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Investigation of ultrastructure and texture changes of fillets of rainbow trout grown under different aquaculture systems

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ABSTRACT

In this study, the physical structure of fillets of rainbow trout (*Oncorhynchus mykiss*) grown under different aquaculture conditions was compared. For this purpose, scanning electron microscopy (SEM) analysis, texture profile (hardness, springiness, cohesiveness, gumminess, chewiness), and color measurements (*L, a, b,* chroma, hue and whiteness) of the fillets of trout taken from two different aquaculture environments were examined. When the texture profile of fillets taken from both environments was compared, it was determined that the hardness (40.30–69.80 N), gumminess (26.85–41.89 N) and chewiness (25.37–36.82 N) values of the fish samples taken from the extensive culture were higher than the samples taken from the recirculated system. The difference between other values was not found to be significant. In parallel with the hardness results, when the SEM images were examined, it was determined that the fillets of the fish taken from the extensive system had a thicker fibril ultrastructure than those from the RAS. The variable environmental parameters and aquaculture duration were observed to have an effect on muscle development, and that especially the long breeding period in the extensive system has a positive effect on the meat structure of the fish. Cultivation in different environments was not determined to have a significant effect on the color values of either the skin or fillet samples. As trout is the leading fish in freshwater production, it is very important for aquaculture to determine the physical changes in the flesh structure of trout according to the growing conditions.

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Introduction

Rainbow trout (*Oncorhynchus mykiss*) are members of the Salmonidae family, which live in cold, clear, well-oxygenated rivers and lakes, generally with a water temperature of 20–21ºC. *Oncorhynchus mykiss* is one of the most well-known fish species in North America (1). It is widely farmed in many countries because of its rapid growth and high value as food. The rainbow trout is one of the most economically important freshwater cultured fish species. The statistics for 2021 show that 134,147 tons of rainbow trout were cultured in Turkey, and the annual trout aquaculture in marine water is 31,509 tons/year (2).

Two different systems are used in trout farming, namely extensive and intensive conditions. The main goal of these two systems is to obtain products with maximum efficiency from the minimum area, both qualitatively and quantitatively (3). Closed Circuit Systems (RAS) have been developed for this purpose, based on the principle of continuously providing the optimum conditions (O2, temperature, photoperiod, etc.) needed by living organisms, in line with technological developments. These systems aim to meet all the needs of the creature, from the oxygen dissolved in the water, which is the first requirement, to the purification of disease-causing pathogens and the provision of highly nutritious food. These systems can be stocked at a higher rate than extensive cultivation methods (4-5). Instead of traditional farming methods in ponds and canals under environmental influences, these systems raise fish in a "controlled" environment in closed tanks with a high density of stock. The need for a daily 5% water change is an indication that there is little interaction with the environment and that the wastewater released into the environment is minimal (6). With RAS, which is the most advanced system today, environmental parameters can be adjusted and production time is shortened as the feed is given at a rate suitable for the living creature (7). Therefore, fish can reach market size in a shorter time. However, it is thought that reaching the market size in a shorter time compared to extensive farming causes changes in the qualitative characteristics of fish meat. This study aimed to compare the qualitative characteristics of trout produced in the extensive environment with those produced with a super-intensive method in the RAS system.

Although there are studies in the literature on the nutritional quality of rainbow trout (8-19), there is no research providing information on the meat structure and texture profile. In view of these facts, the objective of the present study was to determine the scanning electron microscopy (SEM) profile, textural structure and color changes of rainbow trout grown in different aquaculture conditions. It is very important to investigate the breeding conditions of the mode of production of Rainbow Trout, which is very important for the country's economy, and the changes in the meat structure depending on these conditions.

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Materials and Methods

Fish and aquaculture systems

The study sample comprised rainbow trout (*Oncorhynchus mykiss*) fed in two different aquaculture conditions (RAS & Extensive Pool) on the same farm. Fish weighing 30±2.3 g were randomly placed in 3 kg/m3 concrete trout ponds and a 20 kg/m3 RAS system. Commercial Skretting trout chow was used, with free feeding twice a day. The water change of the concrete pools in the atmospheric environment was adjusted to change completely 5 times a day. Using continuous ventilation, the RAS system, which has a sand filter, biological filter, chiller(Clivet), and UV equipment, was prepared at a constant temperature of 18ºC, with a 12-hour photoperiod. Temperature and dissolved O_2 values were measured with a YSI 55-brand oxygen meter. The oxygen level at the water inlet was not reduced below 9 ppm and daily backwashing was performed. Daily water change was set at 10%. The bacterial density in the biological filter was checked every 15 days. The daily temperature and dissolved O_2 values of the pool and RAS system were recorded. The measurements made from the breeding requests throughout the experiment are shown in Table 1. During the study, the fish were fed with Skretting brand 2 ps trout chow until the fish in the two different aquaculture systems reached approximately the same size. For all analyses, random sampling was made from each experimental group.

