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Research Article
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THE EFFECT OF BIODEGRADATION ON SWEET ORANGE PEEL AND ITS FEED VALUE IN STARTER BROILER CHICK'S DIET

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Abstract: A twenty-eight-day feeding trial was conducted to evaluate maize replacement value of rumen filtrate biodegraded sweet orange peel (SOP) in the starter broiler chick's diet. Sweet orange fruit peels were collected from retailers of peeled sweet orange fruits. Fresh rumen content was collected from a government abattoir, mixed with water at the ratio of 1 kg: 1 liter, and the mixture sieved to obtain rumen filtrate (RF). Rumen filtrate was mixed with sweet orange peels at the ratio of 1 liter: 2.5 kg, poured into polythene bags, tied at the open end, and allowed a 48-hour for biodegradation. The fermented sweet orange peels were sun-dried to about 10% moisture, milled and incorporated into each of five broiler starter diets as a replacement for maize at levels of 0%, 5%, 10%, 15%, and 20% to give diets T1, T2, T3, T4, and T5, respectively. Biodegraded SOP contained 8.80% crude protein, 13.25% crude fibre, 8.65% ether extract, 9.90% ash, and 59.40% NFE, and metabolizable energy of 3720.67 Kcal/kg. The experimental diets had significant effect ($P<0.05$) on daily feed intake, final body weight and body weight gain. There was no significant difference ($P>0.05$) among other performance indices measured across the dietary treatments. Dietary incorporation of SOP meal as a replacement for maize did not support the growth of starter broiler chicks, and further studies are necessary to investigate other processing methods that can further reduce its fibre content, to enhance its feed value as a replacement for maize in the diets of broiler chicks.

Keywords: Sweet orange peel, Biodegradation, Feed value, Growth response, Chicks

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1. Introduction

The poultry industry offers a quick solution for providing the thronging population with the necessary animal protein. Broiler birds are probably the most universal and important of all poultry as producers of meat for human consumption. Animal protein shortage in the diet of the average Nigerian is shown in the consumption of 3.24g per caput which is far below the 35g daily requirement recommended by FAO (Hon et al., 2009). Energy is key to metabolism and if it is limited, dietary protein will be used inefficiently as another source of energy instead of being converted into body protein, hence, adequate energy must be supplied by the diet to make efficient use of dietary protein. Some agro-industrial by-products like composite mango fruits reject (Orayaga, 2016), palm oil sludge (Famurewa and Olarewaju, 2013), citrus by-products like sweet orange peel meal (Oluremi et al., 2018) have been used in non-ruminant animal's diets to partly replace cereals. Sweet orange (*Citrus sinensis*) fruit peel is an agricultural produce waste in Nigeria and with no cost attached to it, and it is high in energy (Oluremi et al., 2010). Rumen content is another important agricultural by-product, in the abattoir industry in Nigeria (Ahemen and Zahradden, 2010) and can be converted into beneficial use by taking advantage of its microbial population rather than its present status as agricultural waste (Oluremi et al., 2010). Its utilisation by taking advantage of its microbial content for the processing of sweet orange fruit (*Citrus sinensis*) peel can result in value addition to the peel to increase its suitability as a dietary energy source for livestock production. This study aimed to determine the effect of partial replacement of dietary maize with graded levels of bovine rumen filtrate-treated sweet orange (*Citrus sinensis*) fruit peel meal on the performance response of starter broiler chicks.

2. Materials and Methods

2.1. Study Area

The study was carried out at the Poultry Unit of the Livestock Teaching and Research Farm of the College of Animal Science, Federal University of Agriculture Makurdi, Benue State, Nigeria. Makurdi is situated in the north-central zone of Nigeria with a latitude of 7°.43´N and a longitude of 8°.53´N (Microsoft Encarta 2008).

2.2. Test Ingredients Collection and Preparation

Sweet orange fruit peels were collected from some sweet orange retail sellers around the Makurdi metropolis. Fresh rumen content was collected from cattle immediately after slaughter at the government-owned Wurukum Abattoir. Rumen content was mixed with water at a ratio of 1 kg: 1 liter, and thereafter sieved to obtain rumen filtrate (RF). The rumen filtrate was mixed with sweet orange peels at the ratio of 1 liter: 2.5 kg, and the mixture put in polythene bags, tied at the open end, allowed a 48-hour biodegradation, and sun-dried to below 10% moisture for safe storage before final use in diet preparation. The sun-dried sweet orange peel material was milled, analyzed for proximate constituent using the standard methods (AOAC, 2015), and used in formulating starter diets replacing maize at levels of 0%, 5%, 10%, 15%, and 20% to give diets T_1, T_2, T_3, T_4, and T_5, respectively as shown in Table 1.

2.3. Experimental Animal, Design and Procedure

A hundred and fifty (150) day-old, unsexed broiler chicks were used for this experiment. The birds were weighed and grouped into five (5) equal numbers and similar live weights. Each group was randomly assigned to one of the five (5) dietary treatments T_1, T_2, T_3, T_4, and T_5. There were 3 replicates per treatment with 10 birds per replicate. Each treatment replicate was randomly allotted to the experimental pens. The experiment was a completely randomized design. The birds were raised in a deep litter of wood shavings. Feed and drinking water were provided ad libitum, and standard routine management practices (feeding, watering, and washing of drinkers, cleaning of feeders, and pen passages) were followed. The birds were vaccinated against Newcastle disease (i/o) at day old, infectious bursal disease at day 14, Newcastle disease (Lasota) at day 21, and infectious bursal disease at day 28 as recommended by the manufacturer, National Veterinary Research Institute, Vom - Jos, Nigeria. An anti-stress supplement which contains vitamins A, B_1, B_{12}, C, D_3, E, Biotin and Niacin, was administered prior to and after each vaccination, and pre-and post-weekly weighing of the birds, to maintain the optimum level of vitamins, reduce mortality due to stress, improve vaccination titre, enhance immune response and improve growth. Coccidiostat was administered at alternate weeks to stem the occurrence of coccidiosis which is endemic in the study environment, and antibiotics was given if and when necessary as prophylactics. Data collected was used for the evaluation of growth performance.

Table 1. Ingredients composition of experimental diets for starter broiler chicks

	Experimental Diets				
Ingredients (kg/100kg)	T1	T2	T3	T4	T5
Maize	49.22	46.76	44.30	41.84	39.38
SOP	-	2.46	4.92	7.38	9.84
Soybean meal	37.08	37.08	37.08	37.08	37.08
Maize offal	4.30	4.30	4.30	4.30	4.30
Brewers dried grain	4.00	4.00	4.00	4.00	4.00
Bone ash	2.00	2.00	2.00	2.00	2.00
Fish meal	0.70	0.70	0.70	0.70	0.70
Limestone	1.20	1.20	1.20	1.20	1.20
Palm oil	0.50	0.50	0.50	0.50	0.50
Broiler Premix*	0.25	0.25	0.25	0.25	0.25
Common salt	0.25	0.25	0.25	0.25	0.25
Lysine	0.20	0.20	0.20	0.20	0.20
Methionine	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00
Calculated analysis					
ME (Kcal/kg)	2855.66	2841.44	2827.23	2813.01	2800.78
Crude protein (%)	22.73	22.70	22.66	22.63	22.60
Ether extract (%)	4.21	4.18	4.14	4.11	4.08
Crude fibre (%)	4.73	4.94	5.16	5.36	5.56
Calcium (%)	1.60	1.60	1.60	1.60	1.60
Avail. P (%)	0.72	0.72	0.71	0.70	0.69
Lysine (%)	1.10	1.09	1.09	1.08	1.07
Methionine (%)	0.37	0.37	0.36	0.36	0.35
Cost/Kg diet ($)	0.38	0.37	0.36	0.35	0.34

SOP= sweet orange peel meal, BDG=body weight gain, T1= 0% maize replacement with SOP (Control diet), T2= 5% maize replacement with SOP, T3= 10% maize replacement with SOP, T4= 15% maize replacement with SOP, T5= 20% maize replacement with SOP.

2.4. Statistical Analysis

Data collected were subjected to a one-way analysis of variance (ANOVA) using SPSS (2012), and the means of significantly different (P<0.05) parameters were separated using Duncan's Multiple Range Test (DMRT) of the same package (Genç and Soysal, 2018).

3. Results

The chemical composition of the biodegraded sweet orange peel meal is presented in Table 2. The SOP contained dry matter (DM) content of 92.5%, 8.80% crude protein (CP), 13.25% crude fibre (CF), 8.65% ether extract (EE), 9.90% ash and 59.40% nitrogen-free extract (NFE). The effect of the experimental diet on the performance response of broiler chicks is shown in Table 3. The initial live weight of the chicks varied from 46.59g to 47.74g with no significant (P>0.05) difference among the treatments. However, there were significant differences (P<0.05) among treatments in the final body weight, average body weight gain and daily feed intake of the birds fed graded levels of biodegraded sweet orange peel meal. The experimental diets did not have a significant effect (P>0.05) on feed conversion ratio, protein intake and protein efficiency ratio and mortality among the treatment groups. The chicks in T1 (control) had the highest feed intake of 36.19g which was significantly different (P<0.05) from chicks in T5 while the chicks in T5 recorded the lowest feed intake of 28.22g. The broiler chicks in T1 performed significantly (P<0.05) better in final body weight than other treatments while, its daily body weight gain was only significantly (P<0.05) better than chicks on diet T4 and T5 containing 15 % and 20 % of biodegraded sweet orange peel meal in their diets respectively.

Table 2. Proximate composition of biodegraded sweet orange peel meal (% DM)

Nutrients (% DM)	Sweet orange peel meal[1]	Maize[2]
Dry matter	92.50	86.50
Crude protein	8.80	9.00
Crude fibre	13.25	1.30
Ether extract	8.65	4.00
Ash	9.90	2.70
Nitrogen free extract	59.40	83.00
[3]Metabolizable energy (Kcal/kg)	3720.67	3432.00

[1]Laboratory Analysis, [2]Aduku (2005), [3]Metabolizable energy as determined using Carpenter and Clegg (1956).

Table 3. Effect of biodegraded sweet orange peel meal on the growth response of starter broiler chick (day old – 28 day old)

	Experimental Diets					
Parameters	T1	T2	T3	T4	T5	SEM
Initial body weight (g/bird)	47.02	47.74	47.29	46.59	46.88	0.42^{ns}
Final body weight (g/bird)	525.37^{a}	468.48^{b}	466.17^{bc}	427.77^{c}	406.10^{c}	14.19*
BWD (g/day/bird)	17.08^{a}	15.03^{ab}	14.96^{ab}	13.62^{b}	12.83^{b}	0.51*
Feed intake (g/bird/day)	36.19^{a}	29.63^{ab}	31.94^{ab}	29.80^{ab}	28.22^{b}	1.07*
Feed conversion ratio	2.12	1.96	2.15	2.19	2.19	0.04^{ns}
Protein intake (g/bird/day)	8.40	6.87	7.39	6.90	6.51	0.25^{ns}
Protein efficiency ratio	2.03	2.20	2.03	1.97	1.98	0.04^{ns}
Mortality rate (%)	0.67	0.00	0.00	0.33	0.00	0.11^{ns}

^{a,b,c}Means with different superscripts in the same row are significantly different (P<0.05), *(P<0.05), ^{ns}Not significantly different (P>0.05), SEM= standard error of mean, SOP= sweet orange peel meal, BDG= daily body weight gain, T1= 0% maize replacement with SOP (Control diet), T2= 5% maize replacement with SOP, T3= 10% maize replacement with SOP, T4= 15% maize replacement with SOP, T5= 20% maize replacement with SOP.

4. Discussion

The proximate composition of biodegraded sweet orange fruit peel meal showed it contained 92.5% DM, 8.80% CP, 13.25% CF, 8.65% EE, 9.90% ash, and 59.40% NFE. Ojabo et al. (2014) reported a DM of 86.20%, 7.40% CP, 8.19% ash, 7.19% EE, 13.50% CF, 62.65% NFE and 3674.44 Kcal/kg ME for sundried sweet orange peel meal. Agu et al. (2010) reported 89.65% DM, 10.74% CP, 7.86% ash, 12.00% EE, 11.90% CF, 56.91% NFE and 3988.70 kcal/kg ME. Also, 7.0 % CP, 12.50% CF, and ME of 3420 kcal/kg were reported by Ashbell and Weinbegger (1999) in Israel for sweet orange peel. The crude fibre level in SOP the test ingredient is high, like what has been reported by some other workers. The nutrient quality of feed ingredients is one of the major prerequisites for the production of good-quality feeds. The basic nutrients that cannot be compromised in the choice of ingredients for

feed formulation are protein and energy. The dry matter of 92.28% in this study was higher than 87.60% for sweet orange peel (SOP) biodegraded with rumen content for 48 hours reported by Oluremi et al. (2008). The possible anti-nutritional factors of sweet orange peels are phytic acid, saponin, tannin, and oxalate, they can interfere with digestive processes and prevents effective absorption and utilization of micro/macro nutrients, but these can be prevented by some processing methods such fermentation, chemical treatments, sun-drying, soaking and various other processing methods which helps to prevent/lower the effect of these antinutritional factors and improve the nutritive value of sweet orange peel.

The SOP meal with a CP of 8.80% was higher than 7.40% reported by Ojabo et al. (2014), and 7.50% by Akpe et al. (2019). The disparity in crude protein composition could be attributed to the type of pasture consumed by the cattle which will affect the type and the population of the ruminal microorganism, the ratio of rumen content to sweet orange peel used for processing, and the stage of digesta degradation in the rumen when cattle was slaughtered. The CP is however slightly lower than CP in maize, a conventional energy feedstuff with 9.10% CP (Aduku, 2005), while crude fibre (CF) of 13.25% in the peel was lower than 13.50 % and 14.60% reported by Ojabo et al. (2014) and Ani et al. (2015), respectively. The slight reduction may be due to the processing method used in this study. The high CF in the peel may reduce its feeding value compared to conventional dietary maize in poultry nutrition, even though it has a high metabolizable energy of 3720.67 kcal/kg. The high CF content in the biodegraded SOP in this study most probably caused the reduction of its NFE, the digestible carbohydrate and energy nutrient in feed ingredients. Hence, the energy yield of biodegraded SOP will be of inferior value compared to that of maize in practical broiler chicken feeding.

The ash content of 9.90% obtained in this study was higher than 4.47% (Ani et al., 2015), and 8.19% (Ojabo et al., 2014). The implication of the high ash content is that it may lower the dietary caloric yield because of the limitation of mineral elements to yield energy in the metabolic process of oxidation. Therefore, the results of the proximate composition of biodegraded SOP meal showed that, while its high crude protein content can be of nutritional benefit to monogastric animals, including broiler chicken, its content of crude fibre and ash can be adverse to the good performance of these farm animals.

The highest feed intake of 36.19 g was lower than 56.16 g reported by Oluremi et al. (2010) who fed fermented sweet orange peel-based diets to broiler chicks, and 37 g reported by Aduku (2005) as the mean daily feed intake for starter broiler chicks. This may be attributed to the low fibre content of the control maize-based diet (T1) compared to the high fibre content in the biodegraded SOP meal-based diets, the overall feed composition, dietary nature, and strain/breed of broiler chicks used. Abbas et al. (2013) also reported dietary fibre effect on broiler chicks fed sweet orange peel-based diet. The body weight gain, like the feed intake of the chicks, significantly ($P<0.05$) decreased as the percent maize replacement with biodegraded SOP increased from 0% to 20%. Consequently, the daily weight gain was highest in T1 (17.08 g) and lowest in T5 (12.83 g). The range was less than 32.44 g to 43.17 g (Medugu et al., 2010) but comparable with 11.97 g to 21.70 g (Oluremi et al., 2010). Furthermore, feed intake appeared to have a direct effect on body weight gain and, thus, a cumulative effect on the final body weight. Feed intake and utilization of the nutrients present are the major factors influencing both body weight gain and feed efficiency in meat-type birds. The final body weight of the chicks was significantly ($P<0.05$) different among the dietary groups decreasing from T1 (525.37 g) to T5 (406.10 g) for the same reason as for body weight gain.

The experimental diets did not have any significant ($P>0.05$) effect on feed conversion ratio, protein intake, protein efficiency ratio, and mortality rate across the dietary groups. This showed that the replacement of maize with biodegraded SOP in the range of 0% to 20% did not negatively impact on the quantitative values of all these performance indicators in the starter broiler chicks. The rate of mortality among the experimental birds in this study was less than 5% regarded as normal for broiler chicks (Oluyemi and Roberts, 2000). Furthermore, since mortality did not show any significant difference among the dietary treatments, biodegraded SOP meal may be a safe ingredient to use in compounding broiler chick diet if its other nutritional limitations can be mitigated.

5. Conclusion

From the result obtained in this study, it was concluded that the rumen filtrate biodegraded sweet orange peel meal is comparable with maize in crude protein and higher in metabolizable energy content but inferior in crude fibre and can thus be transformed from being an agricultural waste into a feed resource in broiler chicken production. The utilization of SOP meal as replacement maize did not support the growth of starter broiler chicks, and further studies are therefore necessary to investigate other processing methods that can further reduce the fibre content of sweet orange peel meal to enhance its feed value as a replacement for maize in the diets of broiler chicks.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	E.T.A.	O.O.	K.C.O.
C	100		
D	40	30	30
S		50	50
DCP	100		
DAI	100		
L	40	30	30
W	50	50	
CR		100	
SR	75	25	
PM	50	50	
FA	100		

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. The experimental procedures were approved by the Local Animal Care and Ethics Committee of the Federal University of Agriculture, 2373, Makurdi, Nigeria.

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Research Article
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THE EFFECT OF DIFFERENT PLANT GROWTH HORMONES AND CONCENTRATIONS ON THE REPRODUCTION OF *ROSMARINUS OFFICINALIS* L. WITH SEEDLING PRODUCTION

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Abstract: To evaluate the proper concentration of plant growth hormone and the suitable plant growth hormone in *Rosmarinus officinalis* L., a significant fragrant, medicinal and herbaceous plant, the study was carried out in a greenhouse during the vegetative period of 2022. In the experiment, peat and vermicompost mixture (3 peat / 1 vermicompost) as the rooting medium, Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA) and 1-Naphthaleneacetic acid (NAA) hormones were used as plant growth regulators and the concentrations of these hormones were 0, 1000, 2000, 3000, 4000 ppm The Randomised Plot Experiment Design were established three times in the Multiple Comparison Test "LSD". In seedlings removed three months after planting, properties such as seedling height (cm), number of roots (pieces), root length (cm), maximum root length (cm), number of laterals (pieces) and lateral length (cm) were examined. The highest seedling length (19.88 cm) and number of shoots (12.60 pcs), 3000 ppm concentration of Indole-3-acetic acid (IAA), root length (16.30 cm), and 3000 ppm concentration of 1-Naphthaleneacetic acid (NAA) at the highest root length (22.82 cm) came to the fore. In terms of root number, the values found at 3000 ppm concentration of indole acetic acid (19.25 pieces) and naphthalene acetic acid (20.09 pcs) were combined into one statistical group and made up the maximum number of roots. The lateral length control seedlings statistically prevented other applications and produced the highest lateral length (1.99 cm). Therefore, it can be said that Indole-3-acetic acid (IAA) and 1-Naphthaleneacetic acid (NAA), both of which have a concentration of 3000 ppm, are the most suited growth hormones.

Keywords: Growth hormones, *Rosmarinus officinalis* L., Vegetative reproduction

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1. Introduction

The rosemary (*Rosmarinus officinalis* L.) plant, known by names such as kusdili, hasalbal, and akpuren according to the regions in Turkish, is an important medicinal, aromatic and herbaceous plant that belongs to the Lamiaceae family (Begum et al., 2013). Rosemary is a coniferous, occasionally leafless shrub that grows in the Mediterranean region's environment (Satya et al., 2017). It is recognized by the European Union as a safe and effective food preservative (E 392) (Andrade et al., 2018) and is a rich source of antioxidants (Nieto et al., 2018). Tea is used in traditional medicine to treat intestinal parasites, coughing, and colds, whereas oil is used to alleviate rheumatism and joint discomfort (Calvo et. al., 2011; Satya et. al., 2017). It is being researched for the treatment of Alzheimer's disease in modern medicine (Habtemariam, 2016).

In medicinal aromatic plants, some species reproduce vegetatively through organs like lateral stems, root parts like rhizome and stolon, onion, tuber, and corm, while some species reproduce generatively through the use of seeds (Baydar, 2019). As a result of the generative reproduction method's prolonged duration from emergence to flowering and maturation in medicinal aromatic plants, slow growth, issues with seed germination, modifications to the plant's morphology, and a change in the amount of active substance content (essential oil composition) are all consequences (Kara et al., 2011, Baydar, 2019). For plants with low seed retention rates, poor germination power, or opening due to foreign fertilization, the vegetative propagation method is widely used (Parađiković et al., 2013; Baydar, 2019). The plant rosemary is also capable of vegetative and generative reproduction. However, seed production is uncommon since the seeds are very hard and small and the germination rate is very low (Baydar, 2019). To produce high-quality seedlings, many growth hormones are used in vegetative production. These hormones are chemical compounds that are used topically to aid in rooting, speed up rooting, increase the quantity of roots and steels, and shorten rooting time (Parlak, 2008; Boyer and Graves, 2009). Gibberellins, and cytokines are classified as growth promoters among these regulators, while abscisic acid (dormines) inhibitors and ethylene

are classified as ripeners (Algül et al., 2016).

Effective rooting hormones from the auxin group include IAA, IBA, and NAA. In today's world, IBA is the hormone most frequently applied and has a long-lasting impact on rooting plants (Boyer and Graves, 2009; Grunewald et al., 2009).

Obtaining quality seedlings depends significantly on the type and concentration of plant growth regulators. The goal of this study was to examine how hormones and concentrations of the auxin class chemicals NAA, IAA, and IBA, which are the most frequently used in aquaculture, affected the growth of the *Rosmarinus officinalis* L plant from cuttings.

2. Materials and Methods

A trial specimen of the *Rosmarinus officinalis* species was taken from the collection garden of the medical and aromatic plants program at Ordu University's Technical Sciences Vocational School, and body steels measuring 12 cm long from the plant were employed. IAA, IBA and NAA concentrations of 0 ppm, 1000 ppm, 2000 ppm, 3000 ppm, and 4000 ppm were utilized as growth regulators of peat and vermicompost mixture (3 peat/1 vermicompost) as rooting medium. On May 16, 2022, the randomized plot experiment design was used to plant the trial in the vials by dipping the hormone concentrations of IAA, IBA, and NAA for 5 seconds with 3 repetitions and 15 sheets of steel in each repetition. According to the needs of the steels, necessary irrigation was made, and the seedlings were removed after three months. The seedling length (cm), the number of roots (pieces), root length (cm), maximum root length (cm), the number of laterals (pieces), and lateral length (cm) were determined.

The study's data were analyzed using the SAS-JMP 10.0 program by the randomized plot experiment design, and one of the multiple comparison test "LSD" was applied.

3. Results and Discussion

Table 1 presents the analysis findings and the standard deviation values demonstrating the impact of hormone administrations on seedling length, root number, root length, maximum root length, number of laterals, and lateral length of steels. The tested characters' responses to different hormone kinds and hormone concentrations were very significant (P<0.01).

3.1. Seedling Length (cm)

The average seedling height was found 19.88 cm for IAA at 3000 ppm and 15.50 cm for NAA at 4000 ppm; this difference was statistically significant (P<0.01). 1000 ppm (17.07 cm), 4000 ppm (18.39 cm) of IAA, 1000 ppm (17.78 cm), 2000 ppm (17.60 cm) and 3000 ppm (18.37 cm) of IBA, and 3000 ppm (16.86 cm) of NAA are in the same group according to the multiple comparison test.

In terms of hormone kinds, the resulting seedling lengths varied between 16.42 (Control) and 19.88 (3000 ppm) cm in IAA, 15.81 cm (4000 pmm) and 18.37 cm (3000 ppm) in IBA application, and 15.50 cm (4000 pmm) and 16.86 cm (3000 ppm) in NAA hormone. The auxin group includes the plant growth regulators employed in the study. These hormones promote growth by boosting cell division and longitudinal extension (Deytieux-Belleau et al., 2007; Algül et al., 2016). According to the study's findings, in line with the results of the researchers, the maximum seedling size was attained at a 3000 ppm concentration of IAA hormone, one of the auxin growth regulators.

3.2. Number of Roots (pcs)

The highest concentration of 20.09 roots and 3000 ppm of NAA also emerged in the control group with the lowest number of 7.28 roots. The highest values, 19.25 roots measured at 3000 ppm IAA hormone concentration and 20.09 roots that emerged at 3000 ppm NAA concentration, were included in the same statistical group. The results of the study showed that, compared to control seedlings, hormone concentrations increased the number of roots.

Table 1. Descriptive statistical values for *Rosmarinus officinalis* L. seedlings

HC (ppm)	SL (cm)	RN (piece)	RL (cm)	LRL (cm)	NL (piece)	LL (cm)
Control	16.42 ± 2.16 ^{b}	7.28 ± 3.48 ^{c}	10.05 ± 2.75 ^{bc}	14.36 ± 4.49 ^{b}	8.28 ± 2.08 ^{c}	1.99 ± 0.54 ^{a}
IAA 1000	17.07 ± 2.58 ^{ab}	16.71 ± 8.42 ^{ab}	13.83 ± 4.965 ^{ab}	17.95 ± 7.14 ^{ab}	9.76 ± 2.98 ^{abc}	1.51 ± 0.38 ^{abc}
IAA 2000	16.55 ± 3.99 ^{b}	12.20 ± 6.36 ^{abc}	12.33 ± 2.95 ^{abc}	15.76 ± 4.48 ^{ab}	10.10 ± 3.16 ^{abc}	1.39 ± 0.38 ^{bc}
IAA 3000	19.88 ± 3.10 ^{a}	19.25 ± 8.33 ^{a}	11.02 ± 4.47 ^{bc}	14.00 ± 5.07 ^{b}	12.60 ± 3.47 ^{a}	1.62 ± 0.46 ^{abc}
IAA 4000	18.39 ± 1.97 ^{ab}	17.00 ± 6.82 ^{ab}	10.68 ± 3.23 ^{bc}	14.44 ± 4.19 ^{b}	10.94 ± 2.94 ^{abc}	1.56 ± 0.58 ^{abc}
IBA 1000	17.78 ± 4.44 ^{ab}	8.22 ± 6.22 ^{bc}	7.77 ± 5.00 ^{c}	11.94 ± 6.72 ^{b}	8.89 ± 1.76 ^{abc}	1.39 ± 0.265 ^{bc}
IBA 2000	17.60 ± 3.75 ^{ab}	15.10 ± 7.59 ^{ab}	12.94 ± 4.73 ^{abc}	17.53 ± 5.98 ^{ab}	11.40 ± 3.17 ^{ab}	1.28 ± 0.33 ^{c}
IBA 3000	18.37 ± 3.24 ^{ab}	14.40 ± 8.22 ^{abc}	11.57 ± 4.50 ^{abc}	15.87 ± 8.60 ^{ab}	10.53 ± 2.59 ^{abc}	1.49 ± 0.80 ^{abc}
IBA 4000	15.81 ± 2.76 ^{b}	15.88 ± 6.44 ^{ab}	10.58 ± 3.85 ^{bc}	15.34 ± 4.12 ^{ab}	9.56 ± 1.86 ^{abc}	1.42 ± 0.39 ^{abc}
NAA 1000	15.90 ± 2.26 ^{b}	12.85 ± 4.56 ^{abc}	8.97 ± 4.56 ^{c}	13.75 ± 4.11 ^{b}	9.15 ± 2.32 ^{bc}	1.38 ± 0.39 ^{bc}
NAA 2000	16.00 ± 3.17 ^{b}	13.81 ± 7.17 ^{abc}	12.32 ± 7.17 ^{abc}	17.03 ± 3.53 ^{ab}	11.38 ± 3.34 ^{abc}	1.88 ± 0.98 ^{ab}
NAA 3000	16.86 ± 2.19 ^{ab}	20.09 ± 9.18 ^{a}	16.30 ± 9.18 ^{a}	22.82 ± 5.33 ^{a}	11.18 ± 3.49 ^{abc}	1.15 ± 0.29 ^{c}
NAA 4000	15.50 ± 2.78 ^{b}	11.75 ± 4.89 ^{abc}	13.28 ± 4.89 ^{abc}	11.69 ± 4.66 ^{b}	8.50 ± 2.62 ^{bc}	1.51 ± 0.32 ^{abc}

HC= hormone concentrations, SL= seedling length, RN= root number, RL= root length, LRL=longest root length, NL= number of laterals, LL= lateral length (cm), ^{a,b,c}The difference between averages without common letters in the same column is statistically significant (P<0.01).

This outcome was in line with the research's findings that hormone administration promoted rooting (Atıcı, 1999; Şekeroğlu et al., 2001; Ilgın and Bulat, 2001; Uysal et al., 2010; Pulatkan et al., 2018; Sarı and Kaçar, 2019). Additionally, Pulatkan et al. (2018) observed that when IBA, IAA, and NAA hormones were provided at dosages of 0, 1000, 3000, 5000, and 8000 ppm upon rooting of *Berberis thunbergii*, the largest number of roots occurred at the dose of 3000 ppm of each hormone.

3.3. Root Length (cm)

The longest root length was determined at 16.30 cm at an NAA concentration of 3000 ppm, and the root length change was between 7.77 cm and 16.30 cm. When the impact of different hormone types and concentrations on root lengths was studied, root lengths ranged from 10.05 cm (control) to 13.83 cm (1000 ppm) in IAA, 7.7 cm (1000 ppm) to 12.94 cm (2000 ppm) in IBA, and 8.97 cm (1000 ppm) to 16.30 cm (3000 ppm) in NAA. It was discovered during the study that applying 3000 ppm of NAA considerably enhanced the quantity of roots compared to the control plants. This result was consistent with 6000 mg/L NAA application in *Bougainvillea* by Memon et al. (2013), 500 mg/L NAA administration in Oleander (*Nerium oleander*) by Akat et al. (2017), Pulatkan et al. (2018) study in where they reported that 3000 ppm NAA concentration increased root length in *Berberis thunbergii* compared to control plants.

3.4. Maximum Root Length (cm)

The maximum root length was calculated to be 22.82 cm at a 3000-ppm concentration of the NAA hormone. The maximum root lengths were then statistically measured at 1000 ppm (17.95 cm), 2000 ppm (15.76 cm) of IAA, 2000 ppm (17.53 cm), 3000 ppm (15.87 cm), 4000 ppm (15.34 cm) of IBA, and 2000 ppm (17.03 cm) of NAA in the same group. According to the root length data, the maximum root length was observed at an NAA hormone concentration of 3000 ppm. Only 3000 ppm of NAA concentration revealed the highest statistical root length. Other applications did not produce a statistically significant difference ($P>0.01$) from the control group when compared to the other hormones, IAA, IBA, and NAA, which are also widely used commercially to promote root formation in plants (Table 1).

3.5. Number of Laterals (pcs)

The maximum number of laterals was 12.60 of 3000 ppm IAA concentration, and the lowest number of laterals was 8.28 found in control plants. Regarding the number of laterals, there was no statistically significant difference ($P>0.01$) between the other hormone concentrations. It is believed that these seedlings do not have a high branching level, and that branching at a concentration of 3000 ppm IAA has increased the number of laterals (12.60 pcs) (Altun et al., 2021).

3.6. Lateral Length (cm)

The highest lateral length was found in the control group (1.99 cm), which received no treatment, in this study, where it was determined that the lateral length was changed by the administered hormone and hormone concentrations at the 0.01 significant level. The lowest lateral length was 1.28 cm at 2000 ppm IBA hormone concentration and 1.15 cm at 3000 ppm NAA concentration.

4. Conclusion

The seedling height and several laterals at 3000 ppm concentration of IAA hormone have come to the fore in this study studying the effect of IAA, IBA, and NAA hormones on steel production in Rosmarinus *officinalis* L. species. The maximum root length was found at a concentration of NAA hormone of 3000 ppm. The control plants that received no treatment had the highest value regarding lateral length. The IAA and NAA roots determined at 3000 ppm concentrations formed the highest number of roots and were included in the same statistical group. According to the research's findings, a quality seedling of the *Rosmarinus officinalis* L. plant should be produced at hormone and concentration concentrations of 3000 ppm for both IAA and NAA.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	M.Y.	E.K.Ö.
C	50	50
D	50	50
S	50	50
DCP	50	50
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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GENETIC VARIABILITY OF SMALL HORSE POPULATIONS FROM GREEK ISLANDS

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Abstract: In this study, we analyzed microsatellite variation in DNA obtained from hair samples collected from 46 local Greek horse populations originating from the islands of Skyros (Skyros Small Horse; n=9), Rhodes (Rodos Small Horse; n=6), Lesvos (with the traditional miniature Midili Small Horse (n=2) and the larger Lesvos Gaiter (n=22)) and Crete (Messara) (n=7). We used 15 autosomal microsatellite markers (VHL20, HTG4, AHT4, HMS7, HTG6, AHT5, HMS6, ASB2, HTG10, HTG7, HMS3, HMS2, ASB17, ASB23 and LEX33) for the genetic characterization of the above populations and exploration of their genetic structure and diversity levels. A total of 120 alleles were detected across the 15 loci with a minimum of 4 alleles in HTG7 locus and a maximum of 13 alleles in ASB17 locus. The total per population number of alleles was 42 (Skyros Small Horse), 20 (Rodos Small Horse), 71 (Lesvos Gaiter), 52 (Messara breed) and 21 (Midili Small Horse). The effective number of alleles (N_e) per locus ranged from 1.47±0.13 (Rodos Small Horse) to 4.67±0.31 (Lesvos Gaiter). The allelic richness (A_r) was between 1.50±0.12 (Rodos Small Horse) and 2.93±0.08 (Lesvos Gaiter) and the average Polymorphism Information Content (PIC) values varied from 0.200±0.035 (Rodos Small Horse) to 0.733±0.026 (Lesvos Gaiter). No significant deviations from H-W equilibrium were found except for three loci (ASB2, HTG10 and LEX33) in Messara and one locus (ASB23) in Lesvos Gaiter. The inbreeding coefficient (F_{is}) ranged from -0.130 (Rodos Small Horse) to 0.042 (Lesvos Gaiter). The observed (H_o) and expected (H_e) multilocus heterozygosity mean estimations were highest in Lesvos Gaiter (0.764±0.027 and 0.783±0.024, respectively) and smallest in Rodos Small Horse (0.300±0.075 and 0.269±0.064, respectively). Across loci, the total genetic diversity H_T was 0.741, the diversity among subpopulations H_S was 0.621 and the multilocus genetic differentiation G_{ST} was 0.161, which was rather high. The population of Rodos Small Horse separated from the remaining horses as shown by factorial correspondence analysis, population assignment and metric multidimensional scaling diagrams. This study highlights the loss of genetic diversity in small isolated horse populations and the urgent need to take protective measures to preserve them.

Keywords: Indigenous Greek horses, Rare breeds, Microsatellites

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1. Introduction

Animal genetic resources make up part of natural and cultural world heritage. The loss of various distinct breeds or populations threatens the genetic variability and adaptation ability of domestic species. Hence, the study and preservation of rare indigenous horse populations is of great importance, both nationally and globally.

Islands of Aegean archipelagos and Crete, with their characteristic warm and dry climate, geographical isolation and rocky ground, are host to several indigenous horse populations that display unique properties and adaptations (Cothran et al., 2010; Bömcke et al., 2011; Kostaras et al., 2021). Most of these are now rare or nearly extinct, with their status classified as endangered or critical. They lack genetic characterization and a systematic preservation plan. Due to their small population size, further difficulties arise for their recognition as breeds and proper management. Modern genetic tools such as microsatellites can play an important role in identification and preservation of horse populations or breeds (Cothran and Luis, 2005; Van de

Goor et al., 2010).

Main documented characteristics of the studied horse populations include exceptional resilience to draught and heat, tolerance to poor nutrition, with physical and behavioral adaptations to fit the challenging mountainous ground and local conditions (from strong feet and small, hard hoofs to alert but self-composed temperament). They also exhibit low susceptibility to parasites, disease and physical injury, compared to other horses on the same environment (Giantsis et al., 2020; Kostaras et al., 2021). The oldest, traditional type has remarkably small body size and has been able to survive for long periods of time without human intervention in mountainous regions, forming feral populations (Papaioannou and Kostaras, 2006). The larger sized, gaiter type breeds are best suited for riding, but have higher maintenance demands. Greek "gaiters", have the ability to perform an extra, high-speed, two-beat lateral gait, also known as "pace", where both limbs from each side of the horse move simultaneously, due to a dominant nonsense mutation in DMRT3 gene affecting locomotion (Promerová et al, 2014).

