tukey's Multiple Comparison Test were used to compare the data according to groups at the significant level of 0.05 (Sümbüloğlu and Sümbüloğlu, 2002).

Results

Size, weight and condition factors (Table 1), colour and texture measurements (Table 2) proximate composition (Table 3), essential amino acid composition (Table 4), essential amino acid intake (Table 5), non- essential amino acid composition (Table 6), and fatty acid composition (Table 7) of rainbow trout from reared different stocks are shown in Tables.

The highest condition factor was found in the B group samples as 1.29±0.12, while the lowest value was detected in the C group samples as 1.22±0.14 (Table 1). Tukey's multiple comparison test showed insignificant differences between measured condition factors. Similarly, insignificant differences were found between the measured sizes and weights.

Table 1: Size, weight and condition factor measurements of rainbow trout from three different reared stocks.

Parameter	Group A	Group B	Group C
Size	24.410 ±2.18[a]	23.924 ±2.16[a]	24.524 ±2.32[a]
Weight	188.714 ±40.41[a]	180.238 ±43.49[a]	183.714 ±47.94[a]
Condition factor	1.24 ±0.10[a]	1.29 ±0.12[a]	1.22 ±0.14[a]

Different letters in the same line for each groups indicate significant differences (p<0.05)

Table 2: Colour and texture measurements of rainbow trout from three different reared stocks.

Colour measurement	Group A	Group B	Group C
L*	53.52 ±2.83[a]	56.73 ±4.0[b]	54.8 ±4.2[ac]
a*	8.39 ±2.88[a]	4.07 ±1.0[b]	5.8 ±2.38[ac]
b*	10.24 ±2.11[a]	9.01 ±2.37[b]	8.9 ±2.0[c]
Texture measurement			
Hardness (N)	1.28 ±0.85[a]	0.93 ±0.65[a]	0.97 ±0.4[a]
Adhesiveness (N.mm)	0.20 ±0.00[a]	0.20 ±0.00[a]	0.10 ±0.0[b]
Resilience	0.06 ±0.03[a]	0.06 ±0.04[ab]	0.8 ±0.4[b]
Cohesiveness	0.32 ±0.10[a]	0.34 ±0.14[a]	0.5 ±0.2[a]
Springiness (mm)	16.99 ±9.86[a]	9.96 ±9.32[b]	10.5 ±9.8[b]
Gumminess (N)	0.41 ±0.27[a]	0.34 ±0.19[a]	0.5 ±0.7[a]
Chewiness (N.mm)	7.70 ±0.01[a]	3.30 ±0.00[b]	3.0 ±0.0[b]

Different letters in the same line for each groups indicate significant differences (p<0.05)

Table 3: Proximate composition of rainbow trout from three different reared stocks.

Proximate composition (%)	Group A	Group B	Group C
Moisture	74.11 ±0.08[a]	70.22 ±0.84[b]	73.80 ±0.58[a]
Ash	1.49 ±0.06[a]	1.67 ±0.16[b]	1.81 ±0.08[c]
Protein	19.19 ±0.54[a]	20.65 ±0.63[b]	20.22 ±0.33[b]
Fat	5.06 ±0.32a	7.15 ±0.78[b]	4.00 ±0.28[c]

*Different letters in the same line for each groups indicate significant differences (p<0.05)

Table 4: Essential amino acid composition (g/100g) of rainbow trout from three different reared stocks.

Amino acids	Group A	Group B	Group C	Amino acid requirements of adults (g/100g/day)*
Phenylalanine + Tyrosine	1.64 ±0.02[a]	1.64 ±0.02[a]	1.74 ±0.01[b]	0.0025
Isoleucine	1.09 ±0.00[a]	1.09 ±0.02[a]	1.16 ±0.00[b]	0.002
Leucine	1.61 ±0.03[a]	1.62 ±0.03[a]	1.70 ±0.00[c]	0.0039
Lysine	1.99 ±0.01[a]	1.94 ±0.01[b]	2.07 ±0.00[c]	0.003
Methionine + Cysteine	0.90 ±0.00[a]	0.88 ±0.00[b]	0.94 ±0.00[c]	0.0015
Threonine	0.91 ±0.00[a]	0.87 ±0.01[b]	0.85 ±0.00[c]	0.0015
Valine	1.15 ±0.01[a]	1.17 ±0.03[a]	1.24 ±0.00[b]	0.0026
Histidine	0.70 ±0.00[a]	0.65 ±0.00[b]	0.68 ±0.00[c]	0.001
Total essential amino acid	**9.29**	**9.21**	**9.70**	**0.018**

Different letters in the same line for each groups indicate significant differences (p<0.05)
*WHO, 2007

Table 5: Non- essential amino acid composition of rainbow trout from three different reared stocks.