After they were caught, the samples were stored on ice for transportation to the laboratory. The averages for the total length of rainbow trout caught from RAS and extensive culture were 30.81 ± 1.75 and 31.14 ± 1.91 cm, respectively, and the total weight was 350.20 ± 22.92 and 362.41 ± 18.85 g, respectively. The fish were then washed and filleted by removing the head, viscera and bones.

Scanning Electron Microscopy images

For SEM analysis, 1-cm^3 samples were taken from the anterior dorsal part of the *linea lateralis* of the rainbow trout fillets (Fig. 1). The samples were hydrated in distilled water and cut from gels into cubes with a side length of 2 to 3 mm for the microscopic examination. They were then fixed in 2% osmium tetroxide (OsO_4) for 2 hours, rinsed with distilled water and dehydrated with a gradual acetone series. The samples were dried at the critical point using liquid carbon dioxide as the exchange medium, mounted on aluminium rods and coated with platinum. The coated samples were examined under a Zeiss Supra 55 SEM (FE-SEM, Germany).

Texture profile analysis

The texture of the rainbow trout fillets was analyzed using the instrumental texture profile analysis (TPA) method for hardness, springiness, cohesiveness, gumminess and chewiness. Texture measurements were taken using a Texture Analyzer TA.XT Plus (Stable Micro Systems, Godalming, England) with a load cell of 50 kg at room temperature. The TPA was measured utilizing a 36-mm diameter cylinder probe. The measurements were made in the anterior region of the dorsal part of the *linea lateralis* of each rainbow trout fillet (Fig. 1). The results were recorded in Newtons.

Color measurements

Colorimetric measurements were taken according to the Calder method(20). The sample color was measured using a portable Hunter Lab color analyser (Hunter Associates Laboratory, Inc., Reston, VA, USA). The sensor was standardised with white and black tiles for analysis. *L*, a** and *b** values were recorded. The *L** variable represents lightness (*L**=0 for black, *L**=100 for white), *a** scale represents red/green, +*a** intensity in red and -a* intensity in green. The *b** scale represents yellow/blue, +*b** intensity in yellow and -b* intensity in blue. The color was measured in three different parts of the fillet pieces, and then chroma, hue and whiteness values were calculated using the following equations:

$$Chroma = (a^{*2} + b^{*2})^{1/2} \quad [1]$$

$$Hue = Arctan(b^* / a^*) \quad [2]$$

$$Whiteness = 100 - ((100 - L^*)2 + a^*2 + b^*2)\ ½ \quad [3]$$

The chroma value shows the distance from the neutral axis and is a measure of saturation. Angles ranging from 0 to 360 degrees represent the hue value.

Statistical analysis

The data of the texture and color parameters were subjected to analyses of variance at the 0,05 level using SPSS (SPSS Inc., Chicago, IL, USA) software, and the t-test was performed to determine differences between mean values.

Results

Scanning Electron Microscopy

As a result of the histological examination, it was de-

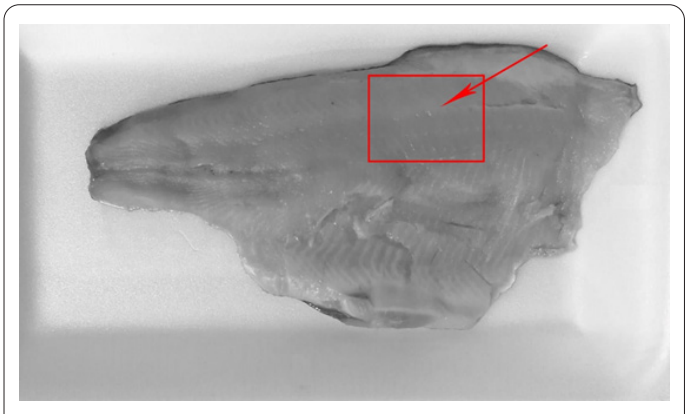

Figure 1. Sampling region for SEM analysis and texture profile.