Attempts have been conducted to study the horse populations in Greece using DNA markers such as RAPDs (Apostolidis et al., 2001), microsatellites (Cothran et al., 2010) or microsatellites and allozymes (Bömcke et al., 2011).

For this study, five indigenous horse populations were considered. Three of them are traditional miniature horses (Skyros Small Horse, Rodos Small Horse and Midili Small Horse) and two are larger gaiter types (Lesvos and Messara). From the above populations, only Skyros Small Horse and Messara horses are officially recognized as breeds and have studbooks (Centre of Animal Genetic Resources of Athens, 2019). This preliminary study of local Greek horse populations aims to support their conservation by determination of their genetic characterization. The objective is to study their genetic structure by defining the number of alleles, testing for Hardy-Weinberg equilibrium and estimating genetic diversity indices.

2. Materials and Methods

2.1. Animal Materials

The sampled horses were chosen according to previous breed/population descriptions and parentage information (Scherf, 2000; Kostaras et al., 2021). They form two distinct phenotypes: miniature, non-gaiting type with withers height (WH) ranging from 105-115 cm (Skyros Small Horse, Rodos Small Horse and Midili Small Horse) and larger gaiter type with a WH of 130 to 150 cm (Lesvos Gaiter and Messara breed). For this study, 46 hair root follicle samples were used (10 to 20 rooted hairs from each horse) from 5 local horse populations (Figure 1): Skyros Small Horse (n=9), Rodos Small Horse (n=6), Midili Small Horse (n=2), Lesvos Gaiter (n=22) and Messara breed (n=7). The Skyros Small Horse breed with around 160 purebred individuals is officially recognized as a breed and under protection (Centre of Animal Genetic Resources of Athens, 2019; Kostaras et al, 2021).

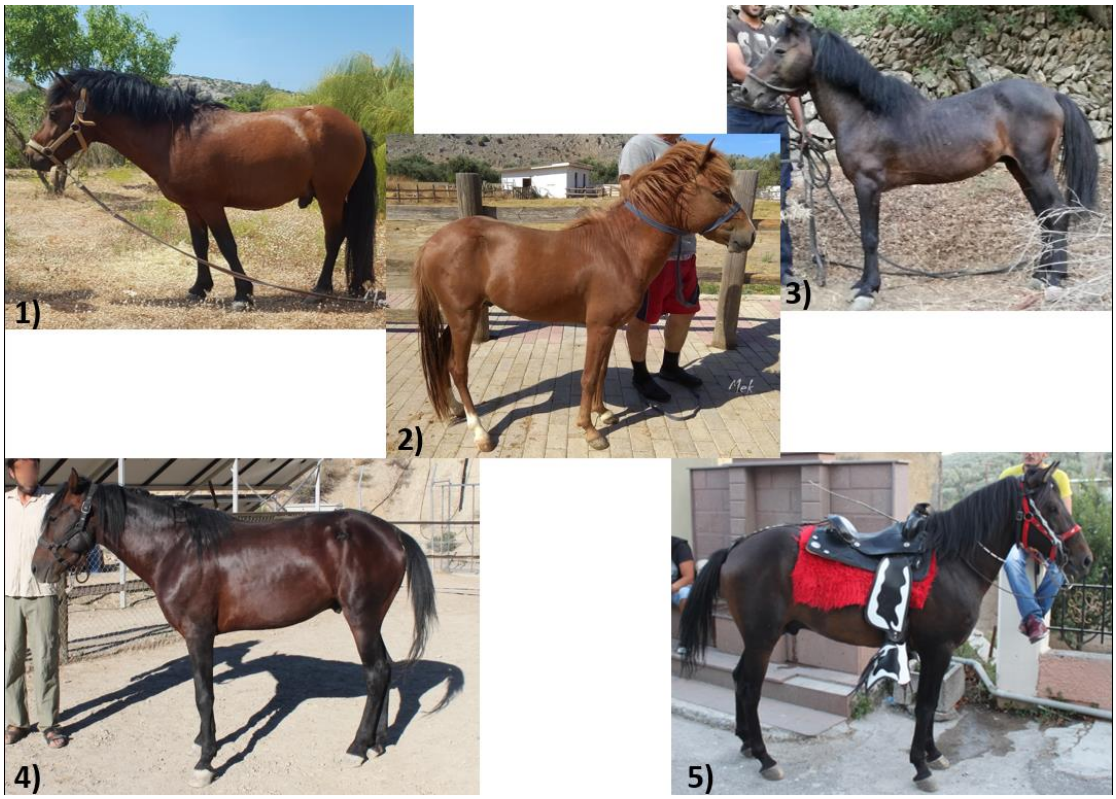

Figure 1. Phenotypic characteristics of 1) Skyros Small Horse breed, 2) Rodos Small Horse, 3) Midili Small Horse, 4) Messara breed and 5) Lesvos Gaiter.

Messara horse is a gaiting breed from the island of Crete, officially recognized by the state, with an estimated population of 100-200 horses (Centre of Animal Genetic Resources of Athens, 2019; Kostaras et al., 2021). Rodos Small Horse population consists of 10 reproductive horses from the homonymous island. Its population has passed through a recent severe bottleneck of 3 females and 3 males (Kostaras et al, 2021). Last feral herd was captured two decades ago and has been bred for preservation since then (Papaioannou and Kostaras, 2006). Rodos Small Horse horses are not recognized or protected by the state. Midili Small Horse population, from Lesvos Island, was considered extinct until recently when the presence of some small feral herds was reported (population size is estimated from 5 to 20 horses). The two sampled Midili horses are remains of an old population and the only unrelated individuals from two different feral herds, each representing a separate herd. Lastly, Lesvos Gaiter is not recognized as a breed by the state and its population size is unknown. The geographical location of the studied horses is shown in Figure 2.

Figure 2. geographical location of the studied horse populations.

The collected rooted hair was stored in plastic bags and submitted to Texas A&M University Animal Genetics Laboratory. DNA was isolated from the hair follicles using PUREGENE DNA purification kit according to the manufacturer's protocol and analyzed for 15 species-specific horse microsatellite loci (VHL20, HTG4, AHT4, HMS7, HTG6, AHT5, HMS6, ASB2, HTG10, HTG7, HMS3, HMS2, ASB17, ASB23 and LEX33) using multiplex PCR. Table 1 shows the analyzed microsatellite loci, their chromosome location and length in base pairs. Microsatellite analysis was achieved according to the procedures described by Juras et al. (2003), using the nomenclature of the International Society for Animal Genetics.

Table 1. Fifteen horse-specific microsatellite loci used in this study

Locus	Amplicon Length (bp)	Chromosome Location
VHL20	89-109	30
HTG4	127-141	9
AHT4	148-164	24
HMS7	173-187	1
HTG6	84-106	15
AHT5	130-146	8
HMS6	159-171	4
ASB2	222-254	15
HTG10	93-113	21
HTG7	120-130	4
HMS3	150-172	9
HMS2	284-304	10
ASB17	93-121	2
ASB23	183-217	3
LEX33	195-221	4

2.2. Statistical Analysis

Statistical analysis of the genotypic frequencies was conducted using the following programs: Create (Coombs et al, 2008), GENEPOP (Rousset, 2008), GeneAlEx (Peakall and Smouse, 2012) and GENETIX (Belkhir, 2004). We estimated allelic frequencies, number of alleles (N_a) and effective number of alleles (N_e) using GENETIX. Polymorphic information content (PIC) for each locus was calculated. We tested the Hardy-Weinberg equilibrium calculating F_{is} values in each locus with GENEPOP. Allelic richness (A_r) (El Mousadik and Petit, 1996) was calculated on the basis of the minimum sample size of 2 diploid individuals using HIERFSTAT (Goudet, 2005) in R (R core team, 2020). Estimators of genetic diversity included: the mean observed and expected heterozygosity [H_o, H_e (unbiased estimation according to Nei, (1978)], the mean genetic diversity within subpopulations (H_S), the total genetic diversity (H_T) and the proportion of genetic diversity that resides among populations (G_{ST}), which is equivalent of F_{ST}, calculated, applying the corrections of Nei and Chesser (1983) and Nei (1987) for small sized populations and inbreeding applied in the calculations of H_S and H_T. Furthermore, we applied three methods to investigate genetic structure of the studied populations: (i) the factorial correspondence analysis based upon allelic frequencies (GENETIX software), (ii) the population assignment method based upon genotypic frequencies of the studied populations according to Paetkau et al. (1995) and Paetkau et al. (2004) through GeneAlEx software and (iii) the metric multidimensional scaling (MDS) which was calculated with default distribution of R program (command cmdscaling; R core team, 2020) to project the multidimensional D_{PS} genetic distance matrix onto a two-dimensional (2D) plot for the 5 populations. The matrix of D_{PS} genetic distances between all pairs of individuals was estimated as D_{PS} = 1-PS. The proportion

of shared alleles (PS) was calculated with Adegenet 2.0.0 (Jombart, 2008).

3. Results and Discussion

In total, 120 alleles were found across the 15 analyzed microsatellites and their allelic frequencies are presented in Supplementary Table 2. A minimum of four alleles was detected in HTG7 locus while a maximum of 13 alleles was found in ASB17 locus. The total number of alleles detected in each population was: 42 alleles for Skyros Small Horse, 20 alleles for Rodos Small Horse, 71 alleles for Lesvos Gaiter, 52 alleles for Messara breed and 21 alleles for Midili Small Horse (Supplementary Table 2). Genetic variability indices per locus and population are shown in Table 3. The mean number of alleles Na ranged from 1.73±0.15 (Rodos Small Horse) to 7.00±0.39 (Lesvos Gaiter). The effective number of alleles Ne ranged from 1.47±0.13 (Rodos Small Horse) to 4.67±0.31 (Lesvos Gaiter) and the PIC was between 0.200±0.035 (Rodos Small Horse) and 0.733±0.026 (Lesvos Gaiter). Because of the different sample sizes, we calculated allelic richness Ar, which ranged between 1.50 (Rodos Small horse) and 2.93 (Lesvos Gaiter). All estimators of genetic diversity reached highest values in Lesvos Gaiter in comparison with the other populations with the highest PIC (mean=0.733±0.026), N_e (mean=4.668±0.309) and A_r (mean=2.93±0.08). The Skyros Small Horse (mean PIC=0.571±0.047 and N_e=3.051±0.290 and Messara breed (mean PIC=0.664±0.020, N_e=3.559±0.186) had intermediate levels of genetic variability. The Rodos Small Horse had the lowest levels (mean PIC=0.200±0.035, N_e=1.469±0.134) and five monomorphic loci. The Midili Small Horse was excluded from further analysis due to inadequate sample number. The Skyros Small population showed slightly lower PIC values (0.571) than in 2011 (0.598) (Bömcke et al, 2011) but our sample was smaller. As seen on Table 4, Hardy-Weinberg (H-W) equilibrium was not violated on most populations. Messara breed showed the highest number of loci that deviated from H-W equilibrium (ASB2, HTG10, LEX33) but sample size was low. The mean multilocus observed heterozygosity (H_o) was highest on Lesvos Gaiter population (0.764±0.0.027), followed by Messara breed (0.733±0.052), and then Skyros Small (0.689±0.0.051). The Rodos Small Horse had the lowest heterozygosity values as expected (0.300±0.075) due to its small size and population history. The Skyros Small Horse population from this study showed slightly higher heterozygosity values (expected heterozygosity He=0.656±0.049 and observed heterozygosity Ho=0.689±0.051) than in 2011 (0.621 and 0.647, respectively) (Bömcke et al., 2011) despite lower PIC values, which could possibly be due to sampling or analysis variation. It is worth mentioning that Bömcke et al. (2011) used nearly the same set of microsatellites as we did, except LEX33, plus they used HMS1 and CA425, and they had 99 Skyros samples. The above heterozygosity estimations are within the range of values reported in rare horse populations around the world. From the literature review examples given are the Japanese Tsushima (n=25; Ho=0.66), Kiso (n=55; Ho=0.67) and Tokara (n=110; Ho=0.44) breeds (Kakoi et al, 2007; Takasu et al, 2012) or the Knabstupper horse from Denmark (n=170; Ho=0.71) (Thirstrup et al., 2008) and other breeds (Scherf, 2000). In relation to the above, the Rodos Small Horse, with its extremely small and isolated population, had low heterozygosity estimations. F_{is} values (Table 5) showed that among the main four populations Rodos Small Horse had an excess of heterozygotes (F_{is} = -0.130), which is in contrast with the known history of genetic isolation, inbreeding and a severe bottleneck effect of this population. In many cases Fis index is affected by the extremely small sample size (<10) and from the fact that the sampled individuals Rodos Small Horse are all relatives.

G_{ST} values representing a multiallelic expansion of Wrights F_{ST} ranged between -0.004 (HTG6 locus) and 0.362 (HTG4 locus), indicating from low (less than 0.150) to strong (more than 0.250) differentiation among populations, depending on the locus. The average G_{ST} value among Skyros Small Horse, Rodos Small Horse, Lesvos Gaiter and Messara breed populations was high at 0.161, showing that 16% of the total genetic variability was explained by population differentiation. It is worth mentioning that this differentiation also has a geographical component as each of these populations come from different islands.

The factorial correspondence analysis based upon allelic frequencies (Figure 3), the population assignment diagram (Figure 4) and the metric multidimensional scaling (MDS) (Figure 5) showed the same pattern of distribution at the individual level. The above analyses reveal two clusters, with the individuals of Rodos Small Horse separated from the remaining populations. In the second cluster, the different colors for individuals of Lesvos, Messara and Midili are combined, while Skyros individuals within this cluster were more distinct. The above analyses confirm the distinctiveness and relative homogeneity of Rodos and Skyros Small Horses. On the other hand, Lesvos Gaiter and Messara breed, the populations with the highest genetic diversity estimates as previously noted, seem quite heterogeneous as their individuals are not separated from each other and occupy a common area on the plot (Figure 3). The clear differentiation between the Skyros Small Horse and Messara breed (Crete) populations found here, is in agreement with previous findings using RAPD DNA markers (Apostolidis et al., 2001). The two unique Midili Small Horse individuals seem related to Lesvos Gaiter individuals, not surprisingly, since they are from the same island. Using two different analyses the same two clusters are depicted in the population assignment and MDS diagrams (Figure 4 and Figure 5).

Table 3. Genetic variability estimations per locus and population

	Skyros Small Horse n=9				Rodos Small Horse n=6				Lesvos Gaiter n=22				Messara Breed n=7				Midili Small Horse n=2			
locus	PIC	N_a	N_e	A_r	PIC	N_a	N_e	A_r	PIC	N_a	N_e	A_r	PIC	N_a	N_e	A_r	PIC	N_a	N_e	A_r
VHL20	0.687	6	3.6	2.86	0.368	2	1.9	1.92	0.753	8	4.6	2.99	0.701	6	3.8	2.96	0.305	2	1.6	2.00
HTG4	0.194	3	1.3	1.44	0.141	2	1.2	1.33	0.701	5	3.9	2.80	0.453	3	2.2	2.15	0.375	2	2	2.00
AHT4	0.286	2	1.5	1.67	-	1	1	1	0.764	8	4.8	3.03	0.759	7	4.7	3.19	0.375	2	2	2.00
HMS7	0.789	6	5.4	3.24	-	1	1	1	0.806	7	5.8	3.18	0.655	4	3.4	2.78	0.375	2	2	2.00
HTG6	0.448	3	2.2	2.11	0.375	2	2	1.94	0.518	5	2.5	2.26	0.626	4	3.2	2.68	0.375	2	2	2.00
AHT5	0.749	4	3.8	3.07	0.555	3	2.7	2.46	0.723	6	4.2	2.88	0.664	5	3.1	2.83	0.305	2	1.6	2.00
HMS6	0.327	3	1.6	1.78	-	1	1	1	0.744	6	4.5	2.94	0.657	5	3.4	2.78	0.375	2	2	2.00
ASB2	0.624	4	3.2	2.63	0.239	2	1.4	1.58	0.799	8	5.6	3.18	0.617	5	3.1	2.66	0.375	2	2	2.00
HTG10	0.592	4	2.8	2.53	0.368	2	1.9	1.92	0.827	9	6.5	3.27	0.719	6	4.1	3.03	0.375	2	2	2.00
HTG7	0.512	4	2.5	2.30	0.141	2	1.2	1.33	0.479	4	2.1	2.14	0.585	4	2.8	2.55	0	1	1	1.00
HMS3	0.565	4	2.8	2.45	0.305	2	1.6	1.75	0.806	7	5.8	3.18	0.671	5	3.5	2.84	0.375	2	2	2.00
HMS2	0.689	6	3.6	2.86	0.368	2	1.9	1.92	0.781	8	5.1	3.09	0.754	6	4.7	3.16	0.305	2	1.6	2.00
ASB17	0.708	6	4	2.93	-	1	1	1	0.761	9	4.7	3.02	0.657	5	3.4	2.80	0.305	2	1.6	2.00
ASB23	0.718	6	4.1	2.97	-	1	1	1	0.790	8	5.4	3.12	0.754	6	4.7	3.16	0.305	2	1.6	2.00
LEX33	0.671	4	3.6	2.78	0.141	2	1.2	1.33	0.742	7	4.5	2.94	0.685	5	3.6	2.90	0.375	2	2	2.00
mean	0.571	4.33	3.05	2.51	0.200	1.73	1.47	1.50	0.733	7.00	4.67	2.93	0.664	5.07	3.56	2.83	0.327	1.93	1.80	1.93
±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
s.e.m	0.047	0.35	0.29	0.14	0.035	0.15	0.13	0.12	0.026	0.39	0.31	0.08	0.020	0.27	0.19	0.07	0.025	0.07	0.08	0.06

PIC= polymorphism information content, Na= number of alleles, Ne= effective number of alleles, Ar= allelic richness.
"-"= the PIC value in monomorphic loci. s.e.m= standard error of the mean

Table 4. Genetic diversity estimations per locus and population. (expected heterozygosity He, observed heterozygosity Ho, diversity within subpopulations HS, total diversity HT, diversity that resides between subpopulations GST)

Locus	H	Skyros Small Horse n=9	Rodos Small Horse n=6	Lesvos Gaiter n=22	Messara Breed n=7	Genetic diversity per locus (total estimates)		
						H_S	H_T	G_{ST}
VHL20	H_e	0.765	0.530	0.801	0.791	0.721	0.831	0.132
	H_o	0.667	0.833	0.818	0.714			
HTG4	H_e	0.216	0.167	0.760	0.582	0.435	0.681	0.362
	H_o	0.222	0.167	0.818	0.571			
AHT4	H_e	0.366	0	0.810	0.846	0.508	0.726	0.301
	H_o	0.444	0	0.773	1			
HMS7	H_e	0.863	0	0.848	0.758	0.620	0.786	0.211
	H_o	0.889	0	0.909	0.857			
HTG6	H_e	0.582	0.546	0.610	0.736	0.612	0.610	-0.004
	H_o	0.778	0.667	0.545	0.857			
AHT5	H_e	0.830	0.682	0.778	0.747	0.760	0.821	0.075
	H_o	0.778	0.667	0.773	0.857			
HMS6	H_e	0.386	0	0.797	0.758	0.492	0.583	0.157
	H_o	0.444	0	0.727	0.714			
ASB2	H_e	0.726	0.303	0.841	0.725*	0.660	0.775	0.148
	H_o	0.667	0.333	0.864	0.286			
HTG10	H_e	0.686	0.530	0.865	0.813*	0.721	0.797	0.092
	H_o	0.667	0.500	0.864	1			
HTG7	H_e	0.628	0.167	0.532	0.692	0.503	0.665	0.243
	H_o	0.667	0.167	0.546	0.857			
HMS3	H_e	0.673	0.409	0.848	0.769	0.681	0.797	0.146
	H_o	0.667	0.500	0.818	0.571			
HMS2	H_e	0.765	0.530	0.825	0.846	0.748	0.774	0.033
	H_o	0.778	0.500	0.682	0.714			
ASB17	H_e	0.791	0	0.808	0.758	0.591	0.750	0.213
	H_o	1	0	0.773	0.857			
ASB23	H_e	0.797	0	0.833*	0.846	0.629	0.780	0.193
	H_o	0.778	0	0.727	0.714			
LEX33	H_e	0.765	0.167	0.794	0.780*	0.634	0.734	0.136
	H_o	0.889	0.167	0.818	0.429			
Multi-locus (mean±s.e.m)	H_e	0.656±0.049	0.269 ±0.064	0.783±0.024	0.763±0.023	0.621 ±0.026	0.741 ±0.019	0.161 ±0.025
	H_o	0.689±0.051	0.300±0.075	0.764±0.027	0.733±0.052			

*The significant differences between H_e and H_o, s.e.m= standard error of the mean

Table 5. Fis values by locus and population*

Locus	Skyros Small Horse	Rodos Small Horse	Lesvos Gaiter	Messara Breed
VHL20	0.195	-0.667	-0.022	0.104
HTG4	-0.032	-0.000	-0.078	0.020
AHT4	-0.231	-	0.047	-0.200
HMS7	-0.032	-	-0.074	-0.143
HTG6	-0.366	-0.250	0.108	-0.180
AHT5	0.067	0.024	0.007	-0.161
HMS6	-0.164	-	0.089	0.062
ASB2	0.086	-0.111	-0.027	0.625
HTG10	0.030	0.063	0.001	-0.254
HTG7	-0.067	-0.000	-0.026	-0.263
HMS3	0.010	-0.250	0.036	0.273
HMS2	-0.018	0.063	0.176	0.167
ASB17	-0.286	-	0.044	-0.143
ASB23	0.026	-	0.130	0.167
LEX33	-0.174	-0.000	-0.031	0.471
mean	-0.054	-0.130	0.026	0.042

*Fis value="-" in monomorphic loci. (Midili was excluded)

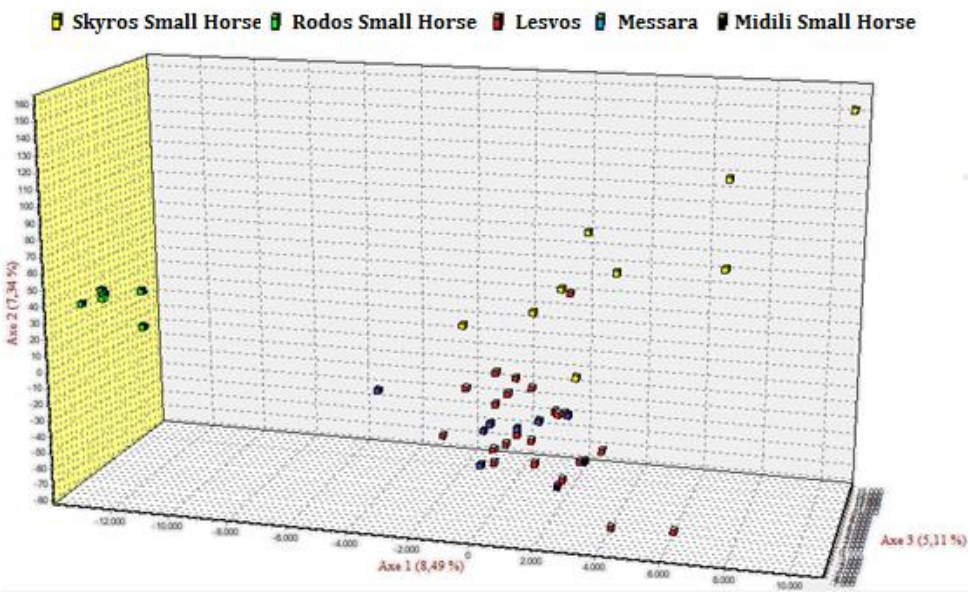

Figure 3. Diagram of factorial correspondence analysis of the studied horses.

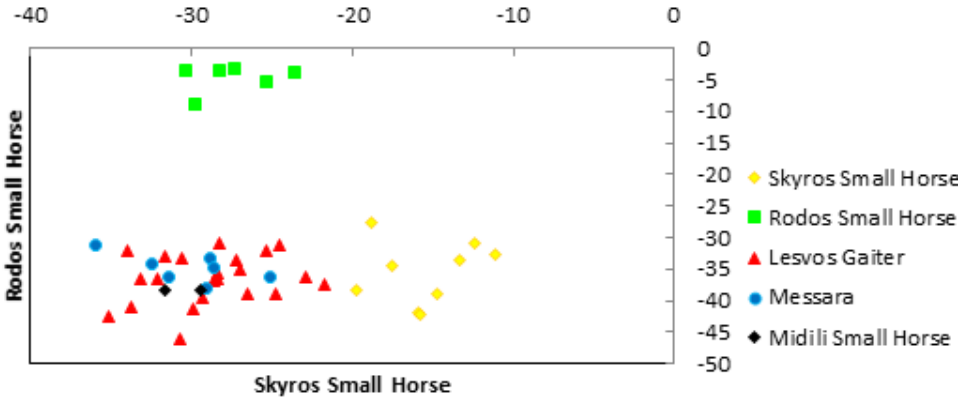

Figure 4. Population assignment diagram of the studied horses.

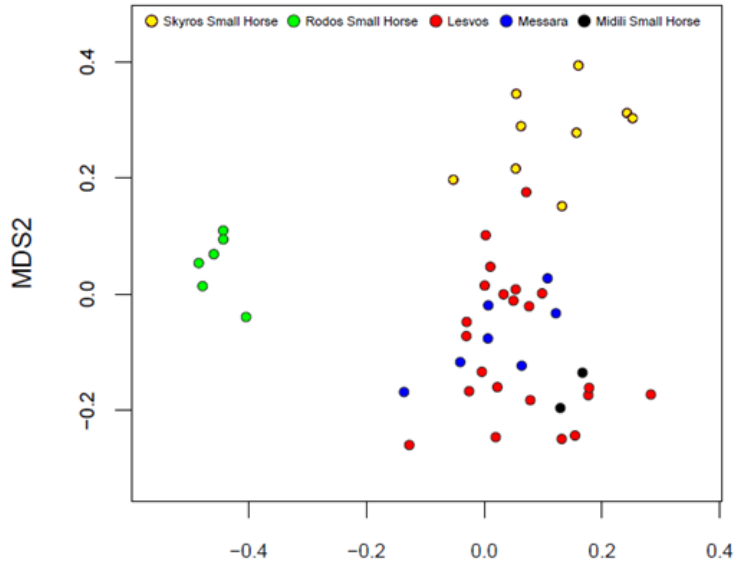

Figure 5. Diagram of metric multidimensional scaling (MDS) analysis of the studied horses.

It is worth mentioning that miniature horses like the Skyros Small (probably the most studied Greek breed) (Apostolidis et al., 2001; Bömcke et al., 2011; Laliotis and Avdi, 2017), Rodos Small and Midili Small were abundant on the islands until two centuries ago. Then they were practically abandoned after the onset of larger breeds for riding and their use as working animals was reduced. The surviving herds were then discarded and their numbers declined significantly. Although almost extinct now, these horses seem to have contributed significantly to modern Greek horse populations. Lesvos Gaiter for example seems to have evolved from crossings between the local Midili Small Horse and Greek or foreign gaiters. On the other hand, Lesvos and Messara gaiters are much appreciated by locals, who favor their gait and cross them in an effort to preserve them by their own initiative, without any specific, organized plan of mating. The above horse populations are often confused with each other and they are crossed with other gaiting breeds of Greek or foreign origin, a practice that puts efforts to preserve and establish them as breeds at risk while eroding their genetic character.

Summarizing our findings, Lesvos Gaiter population showed sufficiently large amounts of genetic variability and this likely is due to its heterogeneous origin. It is suggested that a registry for the Lesvos be set up with clearly defined breed standards. Removal of individuals that do not meet these standards will help create uniformity within the population but it is important that genetic diversity be maintained to the greatest degree possible. Avoiding crosses with other breeds is a necessity. The Messara breed and Skyros Small Horse had intermediate levels of heterozygosity and high PIC values relative to their medium low population size and are therefore possibly not at immediate risk of genetic erosion and extinction. Finally, Rodos Small Horse and Midili Small Horse are at the verge of extinction with population sizes of less than 20 individuals and largely inbred, as suggested by our findings. The two populations were found to have low levels of genetic variability that would possibly still allow their survival if conservation efforts were to be urgently fortified, focusing on controlled breeding practices to avoid any further loss of variability.

In general, the insufficient individual ID tracking (by microchip or other) of the animals and lack of pedigrees (except Skyros Small Horses) in Greece are prominent problems that strongly affect the efforts of protection of indigenous breeds. The onset of widely acceptable, objective breed standards and further study of the Greek breeds would be of great importance for their official recognition and preservation.

4. Conclusion

From our analysis, the estimations of genetic variability showed promising levels for Lesvos Gaiter, Messara Breed and Skyros Small Horses that seem to have good chances of preservation if properly managed. Rodos Small Horse and Midili Small Horse are clearly in danger of extinction and only well-planned and immediate protective measures could rescue them.

In the future, we plan to add more samples from these and other Greek horses as well as foreign breeds to our dataset in order to have a better depiction of the genetic relationships among these populations.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	M.E.K	D.P.	N.K.	G.P.L.	I.B.	E.G.C.	R.J.	P.K.
C			34		33			33
D			25		25	25		25
S			25			25	25	25
DCP	20		20	20	20		20	
DAI	34	33						33
L	25	25		25				25
W	50							50
CR				20	20	20	20	20
SR	50				25			25
PM					25	25	25	25
FA			25		25	25	25	

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

All experimental procedures were approved by the Bioethical Committee of the Agricultural University of Athens under the guidelines of Council Directive regarding the protection of animals used for experimental and other scientific purposes (protocol code: 86/609/EEC and date of approval: April 14, 2021).

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THE EFFECT OF SALT STRESS ON THE GERMINATION AND SEEDLING GROWTH PARAMETERS IN BIRDSFOOT TREFOIL (*Lotus Corniculatus* L.)

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Abstract: This study was carried out to determine the response of birdsfoot trefoil to salt stress during germination and seedling growth. The seeds of birdsfoot trefoil (Sarıyıldız variety) was used as material. In the study; 6 different doses of NaCl (pure water (control), 250 ppm, 750 ppm, 2500 ppm, 5000 ppm, 10000 ppm) was applied to birdsfoot trefoil seeds during germination and emergence. Germination experiment was carried out in darkness (20±2 °C) in the petri dishes according to the completely randomized design with 4 replications. Observations were made every day at the same time, seeds with radicle length exceeding 2 mm were considered germinated. According to the results of the research, salt concentrations in many features in terms of germination and seedling development were statistically significant. Increased salt concentrations negatively affected germination and seedling growth. In terms of all the properties examined the lowest values were obtained in 10000 ppm application.

Keywords: Salt tolerance, Birdsfoot trefoil, Forage crops

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1. Introduction

The growth and devolepment of plants in the environment they live in can be adversely affected by biotic or abiotic elements which are considered stress factors. Salinity, which is one of the leading abiotic stress factors, is defined as the accumulation of salt on the soil surface and near the surface as a result of the rising of the salts, which are washed into the groundwater by washing, especially in regions with arid and semi-arid climate characteristics, to the soil surface through capillarity with high ground water and the separation of water from the soil by evaporation (Kwiatowsky, 1998; Kara, 2002). Salinity stress causes significant losses in plant yield and quality, especially by changing the soil structure. Salinity, which is one of the limiting factors in increasing crop productivity and yield potential in the areas agricultural production, is a problem such as low precipitation, high evapotranspiration, high ground water, salty irrigation water and incorrect irrigation practices in arid and semi-arid regions where the amount of precipitation is low and the temperature is high. This situation emerges as one of the most important problems threatening food security for societies whose livelihood is plant and animal production (Cowie et al., 2018; Tietel et al., 2019). In our country salinity is a problem which is seen in 1,518,746 ha of the land and 867,405 ha of the agricultural lands moreover on a worldwide basis this ratio follows as 12,781 ha of agricultural land (Karaoğlu and Yalçın, 2018; Dursun and Mikailsoy, 2020). It is also said that the area of saline soils in the world today continues to increase steadily (Athar et al., 2009). It is recommended that the germination period is the most sensitive period of the life of plants to salt stress (Ahmad et al., 2013), and in the salinity studies, when the development periods of the plant are compared, germination and seedling development periods should be emphasized more and these developmental stages should be taken into consideration more in determining the salt responses of the species (Van Hoorn 1991; Ghoulam and Fares 2001). The response of plants to salt stress varies according to plant species, as well as differences within the same species. As a result of many studies conducted to date, it has been revealed that salinity significantly reduces germination and even completely prevents germination, and it has been reported that this effect varies depending on the plant type, variety and salt dose (Acar et al., 2011; Şentürk and Sivritepe, 2015; Önal Aşcı and Üney, 2016; Uslu and Gedik, 2020, Kızılşimşek and Süren, 2020). In the case of salt in the soil where most cultivated forage crops are grown, which will limit their growth and development, their yields decrease greatly. Especially in arid and semi-arid regions, saline soils limit yields in areas where forage crops are grown (Ateş and Tekeli, 2007). It has been reported that if the amount of salt in the soil is more than 1.5% of the dry soil weight,

most forage crops cannot be grown (Gökkuş, 2009). On the other hand, it has been reported that high salt concentrations such as 200 mM NaCl cause very significant decreases in germination and seedling development of *Trifolium incarnatum*, *Trifolium repens* (Gravandi, 2013), *Trifolium resupinatum* (Ates and Tekeli, 2007), *Trifolium fragiferum* (Can et al., 2013), *Trifolium repens, Trifolium alexandrinum* (Saberi et al., 2013), *Medicago sativa* (Zhanwu et al., 2011; Kaplan et al., 2015), *Trifolium pratense* (Tolan et al., 2017), *Melilotus officinalis* (Ghaderi-Far et al., 2010), *Onobrychis sativa* (Majidi et al., 2010), *Pisum sativum* (Demirkol et al., 2019), *Vicia* spp. seeds (Uslu and Gedik, 2020). Since the reclamation of salty soils is a difficult and costly process, it is more appropriate to grow salinity-resistant plants to increase crop production in these areas (Turhan and Şeniz, 2010; Önal Aşcı, 2011). Therefore, in recent years, studies have focused on the salinity resistance of plant species and varieties. Determination of plant species and varieties that are tolerant to high soil salinity and that can produce economically have been expressed as the primary biotic approach in bringing the areas with salinity problems into cultivation (Ashraf and Harris, 2004). Birdsfoot trefoil (*Lotus corniculatus* L.) is a medium-perennial, perennial, easily self-renewing special legume widely found in different parts of the world. With a high protein content (15-28%), birdsfoot trefoil can be grown mostly for grass, silage and cover plant, or in rangeland pure or mixed. It is also considered a valuable forege crops that improves the performance of ruminants (Hannaway and Myers, 2004; Waghorn, 2008; Anonymous, 2014). Birdsfoot trefoil is a rangeland plant that is resistant to drought and cold, adapts to a very different soil structure in terms of acidity and moisture, is palatable, has no swelling feature, can easily renew itself in pastures due to its seed pouring feature, forms a good mixture with kentucky bluegrass, smooth broom, cocksfoot and timothy for grazing purposes (Açıkgöz, 2001). Birdsfoot trefoil is an N-stabilizing legume, an important part of sustainable agriculture and organic production (Tomic et al., 2007). It has the property of tolerating wet acidic soil (pH = 4.5), soil salinity and some drought conditions (Karadağ et al., 2017). For this reason, it is very important to determine the salt resistance of legume forage crop species and varieties that can grow efficiently with grasses in salty areas (Rogers, 1997). Although birdsfoot trefoil species are seen in natural vegetation in almost every region of our country, the fact that there are not enough studies on these species is considered to be a great deficiency for our country. The current state of rangelands and cultivation of forage crops, and the Mediterranean climate zone, in which our country is a part, reveal the need to give priority to this plant at least as much as alfalfa and Trifolium spp. or even more so according to the situation (Uzun et al., 2008). In this respect, it is necessary to evaluate the types of birdsfoot trefoil. Therefore, in this research, the resistance of birdsfoot trefoil, which is a productive and high quality forage crop, to salt stress during germination and seedling period was investigated.