Amino acids (g/100g	Group A	Group B	Group C
Alanine	1.21 ±0.01[a]	1.20 ±0.00[a]	1.21 ±0.00[a]
Arginine	1.43 ±0.01[a]	1.42 ±0.00[a]	1.43 ±0.00[a]
Glycine	1.11 ±0.01[a]	1.09 ±0.01[a]	1.11 ±0.00[a]
Aspartic acid	2.09 ±0.01[a]	2.11 ±0.02[a]	2.17 ±0.01[b]
Glutamic acid	3.00 ±0.01[a]	2.98 ±0.02[a]	3.11 ±0.01[b]
Proline	0.65 ±0.01[a]	0.72 ±0.01[b]	0.67 ±0.01[a]
Serine	0.85 ±0.00[a]	0.75 ±0.00[b]	0.68 ±0.00[c]
Total N-EAA	10.34	10.27	10.38

Different letters in the same line for each groups indicate significant differences (p<0.05)

Table 6: Fatty acid composition (%) of rainbow trout from three different reared stocks.

	Group A	Group B	Group C
$C_{14:0}$	2.87 ±0.19	2.94 ±0.00	2.72 ±0.29
$C_{15:0}$	0.22 ±0.03	0.22 ±0.02	-
$C_{16:0}$	14.13 ±0.10	14.15 ±0.22	12.40 ±0.09
$C_{18:0}$	3.37 ±0.06	3.27 ±0.03	3.03 ±0.02
$C_{22:0}$	2.55 ±0.01	2.70 ±0.07	2.88 ±0.04
$C_{24:0}$	1.41 ±0.01	1.32 ±0.01	1.34 ±0.04
Total SFAs	**24.55 ±0.07[a]**	**24.60 ±0.06[a]**	**22.37 ±0.10[b]**
$C_{16:1}$	4.23 ±0.04	3.94 ±0.20	4.01 ±0.16
$C_{18:1}$ n-9	34.69 ±0.31	34.70 ±0.66	35.68 ±0.27
$C_{20:1}$ n-9	4.00 ±0.18	3.89 ±0.53	3.99 ±0.50
Total MUFAs	**42.92 ±0.18[a]**	**42.55 ±0.46[a]**	**43.68 ±0.31[b]**
$C_{18:2}$ n-6	14.77 ±0.08	15.96 ±0.86	15.43 ±0.17
$C_{20:2}$	0.89 ±0.06	0.77 ±0.07	0.98 ±0.01
$C_{22:2}$	-	0.50 ±0.30	0.53 ±0.03
$C_{18:3}$ n-3	2.71 ±0.04	2.68 ±0.09	3.02 ±0.21
$C_{18:3}$ n-6	-	-	-
$C_{20:3}$ n-3	0.39 ±0.03	0.40 ±0.00	0.44 ±0.02
$C_{20:3}$ n-6	0.44 ±0.02	0.41 ±0.02	0.44 ±0.02
$C_{20:4}$ n-6	0.27 ±0.02	0.27 ±0.01	0.31 ±0.01
$C_{20:5}$ n-3	2.72 ±0.02	2.55 ±0.14	2.72 ±0.04
$C_{22:6}$ n-3	8.67 ±0.10	7.71 ±0.09	8.30 ±0.09
Total PUFAs	**30.86 ±0.05[a]**	**31.25 ±0.18[b]**	**32.17 ±0.07[c]**
ND	**1.67**	**1.60**	**1.78**
Total n-3	14.49 ±0.05	13.34 ±0.08	14.48 ±0.09
Total n-6	15.48 ±0.04	16.64 ±0.30	16.18 ±0.07

Different letters in the same line for each groups indicate significant differences (p<0.05)

Discussion

Stocking density (SD) is a significant factor that determines production in ponds. Understocking results in failure to make the maximum possible utilization of the space, and overstocking may result in stress that may lead to enhanced energy requirements causing reduced growth and feed utilization (North *et al*., 2006b). Such guidance available to rainbow trout farmers varies from 2 to 80 kg fish m^{-3} depending on type of holding systems and size of fish, with the normal range thought to be 15–40 kg fish m^{-3}, with 60 kg fish m^{-3} being seen as a maximum (Ellis *et al*., 2002). A rare reference to SD practices of commercial trout farms in the UK suggested the range to be 3–30 kg fish m^{-3} (Shepherd, 1975), but there is a paucity of up-to-date information.