Table 1. Aquaculture parameters of the extensive system and RAS.

		Extensive system	RAS
O_2 changes		Between 7.2-9.6 ppm	9 -10.2 ppm
Temperature		13.6-19.2	18ºC
Photoperiod (Light/Dark)	Beginning	11/13	12/12
	End	9.5/14.5	12/12
Daily water change amount		5	10%

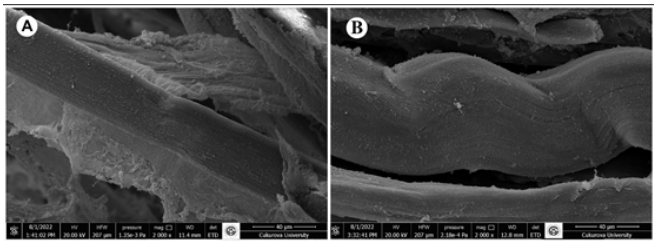

Figure 2. Ultrastructure of rainbow trout muscle fibril, A-Extensive system, B- RAS.

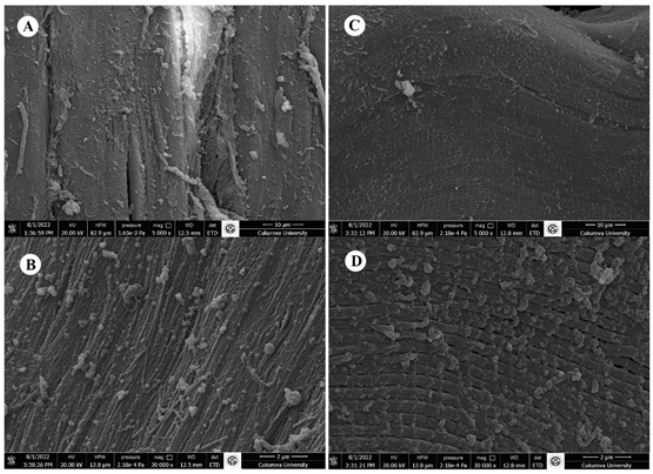

Figure 3. Ultrastructure of rainbow trout muscle myofibril, A-B, Extensive system, C-D, RAS.

Table 2. Measurements of the fibre and myofibril thickness in the fillets of rainbow trout grown under different aquaculture conditions.

	Extensive system	RAS
Fibril(µ)	65.44±2.71µ*	37.30±3.02 µ
Myofibril	0.46±0.06 µ*	0.22±0.02 µ

± Standard deviation, * The values are significantly different at p <0.05.

termined that the muscle fibres and myofibrils of the fish fed in the extensive system were of considerably greater thickness than those fed in the RAS (Figs. 2-3). The fibril thickness was determined as a mean of 65.44 µ in the samples taken from the extensive system, and as 37.30 µ in the samples from the RAS system. Myofibril measurements were made from the same regions as the fibril measurements and were determined to be 0.46 µ and 0.22 ± 0.02 µ for natural and aquaculture microfibril thickness, respectively (Table 2). A statistically significant difference was determined between the fibril and myofibril metric measurements ($p < 0.05$).

Texture profile

The texture profile results of the fillets of trout raised in RAS and extensive systems are presented in Table 3. As a result of the tests performed on the texture analyser, the values of hardness, springiness, cohesiveness, gumminess and chewiness were recorded. The results of the texture analyses in the fillet of rainbow trout from the extensive system were 69.80, 0.87, 0.59, 41.89 and 36.82 for hardness, springiness, cohesiveness, gumminess and chewiness, respectively. The results of texture analysis in the fillet of rainbow trout from the RAS were 40.30, 0.94, 0.68, 26.85, 25.37 for hardness, springiness, cohesiveness, gumminess and chewiness, respectively.

Table 3. Texture profile of fillets of rainbow trout grown under different aquaculture conditions.

	Extensive system	RAS
Hardness	69.80±12.46*	40.30±8.78
Springiness	0.87±0.01	0.94±0.04
Cohesiveness	0.59±0.01	0.68±0.14
Gumminess	41.89±7.82*	26.85±2.57
Chewiness	36.82±2.03*	25.37±2.63

± Standard deviation, *The values are significantly different at $p < 0.05$.