2. Materials and Methods

This study was carried out in Muş Alparslan University Central Laboratory to determine the effects of salt concentrations applied to the birdsfoot trefoil on germination and seedling growth characteristics. Seeds are subjected to germination testing to obtain information about the viability of the seed, calculate the amount of seed to be sown and compare different seeds about biological value. In order for plants to develop in a healthy way, the seed, which is the first stage of production, must germinate in a uniform way. Before starting to work for this, the seeds of the Sarıyıldız variety of the birsfoot trefoil, which was first procured from the Central Black Sea Transition Zone Agricultural Research Institute Directorate, were subjected to germination testing with four repetitions and 50 seeds per repeat. As a result of the germination tests, the germination rate of the birdsfoot trefoil seeds was determined as 65% on average. Among the reasons for the low germination rates of birdsfoot trefoil, it has been reported by studies conducted in different locations that there is a significant hardseededness in the seeds of birdsfoot trefoil and that it is affected by climatic events (Gençkan, 1992; Hatipoğlu and Avcıoğlu, 2009). In another study, it was reported that there were 92.3% hard seeds in birsfoot trefoil seeds, which adversely affected the germination rate and average germination time. From this point of view, before the seeds are exposed to salt stress, the seed pods are eroded with the help of sandpaper in order to increase germination rates and to obtain more accurate results. The sanded seeds were subjected to germination testing with four repetitions and 50 seeds per repeat before the trial. As a result of the test, the average germination rate was determined as 95% and thus the seeds whose hardseededness properties were broken were prepared by sanding again for the experiment. Before the germination trial, the seeds were kept in a 1% sodium hypochlorite solution for five minutes for surface sterilization, then rinsed three times with pure water (Özkurt ve ark., 2018). The rinsed seeds were dried in air on filter paper and placed in petri dishes with a diameter of 9 mm in filter paper so that there were 50 seeds each. It is prepared so that the salt concentrations to be applied to the seeds are 0, 250, 750, 2500, 5000, 10000 ppm (Uslu ve Gedik, 2020). The petri dishes with added concentrations were followed in the dark at a temperature of 20±1 °C for a 15-day germination period (Azarafshan and Abbaspour, 2014), and the seeds that produced 2 mm of radicle were considered germinated. The germination rate was calculated by dividing the germinated seeds by the total number of seeds and then multiplying them by 100 (Maquire, 1962). For seedling lengths, radicle and plumule lengths were measured

separately, and then the seedling length was determined by adding both lengths (ISTA, 1984). The radicle and plumule were weighed as fresh and the fresh weights of the seedlings were determined. The dry weight of the seedlings dried by soaking at 70 °C for 48 hours in the oven was determined (ISTA, 1984). The Vigor index value was calculated by multiplying the seedling length by the germination rate (Abdul-Baki and Anderson, 1973; ISTA, 1983). To determine the difference between doses in terms of the tolerance of birdsfoot trefoil to salinity, the salt tolerance index (%TTI) as a function of seedling fresh weight was calculated with the following formula (Bağcı et al., 2003): Salt tolerant indeks = (total fresh weight in salt concentration / total fresh weight in control application) * 100.

The data obtained in the study were subjected to analysis of variance in accordance with the randomized design with four replication using JMP-13.0 statistics package program to compare significant differences among treatments. Duncan's multiple range test was applied to compare the means if there were any significant differences.

3. Results and Discussion

The effects of salt doses on germination rate were given in Figure 1 and it was determined that there was a statistical difference between salt doses in terms of germination rate (P<0.01). Salt stress often reduced germination relative to control, but the increase in salt dose did not always reduce germination rate linearly (Figure 1). Therefore, the dose of 250 ppm salt was statistically included in the same group as the control. In the study, the germination rate ranged between 13.00-98.00%, and the lowest germination rate (13.00%) was realized at the highest salt dose (10000 ppm). While there was no significant reduction in the germination rate up to 5000 ppm application, the obtained values were found to be lower than the control. Salinity has been reported to adversely affect germination by creating osmotic stress in plants (Doğan and Çarpıcı, 2016). As the salt concentration in the germination environment increases, the osmotic pressure increases and therefore the seed in the environment does not receive enough water for germination. As a matter of fact, osmotic stress due to salinity is clearly seen at the dose of 10000 ppm, where the lowest germination occurs. Findings that germination rate decreases as salt concentration increases in many plants under salt stress support the study results (Tolan et al., 2017; Özkurt et al., 2018; Demirkol et al., 2019; Okcu 2020; Uslu and Gedik, 2020).

The effect of salt doses on the mean germination time is given in Figure 2 and it was determined that there was a statistical difference between the salt doses in terms of the average germination time (P<0.01). Salt stress often increased the mean germination time compared to the control, but the increase in salt dose did not always increase the mean germination time linearly.

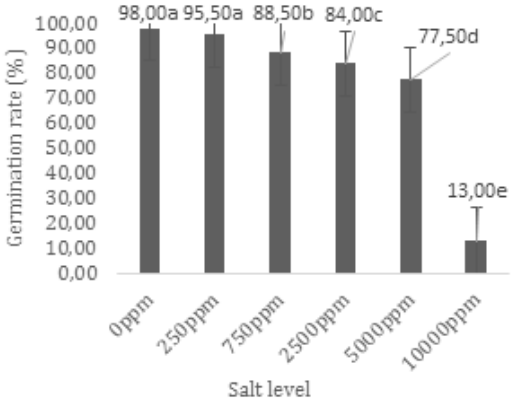

Figure 1. Effect of salt level on germination rate.

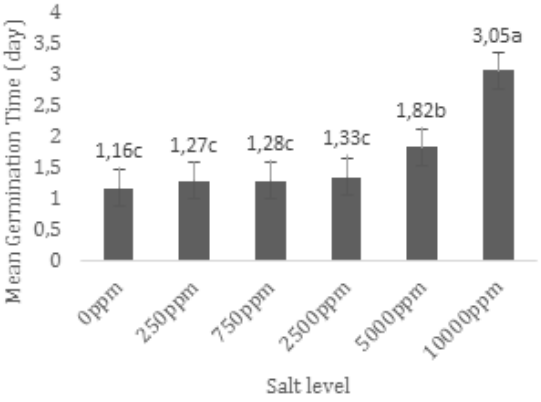

Figure 2. Effect of salt level on mean germination time.

Therefore, salt doses of 0 (1.16 days), 250 (1.27 days), 750 (1.28 days), 2500 (1.33 days) ppm were statistically in the same group in terms of mean germination time, that is, germination showed germination for similar periods, and germination in the longest time (3.05 days) was realized in 10000 ppm salt application. The observed increase in mean germination times due to increased salt doses coincides with the findings of various researchers (Önal Aşçı and Üney, 2016; Tolan et al., 2017; Şimşek Soysal et al., 2018; Şimşek Soysal et al., 2021).

The effects of salt doses on germination index were given in Figure 3 and it was determined that there was a statistical difference between salt doses in terms of germination index (P<0.01).

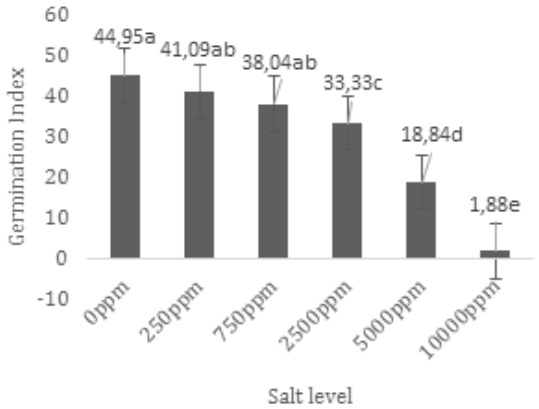

Figure 3. Effect of salt level on germination index.

As a result of the salt doses applied to the birdsfoot trefoil seeds, the germination index varied in the range of 1.88-44.95, and the highest values were obtained from the control application and the lowest values were obtained from the highest salt concentration of 10000 ppm. It has been stated that the high germination index is an indicator of high seed strength (Maquire, 1962). In other words, as the salt dose increases, it has been observed that the decrease in the germination index also reduces the seed strength. In similar studies, it has been reported that the germination index decreases as salt stress increases (Bilgili et al., 2018; Ertekin et al., 2017; Beyazçiçek and Yılmaz, 2020).

The effects of salt doses on germination energy were given in Figure 4 and it was determined that there was a statistical difference between salt doses in terms of germination energy (P<0.01). As a result of the salt doses applied to the birdsfoot trefoil seeds, the germination energy ranged from 0.0-21.37 and the highest values were obtained from the highest values control application and the lowest values were obtained from the highest salt concentration of 10000 ppm. Germination energy is one of the most important parameters in which the strength and quality of the seed is evaluated, and high germination energy indicates that the quality and strength of the seed is high. Although salt stress up to 2500 ppm can be tolerated, the values obtained after 5000 ppm have caused the strength of the seed to decrease greatly.

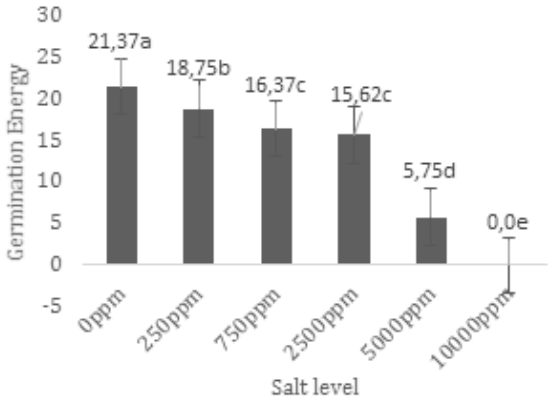

Figure 4. Effect of salt level on mean germination energy.

In the study, plumule length, radicle length, seedling length, seedling length, seedling fresh and dry weights were examined to determine the effects of salt doses on seedling development. The effect of salt doses on the development of plumule and radicle of birdsfoot trefoil seedlings was found to be statistically significant (P<0.01). Plumule and radicle lengths are important parameters in terms of salt stress. From these parameters, the radicle is taken to the plant by direct contact with soil and water and contributes to plumule development (Haileselasie and Gselasie 2012). Therefore, radicle and plumule lengths give the first information about the level of salt exposure of plants.

The plumule lengths of birdsfoot trefoil varied in the range of 0.7525-4.5675 mm. While 250 ppm salt administration was statistically in the same group as the control, salt doses of 750 ppm and above adversely affected plumule development. The radicle lengths of the birdsfoot trefoil ranged from 0.4325 to 2.32 mm. The highest radicle length was obtained from the control group and the lowest was obtained from the salt dose of 10000 ppm. Salt doses of 250 ppm and above adversely affected the development of the radicle. The development of the plant to the radicle is important in terms of salt resistance. It is known that salt has an inhibitory effect on the development of radicle during germination. This study reveals that salinity has a negative effect on radicle development, and studies by different researchers support this (Ahmed et al., 2017; Bose et al., 2018). The seedling length of the birdsfoot trefoil varied in the range of 1.1850-6.8875 mm, while the application of 250 ppm salt was statistically in the same group as the control, salt doses of 750 ppm and above adversely affected the length of the seedlings. The fact that the roots that first come into contact with the salt concentration under salt stress conditions are adversely affected by this situation also adversely affects the intake of water and other nutrients to the plant. The development problem that starts from the radicle component prevents the plant from feeding enough and this situation adversely affects plumule development. It is thought that the reason for the decrease in plumule, radicle and seedling length compared to increasing salt concentrations is due to the inhibition of cell division and elongation due to salt stress and the toxic effect of these salts (Van Horn 1991; Delgado and Sanchez-Raya 2007).

Various studies investigating the effects of salt stress on the birdsfoot trefoil plant also confirm that salt stress has a negative effect on plumule, radicle and seedling development (Teakle et al., 2006; Galloway et al., 2010; Azarafshan and Abbaspour, 2014).

Fresh weights and dry weights of birdsfoot trefoil are 5.22-18.32 mg/seedling, respectively; it varied in the range of 0.74-1.17 mg/seedling. In terms of seedling age weights, 250 ppm salt application was statistically in the same group as the control group, while salt doses of 750 ppm and above adversely affected the fresh seedling weight. In terms of dry seedling weights, salt applications after 250 ppm adversely affected the seedling dry weight compared to the control application, and dry seedling weight exhibited the lowest values in 10000 ppm salt applications. Salinity causes physiological drought and as a result, plants do not get enough water (Goertz and Coons, 1989). When the water lost by transpiration cannot be met, the turgor pressure in the cells decreases and plant growth is limited (Ashraf, 1994). As a result of the inability of plants to get water, a decrease in seedling weight occurs. It has been reported that salt stress as an abiotic factor causes inhibition of the development of root and above-ground organs in plants and reduces the dry weight of roots and stems (Epstein, 1985). In the current study, as salt concentrations increased, the

seedling fresh and dry weights of birdsfoot trefoil decreased. Many plants under salt stress have been reported by different researchers to have significant reductions in seedling fresh and dry weight (Saboraa et al., 2006; Karakullukçu and Adak, 2008; Benlioğlu and Özkan, 2015; Akçay and Tan, 2018).

The vigor index is a value that indicates the vitality and performance level of seeds during germination and development of seedlings (Uslu and Gedik, 2020).

The effect of salt doses on vigor index in birdsfoot trefoil is seen in Figure 5. The vigor index values of the birdsfoot trefoil against the applied salt doses varied between 17.23-674.97, and the vigor index value decreased as a result of the increased salt applications and this decrease was found to be statistically significant. In similar studies, similar to the current study, it has been found that the vigor index decreases with the increase in salt concentrations (Özkurt et al., 2018; Uslu and Gedik, 2020).

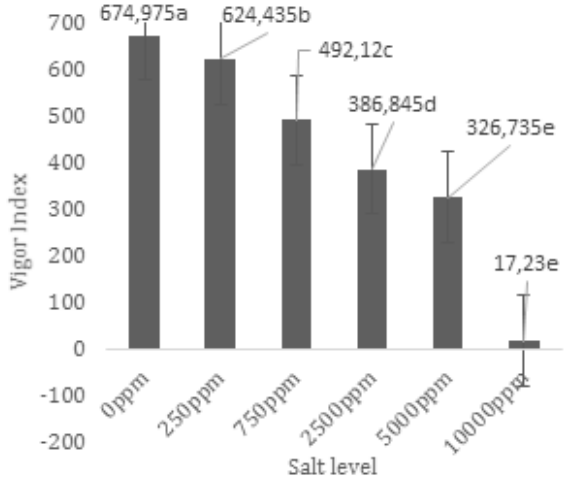

Figure 5. Effect of salt level on vigor index.

The effects of salt doses applied to birdsfoot trefoil on salt tolerance index are given in Figure 6 and it is determined that salt tolerance index decreases as salt dose increases and this decrease is statistically significant.

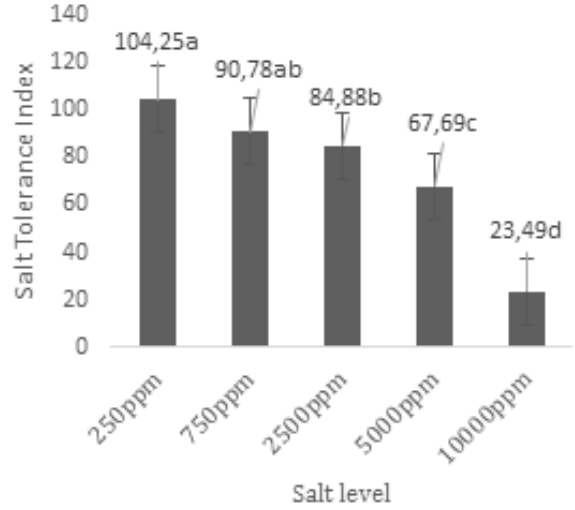

Figure 6. Effect of salt level on salt tolerance index.

While the highest level of salt tolerance birdsfoot trefoil was determined to be at a dose of 750 ppm (90.78) and 2500 ppm (84.88) salt, which are in the same statistical group with the highest 250 ppm (104.25), tolerance to salt at a dose of 10000 ppm was considerably reduced. It has been stated that when plants are grown in salty environments, the Na^+ and Cl^- ions they absorb accumulate in the roots, stems and leaves, and the accumulation of Na^+ and Cl^- in various organs of the plants slows down the development and seriously affects their tolerance to salt (Salisbury and Ross, 1992). Similar studies have also reported that increased salt concentrations reduce the salt tolerance index (Kökten et al., 2010; Avcı, 2019; Uslu and Gedik, 2020; Güleç Şen, 2021).

4. Conclusion

In this study, the effect of different salt concentrations on germination and seedling development parameters in birdsfoot trefoil was investigated. It has been determined that increasing salt concentrations significantly reduce the germination rate, germination index, germination energy, plumule length, radicle length, seedling length, seedling fresh and dry weights, vigor index, salt tolerance index (0 ppm) of the birdsfoot trefoil compared to control applications and increase the average germination time. When the findings are evaluated in their entirety, they show that the germination and seedling parameters considered together with the increase in salt concentration are also adversely affected, and this negative effect increases as the salt concentration increases. It has been observed that the germination rate of birdsfoot trefoil, especially at 10000 ppm, which is the highest salt concentration, is significantly reduced. The first negative effect of salt stress in terms of germination rate was seen after 5000 ppm, but it is thought that when plants complete germination and have poor development in their first development cycle, they may not maintain their subsequent vegetative development well and may cause a decrease in their yield. As a matter of fact, salt stress causes the sensitivity of plants to biotic stressors that they will encounter in their future development periods to increase. As a result of the study, although the germination rate in the birdsfoot trefoil can be tolerated up to 5000 ppm, it was seen that other parameters decreased as the salt dose increased according to the control application. It has also been determined that birdsfoot trefoil can tolerate salt doses of 250 ppm, 750 ppm, 2500 ppm. At the same time, it is thought that in the areas where the salinity problem of the studied variety is experienced, it will be important to determine the yield and quality performance in field conditions in terms of evaluating the salty areas. However, in order to make healthier recommendations, it will be useful to carry out new studies carried out in pot and field conditions of this study.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	S.B.	M.Y.	A.N.K.
C			100
D			100
S			100
DCP	25	25	50
DAI	25	25	50
L			100
W			100
CR			100
SR			100
PM			100
FA			100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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THE EFFECT OF REGION ON NUT AND BIOCHEMICAL TRAITS OF MINCANE HAZELNUT CULTIVAR

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Abstract: This study investigated the effect of region on the nut and biochemical traits in Mincane hazelnut cultivar. The study was carried out in the Trabzon (Black Sea Region) and Sakarya (Marmara Region) districts, in 2021 and 2022. The material of the study consisted of the nut of Mincane hazelnut cultivar grown in both regions. Depending on regions, nut weight ranged from 1.89 (Black Sea) to 2.14 g (Marmara), while kernel weight ranged from 0.96 (Black Sea) to 1.06 g (Marmara). The nuts obtained from the Black Sea region yielded the highest total phenolics (118.1 mg 100 g^{-1}). Marmara region's nuts had the highest total flavonoids (8.1 mg 100 g^{-1}) and antioxidant activity (1027.8 and 738.1 µmol 100 g^{-1} according to DPPH and FRAP assays, respectively). The results demonstrated the significance of the growing region on the investigated nut and biochemical traits and the superiority of the Marmara region on many quality traits.

Keywords: *Corylus avellana*, Antioxidant, Phenolic, Kernel weight, Nut size

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1. Introduction

The hazelnut is one of the most important nut species. Approximately 1.1 million tons of hazelnuts are produced on 1 million hectares (ha) in the world. Türkiye is leads the world in hazelnut production, with 665 thousand tons on an area of 734 thousand ha. Italy, USA, Azerbaijan are other important hazelnut producers (Anonymous, 2022a). The Black Sea Region has the most suitable ecological conditions for hazelnut cultivation in the Türkiye (Serdar and Demir, 2005; Ercisli et al., 2011). Hazelnut is cultivated in the area up to 80 km inland and up to 1000-1200 m altitudes in the region. The cultivation zones are classified as old and new production zones (Karadeniz et al., 2009). Tombul, Palaz, Çakıldak, Mincane, Foşa and Kalınkara cultivars are common grown in the old production areas, whereas Çakıldak, Mincane, Foşa and Karafındık cultivars are grown in the new production areas.

Hazelnut is essential in human nutrition and health, with high nutritional value thanks to being rich in fat, protein, vitamins, carbohydrates, minerals, fatty acids, phenolics, and antioxidants (Cosmulescu et al., 2013). It promotes human health and reduces the risk of many chronic diseases such as cancer, diabetes, neurodegenerative and inflammatory, especially cardiovascular diseases (Contini et al., 2011; Di Nunzio, 2019). Hazelnut also lowers the risk of heart disease and the adverse effects of hypertension due to the mono and polyunsaturated fatty acids it contains (Yücesan et al., 2010).

Many factors such as genetic structure (Balta et al., 2006), ecology (Amaral et al., 2010; Karakaya, 2022), technical and cultural practices (irrigation, fertilization, pruning etc.) (Bak and Karadeniz, 2021), harvest time (Cristofori et al., 2015), storage conditions (Turan and İslam, 2018), altitude and orchard direction (Bostan, 2003; Beyhan et al., 2011; Kul, 2020) affect the chemical composition of hazelnut. In particular, ecological conditions impact primary and secondary metabolites (Bacchetta et al., 2013; Mezni et al., 2018). Phenolics, flavonoids, and antioxidants in Çakıldak vary significantly depending on eco-geographic regions (Kul, 2020). Similar phenomena were reported in different hazelnut cultivars (Bacchetta et al., 2018; Karakaya, 2022).

Very few studies have been conducted on the effect of region on the biochemical characteristics of widely grown hazelnut cultivars in Türkiye. These studies include mainly the Tombul, Çakıldak, and Kalınkara cultivars (Bostan, 2003; Kul, 2020; Karakaya, 2022). No studies evaluated the effects of the growing region on biochemical properties in the Mincane, widely grown in both old and new production areas. The sole research on this cultivar determined the vitamin and mineral content (Açkurt et al., 1999). This study investigated the effect of growing regions on the nut and biochemical traits in Mincane hazelnut cultivar.

2. Material and Methods

2.1. Plant Materials

The research was conducted in Trabzon (Black Sea Region) and Sakarya (Marmara Region) provinces, in

2021 and 2022. The material of the study was Mincane hazelnut cultivar grown in the regions. The trial orchard (about 50 years old, 610 m altitude) in the Trabzon was established in the 'Ocak' (8-10 stems per 'Ocak') training system in south direction, and were planted spacing 3 m in the row and 3.5 m between rows. The trial orchard (about 35 years old, 150 m altitude) in the Sakarya was established in the 'Ocak' (9-12 stems per 'Ocak') training system in south direction, and were planted spacing 3.5 m in the row and 4 m between rows. In the trial orchards, technical and cultural practices (except irrigation) were regularly performed. A total of 350-450 g compound fertilizers [N-P-K (12-18-12) + (10 SO_3) + 1 Zn + 0.2 B] and 500-600 g nitrogen (N) were supplied per 'Ocak' in the orchards. Chemical control was performed to against the diseases and pests. Weed control was carried out twice a year and branch thinning was performed in the winter period.

In the Trabzon region, the average annual temperature is 8.9 °C. The warmest month is August (18.1 °C), and the coldest month is January (-1.7 °C). The minimum temperature is between -5.2 °C (January) and 15.1 °C (August). The maximum temperature is from 2.4 °C (January) and 21.4 °C (August). Annual rainfall is 1492 mm and relative humidity is 81%. In the Sakarya region, the average annual temperature is 12.5 °C. The warmest month is August (22 °C), and the coldest month is January (3.2 °C). The minimum temperature is between 0.1 °C (January) and 18.5 °C (August). The maximum temperature is from 6.7 °C (January) and 25.1 °C (August). Annual rainfall is 953 mm and relative humidity is 79% (Anonymous, 2022b).

2.2. Methods

The research was designed according to the randomized blocks experimental design with three replications and three 'Ocak' in each replication. At harvest time (in 10-15 August according to regions and years), approximately 500 g of nut were collected from each 'Ocak'. Harvested nut were naturally dried until the moisture content decreased to 6% after being separated from their husk. Then, the nut and biochemical properties were studied.

Thirty nut were used in nut traits evaluation. Nut and kernel weight were determined using a digital precision balance (Radwag, Poland). The shell thickness, nut and kernel dimensions were measured with a digital caliper (Mitutoyo, Japan). The kernel ratio and nut and kernel size were calculated using the following equation 1, 2 and 3 (Balta et al., 2018a; Guler and Balta, 2020):

$$\text{Kernel ratio} = (\text{kernel weight}/\text{nut weight}) \times 100 \quad (1)$$

$$\text{Nut size} = \sqrt{\text{kernel dimensions (length, width, thickness)}} \quad (2)$$

$$\text{Kernel size} = \sqrt{\text{kernel dimensions (length, width, thickness)}} \quad (3)$$

Total phenolics and total flavonoids were determined by the method of Yılmaz et al. (2019), using a UV-Vis spectrophotometer (Shimadzu, Japan) at 760 nm and 415 nm, respectively. The results for total phenolics and total flavonoids were expressed as mg 100 g^{-1}. The antioxidant activity was detected by modifying the DPPH and FRAP assays previously described by Blois (1958) and Benzie and Strain (1996), reading the absorbances of in the spectrophotometer (Shimadzu, Japan) at 517 nm and 700 nm, respectively. The quantities for DPPH and FRAP were expressed as µmol 100 g^{-1}.

2.3. Statistical Analysis

JMP 14 (trail) statistical software was used in the statistical analysis. Data from two consecutive years were averaged, and the LSD multiple comparison method was used to determine the differences between the means. The PCA analysis was used to examine the interrelations between traits and the growing regions illustrated by a biplot graph (Putra et al., 2020).

3. Results

The region significantly affected nut and kernel weights, shell thickness, nut width and thickness, kernel width and size, while the effect on kernel ratio, nut length and size, kernel thickness and length traits was insignificant ($P<0.05$). The highest nut and kernel weight (2.14 g and 1.06 g, respectively) were determined in the Marmara region. In contrast, the highest kernel ratio (50.7%) and the thinnest shell (1.07 mm) were obtained from the Black Sea region (Table 1).

The highest values in nut dimensional characteristics were determined in the Marmara region, except for nut and kernel lengths. The nut size ranged from 16.15 mm (Black Sea) to 16.68 mm (Marmara), while the kernel size was determined as 12.57 mm (Black Sea) and 13.05 mm (Marmara) (Table 1).

The region significantly impacted bioactive compounds ($P<0.05$). The nuts grown in the Marmara region possessed the highest bioactive compounds, except for total phenolics. Total phenolics were 105.0 mg 100 g^{-1} in the Marmara and 181.1 mg 100 g^{-1} in the Black Sea, while total flavonoids were 3.0 mg 100 g^{-1} in the Black Sea and 8.1 mg 100 g^{-1} in the Marmara (Table 2).

The DPPH assay determined antioxidant activity as 128.8 µmol 100 g^{-1} in the nuts grown in the Black Sea and 1027.8 µmol 100 g^{-1} in the nuts grown in the Marmara. The antioxidant activity was determined as 152.4 µmol 100 g^{-1} in the nuts obtained from the Black Sea and 738.1 µmol 100 g^{-1} in the nuts of the Marmara region by the FRAP assay (Table 2).

According to principle component analysis, the first two components explained 87.9% of the total variation in the data. PC1 accounted for 72.8% of the total variation and was highly related to nut and kernel weight, shell thickness, nut and kernel dimensions (except for kernel length), bioactive compounds. PC2 was related to nut and kernel length traits, and accounted for 15.1% of the total variation. When evaluated by regions, the Black Sea region grouped with nut length, kernel length, kernel ratio, and total phenolics, while many other traits were grouped with the Marmara region (Figure 1).

Table 1. Mincane hazelnut cultivar's nut traits grown in the Black Sea and Marmara regions

Traits / Regions	Black Sea	Marmara	Significance	LSD (0.05)
Nut weight (g)	1.89 b*	2.14 a	*	0.18
Kernel weight (g)	0.96 b	1.06 a	*	0.09
Kernel ratio (%)	50.7 a	50.0 a	ns	1.32
Shell thickness (mm)	1.07 b	1.23 a	**	0.08
Nut width (mm)	16.27 b	16.94 a	*	0.45
Nut thickness (mm)	14.09 b	15.11 a	*	0.64
Nut length (mm)	18.38 a	18.14 a	ns	1.07
Nut size (mm)	16.15 a	16.68 a	ns	0.55
Kernel width (mm)	12.54 b	13.11 a	*	0.48
Kernel thickness (mm)	11.04 a	11.89 a	ns	0.88
Kernel length (mm)	14.34 a	14.28 a	ns	0.55
Kernel size (mm)	12.57 b	13.05 a	*	0.46

*The difference in between same letters in the same row is statistically insignificant (P<0.05).

Table 2. Biochemical properties of Mincane hazelnut cultivar grown in Black Sea and Marmara regions

Traits / Regions	Black Sea	Marmara	Significance	LSD (0.05)
Total phenolic (mg 100 g^{-1})	181.1 a*	105.0 b	***	15.3
Total flavonoid (mg 100 g^{-1})	3.0 b	8.1 a	***	0.31
DPPH (µmol 100 g^{-1})	128.8 b	1027.8 a	***	72.3
FRAP (µmol 100 g^{-1})	152.4 b	738.1 a	***	182.1

*The difference in between same letters in the same row is statistically insignificant (P<0.05).

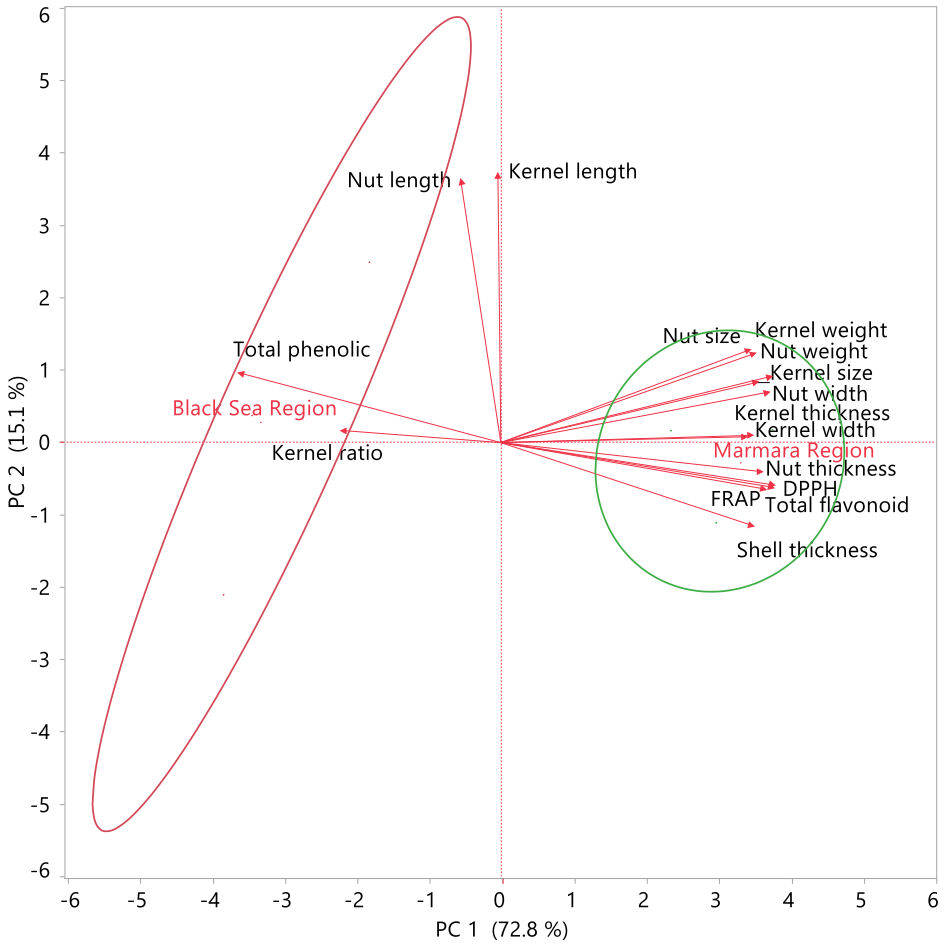

Figure 1. Biplot graph based on nut and biochemical traits of Mincane hazelnut cultivar.

4. Discussion

Nut and kernel weight, shell thickness, and kernel ratio are crucial quality traits in hazelnut (Balta et al., 2018b). Thin-shell and high kernel ratio are desirable in the hazelnut processing industry. Large-sized nut is evaluated for in-shell consumption, while small and medium-sized nut are used in confectionery industry. Many factors such as genotype, variety, ecological conditions, technical and cultural applications (fertilization, irrigation, pruning etc.) are effective on these properties (Beyhan and Demir, 2001; Külahcılar et al., 2018; İslam and Çayan, 2019; Bostan and İsbakan, 2020). In the current study, while region had a significant effect on nut and kernel weight, shell thickness, nut thickness and width, kernel width and size, it had no effect on kernel ratio, nut length and size, kernel length and thickness. Except for kernel ratio, nut and kernel dimensions (length, width, and thickness), no study has been found that investigated the effect of region on other traits in Mincane hazelnut cultivar, and it has been reported that the investigated traits vary significantly depending on the region. The highest kernel ratio and nut dimensions were reported in the west Black Sea region (Köksal et al., 2012). In Kalınkara cultivar grown in Ordu, Samsun, and Sakarya region, the highest nut and kernel weight, shell thickness, kernel ratio, nut and kernel dimensions values were found in Sakarya (Marmara region) (Karakaya, 2022). In the current study, except for kernel ratio, the highest values were recorded in the Marmara region in terms of other traits investigated. Although the highest kernel ratio was found in the east Black Sea region, it was statistically insignificant. Except for kernel ratio, the results obtained for other nut traits similar with the findings of the researchers. The observed differences in terms of kernel ratio could be attributed to environmental conditions, cultivar, cultivation conditions (Balta et al., 2018b; Gülsoy et al., 2019; Karakaya, 2022).

Phenolic compounds are secondary metabolites. They are crucial for the determine health benefits and quality of fruits, and influence sensory traits such as taste, aroma, color of foods (Haminiuk et al., 2012). Furthermore, polyphenols are beneficial to human health and play a crucial role in disease prevention. The synthesis of primary and secondary metabolites is influenced by climatic conditions (Bacchetta et al., 2013; Mezni et al., 2018). Phenolic synthesis increased is associated with extreme temperatures, UV radiation, parasite and pathogen damage (de Abreu and Mazzafera, 2005; Del Valle et al., 2020; Ozdemir et al., 2022; Karakaya et al., 2023).

In the current research, the influence of region on bioactive compounds was significant. Except for total phenolics, the highest bioactive compounds values were determined in the Marmara region. Although the influence of region on the vitamin and mineral content of the Mincane hazelnut cultivar has been studied (Açkurt et al., 1999), no research on the influence of the region on bioactive compounds has been conducted. Researchers reported that the vitamin and mineral content varied significantly depending on the region, and the highest vitamin and mineral values determined in the eastern and western Black Sea regions, respectively (Açkurt et al., 1999). Bioactive compounds have been found to differ in Tombul cultivar grown at different altitudes in the same location (Şengül, 2019). Similar findings were reported in the Çakıldak cultivar (Kul, 2020). In the Kalınkara cultivar grown in different regions, the highest total phenolics and total flavonoid were reported in Sakarya (Marmara), and the highest antioxidant was reported in Ordu (Black Sea) (Karakaya, 2022). Also, Solar et al. (2022) reported that phenolics and flavonoids in the Barcelona, Pauetet, Merveille de Bollwiller, Tonda di Giffoni, and Tonda Gentile delle Langhe hazelnut cultivars varied significantly depending on the region. In the current study, except for total phenolics, the highest bioactive compounds values were determined in the Marmara region. Except for total flavonoids, the results obtained for other bioactive compounds differed from the findings of Karakaya (2022). The observed differences could be attributed to environmental conditions, cultivar, cultivation conditions (Yılmaz et al., 2019; Tonkaz et al., 2019).

5. Conclusion

The region significantly influenced the nut traits and bioactive compounds of the Mincane hazelnut cultivar. The Marmara region produced the best results in terms of nut and kernel weight, which are crucial quality characteristics of hazelnut. The Black Sea region had the best kernel ratio and shell thickness. Except for total phenolics, the highest bioactive compounds were determined in the Marmara region. Overall, the best results were found in a Mincane cultivar grown in the Marmara region in many traits. These findings provide essential information for the hazelnut industry and processors in Trabzon and Sakarya, where the Mincane cultivar is widely grown.

Author Contributions

The percentage of the author contributions is present below. The author reviewed and approved final version of the manuscript.

	O.K.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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Research Article
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PARTIAL PURIFICATION OF CATALASE ENZYME FROM MUSCLE TISSUE OF DUSKY SPINEFOOT (*Siganus Luridus*)

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Abstract: Catalase, one of the antioxidant enzymes, decomposes hydrogen peroxide into water and oxygen. From the discovery of catalase, several studies have been carried out to reveal its importance in health, food and cosmetics industries etc., and these studies are still ongoing. In this study, catalase enzyme was partially purified from muscle tissue of Dusky spinefoot (*Siganus luridus*). Purification procedure consisted of homogenate preparation, ammonium sulfate precipitation and dialysis. The enzyme was precipitated in the range of 40-60 % Ammonium Sulfate concentration. The optimum buffer was determined as 200 mM phosphate buffer, optimum pH 8.0 and optimum substrate concentration 24mM for H_2 and O_2, respectively.