L* value in rainbow trout were 53.52±2.83 for A group, 56.73±4.90 for B group and 54.08±4.82 for C group. Tukey's multiple comparison test showed insignificant differences between measured L values of A and C groups. The highest a* value was found in the A group samples as 8.39±2.88, while the lowest value was detected in the B group samples as 4.07±1.60. There was no difference between the A and C groups. Tukey's multiple comparison test showed significant differences between measured b values. Tukey's multiple comparison test showed insignificant differences between measured hardness, adhesiveness, cohesiveness and gumminess values of A and C groups. There was differences between the springiness and chewiness value of A-C and A-B groups. Colour is an important sensory attribute of foods and may have a direct effect on the price of sea foods (Metusalach *et al*., 1997, 1999). Texture is a complex trait involving parameters of hardness, springiness, cohesiveness, gumminess, and mouth feel (Haard, 1992). The texture of fish is another important quality characteristic, and soft fillets are a problem for the fish industry (Hultmann and Rustad, 2004). Depending upon storage time and nutritional state, among other factors, fish flesh can vary in firmness. Generally, consumers prefer firm and elastic fish flesh (Rasmussen, 2001).

The lowest moisture values of rainbow trout were determined in B group samples (70.22±0.84%). The highest fat values of rainbow trout's were measured in B group samples (7.15±0.78%). Protein values in fishes were found between 19.19–20.65%. Tukey's multiple comparison test showed insignificant differences between measured protein values of B and C groups. Ash values in rainbow trout are varied from 1.49 to 1.81 %. The maximum level was observed in C group and minimum level in A group samples.

Amino acids are important in healing processes and its composition in fish is similar to that in man and people can therefore acquire the essential and non-essential amino acids in abundance and proper balance by eating fish. The essential amino acids cannot be manufactured in human bodies, but can be obtained from food. Eight amino acids are generally regarded as essential for humans: phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine and lysine. Additionally, cysteine (or sulphur-containing amino acids), tyrosine (or aromatic amino acids), histidine and arginine are required by infants and growing children. Deficiency in these amino acids may hinder healing recovery process (Smith *et al*., 2005; Li *et al*., 2009; Mach *et al*., 2010). Estimates of amino acid requirements for adults have been reported as follows. (Phenylalanine+ tyrosine: 25 mg/kg per day, Isoleucine: 20 mg/kg per day, Leucine: 39 mg/kg per day, Lysine: 30 mg/kg per day, Methionine+ Cysteine: 15 mg/kg per day, Threonine: 15 mg/kg per day, Valine: 26 mg/kg per day, Histidine: 10 mg/kg per day (WHO, 2007). According to the results of this study, the amino acid /total essential amino acid content in all groups are more than 500 times of amino acid requirement of adults. Group C samples have the highest total amino acid content.

Table 5 shows the non-essential amino acid (N-EAA) compositions for each stock density. Glutamic acid and aspartic acid constituted the highest N-EAA concentration in C group samples, while serine, proline had the highest concentration of N-EAA in B group samples. Essential amino acids are responsible for the taste and flavour in fish flesh. Group C samples were found to have the highest content of non-essential amino acid, followed by Group A and B samples.

Lipid content is a key factor in the diet for human consumption and aquatic species. High lipid levels serve to save most of the protein and achieve excellent growth. Lipid deposition and fatty acid profile can be influenced by the rearing system, season, and the geographic source (Gonzales *et al*., 2006; Jankowska *et al*., 2007; Mairesse *et al*., 2006, 2007). Fatty acids, especially ones in fish lipids, are very important nutritional elements for human health. The beneficial effect of fish consumption on human health has been related, among other factors, to the high content of n-3 fatty acids (especially eicosapentaenoic acid (EPA: $C_{20:5}$ n-3) and docosahexaenoic acid (DHA: $C_{22:6}$ n-3) (Zlatanos and Laskaridis, 2007). It is known that n-3 fatty acids are essential for neural development in the infant in uterus and during the first few years after birth. The n-3 polyunsaturated fatty acids have been reported to have various beneficial health effects that include reducing the risk of cardiovascular diseases (Candela *et al*., 1997; Ferraro *et al*., 2010). The fatty acid profile of C group samples was dominated by polyunsaturated fatty acids, especially n-3, DHA and EPA (Table 6).

According to the results of this study, texture, color quality and amino acid/fatty acid composition in relation to the stocking density of cultured rainbow trout the stocking density of 25 kg fish m^{-3} can be recommended.

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