Color

*L**(lightness or darkness), *a**(redness or greenness) and *b**(yellowness or blueness) values were measured from color measurements made to monitor the changes in the physical parameters of trout samples grown in RAS and extensive media, and the whiteness, hue and chroma values were calculated from these values. The results of the color analysis on the meat of the fish fillets are given in Table 4 and the analyses of the skin surface are given in Table 5.

There was no significant difference between all the fillet color values of trout grown in the extensive system and RAS ($p > 0.05$). The *L*-value was determined as 51.05–51.29, the *a* value as 3.00–4.31, and the *b* value as 15.81-17.11. No significant difference was determined between the two environments in respect of the color values of the trout skins ($p > 0.05$). The *L*-value was determined as 47.60–48.91, the *a* value as 0.48–1.61, and the b value as 10.52-12.84.

Discussion

Physical growth is associated with skeletal muscle growth through cell proliferation and differentiation in

Table 4. Color parameters of fillets of rainbow trout grown under different aquaculture conditions.

	Extensive system	RAS
L	51.29±0.55	51.05±0.39
a	4.31±1.77	3.00±1.52
b	17.11±0.95	15.81±0.65
Chroma	17.69±1.32	16.15±0.65
Hue	1.33±0.08	1.38±0.09
Whiteness	48.07±0.90	48.40±0.25

± Standard deviation.

Table 5. Color parameters of the skin of rainbow trout grown under different aquaculture conditions.

	Extensive system	RAS
L	48.91±6.81	47.60±1.97
a	0.48±0.12	1.61±0.99
b	12.84±1.93	10.52±3.30
Chroma	12.85±1.93	10.69±3.19
Hue	1.53±0.00	1.40±0.12
Whiteness	47.31±7.00	46.45±2.52

± Standard deviation.

many animal tissues (21). There is no clarity as to whether the size and number of muscle bundles that make up the muscles of fish, which are vertebrates, are constant or variable (22-24). It has been reported that muscle development develops in two ways: hyperplasia and hypertrophy (25). Other authors have reported that the development of muscles depends on factors such as genetics, nutrition, temperature, photoperiod, dissolved oxygen and ammoniac (26-27). Rowlerson and Vegetti (28) reported that fibril sizes in fish vary between 100 and 200 µ, whereas the current study results conclude that it may be much smaller. It is thought that these differences are due to the species differences of the fish, nutrition and environmental parameters. It was concluded that the natural environment is more suitable for the muscle development of the fish, as the fish grown in the natural environment where the nutritional and environmental parameters are variable have a thicker fibril structure than the fish grown in the RAS system, in conditions that the fish can tolerate.

The hardness, gumminess and chewiness values were found to be significantly higher in fish grown in the extensive medium than in fish grown in the RAS medium ($p < 0.05$). Similar to the present study, Vacha et al.(29) reported that tench *(Tinca tinca)* meat grown in extensive media is harder, springier, more cohesive, gummier and chewier than meat grown in intensive media. When previous studies on the tissue structure of many different fish species are examined, it can be seen that all were studies on the effect of storage conditions on the tissue structure (30). There have been very few studies on the tissue structure of fish in different aquaculture systems.

Texture measurements and evaluations for fish and fish fillets are important in the seafood industry, in product development with quality control and assurance of freshness. These factors contribute to consumer acceptance and thus to the marketability of the final product (31).

The color of seafood is one of the most important sensory/visual acceptability criteria of the consumer. Color is one of the primary parameters in the marketing of fish meat, both hunted from natural environments and grown because its appearance comes first. The results of the present study showed that the fish grown in both systems were in good condition in terms of color values, especially in terms of the *L* value, which expresses brightness.

In the current study, comparisons were made of the physical quality of the fillets of trout grown and brought to market size in an extensive system and RAS media. The SEM and texture results showed that the meat of the trout reared in the extensive system was harder and tighter than the meat of the trout farmed in RAS. The results of both analyses supported each other. There was no difference between the groups in the color results.

Trout has a very important place in freshwater aquaculture, both in Turkey and throughout the world. Therefore, breeding conditions are also very important. The results of this study can be considered very important in terms of comparing the meat quality of fish grown in different environments with different parameters. Moreover, these results will be of guidance for further studies of other fish species

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Interest Conflict
The author declares no conflict of interest/competing interests

Authors' contributions
Submission is a single author

Ethical Approval
This study was carried out with ethical approval given by Cukurova University Health Sciences Experimental Practice and Research Center (SABIDAM). SABIDAM registration number is CUSABIDAM/2022/12/08.

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