Keywords: Purification, Catalase, Characterization, Enzyme

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1. Introduction

Oxidative substances such as reactive oxygen species and free radicals and their undesirable biological effects are eliminated by enzymatic and non-enzymatic antioxidant defence systems. Enzymatic defence is provided by many enzyme systems such as glutathione peroxidase, glutathione reductase, superoxide dismutase, glutathione S-transferase, catalase and DNA repair enzymes. Non-enzymatic antioxidant defence systems include transferrin, lactoferrin, ceruloplasmin, uric acid, GSH and cysteine. Antioxidants are substances that can scavenge for free radicals and prevent cell damage (Shinda et al., 2012, Gelen et al., 2021, Ustundag et al., 2021).

The defence systems that function in the organism to prevent the formation of reactive oxygen species ward off the damage caused by these substances, and provide detoxification are called "antioxidant defence systems" or "antioxidants".

Catalase (CAT: EC 1.11.1.6) is a characteristic enzyme abundant in cells. This enzyme is widely present in animals, plants and microorganisms. It also plays an essential role in eliminating toxic hydrogen peroxide from cells (Masters et al., 1977).

Catalase enzyme catalyzes the removal of H_2O_2, one of the reactive oxygen species, which causes cellular damage, has a toxic effect and is one way to water. The enzyme uses H_2O_2 as a substrate, both as an electron acceptor and electron donor (Lanir and Schejter, 1975; Jones and Masters, 1976).

Siganus luridus is one of two species of siganus on Israel's Mediterranean coast. Both are migratory fish from the Red Sea described by Ben-Tuvia. This species migrated from the Red Sea along the Suez Canal to the Mediterranean, where it was first recorded in 1956 along the respective coasts of Israel. Later, they spread rapidly to the west and north and became widespread in Lebanon, the Turkish Republic of Northern Cyprus, the shores of Türkiye, Rhodes and the Central Aegean Sea. Since then, they have established significant populations in the Mediterranean and have acquired high economic importance. There are two species belonging to the *Siganidae* family in our country (*S. luridus* and *S. rivulatus*). The primary food of these two species in the *Siganidea* family is algae. The most defining feature that distinguishes these two types from each other is; *that S. luridus* has a caudal fin close to a flat shape, while *S. rivulatus* has a forked caudal fin. The length of both species can be around 25-30 cm. *S. luridus* has spines on its dorsal and ventral fins. These sharp and strong spines are covered with mucus mixed with venom and can cause painful wounds. They may lose their colour at dusk and change colour if threatened, but usually have a brown back, light brown abdomen and fine yellow stripes on both sides. They generally prefer coastal waters that are not deeper than 40 meters and on the rocks covered with vegetation. They live their entire lives without migrating to distant places or leaving their areas (Castriota and Andaloro, 2008).

2. Material and Methods

In this study, 8 grams of muscle tissue of the used Dusky spinefoot was weighed and used to prepare homogenate.

The muscle part taken from the fish was thoroughly pounded in a mortar with the help of liquid nitrogen and turned into flour, and the prepared homogenate was taken into a 50 ml tube and made up to 40 ml by adding 200 mM KH_2PO_4+0.5 mM EDTA+%PVP (pH: 8). It was then centrifuged for 20 minutes at 15000 g at 4 °C.

Solid ammonium sulfate precipitation process was applied to homogenates obtained from muscle tissue of Dusky spinefoot in the range of 0-20%, 20-40% and 40-60%, respectively. In this process, the homogenate is kept in ice and placed on a magnetic stirrer. Then, solid ammonium sulfate was carefully added to the homogenate at intervals of 45 seconds, not exceeding 1 gram. Centrifugation was performed for 10 minutes at 10000 g at 4 °C at each concentration interval.

Dialysis is performed to remove salts from the protein solution. In this study, dialysis was applied to remove the salt around the precipitated proteins at 40-60% ammonium sulfate concentration. For dialysis, 200 mM phosphate buffer was prepared, and the sample containing the precipitated proteins was placed into the membrane and left in the prepared buffer solution for 2 hours to remove the salts around the proteins.

Tris and phosphate buffers were prepared at 10mM, 50 mM, 100 mM, 200 mM and 300 mM concentrations, and activity measurements were made with these buffers at different concentrations.

In order to determine the optimum pH after buffer characterization, phosphate buffer was prepared at pH ranges of 5.5-6, 6.5-7, 7.5-8, 8.5-9 and activity measurement was made at these intervals.

In order to determine optimum substrate concentration, activity measurements were made for optimum substrate concentration using 3 mM, 6 mM, 12 mM, 18 mM, and 24 mM H_2O_2 substrate.

Determination of the optimum enzyme concentration was carried out by observing activity measurements using 25µl, 50µl, 100µl, 150µl and 200µl of enzymes.

3. Results

Ammonium sulfate was precipitated in the ranges of 0-20%, 20-40% and 40-60%, respectively, into the homogenate prepared for purifying catalase enzyme from the muscle tissue of Dusky spinefoot. In the results obtained after the activity measurements, it was determined that the value giving the highest activity was between 40-60% ammonium sulfate concentration.

KH_2PO_4 measurements were made at different concentrations to purify catalase enzyme from Dusky spinefoot muscle tissue. As a result of the activity measurements, it was determined that the most suitable ionic strength for catalase enzyme from stingray tissue was in 200 mM KH_2PO_4 buffer.

Catalase enzyme activity in shaded Dusky spinefoot muscle tissue was measured by spectrophotometer at pH 5.5-9.0 using 200 mM phosphate buffer. The optimum pH value of catalase enzyme obtained from Dusky spine foot muscle tissue was determined as pH 8.0 in 200 mM phosphate buffer.

The optimum substrate amount for catalase enzyme was measured using 200 mM KH_2PO_4 (pH 8.0) between 3-24 mM from Dusky spinefoot tissue. The optimum amount of substrate was determined as 24 mM.

4. Discussion

Catalase is one of the important antioxidant enzymes that significantly reduces oxidative stress by destroying cellular hydrogen peroxide to produce water and oxygen. It is assumed that catalase deficiency is associated with the pathogenesis of many age-related degenerative diseases such as diabetes, hypertension, anemia, vitiligo, Alzheimer's disease, Parkinson's disease, bipolar disorder, cancer and schizophrenia. Therefore, efforts are being made in many laboratories to investigate its use as a potential drug in treating such diseases (Nandi et al., 2019). In the process of partial purification of catalase enzyme from Dusky spinefoot (*Siganus luridus*) muscle tissue, the optimum ionic strength was determined in 200 mM potassium phosphate buffer. Maximum protein precipitation in ammonium sulfate precipitation was determined in the range of 40-60% concentration. Optimum pH: 8, optimum substrate amount was determined as 24 mM. When we compare the results of some studies in the literature on the purification process of the catalase enzyme; Optimum ionic strength was determined in 50 mM potassium phosphate buffer in the purification of catalase enzyme from hazelnut fungus (*Lactarius pyragalus*). Maximum protein precipitation in ammonium sulfate precipitation was determined at 60% concentration. Optimum pH determined as 8 (Sökmen and Ahıskalı, 2017). In the purification process of catalase enzyme from walnut (*Juglans regie*), maximum protein precipitation was determined at 40% concentration in ammonium sulfate precipitation process. Optimum pH is determined as 8 (Akar, 2015). In the purification process of catalase enzyme from celery (*Apium graveolens*), optimum ionic strength was determined in 50 mM potassium phosphate buffer. Maximum protein precipitation in ammonium sulfate precipitation was determined at 30% concentration. Optimum pH determined as 7.5 (Güngör, 2015). Our study is in accordance with the studies in the literature.

5. Conclusion

We aimed in this study for purification of catalase enzyme from Dusky spinefoot, determination of structural properties and characterization. We hope that results of this study will contribute to the elucidation of the function of the catalase enzyme in Dusky spinefoot, we think that our research can help future studies on catalase enzyme and antioxidant enzyme systems.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	B.Ç.	S.B.	D.E.
C	10	10	80
D			100
S			100
DCP	25	25	50
DAI	30	30	40
L	30	30	40
W	30	30	40
CR	30	30	40
SR	30	30	40
PM	30	30	40
FA	30	30	40

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans. In the study, muscle samples were used as experimental materials, which were used in the B.Sc. Graduation Thesis of first author entitled "Purification of the catalase enzyme from the muscle part of Dusky spine foot (*Siganus luridus*)" and stored at -80 °C. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to.

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Research Article
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INTESTINAL FATTY ACID BINDING PROTEIN GENE VARIATION IN EUROPEAN SEA BASS (*Dicentrarchus Labrax*)

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Abstract: Fatty acid binding proteins (FABPs), which belong to the multigene family, play an important role in homeostasis, lipid uptake and transport in tissues. Intestinal fatty acid binding protein (I-FABP) is a small cytosolic protein and is highly active in intracellular fatty acid metabolism in fish gut. The European Sea bass (*Dicentrarchus labrax*) is an important commercial marine fish species in the Mediterranean region. In the present study, the partial I-FABP gene region of European sea bass was sequenced for detecting single nucleotide polymorphism (SNP) using DNA sequencing. We identified one SNP (g.2450T>C) in the noncoding region of the I-FABP gene in European sea bass. In this study, the relationship between the g.2450T>C locus of the I-FABP (fabp2) gene and body length, post-anal length, body weight and fillet weight was found significant (P<0.05). According to these results, the g.2450T>C locus in I-FABP which could affect growth and muscle fat content, can be used for marker-assisted selection (MAS) studies in European sea bass.

Keywords: SNP, FABP2 gene, Teleost, Variation

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1. Introduction

Growth and development in animals are affected by the intracellular transport of long-chain fatty acids (FABPs) which are special-formed proteins of the lipid-binding protein (LBP) family (Besnard et al., 2002; Kaitetzidou et al., 2015; Zhang et al., 2019). FAPBs, long-chain fatty acids, consists of 126 to 137 amino acids and their molecular weight is around 150 kDa (Chen and Shi, 2009; Venold et al., 2013). These proteins are involved in fatty-acid metabolism, including absorption, transportation and regulation of the concentration gradient across cellular membranes (Wang et al., 2005). Total of 12 FABP genes have been identified in vertebrate and invertebrate species (Kaitetzidou et al., 2015; Venkatachalam et al., 2017; Zhang et al., 2020). These proteins were named according to the tissue from which they were initially isolated, e.g. liver type fatty acid-binding protein (L-FABP), brain-type fatty acid-binding protein (B-FABP), intestinal-type fatty acid-binding protein (I-FABP or *fabp2*), etc. (Alves-Costa et al., 2008). The intestinal fatty acid binding protein (I-FABP) gene is involved in the synthesis, uptake and intracellular transport of triglyceride-rich lipoproteins in the intestine (Chen and Shi, 2009; Levy et al., 2001). The fatty acids and dietary lipids supply most of the energy needed for vital activities such as the growth, development, swimming and reproduction of fish (Andre et al. 2000). I-FABP is expressed in many tissues such as intestine, brain and muscle in fish (Sharma et al., 2004; Venold et al., 2013; Zhang et al., 2019).

Improving growth and meat quality characteristics is the primary focus of livestock and fish breeding studies. Single nucleotide polymorphisms (SNPs) in candidate genes that regulate yield traits are identified and widely used as markers in breeding studies, for example, gene mapping, association analyses, etc. (Wang et al., 2014). Although a significant correlation between I-FABP gene and fatty acid content in cattle has not been reported before, this gene has been reported as a candidate gene for meat quality as a result of the genome-wide association study (GWAS) (Dawood et al., 2021). Besides, there are significant associations between the single nucleotide polymorphisms (SNPs) of the I-FABP gene and growth characteristics of fish species such as growth, body thickness and etc. (Xia et al., 2013; Zhou et al., 2019).

European sea bass, which belongs to the Moronidae family, lives from the north-eastern Atlantic Ocean to the Mediterranean and the Black Sea (Vandeputte et al., 2019). Türkiye was the largest producer of farmed sea bass and the largest exporter of sea bass products in the worldwide. Türkiye has produced 149.000 tonnes European sea bass in 2020 (FAO, 2022). World aquaculture production of European sea bass was

276.000 tonnes in 2020 (FAO, 2022). Increasing the growth rate and muscle fat content of *D. labrax* stocks is important for sustainable aquaculture. Molecular markers are very effective tools in breeding programs of aquaculture species.

The aim of this study is to reveal the SNPs in the I-FABP gene region by DNA sequencing method and their associations with the growth traits of 80 European sea bass individuals.

2. Materials and Methods

A total of 80 European sea bass samples were randomly taken from a processing factory in Urla- İzmir. These fish samples were reared in the same cage environment from Çeşme-İzmir. The standard length (SL, cm), head length (HL, cm), body length (BL, cm), pre-anal length (PAL, cm), abdominal length (AL, cm), post-anal length (POSTAL, cm), head width (HW, cm), body width (BW, cm), total weight (TW, g) and fillet weight (FW, g) of fish samples were measured. Muscle tissue samples were collected from each fish and preserved at -20°C until DNA isolation. Genomic DNA was extracted by using the High Pure PCR Template Kit (Roche, Germany) following recommended protocols in the Ege University, Faculty of Fisheries, Laboratory of the Molecular Genetics and Fish Breeding. The concentration and purity of the genomic DNA samples were measured by spectrophotometer (MaestroGenNano).

The primer sequences of partial region of I-FABP gene were designed for European sea bass based on whole genome shotgun sequence (Accession number CBXY010015347) using Primer-BLAST algorithm (https:// www.ncbi.nlm.nih.gov/tools/primer90blast/) from GenBank using the Primer3 program (http://bioinfo.ut.ee) (NCBI, 2022). Primer sequences of I-FABP gene are F: 5'- TCCAGGGTGCGGAATTTACT -3' and R: 5'- CCTTCAACGGCAACTGGAAA -3'. PCR was performed in a 50-µL volume containing 50 ng genomic DNA, 0.5 µM of each primer, 2× MyTaq Mix (Meridian Bioscience, USA), 0,5 U Taq Hot Start DNA (Bioline) polymerase and distilled water. The thermal profile consisted of initial denaturation at 95°C for 4 min; 37 cycles of amplification, including 95°C for 45 s, 56°C for 45 s and 72°C for the 90 s and final extension at 72°C for 10 min. The PCR products were checked on 2% agarose gel using horizontal electrophoresis and the gels were stained using RedSafe (iNtRON) (Figure 1).

The genotyping of the SNPs in the partial region of the I-FABP gene was performed by 3500XL Genetic Analyzer System (Applied Biosystems, USA). The sequence results were aligned and controlled by ChromasPro Version 2.1.10 (Technelysium Pty. Ltd. Australia). Differences of gene sequences between individuals were detected using BioEdit (Hall, 1999). The Hardy–Weinberg equilibrium of the population was estimated using the 'HardyW'einberg' package in R (R Core Team, 2013). The associations between genotypes, haplotypes and growth traits were analysed (via SPSS Inc. V. 18.0, IBM, Chicago, IL, 2009) using the general linear model (GLM; equation 1) and an alpha value of 0.05 was considered significant.

$$\text{Linear Model I} = Yjk = \mu + Gj + ejk \quad (1)$$

Where;

Yijk represents the traits; µ represents the intercept; Gj represents the fixed effect of the I-FABP genotype or each haplotype and eijk is the random error. The significance of differences between genotypes of the locus was determined using Bonferroni multiple range test. The thresholds for significant differences were $P<0.05$.

The sequence data obtained for I-FABP gene region and the reference sequences taken from GenBank were used in the reconstruction of the phylogenetic tree based on Maximum Likelihood (ML) method applying HKY nucleotide substitution model. The nucleotide substitution models were selected based on the results obtained from ModelTest implemented in the software MEGA. In order to test the reliability of the tree topology, bootstrapping (×1000) was performed. Phylogenetic evolutionary analyses of I-FABP gene region of European sea bass were conducted using MEGA version 11 (Tamura et al., 2021).

3. Results and Discussion

European sea bass I-FABP gene contains four exons and three introns, that encode 132 amino acids (KJ130030) (NCBI, 2022). The genetic variation at 754 bp of the partial I-FABP gene was amplified by PCR and it was shown in Fig. 1. The amplified gene region in this study is located between 1845 and 2598 bp in the reference sequence (CBXY010015347) (NCBI GenBank).

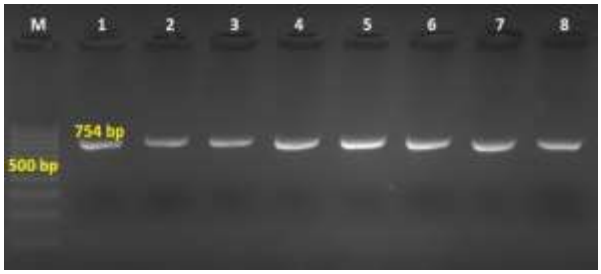

Figure 1. Electrophoresis image of the PCR amplicons of I-FABP gene for the 8 European sea bass samples. M= marker.

In this study, PCR products of the I-FABP gene region were investigated with the Sanger sequencing method and a g.2450T>C change was detected in the noncoding region (Figure 2). Generally, in the literature more polymorphisms are found in intronic regions because they are under less selection pressure than exonic regions of genes (Özcan Gökçek and Işık, 2020; Tran et al., 2021). Moreover, SNPs in non-coding regions can affect transcription and translation of mRNA splicing and regulate gene expression (Pagani and Baralle, 2004). Gene expression level should be analyzed, to detect if it is affected from SNP or not. In this study, the g.2450T > C

locus of I-FABP gene was in Hardy–Weinberg equilibrium. The genotype and allele frequencies of the European sea bass I-FABP gene were shown in Table 1.

In the current study, we detected significant associations between the g.2450T>C locus of the I-FABP gene and body length, post-anal length, body weight and fillet weight of European sea bass (P<0.05) (Table 2). According to the results, the CC genotype is superior for all these traits. Similarly, in Asian sea bass (*Lates calcarifer*), Xia et al. (2013) reported that a SNP (SNP1245) in the exon 3 of the IFABP-a gene has a significant relationship with the growth characteristics of 6- and 9-month-old fish by using QTL mapping and association analysis. It has been reported that heterozygosity (CG) is quite high in the large-size fish group for the IFABP-SNP1245 locus (P<0.01). Besides, Wan et al., (2018) performed a genome-wide association study (GWAS) for highly unsaturated fatty acids (HUFA) and eviscerated weight (EW) traits in the large yellow croaker (*Larimichthys crocea*) population. They reported that the FABP gene is one of the candidate genes associated with n-3 HUFA and EW traits in large yellow croacker. Also, Zhou et al. (2019) found that I-FABPa gene on chromosome 24 affects the growth and body thickness (BT) of *L. crocea* with GWAS. The I-FABP gene has the potential to be a candidate gene that affects growth due to its role in the regulation of vertebrate and fish metabolism, such as digestion and intracellular transport of dietary fatty acids (Sharma et al., 2004; Xia et al., 2013).

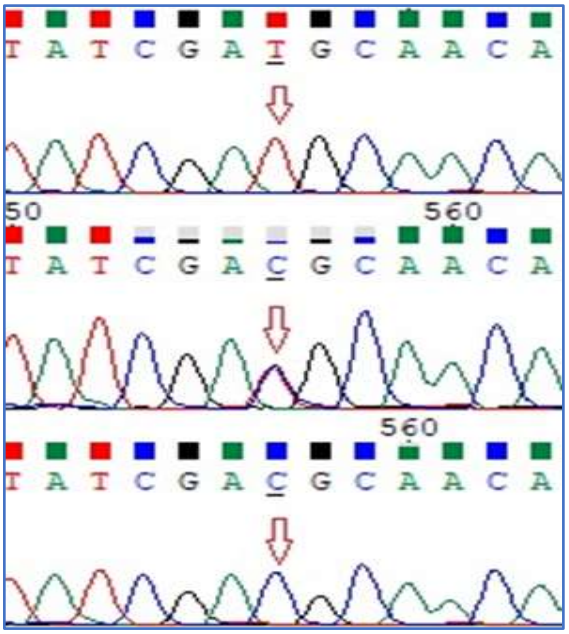

Figure 2. Partial chromatogram for SNPs g.2450T>C in noncoding region of I-FABP gene.

Table 1. Allele and genotype frequencies of I-FABP gene region in European sea bass

Loci		I-FABP Genotypes			Allele Frequency		χ2*
		TT	TC	CC	T	G	
g.2450T>C	O	50	25	5	0.78	0.22	0.58
	E	48.83	27.34	3.83			

*χ2 (0.05; 1), P<0.05

Table 2. Associations between g.2450T>C genotypes of growth traits of *Dicentrarchus labrax* (mean±SE).

	I-FABP Genotypes			
Trait	TT	Y	CC	P*
SL	29.03 ± 0.52	30.87 ± 0.47	32.68 ± 0.78	0.012
HL	7.90 ± 0.17	8.33 ± 0.19	8.59 ± 0.23	0.178
BL	21.16 ± 0.38[a]	22.64 ± 0.37[a]	24.19 ± 0.63[b]	0.005*
PAL	21.11 ± 0.42	22.29 ± 0.38	23.65 ±0.83	0.043
AL	13.49 ± 0.27	14.26 ± 0.26	15.40 ± 0.62	0.038
POSTAL	8.94 ± 0.16[b]	9.68 ± 0.19[a]	10.08 ± 0.28[a]	0.006*
HD	4.96 ± 0.10	5.35 ± 0.12	5.30 ± 0.18	0.063
BD	8.66 ± 0.16	7.85 ± 0.14	7.32 ± 0.35	0.005
BW	434.91 ± 22.38[a,b]	531.37 ± 21.98[a]	676.90 ± 16.46[b]	0.000*
FW	226.05 ± 11.61[a]	284.40 ± 11.27	362.98 ± 7.73[b]	0.000*

*Values with different superscripts (a, b) within the same row differ significantly at P<0.05. SL= standard length, HL= head length, BL= body length, PAL= pre-anal length, AL= abdominal length, POSTAL= post-anal length, HD= head depth, BD= body depth, TW= total weight, FW= fillet weight.

According to ML analysis of the evolutionary relationship of the I-FABP sequences obtained from the present study with the other fish species retrieved from GenBank are shown in Figure 3. The ML tree based on HKY nucleotide substitution model revealed *D. labrax, Epinephelus fuscoguttatus, Larimichthys crocea* and partial region (1845-2598) of *D. labrax* whole genome shotgun sequence in the same clade. Kaitetzidou et al. (2015) identified that European sea bass I-FABP gene has 2 paralogs, fabp2a and fabp2b. In this study, the fabp2a gene region was amplified which localized on LG7.

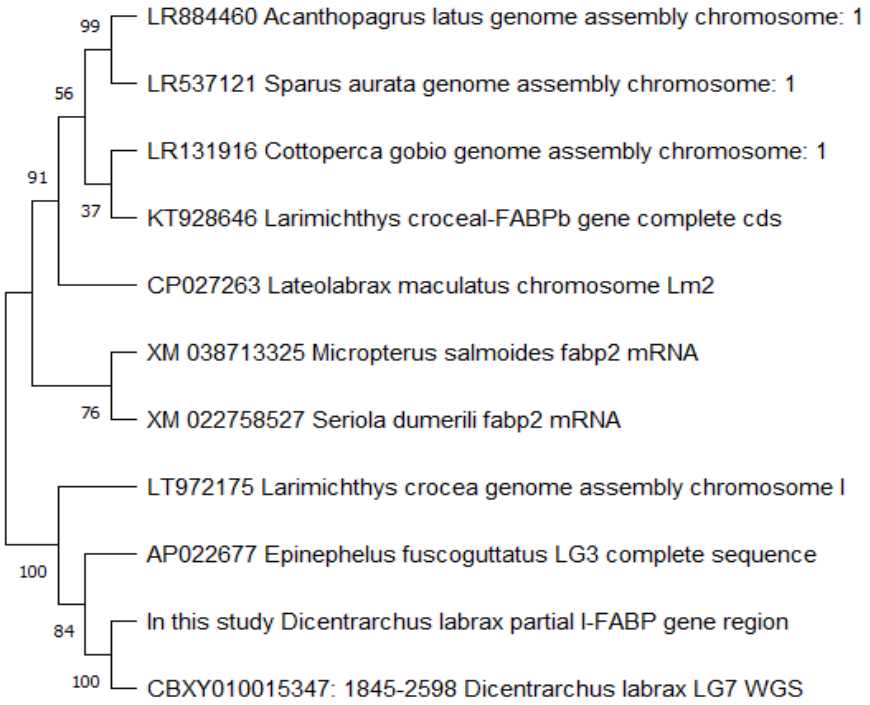

Figure 3. The phylogenic tree of the I-FABP (fabp2) gene sequences retrieved from GenBank database for different species (*Dicentrarchus labrax, Lateolabrax maculatus, Acanthopagrus latus, Epinephelus fuscoguttatus, Sparus aurata, Larimichthys crocea, Cottoperca gobio, Micropterus salmoides, Seriola dumerili, Larimichthys crocea*).

4. Conclusion

The SNP (g.2450T>C) detected in the current study and potential SNPs that can be found in other regions of the I-FABP gene of European sea bass and their relationships with harvest traits such as growth, muscle fat content and body thickness should be investigated with large sample size. The results of this study show that the I-FABP (fabp2) gene has a high potential for MAS studies.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	E.Ö.G.	R.I.
C	50	50
D	50	50
S	50	50
DCP	50	50
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on live animals or humans. Ethics committee approval was not obtained because of the dead fish samples were taken from a private facility that breeds and processes fish for commercial purposes. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to.

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Research Article
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E-FOOD TRADE EFFECTS ON PURCHASING APPROACH REGARDING CUSTOMER BEHAVIOR

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Abstract: Our world is changing too fast. The distances have been shortened and communication has been increased by the invention and widespread use of the internet. Our habits started to change in many aspects of our lives by the development of digitalization. Especially during the pandemic Covid-19 that has started at the end of 2019, it's observed that mobility of people have been decreased and however, they began to have difficulties in meeting the needs. There has been a great demand for companies that sell over the internet. In particular, the tendencies of people to purchase food items which are their vital need over the internet have increased to a great extent. Looking at the e-commerce market on a sectorial basis during the pandemic, the biggest increase was seen in e-food trade with a growth of approximately 400%. Within that period, it is predicted that our purchasing habits will change rapidly and virtual markets will become much more important in our lives. The delivery of food products to the consumer has always been a big problem. The risk of deterioration of the products in a short time and the physical destruction of the products during their transportation has increased the importance of food logistics. The logistics channels are needed to be developed in order to increase e-food trade. For this reason, contemporary logistics channels have started to be used by integrating developed logistics applications to the traditional logistics channels. It will be much faster and cost-efficient to deliver the products to the consumer with the new logistics channels in the near future. Yield penalty due to transportation will decrease, and since the number of stock brokers is reduced, the consumer will be able to reach the product they demand at a more affordable price from the producer that they have chosen. In the future, physical stores will be replaced by logistics-supported virtual markets. The agriculture and food sector should also start working in order to accommodate quickly to the new trade order.

Keywords: Online purchasing, E-trade, Consumer, Contemporary logistics

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1. Introduction

In the modern world, agriculture and food trade is changing rapidly and e-commerce usage rates of consumers are increasing. E-Food trade also has an important share in the food market. Especially, due to increase of widespread use of the internet, the consumers' desire for reaching needed food products with a fair price and faster grows. In accordance with the impact of technological innovations, the development of the impact of product packaging facilities and logistics companies in product distribution accelerates the delivery of products produced in the field to the consumer. Price advantages arise in selling of a product by effective use of logistics, since the transportation network between the producers through the consumer is shortened.

Wen (2007), the most important effect of electronic commerce is that it provides direct communication between the manufacturer and the customer by creating a disintermediation. In this way, the intermediates are eliminated, transaction costs are reduced and the farmers are able to achieve high profit rates at low prices. It has been stated that by virtue of information technologies, a market which is based on effective and competitive prices has emerged in terms of electronic commerce. By this way, the food products can reach the consumer much faster. For this reason, the rate of deterioration resulting from product storage conditions is reduced. Thus, a positive contribution is made to the countries' economy by decreasing the casualty rate.

Leroux et al. (2001) Three main factors that have an impact on business-to-business (B2B) electronic commerce are stated as the structure of the agricultural industry; the complexity of the product and the role of face-to-face consumer communication in trade. The research which states that consolidation is an important situation in the agricultural industry, stated that this may have a negative impact on electronic commerce.

In addition to all these, the consumer also has a more affordable price for the product. Also, the rate of being unrecorded work in the food economy reaches a minimum level with the development of e-food trade. This situation increases the tax collection rate; as well as contributes to the effective implementation of agricultural policies.

It is aimed to improve the necessary conditions for our

country to play a more active role on a global scale in the changing and developing e-food trade nowadays, and to pave the way for the food products which are produced in our country to open up to the world markets more effectively with the spread of e-food trade. By virtue of the future outlook perspective that will be revealed by the results of this study, public or private agricultural executives in our country will have the opportunity to act early in shaping agricultural policies. It will be possible for larger domestic companies to emerge in the global market.

Canavan et al. (2007) investigating the marketing of specialty food products over the internet in Ireland, the most common use of the internet was; information, communication and shopping; It was stated that internet use for purchasing food is not common. Specialty foods are defined as unique food products that are produced in small quantities and are not subject to mass production, and they can be attractive in terms of sales over the internet. After the widely using e-retailing Kızılaslan and Gönültaş (2011) emphasized that there are new developments, that with the introduction of the internet into this field, new supply chains are formed, the transportation sector has developed and access to agricultural information has become easier. While the increasing and spreading information affects the expectations in the market, new approaches should be put forward to meet these expectations. Researchers emphasized that; electronic commerce applications in the field of agriculture will develop in Türkiye by means of elimination in taxation, governmental infrastructure and other obstacles.

Logistics management is one of the most important factors of e-food trade. In our constantly developing world, it is necessary to plan the flow of goods, services, information and capital simultaneously in business life. These plans can be made by using complex information, communication and control systems simultaneously by the force of logistics.

2. Materials and Methods

Research data collected online from consumer groups (by considering gender, age, occupation, etc.) who have purchasing potential for e-trade products within the municipality of Istanbul. A form consisting of 15 questions that includes gender, age, marital status, occupation, education level, number of household members, household monthly income, e-food shopping status information, reasons for choosing e-commerce, product information bought online through e-commerce, online shopping payment types, online shopping cost information, and another form consisting of 26 questions as Consumer Survey were applied to consumers which are included in the study and recorded. The study was carried out between November 2019 and December 2020.

According to the statistical analysis used in the study; after the surveys and data which were used in the research were collected and checked, the analysis phase has been carried out. Statistical package program was used for data analysis. In the first stage of the analysis, the reliability analysis of the scale was carried out. Cronbach's Alpha technique, which is an internal consistency coefficient technique, was used in order to calculate the reliability of the scale.

The summary statistics of the qualitative variables were given as frequency and percentage, and besides the analysis of whether the quantitative variables fit the normal distribution was made with the "Shapiro-Wilk Test".

Summary statistics of quantitative variables are given as mean (±) standard deviation for those have normal distribution, and median and min – max values for those don't have normal distribution. In the analysis of the significance of quantitative variables according to qualitative variables, the analysis between two groups that were not normally distributed was made with the "Mann-Whitney U Test", and the analysis between more than two groups was made with the "Kruskall-Wallis Test" (Önder, 2018).

In order to find out which group was significant after the significance detected in multiple groups, one of the subgroup tests, the "Homogeneous Subset Differences Test (HSD Test)", was applied (Genç and Soysal, 2018). "Spearman's Rho Rank Difference Correlation Coefficient" was used in the analysis of two numerical variables that were detected as inconsistent to normal distribution. In the interpretation of the correlation coefficient, "very weak correlation if <0.2", "weak relationship if between 0.2-0.4", "moderate relationship if between 0.4-0.6", "if between 0.6-0.8, high-grade relationship" criteria, if "0.8> very high-grade relationship" criteria were used. The significance level was taken as $P<0.05$; $P<0.01$ and bidirectional hypothesized.

3. Results and Discussion

In recent years, online food trade (e-food trade) has gained importance and its prevalence has increased considerably. Especially the great developments in the technological infrastructure over time and the increase in the use of computers in Türkiye have brought the popularity of online food shopping applications to the agenda. Supports and actions to be taken in order to boost the e-food trade will create a potential that will further expand the use of the internet in the field of food.

The majority (68.6%) of the consumers participating in the research are men. There are various studies in the literature similar to our findings. It was stated that 53.4% of the consumers were male and 46.6% were female in the field study in which the factors determining consumer behavior in online shopping in our country and the applicability of the theory of planned behavior were examined (Turan, 2011). In another study, Akçi and Annaç (2015) examined approaches to e-commerce perceptions of consumers and found that 53.1% of the

participants were male and 46.9% were female. In a study examining the attitudes of consumers shopping online in the province of Tokat, it was stated that male participants were 73% (Sayılı and Büyükköroğlu, 2012).

It was determined that the majority of the consumers that had participated in the research were under the age of 40 (54.9%). Ayden and Demir (2011) stated that most of the participants were young in their study, in which they examined how consumer preferences and behaviors are shaped in e-commerce. In another study in the literature, Saygılı and Büyükköroğlu (2012) analyzed the factors affecting the attitudes of consumers shopping online and stated that the majority of consumers (67%) were under the age of 40.

It is seen that there are other studies in the literature that are similar to the findings of the study (Aksoy, 2006; Turan, 2011; Akçi and Annaç; 2015, Tatlı and Korkut, 2015). The marital status of the consumers participating in the research was questioned and it was found that the rate of married people were 66.9%. When the literature is examined, Farinnia (2011) stated that in her study which had examined online shopping attitudes of consumers; married consumers were more than single consumers.

Majority (76.7%) of the consumers participating in the research had bachelor's degree and master's degree education levels. When the studies in the literature are examined, Candan and Kurtuluş (2003) stated in their study that 65% of internet shoppers were those with bachelor's degree education and 35% were those with master's degree education. Akçi and Annaç (2015) in their study in which they examined the attitudes of consumers living in the eastern region of eastern Türkiye about e-commerce, stated that 93% of the participants had bachelor's degree and master's degree education levels. In the light of the findings, it can be said that consumers with a high level of education make online purchases because they are in the computer age and due to the average age of these consumers, they grow together with technology.

Regarding the number of household members, more than half (54.7%) of the respondents in the research have 4 or more family members. In a study examining the food purchase attitudes of consumers through e-commerce, it was stated that the majority of consumers (78%) had 3 or more family members (Sayılı and Büyükköroğlu, 2012).

Approximately half (51.1%) of the consumers have 7500 TL or more household monthly income. In a study searching how behaviors of the consumers are shaped in e-commerce, it is stated that most of the participants have middle and high income levels (Ayden and Demir, 2011).

It has been determined that 36.2% of the consumers that has participated in the research shop online once or twice a month; the vast majority (55.6%) do not shop for food online, while the remaining consumers shop for food once a week to once or twice a year. If they had the opportunity to do all their food shopping online, 33.3% of the participants stated that they would do it. It was stated that more than half of the participants (50.4%) preferred traditional e-commerce sites in the preferred e-commerce shopping models. As the second model, it was determined that 36.4% of the participants preferred shopping through the official websites of the company. Participants did shopping mostly for clothing (41%), secondly for travel tickets (12.7%), and thirdly for electronic products (10.6%) and 87.9% of consumers paid 750 TL or less for online shopping. Almost all of the consumers (96.4%) stated that they take customer comments during online shopping. Tatlı and Korkut (2015) examined the e-commerce usage of consumers in their study and found that less than half of the participants (45%) do not use online shopping; the vast majority of consumers (94%) do online shopping monthly and annually; the most purchased products from the internet are respectively clothing, technological products, books, magazines and other tools. In another study that has surveyed online shopping, the products purchased by the majority of the participants were electronic goods, car accessories and cassette-cd, on the other hand, it was stated that the rate of those who done online shopping for food was low (12%) as mentioned in this study. In another study examining the rate of online shoppers, it was stated that the majority of consumers (54%) did not prefer to online shopping (Turan, 2011). In another e-commerce study conducted in Bursa, it was stated that more than half of the participants did online shopping at least once and they wanted to do other food shopping online also (Özkan, 2012). In a study conducted by TÜSİAD, it was determined that the majority of consumers (62%) bought clothing and sports equipment (Kantarcı et al., 2017). When all this literature review is examined, it is seen that these studies that has been carried out, support the findings.

It has been indicated that the majority of the consumers that has participated in the research have adopted the online payment method with a credit card for their internet commerce; and secondly, the cash on delivery method. In a study conducted in Iran by Farinnia (2011), he stated that the most used method in e-commerce is the cash on delivery option.

The importance given to e-commerce by the consumers participating in the research was determined as the reliability / awareness of the e-commerce site, the price of the product and the delivery time of the product, respectively. Papathanassiou et al. (2003) in a field study in Greece, they determined that the most important issues for consumers in online food shopping are products' delivery on time, product quality and product warranty disclaimer

It has been determined that according to the reasons why the consumers prefer online shopping, the product mostly preferred (54.4%) when the it is found at a discount or low priced, and secondly (24.9%), it is preferred when they do not have time to buy it from

stores. Uzel and Aydoğdu (2010) in their study in which they examined the reasons for preferring e-commerce, stated that there are reasons such as the convenience provided to people who are short of time, the convenience of online shopping and the opportunity for shopping at any time of the day.

It was determined that the majority of the consumers (68.6%) who participated in the research would like to order natural and organic products over the internet if they had the opportunity to order, and the reasons for preferring online shopping for organic products were determined as natural village products, local products, pulses and cereals, respectively.

The reliability of the consumer questionnaire applied to the consumers participating in the research was obtained as (96.3%) and explanatory factor analyzes were carried out in this direction. As a conclusion of the explanatory factor analysis, it was determined that the scale was divided into three factors and the rate of explanation of the scale by these factors was 74.6%.

Scale factor levels were named based on the meanings of the items. Within this context, the first factor level was named “Customer Expectations”, the second factor level was named “Web Site Features” and the third factor level was named “Customer Satisfaction”. The averages of the factor levels were examined and it was determined that the consumers agreed with the factor levels.

After the factor levels of the consumers that has participated in the research were created, their statistical relevance was examined and there were statistical differences between "Customer Satisfaction" according to age groups, "Customer Expectations and Website Features" according to occupational groups, "Customer Satisfaction" according to the number of people in the household, and there were statistical differences between factor levels. It was found that there were moderate correlations.

In this section, the findings regarding the sub-problems created according to the problem status of the research conducted by including 417 consumers who meet the inclusion criteria in the study were evaluated on 185 consumers who stated that they shopped online for food.

Reliability, which is one of the most important features that measurement tools should have, is an indicator of the stability of measurement values obtained in repeated measurements under the same conditions with a measurement tool (Öncü, 1994). The reliability of the scales is examined in different ways. The alpha coefficient method developed by Cronbach (1951) is a technique that is frequently used to estimate the internal consistency of especially likert-rated scales. The reliability analysis of the data obtained by the application of the Consumer Questionnaire used in the research to 185 consumers was examined and given in Table 1.

The reliability analysis of the consumer survey, which included 26 statements, was made on the data and it was found to be 0.963 (Table 1) and showed that the scale was quite reliable.

Table 1. Findings on the reliability of the consumer survey

Cronbach's Alpha	Item
0.963	26

Exploratory Factor Analysis (EFA) is a multivariate analysis technique that allows the interpretation of a large number of variables that are thought to be related with a smaller number of variables or variables that cannot be directly observed (Şencan, 2005; Çolakoğlu and Büyükekşi, 2014).

Before the EFA phase, the convenience of the data for factor analysis was tested with the Kaiser-Mayer-Olkin (KMO) and Bartlett Tests. The KMO value is defined as moderate between 0.5 and 07, good between 0.7 and 0.8, very good between 0.8 and 0.9, and excellent above 0.9 (Çolakoğlu and Büyükekşi, 2014). The findings regarding the convenience of the consumer questionnaire for factor analysis are given in Table 2.

Table 2. KMO and Bartlett test results of the consumer survey

KMO		0.960
Bartlett's Test	Chi-square	7828.339
	SD	190
	P value	<0.001

In this study, the KMO value was obtained as 0.960 (Table 2). In other words, it was determined that the sample size of the study was sufficient. With the Bartlett test which performed as to measure the normal distribution level, the result was 7828.339 and it was found to be statistically significant at the P=0.000<0.01 level. Therefore, it was decided that the data set used in the study was suitable for EFA and the analysis phase was started.

In this framework, Principal Components Analysis (PCA) was conducted in the first stage to determine the factor structure of the scale. Due to convenience of the scale for a three-factor structure, the Varimax rotation technique was used as the rotation technique. Accordingly, after removing the items 1, 2, 8, 21, 22, and 23 with an item load value below 30 (Kalaycı, 2006), the process was repeated on the remaining 20 items, and the results are given in Table 3.

As a result of EFA, 3 factors were obtained explaining 74.602% of the total variance with factor loads above 30 and eigenvalues above 1. The resulting factors were named as “customer expectations”, “website features” and “customer satisfaction”. The factor loads of the items constituting these dimensions are given in Table 3.

The analysis findings of the data obtained by the application of the Consumer Questionnaire used in the research to 185 consumers are given in this section. The intervals were used in the evaluations made according to the arithmetic averages (Balcı, 2001).

When the average and standard deviations of the

answers given to the sub-factors of the consumer survey according to the gender of the consumers participating in the study were examined; the average of men's responses to the "Customer Expectations" sub-factor is determined as 4.22±0.91, the average of their responses to the "Website Features" sub-factor is determined as 3.82±0.97; the average of their responses to the sub-factor "Customer Satisfaction" is determined as 3.60±1.10; the average of women's responses to the "Customer Expectations" sub-factor is determined as 4.20±0.98, the average of their responses to the "Website Features" sub-factor is determined as 3.76±0.92, the average of their responses to the "Customer Satisfaction" sub-factor is determined as 3.77±1.11 (Table 4).

When the average and standard deviations of the answers that are given to the sub-factors of the consumer survey by the age groups of the consumers who participated in the study are examined; the average of the responses of consumers who are 40 years old and under to the "Customer Expectations" sub-factor is determined as 4.17±1.01; the average of their responses to the "Website Features" sub-factor is determined as 3.79±0.96; the average of their responses to the "Customer Satisfaction" sub-factor is determined as 3.77±0.14; the average of the responses of consumers who are over the age of 40 to the "Customer Expectations" sub-factor is determined as 4.26±0.84; the average of their responses to the "Website Features" sub-factor is determined as 3.82±0.95; the average of their responses to the "Customer Satisfaction" sub-factor is determined as 3.51±1.04.

Table 3. The sub-dimensions of the consumer survey obtained as a result of the EFA

	customer expectations	website features	customer satisfaction
Q13- If the order processing error of the company is too much, such as missing or wrong product, it affects my shopping decision.	0.827		
Q5- The fact that the product that is not practically in stock, seems to be being prepared and afterwards cancellation of my order negatively affects my attitude towards the company.	0.808		
Q9- Mistakes that made in the image of the product while defining on the shopping site will negatively affect my shopping satisfaction.	0.780		
Q4- Proper operation of the return procedure increases my e-shopping frequency and amount.	0.773		
Q11- I expect the product to be picked up from my home by a free courier during the course of product return.	0.762		
Q3- I would like to be able to monitor the status of my order online.	0.759		
Q12- If the product is sent wrong; I expect the mistake to be compensated with opportunities such as gift certificates, discount coupons and free shipping.	0.740		
Q6- The fact that the product is delivered on the date and time which I determined, increases my online shopping frequency.	0.704		
Q10- I prefer shopping sites that have flexible rules for product returns and exchanges.	0.693		
Q16- The site I shop online has superior knowledge of the goods and services it offers.		0.803	
Q17- This site, which offers online shopping service, strives to achieve excellence.		0.786	
Q18-. The wide range of products and services offered by the online retailer fulfil my needs.		0.731	
Q20- The design of shopping sites usually looks nice.		0.725	
Q15- The product and service I buy online is of the promised quality and at the right price.		0.723	
Q19-. My preferred shopping sites not only sell me products and services, but also entertain me.		0.718	
Q14-Generally, I am satisfied with the prices of the site I shop from.		0.689	
Q25- I enjoy recommending shopping sites and products to others.			0.810
Q24- I think that the best way to get information about the products/services of shopping sites is by using product reviews, surveys, or chat rooms.			0.729
Q26- If I am satisfied with the shopping site service, I try to respond with feedback.			0.728

Table 4. The mean and standard deviations of the responses of the consumers to the sub-factor levels of the consumer survey according to their demographic data

	Customer Expectations	Website Features	Customer Satisfaction
Gender	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$
Male	4.22±0.91	3.82±0.97	3.60±1.10
Female	4.20±0.98	3.76±0.92	3.77±1.11
Age			
40 years of age and under	4.17±1.01	3.79±0.96	3.77±0.14
Over 40 years of age	4.26±0.84	3.82±0.95	3.51±1.04
Occupation			
Government official	4.41±0.73	4.00±0.80	3.73±1.02
Worker	3.93±1.12	3.54±1.12	3.43±1.21
Not working	4.37±0.76	3.89±0.74	4.01±0.93
Educational Status			
High School – Associate's Degree	4.13±1.04	3.87±1.03	3.83±1.16
University (Bachelor's Degree)	4.09±1.07	3.70±1.05	3.55±1.16
Postgraduate	4.44±0.52	3.93±0.72	3.73±0.95
Household number of persons			
4 ve above	4.38±0.69	3.96±0.76	3.84±0.93
3 ve below	4.03±1.12	3.63±1.11	3.44±1.23
Household Monthly Income Level			
7500 TL ve below	4.15±1.07	3.74±1.04	3.62±1.21
7500 TL above	4.26±0.82	3.85±0.88	3.68±1.01

Table 5. Comparison of consumers' responses to consumer survey sub-factor levels according to demographic data

	Customer Expectations	Website Features	Customer Satisfaction
Gender	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$
Male	4.50 (1.00-5.00)	4.00 (1.00-5.00)	3.67 (1.00-5.00)
Female	4.40 (1.00-5.00)	3.86 (1.00-5.00)	4.00 (1.00-5.00)
P value	P^a=0.991	P^a=0.548	P^a=0.235
Age			
Age of 40 and under	4.50 (1.00-5.00)	3.86 (1.00-5.00)	4.00 (1.00-5.00)
Over 40 years of age	4.40 (1.00-5.00)	4.00 (1.00-5.00)	3.67 (1.00-5.00)
P value	P^a=0.857	P^a=0.594	P^a=0.045*
Occupation			
Government official	4.50 (1.00-5.00)(Y)	4.00 (1.00-5.00)(Y)	4.00 (1.00-5.00)
Worker	4.20 (1.00-5.00)(X)	3.71 (1.00-5.00)(X)	3.67 (1.00-5.00)
Not working	4.50 (2.10-5.00)(XY)	3.86 (1.57-5.00)(XY)	4.33 (2.00-5.00)
P value	P^b=0.011*	P^b=0.020*	P^b=0.077
Education level			
High School – Associate's Degree	4.20 (1.00-5.00)	3.86 (1.00-5.00)	3.67 (1.00-5.00)
University (Bachelor's Degree)	4.50 (1.00-5.00)	3.86 (1.00-5.00)	3.67 (1.00-5.00)
Post-Graduate	4.45 (2.40-5.00)	4.00 (1.71-5.00)	3.83 (1.00-5.00)
P value	P^b=0.481	P^b=0.462	P^b=0.429
Household Number of Persons			
3 and below	4.40 (1.00-5.00)	4.00 (1.00-5.00)	3.67 (1.00-5.00)
4 and above	4.50 (1.00-5.00)	4.00 (1.00-5.00)	4.00 (1.00-5.00)
P value	P^a=0.064	P^a=0.195	P^a=0.049*
Household Monthly Income Level			
7500 TL and below	4.50 (1.00-5.00)	3.86 (1.00-5.00)	3.67 (1.00-5.00)
7500 TL above	4.40 (1.00-5.00)	4.00 (1.00-5.00)	3.67 (1.00-5.00)
P value	P^a=0.753	P^a=0.595	P^a=0.929

*P<0.05, **P<0.01. . [a]the difference between the two groups was evaluated with the Mann Whitney U test, [b]the difference between more than two groups was evaluated with the Kruskal Wallis test, Within-group comparisons were evaluated with the Homogeneous Subset Differences (HSD) Test for non-normally distributed data, X,Y= There is no difference between variables with the same letter.

According to the demographic data of the consumers who participated in the research, it was analyzed with the "Shapiro-Wilk Test" whether the sub-factors of the consumer survey were distributed normally or not, and it was determined that all sub-factors were not normally distributed (P<0.05) according to the demographic data.

With reference to the results of the normal distribution; data has been analyzed whether if there is a difference between the groups that are not normally distributed for two groups (gender, age group, number of households, household monthly income level) with the "Mann Whitney U Test"; has been analyzed with the "Kruskal Wallis Test" for those with more than two groups (occupation, education level); whether if there is a difference between the groups that are not normally distributed, data has been analyzed with the 'Homogenous Subset Differences Test (HSD Test)' and all the results are given in Table 5.

The comparison of the sub-factors of the Consumer questionnaire according to the gender of the consumers participating in the study was examined and it was determined that there was no statistically significant difference (P>0.05) between all sub-factors by gender (Table 5).

Determining that there was a statistically significant difference (P<0.05) between the "Customer Satisfaction" sub-factors according to age groups, and there was no statistically significant difference (P>0.05) between all other sub-factors (Table 5). When the medians of the groups that differ are examined, it can be said that the medians of the consumers aged 40 and below are higher than the medians of the consumers over the age of 40.

It was determined that there was a statistically significant difference (P<0.05), between the "Customer Expectations and Website Features" sub-factors, and there was no statistically significant difference (P>0.05) between the "Customer Satisfaction" sub-factors (P>0.05). When the sub-factors of "Customer Expectations and Website Features" are examined, it can be said that the difference between the two sub-factors according to occupational groups arises from the difference between worker consumers and civil servant consumers. There was no statistically significant difference (P>0.05) among all sub-factors according to educational status.

There was a statistically significant difference between the "Customer Satisfaction" sub-factor according to the number of people in the household (P<0.05), but there was no statistically significant difference (P>0.05) between all other sub-factors. When the medians of the groups that difference are examined, it can be said that the medians of consumers with a household number of 3 and below are less than the medians of consumers with a household number of 4 and above. There was no statistically significant difference (P>0.05) among all sub-factors according to household monthly income level.

The normality of the sub-factors of the consumer survey was examined and it was determined that the sub-factors of the consumer survey were not normally distributed (P<0.05). Therefore, whether there is a relationship between the groups that are not normally distributed was examined with the "Spearman's Rho Rank Correlation Coefficient" and the results are given in Table 6.

When the relationship between the sub-factors of the consumer survey of the consumers who participated in the study is examined, it is seen that there is respectively %56.1 and %50.1 degree positive moderate relationship between the "Customer Expectations" sub-factor and the "Website Features and Customer Satisfaction" sub-factors and was found to be significant.

There was a positive moderate correlation of 58.7% between the sub-factors of the consumer survey which are "Website Features" sub-factor and the "Customer Satisfaction" sub-factor, and it was found to be statistically significant (P<0.01).

Table 6. The relationship status of consumers among the sub-factors of the consumer survey

			Customer Expectations	Website Features	Customer Satisfaction
Spearman's rho	Customer Expectations	r	1.000	0.561	0.501
		P value		P_c=0.000**	P_c=0.000**
	Website Features	r			0.587
		P value			P_c=0.000**

*P<0.05, **P<0.01, the relationship between the two groups was evaluated with Spearman's Rho Rank correlation coefficient.

4. Conclusion

Today, a new world order is being reshaped with the understanding that "the only thing that doesn't change is change itself". It is predicted that much of what we know in the tradition will change step by step in the near future. Foremost among them is the preferences in the purchasing method. Meanwhile, it is assumed that the logistics methods, which are the most important factor in the delivery of products to the consumer, will develop by changing. The delivery of agricultural products to the market is done through traditional channels in the world. The intermediary institutions such as traders, cooperatives and markets at the head of these channels, provide the commercial link between the producer and the consumer. Since there is more than one channel in the current supply chain, the products coming from the manufacturer reach the consumer with a price increase of approximately 4 times higher. Considering the

production costs in this trade pattern, producers earn a lot fewer and the consumers reach the product by paying the ultimate price.

The logistics system has a vital importance in the delivery of agricultural products to the final consumer. Correspondingly with the development of logistics opportunities, it is under consideration that international trade will increase even more in the future. The countries which foresee this situation strengthen their logistics structures in order to transport the products they produce to other markets by using land, sea and air transportation. It is foreseen that logistics costs will be reduced in the near future with the R&D studies on the development of logistics, innovative applications, and the realization of ideas about the future of the logistics industry. Thereby when the logistics costs, which have a direct effect on the product price, are reduced, the consumer will be able to reach the product at a cheaper price.

According to the data in the survey conducted on the consumer, it is seen that the tendency to use e-food commerce is very high, especially due to the difficulty of reaching local and natural products. By means of e-food trade, an important link is established with the producers so that consumers can reach reliable products from the source. In this way, consumers have the opportunity to reach the quality products that they wanted, regional flavors and geographically indicated products, naturally grown products faster and safer. Small and medium-sized producers will have the chance to deliver the products they produce from their fields or production facilities to the consumers in a wide area. By virtue of e-food trade, the consumer will find the advantage of reaching the product at a more affordable price, as the intermediary institutions will be reduced through the trade established directly between the producer and the consumer.

According to the data in the survey conducted on the consumer, it is seen that the tendency to use e-food commerce is very high, especially due to the difficulty of accessing local and natural products. Thanks to the e-food trade, an important link is established with the producers so that consumers can reach reliable products from the source. In this way, consumers have the opportunity to reach the products they want, regional flavors, products with geographical indications, products grown naturally, faster and safer. By establishing close interaction with the producer and consumer e-food trade, there is an opportunity to make a longer-term trade by developing subscriber and/or member systems. Small and medium-sized producers will have the chance to reach the consumer in a very wide area for the products they produce from their fields or production facilities. With the e-food trade and the trade established directly between the producer and the consumer, the consumer will find the advantage of reaching the product at a more affordable price, as the intermediary institutions will decrease.

In the survey conducted with the producers, it is seen that 92% of the producers want to enter the e-food trade. Although manufacturers understand that future trading methods will change, they have realized that e-food commerce will occupy a very important place in their sales channels. Although state authorities have started studies on this issue, it seems that this is not enough. Although the Digital Agricultural Market project initiated by the Ministry of Agriculture is very important for the producers, the desired level of success has not been achieved due to the lack of technical infrastructure, lack of on-site packaging and packaging, and not integrating logistics channels into the system. It is seen that the market will develop very rapidly and in a much more inclusive way with the investments to be made by private companies in the e-food trade in the near future and with pioneering solutions.

The average age of the farmer engaged in agriculture is increasing day by day and this increase turns into a troublesome process for the future of agriculture. The average age of people engaged in agriculture needs to be rejuvenated. Agricultural policy practitioners are working on incentive packages for young farmers in this regard. However, since these are single-channel redirects, success has not been achieved. However, with the more active use of e-food trade, the income of the producers will increase and reverse migration to the villages will increase. Producers with increased income will increase the number of family workers for more production, and expand their production areas by renting neighboring or idle fields if necessary. These model applications will set an example for many people, and a certain part of the population that has accumulated in the city with the expectation of high income from the product they produce will be able to return to their land and start producing.

The spread of e-food trade is of great importance, especially for small-medium producers to compete with monopolistic markets. The legal arrangements made to prevent large producers from reaching the trade volume that will determine the market have not been very successful. Therefore, in order to protect small producers, the channels of reaching the market should be kept open at all times. With e-food trade, producers and farmers will be in contact with consumers without intermediaries. Keeping these channels open with investments by local governments, private companies and public institutions will produce positive results for all stakeholders. Agricultural policy practitioners need to make economic, legal and structural arrangements in order to expand this unity. E-food commerce can reach the desired level as a result of the data analysis and synchronized studies required for the creation of more effective agricultural policies. The development of e-Food trade will be the construction of the future for all agriculture and agriculture-related sectors. In this way, our country will have a stronger agricultural structure, and a process will emerge in which producers, intermediaries and consumers will gain optimum profit.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	R.E.	M.Ö.A.
C	50	50
D	50	50
S	50	50
DCP	50	50
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

This study was conducted with the approval of the Tekirdağ Namık Kemal University Ethics Committee of the Graduate School of Sciences (September 12, 2019/072), the questionnaires were conducted on a voluntary basis.

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GROWTH PERFORMANCE AND SOME SERUM, BONE AND FECAL PARAMETERS OF BROILERS FED WITH DIFFERENT LEVELS OF CALCIUM AND PHOSPHORUS

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Abstract: Aim of the study was to evaluate the effects of diet non-phytate phosphorus (P) and calcium (Ca) level on growth performance and some blood, bone and faeces parameters of broilers. A total of 480 one-day-old Ross 308 broilers were randomly allocated to 3 dietary treatments with 5 replicates for a 42-d study. Corn-soybean meal based diets were consisted of three different Ca and P concentrations for starter and grower periods. High, medium and low Ca and P levels in starter and grower periods were 1.05-0.49, 0.95-0.44, 0.85-0.41% and 0.87-0.42, 0.78-0.38, 0.69-0.34%, respectively. After the first 21-d feeding period, no differences were observed for feed intake (FI) but body weight (BW) and feed conversion ratio (FCR) were higher (P<0.05) in group fed 0.85-0.41% Ca and P. Diet with low Ca and P tended to increase final body weight of the chicks at 42 days old. No differences were observed for FI, FCR and mortality among the treatments. Different Ca and P levels had no effect on internal organ weights (P>0.05). Fecal ash, tibia and sternum weight and sternum ash were not affected from the Ca and P concentrations but tibia ash was lower (P<0.05) in group having low concentration of Ca and P. Decreasing levels of Ca and P had a negative effect on relative breast meat weight (P<0.05). There were no significant differences in Ca, P concentrations and aspartate transaminase (AST), alanine transaminase (ALT) activities in blood serum (P>0.05). However, alkaline phosphatase (ALP) enzyme activity was higher (P<0.05) in group having medium levels of Ca and P. Based on the data, it can be concluded that 8.5 g/kg Ca and 4.1 g/kg P can be used for starter period without any deterioration on growth performance.

Keywords: Broiler, Ca-P level, Growth performance, Serum parameters

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1. Introduction

The requirement of calcium (Ca) and phosphorus (P) of broilers has been the subject of research for decades. Studies conducted to determine Ca and P requirements of broilers has shown that demand of these minerals have changed with the development of genetic, nutrition and environmental conditions. Recent studies have reported that not only Ca and P concentration but also Ca and P ratio is essential for bone development and growth performance (Driver et al., 2005; Rama Rao et al., 2006; Selle et al., 2009; Han et al., 2016). The majority of P in plant source is present in the form of phytate, 28.2% of the total P is called nonphytate P (NPP) (Ravindran et al., 1995). Due to poor solubility, phytate is poorly absorbed by broilers (Wilkinson et al., 2014a). Plants are insufficient to provide the requirements of Ca and NPP for broilers therefore, some inorganic ingredient such as dicalcium phosphate (DCP) and limestone are added to broilers feeds. High acid binding capacity of the limestone leads to poor solubility of proteins and P in the gizzard (Walk et al., 2012). Decreasing Ca concentration of feed have led to develop P utilization but excess Ca decreased P concentration of bone ash (Letorneau-Montminy et al., 2008).

Phosphorus retention may be developed with the early restriction of Ca and NPP. This improvement arises from the adaptation ability of broilers to early restrictions and adaptation also may improve late period performance and P retention (Yan et al., 2005). Some studies have suggested that development of P utilization may be carried out with the arrangements of Ca concentration (Driver et al., 2005; Letourneau-Montminy et al., 2010; Roussea et al., 2012). Low Ca and narrow Ca:P ratios have improved P utilization (Qian et al., 1997; Liu et al., 1998; Brady et al., 2002; Selle et al., 2009). As stated before, diets having sufficient Ca and low NPP can cause similar performance values with diets having relatively high concentration of Ca and NPP (Rama Rao et al., 2006). It is generally accepted that increase of Ca in the diet may increase bone ash content (Driver et al., 2005). However, excess dietary Ca may have a negative effect on birds' performance (Sebastian et al., 1996).

Development of P utilization and determining sufficient concentrations of dietary Ca and NPP requirements for broilers should help to reduce the amount of dietary nonphytate phoshporus (NPP) supplementation needed and also reduce potential environmental pollution (Delezie et al., 2012). Broiler diets are typically formulated to involve between 8.0 and 10.0 g/kg Ca and 4.0 and 5.0 g/kg NPP but advancements in the birds and nutritional strategies indicate that old suggestion cannot be applied at the present time.

The present study was designed to investigate the effect of three different Ca and P levels for both starter and finisher periods on growth performance, bone development with some serum and fecal parameters.

2. Materials and Methods

2.1. Birds and Housing

Commercial feather-sexed 480 one-day-old Ross 308 broilers were randomly allocated to 3 dietary treatments with 5 replicates for a 42-d study in June 2016. Experiment was designed with completely randomized plot design. Chicks were kept at a temperature of 33°C for first day and afterwards this was gradually decreased to 22 °C and between 22 and 42 days 22 °C was maintained. Lightning regime of the study consisted of 23L:1D for 42 days. On day 10 and 14 birds were vaccinated against infectus bursal disease and Newcastle disease, respectively, through drinking water. Birds were checked four times in a day and mortalities were recorded. Feeding and husbandry conditions, except the Ca and P levels, were all same with the company, producing the genotypes.

2.2. Diets and Experimental Procedures

Commercial corn-soybean meal based starter and grower diets were consisted of three different Ca and P concentrations. High, standard and low Ca and P levels in starter and grower periods were presented in Table 1. Ca and P levels were reduced by degrees ranging from 10 to 11% to constitute experimental diets as presented Table 2.

Table 1. Experimental design

	Diet Ca -P levels (%)			
Groups	Starter diet		Grower diet	
High	1.05	0.49	0.87	0.42
Standard	0.95	0.44	0.78	0.38
Low	0.85	0.41	0.69	0.34

Table 2. Ingredients and chemical composition of starter and grower diets

Ingredients (g/kg)	Starter diet			Grower diet		
	High	Std.	Low	High	Std.	Low
Corn	575	571.46	577.96	610.71	612.81	615.7
Soybean Meal (48%)	360	362.11	360.83	314.71	320	320
Vegetable oil	21.58	29.09	27.04	35	35	35
Limestone	14	14	12.3	12.22	11	10
Dicalcium phosphate	18.5	15.5	13.5	14.85	12.38	10.5
Sodium chloride	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin-mineral premix[1]	2.5	2.5	2.5	2.5	2.5	2.5
DL-methionine	2.84	2.84	2.84	2.48	2.48	2.48
Lysine	2.5	2.5	2.5	2.5	2.5	2.5
Chemical composition(%)						
Dry matter	88.40	88.21	88.33	88.13	88.67	88.40
Crude protein	21.91	21.85	21.87	19.85	19.91	19.88
Ether extract	5.14	5.31	5.13	5.72	5.72	5.72
Crude fiber	3.63	3.60	3.61	3.42	3.46	3.47
Crude ash	5.84	5.80	5.71	5.75	5.41	5.14
Calcium	1.05	0.95	0.85	0.87	0.78	0.69
AME (kcal/kg)	3050	3050	3050	3200	3200	3200
Calculated composition(%)						
Available phosphorus	0.49	0.44	0.41	0.42	0.38	0.34
Lysine	1.40	1.40	1.40	1.27	1.27	1.27
Methionine+cystine	1.00	1.00	1.00	0.90	0.90	0.90

[1]Vitamin A, 12000 IU; Vitamin D_3, 2400 IU; Vitamin E, 30 IU; Vitamin K_3, 2.5 mg; Vitamin B_1, 3.0 mg; Vitamin B_2, 7 mg; Nicotin amid, 40 mg; Calcium D-pantothenate, 8.0 mg; Vitamin B_6, 4.0 mg; Vitamin B_{12}, 0.015 mg; Folic acid, 1 mg; D-biotine, 0.045 mg; Vitamin C, 50 mg; Chlorine chloride, 125 mg., Mn, 80 mg; Fe, 40 mg; Zn, 60 mg; Cu, 5 mg; Co, 0.1 mg; I, 0.4 mg; Se, 0.15 mg

A total of 480 one-day-old Ross 308 broilers were randomly allocated to 3 dietary treatments with 5 replicates for a 42-d study. Bird density was 13 chicks per square meter. Each pen was equipped with one hanging feeder. Feed, in mash form, and water were provided to birds as ad libitum throughout the trial. The chemical compositions of diets were determined according to the methods of AOAC (1990). Calculation of metabolic energy was done according to the Turkish Standards Institute (TSE) (1991). Starter and grower diets were offered to birds during days 1 to 21 and from days 22 to 42, respectively.

Feed intake (FI) and BW were recorded weekly. All birds were weighed individually at days 0, 7, 14, 21, 28, 35 and 42 to evaluate body weights. Feed intake was determined for each replicate at these days. Calculation of FCR was made via ratios of FI to BW gain on replicate basis. Mortalities were considered while FCR was calculating. At day 42, ten birds (1 male and 1 female from each replicate) representing average body weights of the group (±5%) were slaughtered from each group. Carcass yield, relative weight of the carcass cuts and internal organ weight were determined. Left tibias and sternums were individually removed and cleaned to determine bone weight and bone ash contents. Bones were waited at room temperature for 6 hours before analysis started. Each tibia and sternum were broken into small pieces, weighed and ashed at 600°C for 12 h.

At day 39, 40 and 41 about 200g faeces from each replicate were collected at the same time every day. Fecal samples were cleaned from all residues. Fecal samples were waited at 105 °C to determine dry matter and thereafter these samples were ashed at 600°C for 6 h to determine ash content.

Blood samples were collected by cardiac puncture and saved into empty collection tubes in order to obtain serum. Serum were separated by centrifugation at 1800xg and promptly analyzed. Serum Ca, P concentrations (Roche Diagnostics), aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) enzyme activities were determined with a spectrometers using commercial kits (Olympus AU-600).

2.3. Statistical Analysis

Data were analyzed by ANOVA using the Minitab 16 package program. An arc-sin transformation was applied to the percentage values (i.e. mortality) before testing for differences. Significant differences among means of treatments were determined by Duncan's multiple range test with 5% probability.

3. Results

Data regarding performance during the overall growth period (1 to 42 d) are presented in Table 3. Calcium and P levels had no significant effect on FI (P>0.05). Low Ca and P levels tended to decrease FI at 42 days of age. Body weight of 14, 21 and 28 days of age were effected from Ca and P levels (P<0.05), the highest body weight was obtained from group fed low Ca and P. Final body weight was not effected from Ca and P levels (P>0.05). Different Ca and P had no significant effect on mortality (P>0.05). Feed conversion ratios of 14, 21, 28 and 35 days of age had a significant difference (P<0.05) and the best FCR values were obtained from the group which was fed with low Ca and P.

Table 3. Effects of different Ca and P levels on feed intake (FI), body weight (BW) and feed conversion ratios (FCR)

Diet						
				FI, g		
				Day		
	7	14	21	28	35	42
High	139.2	529.0	1130.9	2031.0	3156.1	4512.3
Standard	139.3	526.0	1124.6	2050.0	3201.3	4598.4
Low	140.9	543.7	1144.0	2062.8	3163.1	4455.5
SEM	4.17	8,24	15.73	29.56	40.70	60.63
P value	0.954	0.319	0.693	0.787	0.751	0.341
				BW, g		
High	154.2	425.7^{b}	820.2^{ab}	1325.3^{b}	1925.5	2589.5
Standard	158.4	414.7^{b}	789.3^{b}	1313.6^{b}	1936.3	2626.3
Low	161.7	447.0^{a}	851.0^{a}	1394.3^{a}	1983.0	2762.0
SEM	2.17	5.01	10.00	16.13	27,80	54,53
P value	0.099	0.003	0.004	0.009	0.376	0.139
				FCR		
High	1.28	1.39^{b}	1.46^{b}	1.58^{ab}	1.67^{ab}	1.78
Standard	1.23	1.42^{a}	1.51^{a}	1.61^{a}	1.69^{a}	1.78
Low	1.21	1.35^{c}	1.42^{c}	1.52^{b}	1.63^{b}	1.71
SEM[1]	0.02	0.01	0.01	0.01	0.01	0.02
P value	0.440	<0.001	0.007	0.009	0.041	0.112

$^{a, b,c}$ Values within a column not sharing the same superscript are different at P<0.05, SEM= standard error of means.

Data regarding carcass weight, carcass yield and carcass parts are presented in Table 4. Calcium and P levels had no significant effect on carcass yield, thigh and wing weight (P>0.05). However, breast weight was effected from Ca and P levels (P<0.05). Low Ca and P levels caused the lowest breast weight. There was no significant difference between groups in that internal organ weights (P>0.05). Proportional internal organ weights were presented in Table 5.

Serum Ca and P concentrations and ALP, ALT and AST enzyme activity values were presented in Table 6. There were no treatment differences in serum parameters (P>0.05), with the exception of ALP enzyme activity. Medium Ca and P levels caused the highest ALP enzyme activity (P<0.05). There were no treatment differences in faeces ash and dry matter content (P>0.05).

The effects of different Ca and P levels on bone development are presented in Table 7. There were no significant differences in tibia and sternum weights (P>0.05).

Table 4. Carcass weight(CW), carcass yield(CY), proportional and real thigh, breast and wing weight of broilers fed different levels of Ca and P

	Low	Standard	High	SEM	P value
CY, %	76.8	76.5	75.7	0.51	0.355
Breast, %	36.3[b]	38.3[a]	38.3[a]	0.45	0.008
Thigh, %	27.5	26.8	26.4	0.43	0.296
Wing, %	10.0	10.0	9.9	0.20	0.899
CW, g	2125.4	2092.4	2059.1	84.66	0.467
Breast, g	773.7	803.9	790.3	56.16	0.493
Thigh, g	584.4	563.0	545.9	46.41	0.197
Wing, g	213.3	209.7	204.2	13.58	0.335

[a, b] Values within a column not sharing the same superscript are different at P<0.05, SEM= standard error of means.

Table 5. Effects of different Ca and P levels on proportional gizzard, liver, spleen, pancreas and intestine weights (Weight of organ/Body weight)

Diet	Gizzard	Liver	Spleen	Pancreas	Intestine
High	1.612	1.965	0.106	0.245	2.295
Medium	1.503	2.145	0.098	0.256	2.173
Low	1.515	1.864	0.108	0.230	2.438
SEM	0.04	0.08	0.01	0.02	0.10
P value	0.215	0.070	0.789	0.656	0.235

SEM= standard error of means.

Table 6. Effect of different Ca and P levels on serum calcium-phosphorus concentration and aspartate transaminase, alanine transaminase, alkaline phosphatase enzyme activity

Diet	Ca (mg/dL)	P (mg/dL)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
High	11.549	7.203	408.8	2.812	1415[b]
Medium	11.312	7.172	350.7	1.984	3199[a]
Low	11.345	7.054	378.2	1.912	1692[b]
SEM	0.17	0.17	32.73	0.40	253.60
P value	0.619	0.813	0.486	0.142	<0.001

[a,b]Values within a column not sharing the same superscript are different at P<0.05, SEM= standard error of means, AST= aspartate transaminase, ALT= alkaline transaminase, ALP= alkaline phosphatase.

Table 7. Effects of different Ca and P levels on tibia and sternum weights and ash contents

Diet	Tibia		Sternum	
	Weight, g	Ash, %	Weight, g	Ash, %
High	8.205	42.29[a]	3.656	40.71
Medium	8.518	41.67[a]	3.888	40.01
Low	8.424	37.52[b]	3.779	40.99
SEM	0.371	0.960	0.148	0.876
P value	0.835	0.008	0.563	0.737

[a, b] Values within a column not sharing the same superscript are different at P<0.05, SEM= standard error of means.

However, ash content of the tibia had a significant difference between groups (P<0.05). Tibia ash content decreased in proportion with Ca and P level. The lowest tibia ash content was obtained from group fed with low Ca and P. There was no significant difference in sternum ash content (P>0.05).

4. Discussion

It was stated before that not only the amount of the Ca and P in diet but also the ratio between these minerals are essential for performance and bone development of broilers (Driver et al., 2005; Han et al., 2016; Rama Rao et al., 2006; Selle et al., 2009). Amounts of the Ca and P were changed but the ratio between them was tried to be arranged similar in this experiment. It was known that reducing diet Ca and P level can let to obtain the similar performance values if the ratio between them is protected (Rama Rao et al., 2006). Feed intake is related to diet P level and high P and medium Ca levels were said to cause the best feed intake for broilers. In this experiment low starter and grower feeds containing, %0.85-0.69 Ca and 0.41-0.34 P respectively. These levels are similar with the stated values for the best performance and feed intake (Driver et al., 2005; Rama Rao et al., 2006; Hamdi et al., 2015; Rousseau et al., 2016). The highest body weights were obtained from the low Ca and P group at 14, 21 and 28 days of age. Delezie et al. (2012) achieved the highest body weight values with the similar Ca and P levels with this experiment at 14, 21 and 28 days of age. Hamdi et al. (2015) expressed that %0.90 Ca and 0.45 P provided the highest body weight at 14 days of age. Phosphorus digestibility is related to the diet Ca and P level and ratio between these minerals and this mechanism originates from the antagonistic relation in small intestine (Günther and al-Masri, 1988; Al-Masri 1995; Driver et al., 2005; Selle, 2009). Therefore, improvement of the growth performance can be a result of reducing Ca level, it was stated that, low Ca level led to increase availability of some other nutrients (Wilkinson et al., 2014b). Additionally, the results for feed conversion ratio are similar to the findings of earlier studies (Driver et al., 2005; Rama Rao et al., 2006; Delezie et al., 2015; Han et al., 2016).

No differences were observed in carcass yield and carcass parts with the exception of proportional breast weight. Birds fed low Ca and P had the lowest breast proportion of carcass. Age, genotype, sex are the most effective factors on carcass part weights but according to some results genotype and feed composition are both effective on carcass parts and weights (Corzo et al., 2005).

Viveros et al. (2002) determined that different Ca and P levels had a significant effect on internal organ weights. Our results contradict with this earlier study but it is possible to explain this contradiction with the variation of Ca and P level.

Increasing ALP enzyme activity can be explained with the osteoblast activity which is high in young, growing birds and birds, having bone deteriorations. Although it was stated that intestine isoenzyme has the greatest effect on the ALP activity (Campbel and Coles, 1986), ALP is rarity in liver tissue but liver problems lead to increase the activity of it (Zantop, 1997). In some earlier experiment, it was stated that ALP enzyme activity increased when the Ca and P level was inadequate in diet (Rama Rao et al., 2006). Low P can lead to increase serum Ca concentration and increased Ca concentration repress the secretion of parathyroid hormone. Prohibitive effect of parathyroid hormone on phosphate absorption and disposal of Ca by urine decreased (Viveros, 2002). Therefore, according to obtained ALP activity values of this study, it can be concluded that there were no Ca and P deficiency in groups.

ALT activity of serum is slightly found in all tissues of birds (Bogin and Israeli, 1976). However, ALT activity generally increases with the tissue injuries (Zantop, 1997). Unlike mammals, AST is not a liver specific parameter. Activity of AST can increase with a problem in muscle tissues or liver tissue (Lewandowski and Harrison, 1986). Therefore, it can be said that any Ca and P levels in this experiment had a tissue injury.

Low diet P level did not affect serum Ca concentration in this study. Serum Ca and P concentration results coincide with a number of earlier studies (Sebastian et al., 1996; Fernandes et al., 1999; Viveros et al., 2002; Kheiri and Rahmani, 2006).

Many investigations have shown that decreasing diet Ca and P level has led to decrease tibia ash content (Onyango et al., 2003; Rama Rao et al., 2006; Adamu et al., 2011; Mello et al., 2012; Naves et al., 2014; Wilkinson et al., 2014a; Delezie et al., 2015; Hamdi et al., 2015; Han et al., 2016; Rousseau et al., 2016). Imbalance of the diet Ca:P ratio also had a negative effect on ash content of tibia (Driver et al., 2005; Delezie et al., 2012; Rousseau et al., 2016). Decreasing diet Ca and P level had a negative effect on tibia ash content in the present study.

5. Conclusion

From the data of the current experiment, it can be concluded that 0.85 g/kg Ca and 0.41 g/kg P can be used for starter period without any deterioration on growth performance. Gradually increasing cost of DCP, decreasing world P reserves, environmental pollution arising from fecal P and economic reasons for broiler sector must encourage researchers to try finding ways to decrease P level in broiler diets.

Facilities of decreasing P in broiler diets in view of the fact that animal welfare and bone problems with or without phytase enzyme supplementation must be investigated. Relation between serum and bone mineral contents and serum enzyme activity should be a focused subject.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	A.A.	Z.K.
C	50	50
D	50	50
S		100
DCP	100	
DAI	50	50
L	100	
W	100	
CR	50	50
SR	100	

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because this study conducted before 2020.

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DETERMINATION OF TOLERANCE AND SENSITIVITY OF SAFFLOWER GENOTYPES BASED ON GERMINATION INDICES AND COMPARISON OF BIOCHEMICAL CONTENTS UNDER SALT STRESS

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Abstract: Safflower germination and seedling growth stages are extremely sensitive to salinity. The study aimed to identify safflower genotypes' germination, seedling growth responses, and biochemical changes in tolerant and susceptible genotypes in response to salt stress. Total of 28 genotypes were subjected to salt (NaCl) treatments (0, 180, 240 mM), and germination percentage, mean germination time, seedling and root lengths, and vigor index of the genotypes were determined. The genotype, treatments, and interaction effects were significant for germination, seedling, and biochemical parameters. The genotypes' germination percentage, seedling length, root length, and vigor index decreased under salt stress. While the reduction in germination percentage of salt-tolerant genotypes was between 6-21%, it was between 46-65% in sensitive genotypes at 240 mM salt treatment. Five tolerant (Shufu, Sidwill, Finch, Yuyao, Oleic Leed) and sensitive (Huaxian, Linas, 4022, Oker, Rehbein) genotypes were chosen based on reductions in germination percentage and vigor index, and the proline, hydrogen peroxide, and malondialdehyde (MDA) contents of these genotypes were investigated. The proline content of the genotypes increased by 26 to 56 fold at 180 mM salt concentration. The hydrogen peroxide content of sensitive and tolerant genotypes increased at 180 mM salt treatment, but at 240 mM salt treatment, the hydrogen peroxide content of the sensitive genotypes continued to increase by 6-50%, hydrogen peroxide content decreased in tolerant genotypes by 10-30%. MDA contents increased in the sensitive and tolerant genotypes, but the level of increase was higher in sensitive genotypes (307-631%) than the tolerant genotypes (103-323%) at 240 mM salt treatment. The heatmap generated by means of sensitive and tolerant genotypes showed 28 coefficients and 5 of which were significant. These results show that changes in hydrogen peroxide and MDA contents are different between tolerant and sensitive genotypes. They could be useful selection criteria along with germination percentages for determining tolerant and susceptible safflower genotypes at the seedling stage.

Keywords: *Carthamus tinctorius*, Hydrogen peroxide, Lipid peroxidation, Proline, Salinity

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1. Introduction

Safflower (*Carthamus tinctorius* L.) is a member of the Asteraceae family and the only cultivated species in the *Carthamus* genus. Safflower is an alternative crop grown for its flowers and seeds. Safflower seeds contain 20-40% oil and are mainly utilized in the industry for edible oil and dying purposes.

Soil salinity has become a problem for agricultural production, particularly in arid and semi-arid regions, affecting approximately 800 and 1.5 million hectares worldwide and in Türkiye, respectively (Türkan and Demiral, 2009). Soil salinity reduces the amount of water utilized and causes water stress in the plants. Ion accumulation due to soil salinity reduces nutrient uptake, chlorophyll synthesis, and the rate of photosynthesis, increases water loss and causes toxicity, and thus reduces plant growth and yield. It also causes the formation of reactive oxygen species, which causes oxidative stress in the cells, and further impedes the metabolic process (Munns and Tester, 2008; Hussain et al., 2016).

Salinity has detrimental effects on germination, seedling establishment, growth, and yield of crops. Different approaches could be employed to alleviate the effects of salinity, and biotic approaches, such as cultivating salt-tolerant genotypes to grow saline soils, should be preferred because they are sustainable, efficient, and economical (Ashraf and McNeilly, 2004). Plants could be classified based on their performance under saline and non-saline conditions. Therefore, screening genotypes for identification and selection for salt tolerance within the available germplasm resources is necessary. Safflower is classified as tolerant to saline conditions, showing variation in salt tolerance within safflower germplasm (Dajue, 1993; Siddiqi et al., 2007; Kaya et al., 2019). Salt stress affects all developmental stages of safflower

(Irving et al., 1988; Kaya et al., 2003), but germination and seedling development phases are more susceptible to salinity stress (Hussain et al., 2016).

Plants produce or accumulate different solutes and ions under salinity stress, such as proline, hydrogen peroxide, and malondialdehyde (MDA) to maintain the osmotic balance of cells or as a result of oxidative damage. Proline accumulation effectively prevents membrane damage and maintains osmotic balance (Hasegawa et al., 2000; Hosseini et al., 2010). Safflower genotypes under salt stress increase proline content to protect themselves from the effects of salt stress (Hosseini et al., 2010; Karimi et al., 2014). Hydrogen peroxide and MDA accumulation are associated with the production of reactive oxygen species leading to oxidative damage and causing lipid peroxidation. Therefore, the accumulation of these molecules is considered an indicator of cellular damage and seed deterioration in plants (Priestley, 1986; Bailly, 2004).

It is necessary to conduct salt tolerance tests under extreme conditions for identification and differentiation of safflower genotypes' tolerance to salinity (Siddiqi et al., 2007). Determination of biochemical changes occurring under stress conditions could be useful to identify tolerance mechanisms associated with tolerant and sensitive varieties. Therefore, the study aimed to test the response of many safflower genotypes under high salt concentrations during germination and early seedling growth to determine tolerant and sensitive genotypes. Then to study the effects of salt stress on proline, MDA and hydrogen peroxide contents in sensitive and tolerant genotypes to deduce stress responses of these genotypes.

2. Materials and Methods

2.1. Plant Material and Germination Tests

The study was carried out in the Agricultural Biotechnology Department laboratories at Isparta University of Applied Sciences (ISUBU) in 2021. Seeds from 28 safflower genotypes were used as plant material in the study. Enana, Rinconado and 4022 were breeding lines and the rest was registered safflower genotypes. A previous publication provides detailed information about origins, registration status, oil contents and other agronomic characteristics (Erbaş et al., 2016).

Seeds were surface sterilized with 2% sodium hypochlorite solution for 10 min, rinsed under running tap water for 5 min, and dried at room temperature. Seeds (50x4) were placed into two layers of filter papers, moistened with three different (0, 180, 240 mM) NaCl solutions, and put into sealed plastic containers to prevent evaporation. High salt concentrations were chosen because salt-tolerant and sensitive genotypes were distinguished better at stringent conditions (Siddiqi et al., 2007; Tonguç et al., 2021). Germination tests were conducted in a growth cabinet at 25±1 °C under dark conditions for 14 days. Seeds with radicle growth of 2 mm were counted as germinated and allowed to grow further for seedling measurements. Germination percentage and mean germination time were calculated as described (ISTA, 2009). After 14 days of germination, seedling and root lengths (cm) were measured using five seedlings from each replication. The vigor index of genotypes under different salt stress conditions was calculated by multiplying germination percentage and seedling length (Abdul-Baki and Anderson, 1973).

2.2. Biochemical Measurements

Percent reduction in germination percentage along with vigor index of the genotypes at 240 mM salt concentration was used to discriminate salt tolerant and sensitive genotypes. Seedlings were washed with distilled water, dried with paper towels, and used to measure biochemical parameters. Five seedlings (5x4) from each replication were powdered in liquid nitrogen and used to determine proline, MDA, and hydrogen peroxide contents of selected genotypes.

Proline was determined following the protocol of Zhang and Huang (2013). Ground seedlings (0.5 g) was homogenized with 2 mL 3% sulphosalicylic acid to determine the proline content of the samples. The slurry was centrifuged at 5.000 x g for 5 min, the 500 µL supernatant was taken to a new tube, added 500 µL acetic acid and 500 µL ninhydrin solution prepared with glacial acetic acid and orthophosphate. Tubes containing samples were boiled for 45 min and cooled on ice. An equal volume of toluene was added to each sample and vortexed for 1 min, then centrifuged at 1.000 × g for 5 min. The absorbance of the samples was measured at 520 nm by spectrophotometer. The standart curve was generated by known concentrations of proline prepared in 3% sulphosalicylic acid.

The hydrogen peroxide and MDA content of the samples was determined following Velikova et al. (2000), and Zhang and Huang (2013); respectively, and the analyses were performed as described by Önder et al. (2022).

2.3. Statistical Analysis

The experiment was a complete randomized block design with four replications. Each replication contained 50 seeds for germination parameters, and contained five biological replications for biochemical analysis. Germination percentage, mean germination time, and seedling and root lengths were measured. Germination data were transformed for normalization. The vigor index of the genotypes was also calculated using germination percentage and seedling length. Germination, seedling, and biochemical indices were subjected to ANOVA with SPSS 22.0 software (SPSS Inc, USA), and significant effects for genotypes, treatments, and their interactions on all examined germination and seedling indices were detected at $P \leq 0.001$ level of significance. Duncan's multiple range test was used to discriminate the differences between the means. To reveal the relationships between germination, seedling, and biochemical indices, Pearson's linear correlation analysis was carried out using OriginPro software's trial version, and results were presented as a heatmap.

3. Results

Safflower genotypes were tested for their responses at 0, 180, and 240 mM NaCl concentrations (Table 1). Germination percentages varied between 52-93% in the control group. While the genotypes with the lowest germination were Enana, Huaxian, and Olas; the genotypes with the highest germination were Linas, S-517, and 55633 (Table 2). Germination decreased at 180 mM, and 240 mM NaCl treatments, and germination percentages varied between 41-85% among the genotypes at 180 mM NaCl concentration. The lowest germination percentage in this treatment was observed in Olas and Enana, and the highest germination percentage was obtained from Frio, 55633, and S-517. At 240 mM NaCl treatment, germination percentage varied between 24-69%, and Rehbein, Girard, Huaxian, and 4022 had less than 30% germination. Twelve genotypes had higher than 50% germination, and the highest germination percentages were obtained from Frio, Finch, and S-517 at 240 mM salt treatment.

Mean germination times were shorter than two days for all genotypes except Montola 2000, Enana, and Oleic Leed in control. Germination time prolonged under 180 mM NaCl concentration, but germination times of Montola 2000, Enana, and Oleic Leed, contrary to the general trend, decreased. Hartman had over three days, and 13 genotypes had over two days of mean germination time at 180 mM NaCl concentration. At 240 mM NaCl treatment, ten genotypes had over two days, and Rehbein had over three days of mean germination time. With the increased NaCl concentration, the changes observed in the germination times of the genotypes differed, showing a response depending on genotype (Table 2). For example, Shufu, Yuyao, and Sidwill shortened, Linas, 55633, and Rehbein prolonged, and Yenice and Huaxian had the same mean germination times at 240 mM NaCl compared to 180 mM NaCl concentration.

Table 1. Mean squares of ANOVA results for germination and seedling parameters of safflower genotypes

Variance sources	DF	GP	MGT	VI	SL	RL
Genotypes	27	403.95***	0.56***	325285 ***	19.31***	9.27***
Treatments	2	6764.02***	11.67***	33356311***	4528.03***	2310.36***
Interaction	54	80.64***	0.58***	120691***	9.85***	5.95***
Error	252	13.76	0.14	10324	1.51	0.97

GP= germination percentage, MGT= mean germination time, VI= vigor index, SL= seedling length, RL= root length, ***= significant at $P \leq 0.001$ level of significance according to Duncan's multiple range test.

Table 2. Effects of salt treatments on germination percentage, mean germination time and vigor index of safflower genotypes. Values within columns are the means ± standard deviations

	Germination percentage (%)			Mean germination time (day)			Vigor index		
	Control	180 mM	240 mM	Control	180 mM	240 mM	Control	180 mM	240 mM
Shufu	59±6.3^{h-j}	52±6.1^{f-i}	51±2.4^{a-e}	1.4±0.1^{e-h}	1.9±0.2^{b-e}	1.5±0.1^{f}	1079±79^{hi}	381±38^{i-l}	377±31^{f-i}
Saffire	82±6.4^{b-e}	63±5.3^{a-d}	46±1.8^{c-h}	1.8±0.3^{c-e}	2.3±0.4^{b-d}	1.8±0.4^{d-f}	1636±125^{c-e}	630±67^{a-e}	449±61^{d-g}
Sidwill	56±4.0^{j}	51±5.5^{f-i}	50±1.5^{b-f}	1.7±0.3^{c-e}	2.0±0.3^{b-e}	1.6±0.2^{ef}	1177±125^{gh}	484±140^{f-j}	519±75^{a-d}
PCA	63±8.9^{g-j}	47±3.6^{h-j}	45±3.4^{c-h}	1.7±0.2^{c-e}	2.2±0.2^{b-e}	2.0±0.1^{c-f}	1377±127^{fg}	341±34^{kl}	315±20^{h-j}
Frio	76±10.1^{d-f}	69±4.9^{a}	59±4.9^{a}	1.2±0.1^{gh}	2.0±0.2^{b-e}	2.2±0.1^{b-f}	1546±132^{d-f}	700±71^{ab}	555±49^{a-c}
Montola 2000	54±4.0^{j}	44±3.0^{i-j}	38±7.0^{ij}	2.8±0.7^{a}	2.1±0.3^{b-e}	1.9±0.5^{d-f}	901±121^{ij}	291±33^{l}	231±21^{j-l}
Rinconada	82±8.1^{b-f}	58±3.0^{c-f}	52±3.8^{a-d}	1.1±0.1^{gh}	1.9±0.2^{b-e}	1.8±0.2^{d-f}	1603±88^{d-f}	401±43^{h-l}	344±42^{g-i}
Enana	52±2.6^{j}	42±1.5^{j}	37±8.1^{ij}	2.3±0.4^{b}	1.8±0.2^{c-e}	1.7±0.5^{d-f}	981±65^{h-j}	377±32^{j-l}	328±72^{h-j}
Huaxian	52±1.5^{j}	46±2.3^{h-j}	25±3.6^{kl}	1.9±0.1^{b-d}	2.2±0.5^{b-e}	2.2±0.5^{b-e}	795±77^{j}	374±68^{j-l}	207±31^{kl}
Linas	93±5.5^{a}	61±8.2^{b-e}	46±1.8^{c-h}	1.1±0.1^{gh}	1.7±0.3^{c-e}	2.4±1.3^{a-d}	1538±44^{d-f}	674±110^{a-c}	487±54^{a-e}
Dinçer	80±8.6^{b-f}	60±7.6^{b-e}	43±3.5^{f-i}	1.2±0.1^{f-h}	2.0±0.3^{b-e}	1.8±0.4^{d-f}	1610±121^{d-f}	562±144^{c-g}	402±89^{e-h}
4022	78±10.9^{b-f}	59±5.5^{c-f}	27±1.9^{kl}	1.3±0.1^{f-h}	2.5±1.2^{b}	2.7±0.8^{ab}	1405±173^{ef}	560±56^{c-g}	202±12^{kl}
Finch	72±3.7^{e-h}	58±3.5^{c-f}	57±3.6^{ab}	1.3±0.2^{f-h}	1.9±0.2^{b-e}	2.0±0.2^{c-f}	1389±111^{fg}	391±51^{h-l}	391±28^{e-h}
Sahuaripa 88	79±6.3^{b-f}	64±0.9^{a-d}	53±4.1^{a-d}	1.2±0.1^{gh}	2.0±0.3^{b-e}	2.2±0.3^{b-f}	1620±135^{c-f}	624±68^{a-e}	488±59^{a-e}
Oker	87±6.7^{a-d}	65±3.2^{a-c}	42±1.5^{g-i}	1.1±0.1^{gh}	1.9±0.1^{b-e}	1.6±0.2^{ef}	1706±220^{cd}	739±130^{a}	462±75^{c-f}
55633	88±1.5^{a-c}	67±3.2^{ab}	54±2.6^{a-c}	1.1±0.1^{h}	2.0±0.3^{b-e}	2.7±0.5^{a-c}	1943±36^{b}	611±52^{b-f}	588±38^{a}
Yenice	69±7.2^{f-i}	55±6.9^{d-g}	43±1.1^{e-i}	1.3±0.2^{f-h}	1.9±0.3^{b-e}	1.9±0.3^{d-f}	1478±135^{d-f}	515±87^{d-h}	390±26^{e-h}
Leed	70±8.2^{e-i}	50±0.9^{g-i}	45±0.9^{d-h}	1.1±0.1^{gh}	1.7±0.2^{de}	2.0±0.1^{c-f}	1533±109^{d-f}	561±49^{c-g}	480±52^{b-f}
Ole	78±10.8^{b-f}	61±1.8^{b-e}	52±1.8^{a-d}	1.5±0.1^{d-h}	2.4±0.5^{bc}	2.7±0.1^{ab}	1497±332^{d-f}	507±50^{e-i}	490±2^{a-e}
Hartman	73±8.4^{e-g}	61±6.9^{b-e}	54±0.9^{a-d}	1.1±0.1^{gh}	3.2±0.4^{a}	1.6±0.2^{ef}	1548±154^{d-f}	640±92^{a-d}	569±65^{ab}
Ziyang	78±5.5^{c-f}	62±1.5^{a-d}	47±8.6^{c-h}	1.2±0.1^{gh}	1.9±0.3^{b-e}	2.1±0.4^{b-f}	1842±137^{bc}	407±43^{h-l}	332±84^{h-j}
S-517	89±4.9^{ab}	67±5.8^{ab}	59±3.6^{a}	1.1±0.1^{h}	1.5±0.3^{e}	1.9±0.4^{d-f}	2167±212^{a}	447±56^{g-k}	398±66^{e-h}
Yuyao	62±7.6^{g-j}	54±3.2^{e-h}	54±2.8^{a-d}	1.2±0.1^{gh}	2.2±0.4^{b-e}	2.0±0.2^{c-f}	1017±150^{h-j}	353±25^{kl}	354±38^{g-i}
Girard	56±1.8^{j}	51±1.8^{f-i}	29±6.1^{kl}	1.6±0.3^{d-g}	2.0±0.2^{b-e}	1.9±0.3^{d-f}	1042±60^{hi}	505±67^{e-i}	210±104^{kl}
FO-2	58±1.8^{ij}	46±1.8^{h-j}	31±4.4^{jk}	1.4±0.1^{e-h}	2.1±0.2^{b-e}	1.7±0.3^{d-f}	1104±70^{hi}	512±29^{e-h}	346±79^{g-i}
Olas	52±1.8^{j}	41±0.9^{j}	40±3.7^{hi}	1.7±0.4^{c-f}	2.1±0.3^{b-e}	1.6±0.2^{ef}	1050±48^{hi}	421±44^{h-k}	320±58^{h-j}
Oleic Leed	54±3.2^{j}	51±1.8^{f-i}	50±0.9^{b-g}	2.0±0.5^{bc}	1.9±0.1^{b-e}	2.1±0.3^{b-f}	1092±63^{hi}	549±41^{c-g}	378±71^{f-i}
Rehbein	54±2.3^{j}	44±1.5^{ij}	29±3.2^{kl}	1.9±0.4^{cd}	1.8±0.1^{b-e}	3.0±0.4^{a}	1057±113^{hi}	412±332^{h-l}	276±77^{i-k}

Different letters within each column indicate significant differences between the genotypes within treatments.

Table 3. Seedling length and root length of safflower genotypes under different salt treatments. Values within columns are the means ± standard deviations

	Seedling length (cm)			Root length (cm)		
	Control	180 mM	240 mM	Control	180 mM	240 mM
Shufu	18.6±3.1^{h-j}	7.4±0.2^{h-j}	7.4±0.3^{g-j}	11.5±2.5^{f-h}	3.7±0.4^{h-j}	4.2±0.6^{c-h}
Saffire	20.0±0.8^{c-i}	10.1±1.3^{a-f}	9.9±1.2^{a-d}	11.7±0.5^{f-h}	4.7±0.67^{d-h}	4.9±0.6^{a-g}
Sidwill	21.0±1.0^{c-h}	9.5±1.8^{c-g}	10.4±1.6^{a-c}	13.3±1.0^{c-f}	4.6±1.2^{d-i}	5.5±1.1^{a-c}
PCA	22.0±1.1^{b-e}	7.3±0.2^{ij}	7.0±0.2^{h-j}	13.3±1.0^{c-f}	3.7±0.2^{h-j}	3.7±0.1^{e-h}
Frio	20.5±1.3^{c-i}	10.1±0.5^{a-e}	9.5±0.9^{a-e}	13.4±1.7^{c-f}	5.0±0.4^{c-f}	5.0±0.6^{a-f}
Montola 2000	16.7±1.5^{jk}	6.6±0.4^{j}	6.3±0.6^{j}	9.4±1.2^{ij}	3.3±0.3^{j-k}	3.3±0.3^{h}
Rinconada	19.8±1.4^{d-i}	6.9±0.5^{ij}	6.7±0.6^{ij}	12.4±1.3^{d-g}	3.6±0.5^{h-j}	3.5±0.5^{gh}
Enana	18.7±0.7^{g-j}	9.0±0.5^{e-h}	8.9±0.4^{b-h}	11.7±0.8^{f-h}	4.2±0.3^{e-j}	4.4±0.4^{c-h}
Huaxian	15.3±1.5^{k}	8.2±1.4^{g-j}	8.5±2.0^{c-i}	8.3±1.3^{j}	3.8±0.7^{g-j}	4.3±0.9^{c-h}
Linas	16.7±1.1^{jk}	11.1±1.0^{a-c}	10.7±1.2^{ab}	9.8±0.8^{h-j}	6.0±1.0^{a-c}	6.0±0.8^{ab}
Dinçer	20.3±1.0^{c-i}	9.4±1.5^{c-g}	9.4±1.4^{a-f}	12.9±0.9^{c-g}	4.7±0.8^{d-h}	4.6±0.8^{c-h}
4022	18.1±1.0^{ij}	9.6±0.7^{b-g}	7.5±0.2^{f-j}	11.7±0.5^{e-h}	4.7±0.3^{d-h}	3.3±0.2^{h}
Finch	19.3±0.7^{f-i}	6.8±0.7^{ij}	6.8±0.3^{ij}	11.6±1.2^{f-h}	3.2±0.1^{k}	4.6±0.5^{b-h}
Sahuaripa 88	20.5±0.5^{c-h}	9.8±1.1^{a-g}	9.3±1.0^{a-g}	13.4±0.6^{c-f}	5.0±0.7^{c-e}	4.9±0.6^{a-g}
Oker	19.7±1.1^{e-i}	11.3±1.8^{a}	11.0±1.8^{a}	12.5±0.5^{d-g}	5.5±1.1^{a-d}	5.5±1.2^{a-c}
55633	22.1±0.8^{b-d}	9.1±0.4^{e-g}	10.9±0.6^{ab}	14.8±1.0^{a-c}	3.9±0.3^{f-j}	5.3±0.4^{a-c}
Yenice	21.5±0.8^{b-f}	9.3±0.6^{d-g}	9.1±0.4^{a-g}	14.3±0.6^{a-d}	4.8±0.2^{d-g}	4.9±0.1^{a-f}
Leed	22.2±1.8^{bc}	11.2±1.0^{ab}	10.8±1.0^{ab}	13.9±1.6^{b-e}	6.2±0.8^{ab}	6.1±0.8^{a}
Ole	19.0±2.0^{f-i}	8.4±0.9^{f-i}	9.5±0.4^{a-e}	11.0±1.7^{g-i}	3.5±0.2^{j-k}	6.0±0.5^{ab}
Hartman	21.3±0.6^{c-g}	10.5±1.0^{a-e}	10.6±1.1^{ab}	14.3±0.8^{a-d}	5.1±0.8^{c-e}	5.3±0.5^{a-c}
Ziyang	23.6±0.7^{ab}	6.6±0.7^{j}	7.0±0.6^{h-j}	15.6±0.8^{ab}	3.4±0.5^{j-k}	3.8±0.6^{d-h}
S-517	24.5±1.4^{a}	6.7±0.6^{j}	6.7±0.7^{ij}	16.4±1.2^{a}	3.5±0.4^{j-k}	3.6±0.5^{f-h}
Yuyao	16.6±1.9^{jk}	6.7±0.6^{j}	6.6±0.4^{ij}	9.1±1.5^{ij}	3.6±0.4^{i-k}	3.6±0.2^{f-h}
Girard	18.8±1.6^{g-j}	10.0±1.0^{a-f}	7.1±1.7^{h-j}	12.0±1.6^{e-g}	5.2±0.7^{b-e}	3.6±1.3^{f-h}
FO-2	19.2±1.0^{f-i}	11.3±0.3^{ab}	11.1±1.0^{a}	11.6±1.0^{f-h}	6.3±0.4^{a}	6.1±0.8^{a}
Olas	20.4±1.6^{c-i}	10.4±1.0^{a-e}	8.0±1.1^{d-j}	13.4±1.9^{c-f}	5.4±0.8^{a-d}	5.1±0.8^{a-d}
Oleic Leed	20.4±0.6^{c-i}	10.9±1.0^{a-d}	7.6±1.4^{e-j}	13.3±0.8^{c-g}	5.4±0.70^{a-d}	5.1±1.2^{a-e}
Rehbein	19.7±1.6^{d-i}	9.4±0.9^{c-g}	9.7±2.7^{a-d}	12.3±1.4^{d-g}	4.7±0.6^{d-h}	5.4±2.0^{a-c}

Different letters within each column indicate significant differences between the genotypes within treatments.

Seedling and root lengths were measured to observe the effects of salt concentrations on the growth and development of germinated seeds under saline conditions (Table 3). Seedling lengths in the control group varied between 15.3-24.5 cm. Huaxian and S-517 had the control group' lowest and highest seedling lengths, respectively. Seedling lengths decreased with the increased salt concentrations, and as a result, seedling lengths reduced to 6.6-11.3 cm at 180 mM NaCl and to 6.3-11.1 cm at 240 mM NaCl treatments. Seedling lengths of six genotypes (Montola 2000, Rinconado, Finch, Ziyang, S-517, Yuyao) remained below 7 cm at 180 mM NaCl concentration. However, the length of these seedlings varied from 16.7-24.2 cm in the control group. Oker and Linas had the highest seedling length at 180 mM salt concentration. Oker (11.0 cm) still had the highest seedling length at 240 mM NaCl treatment. Montola 2000, Rinconado, Finch, S-517, and Yuyao had the shortest seedling lengths at the same treatment.

Root length measurements of the genotypes also showed that salt concentrations decreased seedling root lengths. While the root length of the seedlings varied from 8.3-14.8 cm in the control group, the root lengths of the seedlings varied from 3.2-6.3 cm and from 3.3-6.1 cm grown at 180 and 240 mM salt concentrations, respectively. Genotypes with the longest root lengths were Ziyang and S-517, and genotypes with the shortest root lengths were Huaxian and Yuyao in the control group. At 240 mM NaCl treatment, 4022 and Montola 2000 had the shortest root lengths with 3.3 cm, while Leed and FO-2 had the longest root lengths.

The vigor index values of the genotypes were between 795-2167. S-517 and Huaxian had the control group's highest and lowest vigor index values, respectively. Montola 2000 and Enana also had low vigor index values. Significant decreases in vigor index values were observed, and vigor indices of the genotypes were reduced to 291-1653 at 180 mM NaCl treatment. The vigor index values decreased further at 240 mM NaCl treatment, and Girard, Montola 2000, Huaxian, 4022 and Rehbein had the lowest, whereas Sidwill, Frio, 55633, and Hartman had the highest vigor index values (Table 2).

After measuring germination and seedling indices, the percent reduction in germination percentage and vigor index values were compared at 0 and 240 mM salt

concentrations. The results were used to select salt-tolerant and sensitive genotypes at the germination stage to investigate biochemical changes within these two groups of plants. Initial germination percentages of Oleic Leed, Shufu, Sidwill, Yuyao, and Finch were between 54-72%, and germination percentages were reduced by 6, 13, 11, 13, and 21% at 240 mM NaCl treatment, respectively. Germination percentages of sensitive genotypes (Huaxian, Linas, 4022, Oker, and Rehbein) were between 52-93% and dropped to 25-46% at 240 mM NaCl treatment, corresponding to 46-65% reduction in germination percentage between 0 and 240 mM NaCl treatments. Girard and Rehbein had the same germination percentages at 0 and 240 mM NaCl treatments, but their vigor index values were significantly different at 240 mM NaCl treatment; therefore, Rehbein was more vigorous and selected for biochemical evaluations.

The proline, MDA, and hydrogen peroxide contents were analyzed using sensitive and tolerant genotypes to determine the biochemical changes caused by salt stress in safflower seedlings grown at three different salt concentrations. Variance analysis revealed that genotypes, treatment, and genotypes x treatment interactions for proline, hydrogen peroxide, and MDA contents were significant at $P \leq 0.001$ levels of significance (Table 4).

1.00 µg g^{-1}among the sensitive genotypes. Shufu and Yuyao among the tolerant and Linas and 4022 among the sensitive genotypes significantly differed for proline content at the control (Figure 1). Salt stress increased proline contents of sensitive and tolerant safflower genotypes, but the increase from 0 to 180 mM treatment was far more dramatic than the increase from 180 mM to 240 mM NaCl treatment. Proline content increased by 26 to 56 fold to reach 25.04 µg g^{-1} and 28.24 µg g^{-1} in Rehbein and Shufu at 180 mM NaCl treatment, respectively. The highest increases in proline contents were observed in Shufu, 4022, and Oleic Leed, and the lowest increase was observed in Rehbein. At 240 mM NaCl treatment, the highest increases in proline contents were observed in 4022 and Rehbein by 1.6 and 1.2 times, respectively. The proline content of Shufu, Sidwill, Yuyao, Linas, and Rehbein was significantly higher at 240 mM salt concentration.

The hydrogen peroxide content of the genotypes was between 73.7-169.3 µmol g^{-1} in the control treatment. Huaxian and Oker had the highest hydrogen peroxide contents, whereas 4022 had the lowest hydrogen peroxide content in the control treatment (Figure 2). Exposure to 180 mM salt concentration increased hydrogen peroxide content among both tolerant and sensitive genotypes. The lowest increase was observed in Huaxian (7%) from 168.1 to 179.6 µmol g^{-1}, and the highest increase was observed in Finch (115%) from 92.9 to 200.1 µmol g^{-1} at 180 mM NaCl treatment. Oker had the highest hydrogen peroxide content (327.8 µmol g^{-1}) among the genotypes at 180 mM NaCl treatment. At 240 mM NaCl treatment, sensitive and tolerant genotypes exhibited different responses to salt stress. While hydrogen peroxide content increased in sensitive genotypes by 6-50%, it decreased in tolerant genotypes by 10-30%. The lowest and the highest increase among the sensitive genotypes was observed in Rehbein from 265.5 to 279.8 µmol g^{-1} and in Linas from 167.8 to 251.4 µmol g^{-1}. Shufu showed the lowest decrease in hydrogen peroxide content from 281.4 to 254.7 µmol g^{-1}. Finch had the highest reduction from 200.1 to 141.4 µmol g^{-1} for hydrogen peroxide content among the tolerant genotypes at 240 mM NaCl concentration.

The MDA content of the genotypes was between 0.89 to 2.10 nmol g^{-1} in the control group. Shufu had significantly higher MDA content than the rest of the genotypes within this group (Figure 3). MDA contents of the genotypes increased with increased salt concentrations, but the increase was higher in sensitive genotypes. MDA content increased by 27-94% in tolerant genotypes, whereas it increased by 132-349% in sensitive genotypes exposed to 180 mM salt concentration. The lowest and the highest MDA contents of tolerant genotypes were between 1.23-3.77 nmol g^{-1} in Sidwill and Shufu, respectively. Sidwill and Yuyao had higher MDA content than the other tolerant genotypes at 180 mM NaCl treatment. On the other hand, the MDA contents of the sensitive genotypes were significantly higher than the MDA contents of the tolerant genotypes, except for Shufu. The highest MDA content was observed in Linas (4.05 nmol g^{-1}), and the lowest MDA content was found in Huaxian (3.15 nmol g^{-1}). MDA contents of the genotypes continued to increase at 240 mM NaCl treatment.

Table 4. Mean squares of analysis of variance for biochemical measurements among the tolerant and sensitive safflower genotypes

Variables	DF	Mean squares		
		Prolin	Hydrogen peroxide	MDA
Genotypes	9	14.02***	23452.17***	8.59***
Treatments	2	7077.73***	82877.53***	134.99***
Interaction	18	11.99***	4324.03***	4.67***
Error	60	0.16	108.35	0.44

MDA= malondialdehyde, ***= significant at P≤0.001 levels of significance according to Duncan's multiple range test.

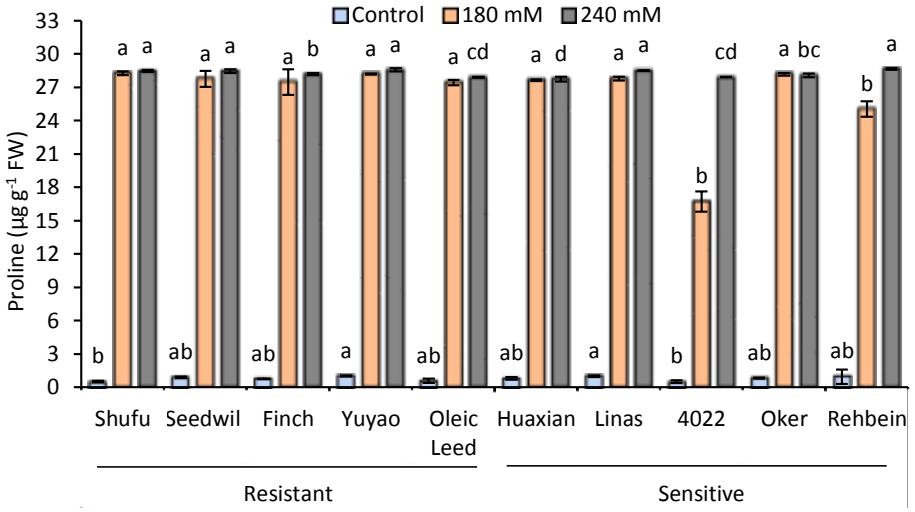

Figure 1. Proline contents of safflower genotypes exposed to three different salt concentrations. Different letters above the bars indicate significant differences between the genotypes within treatments. The vertical lines show standard deviations.

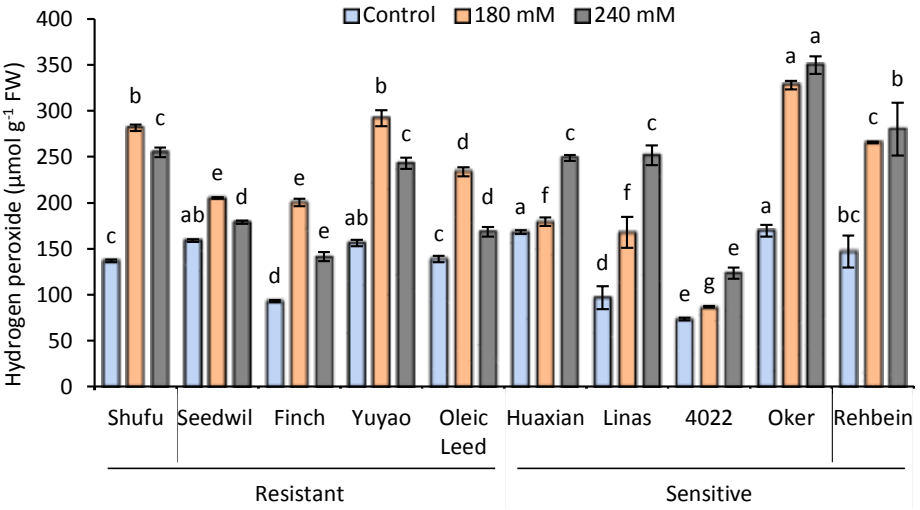

Figure 2. Hydrogen peroxide content of safflower genotypes exposed to three different salt concentrations. Different letters above the bars indicate significant differences between the genotypes within treatments. The vertical lines show standard deviations.

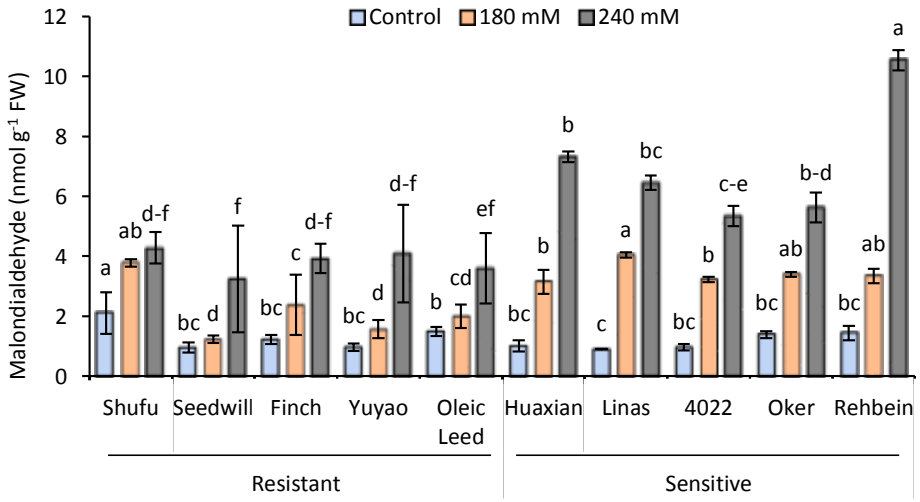

Figure 3. MDA content of safflower genotypes exposed to three different salt concentrations. Different letters above the bars indicate significant differences between the genotypes within treatments. The vertical lines show standard deviations.

The increase in the tolerant genotypes was between 14-166% and was between 61-215% in the susceptible genotypes. Even though Sidwill's MDA content increased by 166% to reach 3.25 nmol g^{-1}, but it still had the lowest MDA content among the genotypes, and Rehbein, the highest MDA content, increased by 215% to reach 10.54 nmol g^{-1} at 240 mM NaCl treatment (Figure 3).

Pearson's correlation analysis was carried out among the tolerant and sensitive genotypes to show the relationships between germination, seedling, and biochemical parameters. The results are given in Figure 4. A total of 28 correlation coefficients were calculated, two were negative, and three were positive and significantly correlated with each other. Germination percentage had a significant negative correlation with mean germination time (-0.83) but had a significant positive correlation with vigor index (0.91). Mean germination time negatively correlated with the vigor index (-0.72). Root length showed positive correlations with seedling length (0.98). Proline content positively correlated with hydrogen peroxide content (0.73).

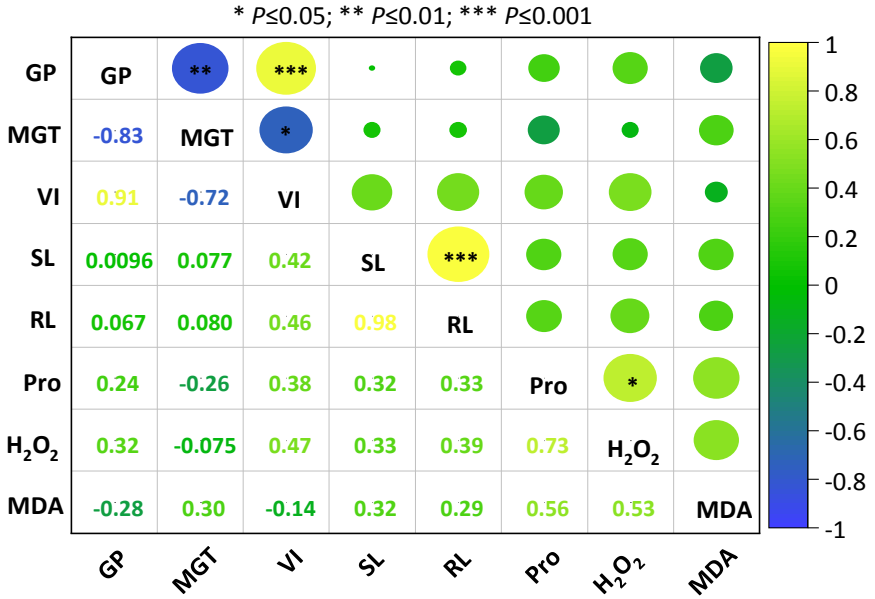

Figure 4. Relationships and correlation between 8 germination, seedling and biochemical parameters generated by a heat map. Color and scale display the intensity of mean values. (GP, germination percentage; MGT, mean germination time; VI, vigor index; SL, seedling length; RL, root length; Pro, proline; H2O2, hydrogen peroxide; MDA, malondialdehyde).

4. Discussion

Salinity, along with drought, is becoming a major constraint for agricultural production in arid and semi-arid regions of the world. Different biological treatments could be employed to mitigate stress conditions exerted by environmental factors, such as different seed treatments and foliar applications (Jabeen and Ahmad, 2012; Ashrafi and Razmjoo, 2015; Turan et al., 2022). Screening and identifying plant germplasm resources for salinity tolerance and cultivation is also another means to maintain reasonable yields under saline conditions (Irving et al., 1988; Siddiqi et al., 2007).

It is recommended that high salt concentrations should be used to differentiate genotypes for salt tolerance (Siddiqi et al., 2007), and we also observed that higher salt concentrations were better for differentiating safflower genotypes (Tonguç et al., 2021); therefore, we have screened safflower genotypes at 0, 180 and 240 mM salt concentrations in the present study. Safflower genotypes exhibited significant variations in germination and seedling indices. Salt stress negatively affected all germination and seedling parameters, especially at 240 mM salt concentration. The germination percentage of the genotypes varied between 52-93% at the control and varied between 27-59% at 240 mM NaCl treatment. The reduction in germination percentages was between 9-65% at 240 mM NaCl treatment (Table 2). Adverse effects of high salt concentrations on the germination of plant species are well known and have been documented for safflower (Kaya et al., 2003; Siddiqi et al., 2007; Çulha and Çakırlar, 2011; Kaya et al., 2019; Tonguç et al., 2021; Kurtuluş and Boydak, 2022). Mean germination time, measuring germination speed, was higher at higher salt concentrations compared to the control, which shows the germination speed was slower under salt stress conditions. In the present study, Montola 2000, Enana, and Oleic Leed had germination times over two days at the control, but 14 genotypes had over two days of germination times at 240 mM salt concentration. Similarly, increased mean germination time with the increased salt concentrations was reported for safflower (Kaya et al., 2019; Tonguç et al., 2021; Kurtuluş and Boydak, 2022). The results presented in the paper confirm that high salt concentrations reduce germination percentage and increase the time necessary for germination.

Germinated seedlings were allowed to grow within filter papers during the experiment to study the effects of salt concentrations on seedling growth. Seedling and root lengths were measured to calculate safflower genotypes' growth performance under stress and non-stress conditions. Plants in the control group had the highest values for these parameters. As the salt concentrations increased, measured values for seedling and root lengths decreased for all genotypes (Table 3). While seedling lengths decreased from 180 mM NaCl treatment to 240 mM NaCl treatment, root lengths of some genotypes, such as Shufu, Sidwill, Ole, and Rehbein, increased at 240 mM NaCl treatment compared to 180 mM NaCl treatment. Changes in seedling and root lengths have also been reported for safflower genotypes exposed to salt stress. In all reported experiments, shoot and root lengths reduced with increased salt concentrations (Kaya et al., 2003; Çulha and Çakırlar, 2011; Erdal and Çakırlar, 2014; Toprak and Tunçtürk, 2018; Kaya et al., 2019; Kurtuluş and Boydak, 2022). However, shoot growth was affected more severely and found more sensitive to salt stress than the root growth in safflower (Kaya et al., 2019). Increased root length under increased salt concentrations was reported for some safflower genotypes (Toprak and Tunçtürk, 2018; Kaya et al., 2019), which was also observed at 240 mM salt concentration in this study.

The vigor index is calculated using germination percentage and seedling length and shows the relative vigor of the genotypes under stress conditions (Abdul-Baki and Anderson, 1973), and genotypes with higher vigor index are considered to be more vigorous. Increased salt concentrations reduced the vigor index values of the genotypes, and the decline was more severe at the highest salt concentration. The vigor index of some safflower genotypes at low salt concentrations increased, but it decreased under higher salt concentrations (Kaya et al., 2019). We have not observed such an increase in vigor index because salt treatments were higher from the beginning. Still, our results for vigor index were similar at higher salt concentrations reported for safflower.

Different selection criteria, such as germination percentage, ion accumulation, ion balance, principal coordinate analysis, and gas exchange rates, are used to discriminate between tolerant and sensitive safflower genotypes to salinity (Siddiqi et al., 2007; Siddiqi et al., 2009; Kaya et al., 2019). We have used reduction in germination percentage as the main selection criteria along with vigor index values for selecting salt sensitive and tolerant genotypes. Based on these results, five tolerant (Shufu, Sidwill, Finch, Yuyao, and Oleic Leed) and five susceptible (Huaxian, Linas, 4022, Oker, and Rehbein) were selected for studying biochemical changes in the sensitive and tolerant genotypes exposed to salt stress.

Proline is accumulated as an osmoregulator to maintain the osmotic balance of cells and prevent the deterioration of proteins. Proline levels of plants increase in response to salt and drought stresses (Hussain et al., 2016). Proline contents of safflower genotypes were very low at the control, but exposure to salt stress increased proline content by 26-56 folds at 180 mM salt concentrations. Only Rehbein and 4022 had lower proline content at 180 mM salt concentration. Further increasing salt concentration caused little change in tolerant genotypes, but it further increased proline contents of Rehbein and 4022 to comparable levels to the other genotypes (Figure 1). Proline accumulation in response to salt stress is a common mechanism and has been reported for safflower (Hosseini et al., 2010; Erdal and Çakırlar, 2014). Dramatic increases in proline levels in response to salt stress, regardless of salt tolerance levels, were reported for safflower (Karimi et al., 2014). Even though the level of increase was very rapid in response to salt stress, proline accumulation did not differ between sensitive and tolerant genotypes and continued to increase in both groups suggesting that proline accumulation may be a common mechanism under stress conditions and may not be a particular part of salinity tolerance in safflower.

Reactive oxygen species are produced under stress conditions, and hydrogen peroxide and other reactive oxygen molecules cause lipid peroxidation (Priestley, 1986). Lipid peroxidation is the main cause of membrane damage, and the level of lipid peroxidation could be measured by monitoring MDA levels (Sharma et al., 2012). Superoxide dismutase scavenges superoxide radicals and converts them to hydrogen peroxide, which is scavenged by catalase and peroxidases. Therefore, antioxidant defense systems play important roles in mitigating oxygen species' effects under stress conditions. Hydrogen peroxide content was lower among the genotypes in the control treatment. Salt stress caused sharp increases in hydrogen peroxide content at 180 mM salt concentration across the genotypes (Figure 2). However, unlike proline contents of sensitive and tolerant genotypes, raising salt concentration to 240 mM did not increase hydrogen peroxide content across the safflower genotypes. Hydrogen peroxide accumulation showed a distinct difference between the sensitive and tolerant genotypes. In sensitive genotypes, hydrogen peroxide accumulation continued. As a result, the hydrogen peroxide content of sensitive genotypes increased by 6-50% at 240 mM salt concentration, whereas the hydrogen peroxide content of tolerant genotypes decreased by 10-30% at the same treatment. Studies showed that salt-tolerant safflower genotypes increase or maintain antioxidant enzyme activity under salt stress in safflower (Hosseini et al., 2010; Erdal and Çakırlar, 2014; Önder et al., 2022). Catalase and peroxidase activity between the salt-tolerant and sensitive safflower genotypes showed that the activity of these enzymes remained high in the tolerant genotype, but their activity ceased at a high salt concentration in the sensitive genotype (Hosseini et al., 2010). Similarly, the activity of different antioxidant enzymes followed the increased salt concentrations (Erdal and Çakırlar, 2014;

Önder et al., 2022). Though we did not determine the antioxidant enzyme activities in the study, these results show that genotypes differed in their abilities to detoxify hydrogen peroxide at 240 mM salt concentration, and the action of enzymes in the antioxidant defense mechanism regulated the level of hydrogen peroxide in tolerant genotypes. We have monitored MDA levels in tolerant and sensitive genotypes to measure lipid peroxidation damage. MDA contents of the genotypes in control were lower. Salt treatment increased the MDA contents of both sensitive and tolerant genotypes, and the increase in the sensitive genotypes at 180 mM NaCl treatment was higher than the increase in the tolerant genotypes at the same treatment (Figure 3). MDA levels of the sensitive genotypes continued to increase, and Rehbein, Huaxian, and Linas had significantly higher MDA levels than the tolerant genotypes at 240 mM NaCl treatment. MDA levels were between 3.25-4.28 nmol g^{-1} and 5.34-10.54 nmol g^{-1} in tolerant and sensitive genotypes at 240 mM NaCl treatment, respectively. MDA levels increase in response to salt stress in safflower (Erdal and Çakırlar, 2014; Önder et al., 2022). Our results show a link between hydrogen peroxide and MDA contents; as hydrogen peroxide content was reduced in tolerant genotypes, MDA levels in tolerant genotypes were also lower than in the sensitive genotypes. Önder et al. (2022) reported that hydrogen peroxide content was significantly associated with MDA, catalase, and antioxidant enzyme activities in two safflower genotypes under salt stress.

Correlation analysis revealed some insights between germination and biochemical parameters. Germination percentage and mean germination times were negatively correlated, as previously reported (Tonguç et al., 2021; Önder et al., 2022). However, our study found that germination percentage did not significantly correlate with MDA, proline, and hydrogen peroxide contents; these parameters were significantly and negatively associated with germination (Önder et al., 2022).

5. Conclusion

According to the results, Germination decreased under salt stress, but decline in the germination of seeds at 240 mM salt concentration was higher. Mean germination times also differed from the control depending on salt concentrations. The vigor index of the genotypes in the control group was higher, and significant decreases in vigor indices occurred with the increased salt concentrations. The highest seedling and root lengths were obtained from the control group, and seedling and root lengths decreased under salt stress. Proline content increased at very high rates in sensitive and tolerant genotypes and remained high under salt stress. MDA levels also increased, but the increase in the tolerant genotypes was lower than the increase in the sensitive genotypes grown under higher salt concentrations. Hydrogen peroxide levels also increased in response to salt stress. However, the hydrogen peroxide levels in the tolerant genotypes decreased, while the hydrogen peroxide levels continued to increase in the sensitive genotypes when the salt concentration increased to 240 mM. The amount of hydrogen peroxide could be a feature that can be used to distinguish between tolerant and sensitive genotypes for their germination ability under high salt concentrations.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	S.Ö.	N.Y.	M.T.
C	50	50	
D	100		
S			100
DCP		100	
DAI		50	50
L	40	30	30
W	50		50
CR	50		50
SR	100		
PM			100
FA		100	

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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SOCIO-ECONOMIC VIEW OF FISHERIES PROCESSING SECTOR EMPLOYEES: BLACK SEA REGION-TÜRKİYE

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Abstract: In this study, the socio-economic status of the employees who work in the seafood processing plants operating in the Black Sea region provinces was examined in order to reveal the social and economic data. 37 enterprises, which have a production permit from the Ministry of Agriculture and Forestry, are located in 7 provinces in the Black Sea region, where 18 provinces are located. It has been studied with 532 interviewers in 28 companies that are actively operating, with a participation of 24 questions each. 19 of the enterprises work for fish and fish products (cold and frozen storage), 4 are for snails, and 5 are for fish meal and oil processing plants. 63.9% of the employees are male. When we look at the distribution of the employees' age groups, the rate of 31-40 years was the highest with a percentage of 36.64%. Regarding the education levels, it is seen that secondary school graduates constitute the highest rate with a percentage of 38.91%. It has been detected that 99.62% of the employees have social security, and all of them take advantage of the social security institution. For the marital status, it is determined that 73.50% of the employees are married. Studies on the seafood sector, which is important in the world and in our country, reveal important data.

Keywords: Seafood, Fish meal, Workpeople, Plant, Questionnaire

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1. Introduction

Aquaculture socio-economically occupies an important business niche, constitutes a significant part of agriculture as well as being a valuable source of quality nutrition. This sector is known to provide raw materials, contribute to rural development, implement employment areas and food manufacturing (Doğan and Yıldız, 2008). Annual aquaculture and capture production is 177.8 tons according to FAO 2020 statistics of which 90.3 million tonnes originate from fisheries and 87.5 million tons from aquaculture practices. Aquaculture and capture production in Türkiye in 2021 reached 799.851 tons. The financial asset for this 2020 production had increased to 13.708.550,105 Turkish Liras. Additionally, 559.932 ton was being consumed as a food resource by human (GDFA, 2021; TSI, 2022). The seafood processing sector, with its developing economic status, takes part in important industries. Seafood processing plant factories are subject to act number 5996 veterinary services, plant welfare, food and feed law for seeking permission. Those that receive permission are registered to the food security information system (FSIS) designed by the Ministry of Agriculture and Forestry of Türkiye. There are 234 seafood businesses registered to FSIS. These are distributed to entire Türkiye; 68 in the Marmara region, 79 in the Aegean region, 38 in the Black sea region, 33 in the Mediterranean region, 12 in the Central Anatolian region and 1 in the South Eastern Anatolia region. From the 234 registered businesses, 97 are certified for exportation to European Union countries (MAF, 2019). Seafood constitutes the only animal product that has been certified for export to EU countries however, socio-economic analysis regarding workers in the processing plant factories is quite limited. Various studies have been carried out inter (nationally) regarding mainly fisheries sector, aquaculture workers, processing plant workers, the owners of the businesses, consumer groups by several researchers (Drewes, 1982; Charles, 1988; Hunte and Oxenford, 1989; Saxena, 1989; Freire and Gracia-Allut, 2000; Supongpan et al., 2000; Yahşi, 2000; Waters et al., 2001; Sabatella and Franquesa, 2004; Villareal et al., 2004; Ünal, 2004; Çolakoğlu et al., 2006; Uzmanoğlu and Soylu, 2006; Yücel, 2006; Emre et al., 2007; Güngör et al., 2007; Kutlu and Balçık, 2007; Çeliker et al., 2006, 2008; Doğan and Yıldız, 2008; Bektaş et al., 2010; Doğan, 2010; Köse et al., 2010; CFRI, 2012; Çağlak et al., 2012; Sarıözkan, 2016; Buruç, 2018).

The Black sea region of Türkiye is the 3rd biggest geographical region, covering 18 cities. The coastal distance of the region is 1685 km thus participating local fisheries up to 50%. The aim of the present study was to exhibit socio-economic status of workers in processing

plant factories in the region which holds significant economic benefits thus obtaining qualitative social datasets. Additionally, the outcomes of the research are anticipated to provide insights for the sector directors, the aquaculture processing industry, and policymakers.

2. Materials and Methods

2.1. Research Area and Processing Plant Factories

Research area, according to the data sets of the Agricultural Economic and Policy Development Institute, 75% of the total supply of Türkiye's wild fishery needs is from the Black Sea region (41.1% East Black Sea region and 34.2% West Black Sea region) (TEPGE/AEPDI, 2021). Timeframe for the study has been expanded to cover peak periods for wild fisheries catchment and shellfish season. In this direction, 7 factories including cities of Sinop, Zonguldak, Kastamonu and 10 factories in Trabzon were surveyed in December 2015, 11 factories in cities of Samsun, Ordu, and Giresun were surveyed in February 2016 (Figure 1). Processing plant companies are registered for the food security information system designed by Ministry of Agriculture and Forestry of Türkiye. Those working in the processing plant factories registered to the system, 28 businesses in total, consisted of the material of the present study. Processing plant factories included crustaceans, cephalopod, fish oil and fish meal, fresh and frozen seafood. The fact that the amount of hunting and partly aquaculture is in the Black Sea has led to the concentration of the sectors in this region.

2.2. Surveys

The survey forms used in the present study consist of 24 questions. Previously applied socio-cultural surveys were redesigned for the purpose of this work (Doğan and Yıldız, 2008; Bektaş et al., 2010; Doğan, 2010).

2.3. Procedure

Exact counting method was applied so as to gather robust dataset regarding processing plant factories. The exact counting method is applied when all participants involved in surveys fill up forms completely (Çapkın et al., 2008; Diktaş-Bulut et al., 2021). The total number of participants, 532 people, included everyone working in processing plant companies that had been surveyed (this study covers the permanent staff; Figure 2).

2.4. Identification of Worker's Socio-Economic Qualifications

Survey included the data generated by the answers to those questions related to socio-cultural and demographic status of the participants follows as; age, marital status, the number of people in the family, educational status, social security status, income of the family, professional satisfaction, socio-cultural activities and food expenses.

2.5. Statistical Analysis

The statistical analysis was carried out using Windows Office 365. Categorical research evidence and percentage distribution were given in tabular format.

Figure 1. Distribution of the facilities by city in the survey study.

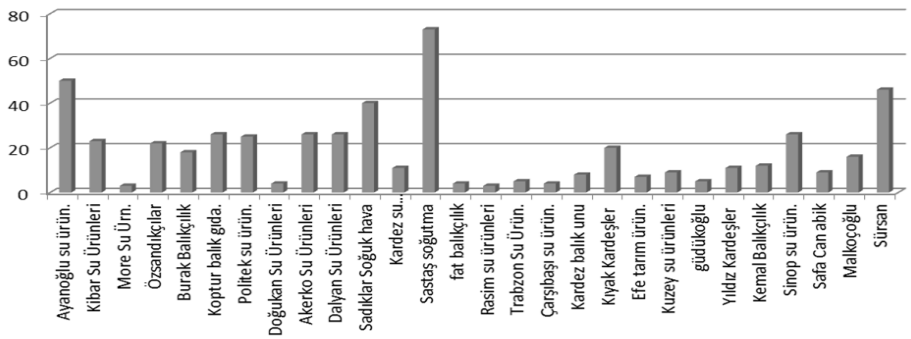

Figure 2. Number of surveyors by facilities.

3. Results

The distribution of factories based on cities, their processing technology, and the number of people working in the factories are given in Table 1. The results revealed that all factories (100%) were company-based businesses. Assessments revealed that all factories had a "freshly frozen seafood section" with a 75% representation. The ratio for crustaceans, cephalopod,

fish oil, and fish meal processing factories was 12.5%. Additionally, one factory possessed more than one processing technology. In total 13 factories had 1-11 personnel, 7 factories had 12-25 personnel, 4 factories had 26-38 personnel, 2 factories had 39-49, 2 factories had 50 and onwards personnel for business operations. The city of Trabzon possessed the highest proportion, 10 in total, for the establishment location of processing plant companies.

Table 1. General properties of seafood processing plants in the Black Sea region

Processing technologies	Piece	(%)
Fresh and frozen seafood	22	75.00
Cephalopods and crustaceans	3	12.5
Fish meal and oil	3	12.5
Number of employed personnel		
1-11 staff	13	46.42
12-25 staff	7	25.00
26-38 staff	4	14.28
39-49 staff	2	7.14
50 ≤ staff	2	7.14

The demographic distribution of workers is presented in Table 2. The dominant sex was detected as male, with 63.3% among workers of processing plant companies. The average age of the workers was older than 30 years old, represented by 68.53%, while 31.47% of the workers were younger than 30 years old; the ratio decreased to 9.16% for 51 years old and onwards. The majority of the workers were married, represented by 71.5%, and singles and widows were represented by 25.94% and 0.56%, respectively. Educational status of the works was detected as follows; secondary school (38.91%) > primary school (35.53%) > two-years degree (11.47%) > literate (5.64%) > Bachelor in Science (3.19%) > Master in Science (1.50%) > nonliterate (0.38%) > Doctor of Philosophy (0.19%). The frequency of newspaper reading revealed that 17.67% of the workers followed the daily posts, 24.06% followed 2-3 days a week, 19.36% followed once in a week and 37.97% followed newspapers once a month. Reading habits of workers were detected as 40.79%, 21.05%, 20.68%, and 17.11% for never reading, one book in a year reading, more than a book in a year reading, and one book in a week reading, respectively.

The use of social media and the internet has increased rapidly, thus the frequency of internet technology utilization is given in Table 3. Internet utilization was 70% among workers of which the majority indicated as 2-3 hours in a day. Social media account holders were represented by 58.83% while 39.29% of the workers did not have a social media account. The number of social media platform use varied among workers; 33.27% indicated as one account use, 33.27% indicated 2 accounts and 10.72% indicated 3 and more accounts on social media. The reason to use the internet was detected as follows; “communication with friends > daily news > researching > listening to music or videos”. The number of workers who did not want to respond to this question was remarkably high thus drawing the authors' attention.

Table 2. Demographic properties of employees in seafood processing plants in the Black Sea region

Social structure of employees	Piece	(%)
Gender status		
Female	192	36.1
Male	340	63.9
Age groups		
<20	20	4.50
21-30	148	27.82
31-40	195	36.64
41-50	121	22.73
51-60	33	6.20
60>	11	2.06
Unanswered	4	0.9
Marital status		
Widow	3	0.56
Single	138	25.94
Married	391	73.50
Education status		
Illiterate	2	0.38
Literate (not educated)	30	5.64
Primary education	189	35.53
Secondary education (including high school)	207	38.91
Associate degree	61	11.47
License education	17	3.19
Post graduate	8	1.50
Doctorate	1	0.19
Unanswered	17	3.19
Newspaper reading frequency		
Everyday	94	17.67
2-3 times a week	128	24.06
Once a week	103	19.36
Once a month	202	37.97
Unanswered	5	0.94
Book reading habit		
Never read	217	40.79
1 time per week	91	17.11
1 time per year	112	21.05
More than once a year	110	20.68
Unanswered	2	0.37

Table 3. Internet and social media usage cases of employees in seafood processing in the Black Sea region

Internet usage frequency	Piece	(%)
2-3 hours a day	209	39.29
2-3 hours a week	106	19.92
1 hour per month	58	10.90
Not using	159	29.89
Do you have a social media account?		
Yes	313	58.83
No	213	39.29
Unanswered	6	1.88
Social media count		
1	177	33.27
2	97	18.23
3	41	7.71
4	10	1.88
5 and above	6	1.13
Not using	200	37.59
Unanswered	1	0.19
Reason for using the internet		
Reading news	102	19.17
Doing research	65	12.22
Communication with friends	116	21.80
Listening to music and watching videos	31	5.83
Unanswered	218	40.98

Table 4 indicated the social security status of the workers of which almost all (99.62%) had social security apart from 2 workers, represented by 0.38%. The types of social security revealed that all (100%) workers were covered under the Social Insurance Institution (SSK). The position of the workers was distributed as 66.73% operatives, 4.32% headmasters, 4.89% engineers, 5.64% technicians, and 18.42% others (drivers, accountants, administrative staff) among workers. The job satisfaction of seafood processing plant workers was detected as follows; 3.38% very satisfied, 45.86% satisfied, 39.47% tolerable (not bad), and 4.89% not satisfied. The salary satisfaction of the workers was detected as 22.74% satisfied, 30.45% not satisfied and 43.05% tolerable (not bad). Monthly salary distribution was identified similarly as 38.72% 1201 TL and lower income and 37.40% 1501 TL and higher income. Similarly, the ratio of income and the number of people was detected comparable; 19.55% (1201-1500 TL) and 20.11% (1501-2000 TL).

Table 4. Social security and economic structures of employees in seafood processing in the Black Sea region

Social security and economic structure of employees	Piece	(%)
Social security status		
Have social security	530	99.62
No social security	2	0.38
Social security institution		
Social insurance institution (SSK)	530	100
Pension fund	-	
Bağkur	-	
Special insurance	-	
Title		
Worker	355	66.73
Foreman	23	4.32
Technician	30	5.64
Engineer	26	4.89
Other (driver, accounting etc.)	98	18.42
Job satisfaction		
Very pleased	18	3.38
Satisfied	244	45.86
So so	210	39.47
Not glad	26	4.89
Unanswered	34	6.40
Salary satisfaction		
Yes	121	22.74
No	162	30.45
So so	229	43.05
Unanswered	20	4.03
Monthly average income (TL)		
<500	31	5.83
501-949	83	15.60
950-1200	92	17.29
1201-1500	104	19.55
1501-2000	107	20.11
2001>	92	17.29
Unanswered	23	4.33

Accommodation types of the workers based on their economic and social security status were provided in Table 5. The rational distribution of the number of people working while sharing the same house was detected as 2 > 1 > 3 > 4 > 5 and onwards. The number of people was found to be three times higher for 4 people and higher (399 persons) compared to 3 persons and lower (133 persons). The ratio for accommodation ownership revealed that 52.91% of the workers own their accommodations, 22.58% are living in rental accommodations, and the remaining 22.92% utilized other facilities. The type of accommodations was detected as follows; 40.04% in the apartment house, 54.51% in a detached house, and 4.89% in others. The sizes of the accommodations were detected as follows; 57 accommodations (10.71%) <90m2, 208 accommodations (%39.10) range between 90m2-120m2, 138 accommodations (%25.94) 120m2-130m2, 84 accommodations (%15.79) 130m2-150m2 ve 38

accommodations (%7.14) 151m2. The heating type of the accommodations was detected as follows; 70.49% stove, 21.24% central heating, 6.95% natural gas central heating, and 1.32% electrical heating.

Table 5. Social security and economic structures of employees in seafood processing in the Black Sea region

Social security and economic structure of employees	Piece	(%)
Number of employees living in the same residence		
1	195	36.65
2	253	47.56
3	62	11.65
4	13	2.44
5 and Above	9	1.7
Number of persons in the household		
2 people	6.58	35
3 people	18.42	98
4 people	33.08	176
5 people	29.70	158
6 and above	12.22	65
Residential ownership		
Host	282	52.91
Tenant	119	22.58
Other (staying with mother, father, grandfather etc.)	121	22.92
Unanswered	10	1.89
Housing type		
Private	290	54.51
Apartment	213	40.04
Other (slum)	26	4.89
Unanswered	3	0.56
Housing size (m^2)		
<90	57	10.71
90-120	208	39.10
120-130	138	25.94
130-150	84	15.79
151 ≥	38	7.14
Unanswered	7	1.31
Heating type of the house		
Stove	375	70.49
Heater	113	21.24
Natural gas	37	6.95
Electricity	7	1.32

4. Discussion

The socio-economic situation of the workers in processing plant factories of the Black sea region was presented in the present study. The surveys conducted in the region revealed all, twenty-eight, companies were run as corporations. Çapkın et al. (2008) reported one company exception in 10 companies investigated in terms of company management in Beyşehir surrounding area. Çağlak et al. (2012) reported all companies run as corporations in terms of management in Balıkesir province. The present study indicated that seafood processing plant companies are generally run as corporations as it was reported by previous studies. Majority of the companies (3/4) operating in the region utilized fresh and frozen seafood technology. Other companies shared equal contributions for their processing type including crustaceans and cephalopods as well as fish oil and fish meal. In a study conducted by Çağlak et al. (2012) reported fresh and frozen seafood products as well as cephalopod and crustacean products were at the forefront in Balıkesir province. The same authors reported bivalve processing technology in the region, although being lower in terms of production volumes. The comparison of these two papers revealed a unique part for the present study in regards to fish oil and fish meal while being similar for the majority of the processing technologies in fresh and frozen seafood as well as cephalopod and crustacean technology. Investigations revealed that companies possessed more than one processing technology. Additionally, it was presumed that companies operating in the region preferred extended self-life compared to investigating new tastes mainly due to the traditional tastes of the consumer's preferences. The highest proportion of workers was detected in companies that had employees ranging between 1 to 11 out of 28 companies that the survey conducted among 532 workers. In a study to reveal the situation, problems, and their solutions of the seafood processing plant companies Duyar and Bayraklı (2005) have previously reported a 50% ratio for employees of 1-11 workers in 6 companies investigated with a total number of 89 workers in Sinop city. Çağlak et al. (2012) reported a 62.5% ratio of employees ranging between 1 to 11 in 5 companies out of 8 seafood processing plants that were surveyed with a total number of 120 workers. The comparison of the literature revealed that the ratio of 1 to 11 workers in seafood processing plant factories was similar to the previous investigations. Thus, in the shed of the current literature, the number of factories employing 1-11 workers is estimated to be 45-60% throughout Türkiye. This is thought to be due to the use of a low number of employees mostly for general operations such as sizing and crating as well as logistical actions instead of delicate processing technologies. The results revealed 340 male employees and 192 female employees among 532 workers being surveyed in the seafood processing plant factories. Çapkın et al. (2008) reported that Çağlak et al. (2012) detected a higher number of female employees. Investigations revealed that such differences were related to the body strength of male employees mostly working in fresh and frozen seafood processing requiring heavy lifting while female employees were assigned to delicate positions such as fillet and crustacean processing due to their familiarity with such operations. As fresh and frozen seafood processing was dominant in the region, in a way, explained the sex differences observed in the gender groups. It was detected that 68.96% of the

workers were under 40 years old. Previous literature revealed that the dominant age group for sea food processing factories were under 40 years old (Yücel, 2006; Çapkın et al., 2008; Doğan and Yıldız, 2008; Tokaç and Dinçer, 2011; Çağlak et al., 2012). Similar to other divisions of aquaculture, seafood processing sector representatives prioritized the employment and labour force thus hiring younger employees. SEKAM (2011) world family symposium indicated that family bonds and marriage is crucial for the social status of Türkiye. The marital status of Türkiye based on Türkiye Statistical Institute (TSI, 2015) data revealed that 62-67% were married and 22-29% were single in the Black sea region. The survey of the present study reported 73.5% married and 25.94% single among participants. The results of the survey revealed that participants cared about marriage as indicated in the report of the symposium and TSI data. TSI (2018) data indicated the graduates of primary school, secondary school including high school degree, university degree (including 2 years and 4 years education), post-graduate degree (masters in science) and, doctor of philosophy in the Black sea region on Türkiye as follows, 38.3%, 35.8%, 13.5%, 1.06%, 0.25% respectively. The results of the educational level detected in the present study were in correspondence with the national statistics. Çağlak et al. (2012) reported the ratio of university degree graduates as 14% in a study conducted on the Balıkesir city processing plant factory employees. The survey results in regard to the ratio of analphabetic employees indicated the development of Türkiye in the field of education. The ratio of university graduates (2 years and 4 years' degree) pointed out the perspective of the sector towards educated manpower while the high ratio found in primary and secondary school level was due to increased need of operatives in the factories. The written media statistics revealed a decrease in the circulation of journals and magazines (TSI, 2017). Çakır et al. (2009) reported an irregular reading of daily newspapers as 28.9% while 9.4% was detected once a weekly basis in the survey conducted in Kayseri city. In the present study, the highest ratio of daily newspaper reading was detected as 37.97% once a month basis, the highest of the previous studies, thus indicating the ratio is decreasing as reported in the literature. This may be due to the increased ratio of online newspaper reading on social media platforms. Such a situation is in accordance with the increased use of the internet. Türkiye was the 86th in the Human Development Report published by the United Nations. In this regard, reading rates are quite low in Türkiye, as detected in the present study's associated answers to the question, listed in Table 2. Türkiye Reading Culture Research revealed that 70% of the participants do not read books, while a more recent study reported a decrease in the ratio down to 36% (OKUYAY, 2019). Çoban et al. (2018) remarked the reading habits of university students completed 1-5 books within one year as 48%. Çağlak et al. (2012) reported the ratio of non-readers as 46% and reads more than one book in a month as 15% among the employees of the processing plant factories operate in Balıkesir city. The present study carried out among the employees of processing plant factories operating in the Black sea region revealed similar results in regard to reading habits with that of entire Türkiye, highlighting reading habits of the country (non-readers as 40.79%, once in a month reader 17.11%, once in a year or more reader 41.73%). Recent studies as well as the present studies suggest reading habits are increasing. The use of the internet is powerful for instant access to updated news, yet the uncontrolled news and addiction to excess use constitute its downsides. Internet addiction is explained by excessive time spending activities on the internet and being incapable of controlling the urge towards it (Leung, 2004; Simkova and Cincera, 2004). The most significant factor of this addiction is the time spent on the internet is much longer than those of non-addicted (Coa and Su, 2007). According to the TSI 2016a datasets, the number of houses with an internet connection was 76.3% while the ratio of internet use was reported as 61.2%. In the present study, the use of the internet was detected as 70.11%. Durak and Seferoğlu (2016) reported daily internet use as 4 h and 37 min from PC and tablets, while mobile data use was indicated as 2 h 51 min. The daily use of the internet for the employees of the seafood processing plant factories was 2-3 h for 39.29%. Internet use was reported as 73.2% according to TSI, 2013 while it reached to 82.4% in 2016. The study indicated the ratio of social media users as 58.83% and the differences were thought to be due to age and educational status. A majority (33.27%) of the employees were detected to use only one social media account, the ratio was down to 1% for multiple social media account users. Aydın (2016), as well as İnce and Koçak (2017), reported more than one social media account. The main reason for internet use was due to communication with friends in the present study. This was followed by daily newspaper reading, researching, and listening to music or videos. TSI (2016a) reported similar outcomes in regard to frequency of internet use; social media, messaging friends, watching videos, online newspaper and magazine reading, and researching health-related topics. The outcomes of the present study associated with internet use and social media were in accordance with TSI datasets. Additionally, no sense of internet addiction was noticed among the participants who indicated the use of only one social media account mostly for communication with friends as well as reading daily newspapers. Legally enforceable rights of the workers revealed that almost all workers (530 persons) were socially and economically covered. Previous investigations carried out in the aquaculture sector revealed that 60-99% of workers were covered by social security status (Çeliker et al., 2006, 2008; Daşdan et al., 2008; Doğan and Yıldız, 2008; Çağlak et al., 2012). Alterations in the social security legislation led to all workers being covered by the Social Insurance

Institution (SII) in the present study. The distribution of workers based on job titles revealed operatives represented the highest proportion, 66.73% as typical in the seafood processing sector. Similarly, previous studies conducted by Duyar and Bayraklı (2005), Çapkın et al. (2008), Çağlak et al. (2012), and Altunel, (2021) reported high numbers of operatives. The cumulative percentage of workers in regard to job satisfaction was up to 85.33% including satisfied and tolerable answers, only 4.89% were not satisfied. A study conducted in the wild fisheries sector revealed 85.9% job satisfaction based on cumulative answers of satisfied and moderate (Güngör et al., 2007). Similar results were observed in a study conducted in Balıkesir city with 88% satisfaction (Çağlak et al., 2012). The outcomes of the present study revealed that seafood processing plant factory workers operate in the Black sea region assessed based on job/labour by the business owners and the results were in accordance with the literature. According to the 2015 and 2016 minimum wage, there was 36.84% gained a salary between 950 TL to 1500 TL, this was confirmed by the number of operatives however for those under gaining under minimum wage, represented as 21.43%, and for those gaining higher than minimum wage, represented as 37.4% indicated that seafood processing sector prioritizes the experience and technical expertise for salary distribution. The results of the present survey revealed that the seafood sector is not different than other sectors in regard to the salary of operatives. In 1989, new legislation was put in place to ensure mutual minimum wage among all sectors including agriculture and forestry thus Committee of Minimum Wage declared a uniform salary range regardless of sectoral differences (Eser and Terzi, 2008). TSI datasets of 2015 and 2016 revealed that the majority of the workers desired an increased salary within the investigation of the poverty threshold. Within this context, only one salary was not sufficient for those living in the same household, thus the ratio of 2 and 3 people working was detected as 59.21% sharing the same household. Türkiye's average household size is 3.5 (TSI, 2016b). In a study conducted to reveal the socio-economic status of fishermen, Sağlam and Karadal (2016) reported owing 0-3 children in 54% of the families, therefore 3-5-person family structure can be estimated. In the present study, the highest proportion of 4-person family structure was detected with 33.08% which was in parallel with the country statistics and previous studies. A significant indicator of the socio-economic status, 52.91% of the participants was house owners. Other studies conducted among the workers of aquaculture sector reported 51.6-76.76% house owners (Çeliker et al., 2006, 2008; Doğan and Yıldız, 2008; Doğan, 2010; Çağlak et al., 2012; Sağlam and Karadal, 2016; Çağlak et al., 2018). The fact that the proportion of house ownership was higher than 50% in all studies, implies the significant perception regarding owning the accommodation. Investigations revealed that detached houses were preferred thus the ratio of 54.51% was detected. Harvesting and agricultural activities have long been a part of the Black sea region's culture. The outcomes of the present study confirmed the situation. In Türkiye, 80% of the houses are at an average of 100 m2 in usable open space (Anonymous, 2016). Çağlak et al. (2018) reported that seafood retailers in Rize lived in an average of 90-130 m2 open space of houses, with a 55% proportion. Surveyors indicated living in 90-130 m2 houses with 65.04%. This finding was in accordance with the previous studies. The heating type of the accommodations was mostly stove, which represented as the highest proportion (70.49%). Çağlak et al. (2012,2018) reported the heating type as a stove in 53% and 77.5% respectively, in two different studies. It is estimated that the higher proportion of the stove as a heating type, higher than the Türkiye average (57.1%), may be due to type of the accommodation, region, and availability of natural gas in the neighbourhood. Additionally, similar results were reported in previous studies.

5. Conclusion

Consequently, the majority of the seafood processing plant factories workers were detected to gain minimum wage as a result of socio-economic investigations carried out. Additionally, the vast majority of the workers were satisfied with their job and almost all were covered under the social security umbrella. The high proportion of workers under social security was observed mainly due to legal obligations in regard to improvements to workers' welfare as well as employer sensitivity. Rental expenses, observed in a low proportion of the workers, caused to stipulate economical drawbacks significantly. As the vast majority of the workers were higher than 30 years old indicating the importance of experience in the seafood processing sector as well as suggesting there may be problems for experienced workers in near future in case of a decrease in new attendees. The vast majority of the workers were male indicating the regional seafood processing factories required mostly body strength, operating in fresh and frozen seafood technologies. Survey results revealed the increasing use of the internet both in Türkiye and globally, led to reading newspapers online thus resulting in a decline in hard copy newspapers. Similar to all sectors, semi-skilled employee requirement is a problem for the seafood processing sector. The number of persons living in the same accommodation was detected as 3-5, as indicated by national reality. The number of persons working in the same house higher than 2 indicated economic problems. As the increase of employees' salaries is dependent on employers' economic power, incentives supporting employers will surely reflect on employees' income. The increase in seafood processing product range will stimulate profitability and employment. Therefore, incentives offered by authorized organizations towards delicate products and novel processing technologies will stimulate the sector for innovator solutions instead of

regular fresh and frozen seafood processing. Seafood technology is an innovative, dynamic sector, if supported financially, has significant potential to eliminate socio-economic problems. Developments in seafood technology-related sectors emphasize the importance of fisheries for both Türkiye and the world.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	M.A.A.	E.Ç.
C	50	50
D	50	50
S	50	50
DCP	50	50
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

It is an article produced from a master study using research data before 2019. For this reason, ethics committee approval is not required.

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ECONOMIC DAMAGE LEVELS OF THE GREEN SHIELD BUG (*Palomena Prasina*, Hemiptera: Pentatomidae) IN TÜRKİYE HAZELNUT ORCHARDS

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Abstract: The green shield bug (GSB) (*Palomena prasina*, Hemiptera: Pentatomidae) is one of the most important pests of hazelnut in Turkish hazelnut orchards. This harmful insect causes serious yield and quality losses by feeding directly on fruits every year. Their feeding on hazelnut fruits may result in nut abortion (empty nuts) in early season and cause shriveled and corked kernels in kernel expansion period. Insect pest management must be decided by depending on insect population level in field according to integrated pest management concept. Economic injury level (EIL) and economic threshold (ET) are the main essential points that must be considered in decision for insect pest control. Thus, it can be possible to protect the natural environment from unnecessary pesticide applications and the growers from high production costs. Therefore, determining of economic decision levels for controlling pests is critical. This is especially important for hazelnuts, which are grown on hundreds of thousands of hectares of land in Türkiye, and for the GSB, a serious pest that requires a couple of chemical applications per year. The economic decision levels vary mainly due to insect species and their damage potential, crop value in the market and control costs which can change over years and countries. This study aimed to calculate the EIL and ET values for GSB control action in hazelnut orchards in Türkiye using new economic market data. Based on previous research, the authors calculated the yield loss caused by one individual of GSB in this study. Direct yield loss, as well as quality and quantity losses from damaged kernels, were calculated separately and then totaled. The data, including crop value and control costs necessary for calculation was updated from free market sources. In the calculation of EIL/ET, the most common formula (EIL= C/ VxbxK) was used. As a result, the economic threshold for a single insecticide application was determined to be3.8 insect/da (=0.1 ha) for K=1 value and 4.76 insect/da for K=0.8 value, for single insecticide application. When ET values were converted in traditional Turkish approach that is special for hazelnut orchards; ET values for K=1 and K=0.8 were 0.76 insect/ 10 "hazelnut "ocak" (traditional growing of hazelnut plants together) and 0.95 insect/ 10 "ocak" respectively. If 2 applications per year for GSB were considered, ET values were doubled up and calculated to be 7.6 and 9.47 insect/da for K=1 and K=0.8 value respectively, and thusly 1.52 and 1.9 insect/ 10 "ocak" for Türkiye. For practical reasons, the ET value for GSB was recommended as 10 insect/da and 2 insects/10 "ocak", for Türkiye, with consideration of 2 chemical applications and K= 0.8.

Keywords: Economic injury level, Economic threshold, Filbert, Insect, Pest, Control

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1. Introduction

Türkiye is the world's main hazelnut producer by a ratio of nearly 62%, producing 665.000 tons per year of in shell hazelnuts over an area of around 735.000 hectares. Türkiye also supplies 75% of the world's hazelnut demand. Hazelnut export earnings contribute significantly to the national economy and the livelihoods of 500.000 hazelnut producer families (Hekimoglu and Altindeger, 2019; Bars, 2021). Despite the fact that Türkiye is the world's primary hazelnut supplier by far, productivity per area is lower than some other hazelnut growing countries. In addition to insufficient and/or inappropriate agricultural practices, harmful mites and insects are among the most important factors causing yield and quality losses in hazelnut production in Türkiye (Tuncer and Ecevit, 1997). There are more than 10 important insect and mite species in Turkish hazelnut orchards. Some of them cause substantial damage every year, but others only in years with high populations. Some pests affect only yearly production and kernel quality, while others threaten the plant's health (Tuncer et al., 2005).

Stink bugs (Hemiptera: Pentatomidae) are one of the most important pests of hazelnut in Turkish hazelnut orchards as well as in other hazelnut-producing countries. These bugs damage hazelnuts during the growing season by feeding on hazelnut fruits and thereby significantly reduce the crop productivity and kernel quality of hazelnuts, thereby causing serious economic damage (Tavella and Gianetti, 2006; Hedstrom et al.,

2014; Ak et al. 2018; Bosco et al., 2018; Ozdemir et al., 2021). More than 15 stink bug species (Hemiptera: Pentatomidae, Coreidae, and Acanthosomatidae) have been found in hazelnut orchards of different hazelnut countries, affecting the hazelnut production qualitatively and quantitatively (Tavella et al., 1997; Tavella et al., 2001; Tuncer et al., 2005; Ozdemir et al., 2022). Stink bugs' damage can result in fruit abortion during the early season, empty and gray–black nuts during the early nut development stage, shriveled kernels during the early kernel development stage, and corked kernels during the kernel expansion period (Kurt, 1975; Tavella et al., 2001; Tuncer et al., 2005; Saruhan and Tuncer, 2010; Hamidi et al., 2022). Their damage is usually unnoticed before harvest since fruit abortion and empty nuts without kernels are not linked with bug activity by most farmers. But after shelling the fruits in the factory, shriveled and corked kernels become very evident, especially the later damage type, which causes problem in exporting because of poor kernel quality (Tuncer et al., 2005). The population density of GSB can reach up to 50 insects/10 plants in some provinces in Türkiye (Saruhan and Tuncer, 2009). Corked kernels differ from healthy kernels in shape, taste, and color, and they lose significant economic value. Tuncer et al. (2005) reported that the corked kernel damage made by stink bugs in Turkish hazelnut orchards could reach up to 20%, with around 5% of the corked kernels as an average of a 5-year sampling from hazelnut factories after harvest. The percentage of corked kernels in hazelnut orchards caused by these bugs has been determined as 6.50, 3.16 and 9.82%, respectively, for 2014-2016, with an extensive sampling throughout Türkiye (Ak et al., 2018).

The most prevalent stink bug species affecting hazelnut production in Türkiye is the green shield bug (GSB) *Palomena prasina* L. (Hemiptera: Pentatomidae), with a density of nearly 85% of total stink bug population, and its population level is generally over the economic damage threshold (Tuncer et al., 2005). Despite the fact that GSB is a polyphagous pest that feeds on a variety of plants, economic damage to other crops is rare. The green shield bug produces one generation per year. Nymphs and adults feeds on hazelnut fruits from early May until harvest time in hazelnut orchards (Saruhan et al., 2022). Chemical application is the only option during bug feeding activity on fruits for control of this insect, and it is recommended to repeat it 2-3 times during the season. Many growers avoid applying the pesticides because of their cost, and in addition, they don't bear the cost of quality loss since they usually sell the hazelnut in shell. But in recent years, many hazelnut trading companies began to consider the kernel quality for price assessment during the buying process. As a result, growers are expected to adopt more chemical control measures in the coming years, in addition to the rising price of hazelnuts in the country. Implementing chemical control for GSB can ensure high productivity and quality in one aspect, but it also means higher control costs for growers and environmental costs for all communities, especially when such a large hazelnut area of the country considered. Hence, determining the action threshold for GSB control in hazelnut orchards becomes more critical.

Before making a control decision for a pest control program, some economic evaluations must be considered because economic decision levels are the keystone of insect pest management programs. Two main evaluations, the economic injury level (EIL) and the economic threshold (ET), which are closely related- are fundamental concepts in an integrated pest management (IPM) approach. The economic injury level is defined as "the lowest numbers of insects that will cause yield losses equal to the insect management costs" and expressed as the number of insects per unit area. The economic threshold is defined as "the pest density at which management action should be taken to prevent an increasing pest population from reaching the economic injury level" (Pedigo, 1996). The economic injury level is usually expressed as a pest density and is generally derived from insect and yield-loss relationships in field studies (Mumford and Knight, 1997). Simply, the value of crop loss caused by one insect can be estimated using the market value of crops, and then EIL is calculated by dividing control cost by the value of crop loss per area so that the smallest number of insects causing yield losses equals management cost. The calculation of ET can be a little complex, but in practical reason, many times the ET may be set equal to EIL or at some fixed point below it. In many studies, the relationship between the yield losses and the insect numbers in that field has been considered for the calculation of EIL for practical reasons (Pedigo, 1996; Mumford and Knight, 1997). Some insect damage and control cost parameters are used in EIL calculations. The damage caused by a single pest is critical data in calculation of EIL and ET, but it is also being by far the most difficult to calculate. The other main parameters are the market value of the crop and management costs needed in EIL calculations. Since economic levels are very dynamic and management costs (pesticide, labor, and amortization of equipment) and the market value of crop may vary due to years and countries, recalculation of EIL and ET values are needed over time (Pedigo, 1996). On the other hand, the evaluation of economic decision levels for some indirect pests may be difficult, while it is easier for direct pests (Mumford and Knight, 1997). Because of difficulties in determining EIL and ET for some pests in practice, nominal decision levels made by experts on related pest can be used (Pedigo, 1996).

The green shield bug is a direct pest on hazelnut, feeding on fruits directly. Therefore, the estimation of crop loss caused by one insect is considerably more applicable than that caused by many other indirect pest species, despite the fact that it is time consuming. Green shield bug damage potential on hazelnut has been quantified directly by field cage studies, and it was determined that GSB caused damage to 175 nuts per insect during the growing season, resulting in direct loss and a negative

impact on nut quality (Saruhan and Tuncer, 2010). In this study, we aimed to calculate EIL and ET values by using injury potential determined before by the authors for GSB depending on the new economic data, including crop value and control costs such as pesticide prices, labor for applications, and other costs.

2. Materials and Methods

2.1. Damage Measurements for GSB

In this study, the yield loss caused by one GSB individual on hazelnut was derived and slightly changed from a previous study done (Saruhan, 2004) by one of the authors. The above-mentioned study used sleeve cages in hazelnut orchards to directly quantify GSB feeding damage because adults and nymphs of GSB feed on hazelnut fruits. In mentioned study; the experiments were carried out in a hazelnut orchard in Samsun in 2002-2003 by using 275 sleeve cages (175 cages for insects and 100 cages for control). After placing the nymphs and adults as pairs into 15-20 different sleeve cages for 15-day at each time of period during the fruit growing season (from May to mid-August), the insects were removed from the cages, and these cages were kept until harvest without insects. With nearly 15-day intervals, this procedure was repeated with different sleeves and insects until harvest, so the fruits in cages objected to insect feeding during the entire growing season. Some sleeve cages were kept without insects as control until harvest. At the harvest time, fruits from each cage were examined and evaluated according to different types of GSB damage on the fruits. Data on damage obtained from cages with insects were corrected for damage types that showed significant difference from control cages by using the Schneider-Orelli formula (Puntener, 1981). Some type of damages was needed to be corrected because they were also observed in control cages naturally but insect feeding increased these types of damages On the other hand corked kernel type of damage is only caused by insect feeding on kernels, not occurring in control cages (Saruhan and Tuncer, 2010) therefore it was not corrected. Consequently, it was determined that one individual of GSB damaged 175 fruits during the whole growing season, including all damage types. Early abortion, empty fruits, and shriveled kernel type of damage (produced the fruits without kernel or non-marketable kernels); therefore, the data for these damage types was considered a direct loss in the calculation. Because corked kernels caused solely by insect feeding do not completely lose their economic value but do lose some of their market value, the percent value loss of the kernels in the market was corrected prior to EIL and ET calculations. Preliminary EIL calculation had been attempted but was not published (Saruhan, 2004). In this study, the data was evaluated again by a slightly different method in order to calculate the damage of GSB.

2.2. Calculation of Economic Decision Levels

Different but mostly similar methodologies are followed to calculate EIL and ET by researchers for practical reasons, although there are some others with theoretical approaches (Kranz, 1992). In this study, the method and formula (EIL= C/VxbxK) given by Pedigo (1996) were used for calculation. Economic data such as the market value of hazelnut and control costs for EIL and ET calculations were derived and used from open market sources for 2018-2022.

3. Results

The Damage potential of GSB, market value of hazelnut and control costs, including pesticide, labor, and amortization of pesticide application equipment are needed to calculate EIL and ET levels. All these determinants must be evaluated and used in the calculation according to the formula provided by Pedigo (1996) as follows in Equation 1:

$$EIL=C/VxIxDxK \quad (1)$$

Where, V= market value per unit of produce (for example, $/kg), I= injury units per insect per production unit (for example, percent defoliation/insect/ha), D= damage per unit injury (for example, kg lost/ha/percent defoliation), C= cost of management per area (for example, $/ha), K= proportionate reduction in potential injury or damage (for example, 0.8 for 80%).

But, with some pests, particularly pierce-sucking insects, the separation of the *I* and *D* variables presents a problem. In those cases, a coefficient *b* represents the loss per insect substituted, and calculation formula changes as follows in Equation 2 (Pedigo, 1996):

$$EIL= C/VxbxK \quad (2)$$

where, *b* = yield loss/ insect.

In this study, since GSB is a direct pest and yield loss per insect (*b*) was determined using sleeve cages in the field, later form of the formula was used in the calculation. But, here, the *b* coefficient was calculated from sleeve cages in which the insects fed on fruits during the growing season, instead of obtaining it from regression analyses of data by using experimental populations and measuring yield losses, as calculated in some studies.

3.1 Market Value of Hazelnut (V)

Of the primary factors, crop value (V) is one of the most variable, and it alone accounts for much of the change in EILs. The relationship between EIL and market value is inverse; as market value increases, EIL decreases and vice versa. As a general rule, estimates for EIL calculation are based on current or past records of crop value (Pedigo, 1996). Since hazelnut prices in the market usually fluctuate depending on years and yield per year, the mean of last five year's market value for in shell hazelnut was considered in the EIL/ET calculation for GSB in this study (Table 1). Following a five-year price evaluation of in-shell hazelnuts on the market, the market value in Türkiye was estimated at 3.3 $/kg and used in the EIL calculation.

Table 1. Market value of hazelnut in shell in last five years in Türkiye (Turkish Grain Board)

Year	Market value of hazelnut in shell ($/kg)
2018-2019	2.93
2019-2020	3.34
2020-2021	3.73
2021-2022	3.50
2022-2023	3.01

Mean market value of hazelnut in shell for five years= 3,30 $

3.2. Management Costs (C)

Management costs include labor (pesticide application), used materials (insecticide), and equipment (insecticide application sprayer). Management costs also tend to be fluctuate over time, especially in developing countries that has high inflation like Türkiye. This fluctuation is generally caused by market inflation and labor costs, depending on economic improvement, but not crop value. The chemical control cost for GSB in hazelnut orchards was calculated in this study using variables gathered from the open market for two main insecticides registered on the market (Table 2). Recommended doses and prices of two registered insecticides on the market for GSB were used in the calculations. The daily labor cost for a pesticide applicator was estimated to be around 27 $ per day (equivalent to 500 TL per day in 2022), and one pesticide applicator can spray 10 da (equivalent to 1 ha) per day. For amortization of spraying equipment, a regular atomizer cost was obtained from the market and it is assumed that economic life of an atomizer is 10 years and that it is used in spraying 100 da/year by any grower.

3.3. Damage per Insect

In EIL calculations, estimating the loss per insect is by far the most difficult. Crude estimates of losses are usually obtained from field observation and experimentation on a crop at specific times, after which yield is measured and losses caused by insects are determined (Pedigo, 1996). Green shield bug damage potential (loss per insect) had been determined before by Saruhan (2004) through semi-field sleeve cage experiments in hazelnut orchards in 2002-2003.

In this study, damage per insect value of GSB on hazelnut was recalculated on an individual insect basis from the mentioned study in order to use it in our calculations. Damage types including “light brown and shrunken at the bottom”, “gray-black nuts without kernels”, empty nuts, shriveled kernels, and corked kernels caused by GSB were considered to calculate damage per insect Because some types of damage occurred in control cages as well, with the exception of corked kernels, the damage values for insect cages were corrected by the Schneider-Orelli formula according to the control if there was a significant difference. If there was no difference between insect and control cages for any type of injury in each year, that injury type was not included in the calculations. Afterwards, mean values of damage per insect were calculated using the result of both years for each damage type.

It was calculated that the mean total direct yield loss (no kernel or no marketable kernel) was 165.01 nuts/insect and corked kernels (quality and quantity loss) was 104.2 per insect (Table 3). Quantitative loss was also calculated for corked kernels using the mean weights of kernels from sleeve cages with insect and control cages. Normal kernel weights, without insect damage were, 0.96, 1.23 and 0.97 gr for çakıldak, palaz and tombul hazelnut cultivars respectively (mean value =1.05 gr/kernel). Corked kernel weight values for these 3 cultivars were 0.79, 0.96, and 0.82 gr (mean value =0.86 gr/corked kernel. As a result, the quantity loss of corked kernels was calculated as follows in Equation 3.

$$\text{Quantity loss ratio of corked kernels} = \frac{\text{Mean normal kernel weight(gr)} - \text{mean corked kernel weight(gr)}}{\text{Mean normal kernel weight (gr) X 100}} \quad (3)$$

Quantity loss ratio of corked kernels = 1.05-0.86/1.05 x 100 = 18%.

Table 2. Chemical control cost parameters for green shield bug management in hazelnut orchards (2022)

Costs for insecticide application	Registered insecticide-I	Registered insecticide-II
	120 g/l Indoxacarb + 12 g/l Beta-cyfluthrin	218 g/l Acetamiprid + 37 g/l Emamectin Benzoate
Recommended dose/da	50 ml/da	50 ml/da
Price/unit	16.13 $	14.78 $
Pesticide cost/da	2.02 $	2.96 $
Labor cost/da	2.7 $	2.7 $
Equipment amortization cost/da	0.5 $	0.5 $
Fuel cost /da	0.5 $	0.5 $
Total cost/da	5.72 $	6.66 $
Average cost/da for single application	6.19 $/da	
Average cost/da for two applications	12.38 $	

Table 3. Different damage types of GSB on hazelnut fruits in sleeve cages (after data corrected with control by Schneider-Orelli formula, the data was modified and recalculated from Saruhan, 2004)

Years	No.of fruits/damage type				
	Light brown and shrunken at the bottom	Gray-black nuts without kernels	Empty nuts	Shriveled kernels	Corked kernels (kernel/insect)
2002	111.5	-	74.85	-	139.5
2003	71.27	21.35	30.68	20.34	68.96
Mean	91.39	10.68	52.77	10.17	104.2
	Total direct yield loss: 91.39+10.68+52.77+10.17= 165.01 nuts				Quality and quantity loss= 104.2 kernels

3.4. Economic Injury Level and Economic Threshold

In this study, *b* value was assessed by using direct quantification through measurements of damage from sleeve cages with insect during growing season (Equation 4).

$$EIL = C / V x\, b\, x\, K \quad (4)$$

where *b* = yield loss/ insect. This formula provided by Pedigo (1996) was modified so that direct nut loss and corked kernel damage caused by GSB were separated, yield loss = direct yield loss + quality and quantity loss of corked kernels (b = b1 + b2), as written in Equation 5:

$$EIL = C / V\, x\, (b1+b2)\, x\, K \quad (5)$$

3.4.1. Calculation of b value

b1= direct yield loss, was calculated as 165.01 nut/insect above (3.3), meaning nut loss without any marketable kernel.

b2 (quality and quantity loss of corked kernels) = corked kernels from GSB feeding have still got market value, even at reduced prices. In the calculation of b2, 25% of market value loss from corked kernels was assumed, plus 18% of weight loss from corked kernels. The number of corked kernels per insect was calculated above as 104.2

In this case;

b2= the number of corked kernels/insect x weight loss induce for corked kernels x ratio of market value loss (%)

b2= 104.2 x 1.18 x 0.25= 30.74 nut loss/insect,

Indices 1.18 for *b2* to add 18% weight loss to the total corked kernel loss were used.

b (Total loss) = *b1* (direct yield loss) + *b2* (quality and quantity loss of corked kernels). In our case;

b= 165.01 + 30.74 = 195.75 hazelnut fruit/ insect.

It was estimated that 1 kg hazelnut in shell consists of almost 535 hazelnut fruits, as a mean of the three main cultivars (çakıldak, palaz and tombul) of Türkiye (Demir, 2004). In this case, the yield loss in kilograms can be calculated as follows:

Yield loss in kg= 195.75/535= 0.37 kg hazelnut in shell loss / insect.

3.4.2. Calculations of EIL and ET (If K value is considered =1)

Here K value (amount of damage avoided because of control action) was considered as equal to 1. Using determinants calculated, EIL can be determined as follows:

EIL= 6.19 $ (cost/da) / 3.3 $ (market value/kg) x 0.37 kg (loss/insect) x 1= 5.07 insect / da

Traditionally ET is given per 10 "ocak" (ocak= a group of plants planted together) and there are almost 50 "ocak" in one decare area, as a mean in Türkiye. It means 10 "ocak" is nearly 0.2 decare. In this case; EIL can be expressed for Türkiye as follows:

EIL = 5.07 insect per da x 0.2 da= 1.01 insect / 10 "ocak"

We can use EIL as our "action levels (=ET)" or we may choose to set ET at levels conservatively below the EIL, say at 75 percent of EIL (Pedigo, 1996);

ET=5.07 x 0.75= 3.80 insect/ da, or

ET=1.01 x 0.75= 0.76 insect/ 10 "ocak", for Türkiye.

EILs values are calculated as 10.14 insect/da and 2.03 insect/10 "ocak", and ETs are values 7.6 insect/ da and 1.52 insect / 10 "ocak", if two insecticide applications are made per growing season (Table 4).

Table 4. Economic decision levels for Green shield bug on hazelnut were calculated for Türkiye

No.of chemical application/season	Economic decision	K-value	Insect/da	Insect/10 "ocak"
Single spray	EIL	1	5.07	1.01
		0.8	6.34	1.27
	ET	1	3.80	0.76
		0.8	4.76	0.95
Two spray	EIL	1	10.14	2.03
		0.8	12.63	2.53
	ET	1	7.6	1.52
		0.8	9.47	1.9

3.4.3. Calculation of EIL and ET (If K value is considered =0.8)

K value (amount of damage avoided because of control action) can be considered equal to 1 in some cases, but it is often hard to determine the real effectiveness of control action in the field. Therefore, we assumed here as another option that K value is equal to 0.8 (80% damage avoided because of control action). In this case;

EIL= 6.19 / 3.3 x 0.37x0.8= 6.34 insect /da

EIL = 6.34 insect /da x 0.2 da= 1.27 insect/ 10 "ocak"

ET= 6.34 x 0.75= 4.76 insect/ da, or

ET=1.27 x 0.75= 0.95 insect/ 10 "ocak",

EILs values are calculated as 12.63 insect/da and 2.53 insect/10 "ocak", and ETs are values 9.47 insect/ da and 1.9 insect / 10 "ocak", if two insecticide applications are made per growing season (Table 4).

4. Discussion and Conclusion

The green shield bug is one of the most important pests of hazelnut in some hazelnut growing countries, such as Türkiye and Italy. This insect pest is abundant in hazelnut orchards during the nut development in season. Therefore, it affects yield seriously as well as kernel quality. Corked kernels, in particular, are the main source of complaints from Turkish hazelnut importers because poor quality of the kernels affects the confectionary products that use hazelnut kernels.

For both reasons- crop yield and quality- proper management of GSB in hazelnut orchards is critical. Unfortunately, chemical control is the only viable option for dealing with this insect. Therefore, the growers currently must rely on chemical control to prevent the damage caused by GSB. In hazelnut orchards, 2-3 applications for GSB are recommended in a year (Anonymous, 2017). Using pesticides incurs additional costs for growers as well as environmental costs for the community. In the IPM concept for agricultural pests, decision-making for chemical control is an essential step to reduce the mentioned costs. For this reason, EIL and ET decision levels are calculated and used to take a control action for any pest. EIL/ET are dynamic action levels and may change due to the market value of the crop and control costs over time. The main critical data that is very important in EIL/ET calculations based on experiments is the damage potential of insects which may vary less overtime. Since damage assessment of any insect in the field to calculate EIL/ET levels is mostly hard, time consuming, and costly, these attempts are very limited with some agricultural pests. Moreover, GSB is a serious pest only in few hazelnut producing countries, so there is only one preliminary attempt (unpublished, Saruhan, 2004) for EIL/ET study about it yet. In this study, the data regarding damage per insect was derived and recalculated from mentioned study.

Currently, in Türkiye the nominal ET accepted for GSB in practice is 1 insect / 10 "ocak" (Anonymous, 2017). For a single insecticide application, ET was calculated to be 3.8 insects per day (0.1 ha) for K = 1 and 4.76 insects per day (0.8 ha) for K = 0.8 in this study. When ET values were converted into the traditional Turkish unit that is special for hazelnut orchards; ET values for K=1 and K=0.8 were 0.76 and 0.95 insect per 10 "ocak", respectively. ET values were naturally doubled when two chemical applications for GSB per year were considered. Here we found that for a single insecticide application, the ET value is about 1 insect/ 10 "ocak" (for K=0.8), similar to the nominal threshold that is currently used in practice.

We propose that two chemical applications in a year and the K= 0.8 option are more realistic approaches to determining the ET value of GSB for the following reasons: Tuncer et al. (2009) found from field experiments on stink bugs that one chemical application was not satisfactory to reduce corked kernel damage, but two applications produced 60-80% effectiveness in the field. Moreover, this insect is very migratory, so it can travel among host plants and orchards, and the damaging period can extend up to three months in hazelnut orchards. On the other hand, hazelnut growing areas are usually rainy, and this can affect the success of chemical control. In fact, another application for GSB is advised in late May to prevent early season losses, but this application time overlaps with the hazelnut weevil control period (that is the key pest of hazelnut in Türkiye and chemical control is made for it) and is rarely used.

Therefore, considering two chemical applications and 80% effectiveness in control (K=0.8) for calculating the ET value for GSB is a more practical approach. Therefore, we recommend the ET value for GSB as 9.47 insect/ da, about 10 insect/da and 1.9 insect/ 10 "ocak" about 2 insect/10 "ocak", for Türkiye.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	İ. S.	M. K. T	C.T.
C	30	30	40
D	30	30	40
S	30	20	50
DCP	60	20	20
DAI	20	40	40
L	20	30	50
W	30	30	40
CR	30	30	40
SR	30	30	40
PM	70	00	30
FA	70	00	30

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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SOME PATHOGENIC BACTERIA ISOLATED AND IDENTIFIED FROM TRADITIONALLY PRODUCED TURKISH WHITE CHEESE

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Abstract: In this study, some pathogens in the microbiota of traditionally produced White Cheese were determined by molecular methods and their phlogenetic similarities were analyzed. Eight different pathogenic species (*Citrobacter braakii, Hafnia paralvei, Klebsiella grimontii, Kosakonia sacchari, Raoultella ornithinolytica, Raoultella terrigena, Serratia liquefaciens, Serratia plymuthica*) were detected in the White Cheese, and *Klebsiella grimontii* was the dominant species. No study was found in the present studies in which *Klebsiella grimontii* was detected in cheese or dairy products. In addition, no study was found in which *Kosakonia sacchari*, another pathogenic bacterium we detected, was also detected in cheese. This study has revealed some pathogenic microflora in traditionally produced White Cheese.

Keywords: White Cheese, Pathogen microflora, Genotypic characterization, *Klebsiella grimontii*

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1. Introduction

White Cheese, also known as Tin Cheese, Pickled Cheese or Edirne Cheese, ranks first among local cheese varieties in Türkiye in terms of both production and consumption (Hayaloglu et al., 2002; Üçüncü, 2013). White Cheese production constitutes 60-80% of the total cheese production, and the average production amount in 2021 is 330.000 tons (TUIK, 2020).

This cheese is produced in many countries around the world and is known by different names. For example, it is called as Feta in the Mediterranean region, in Denmark and Greece, Bjalo Salamureno Sirene in Bulgaria, Domiati in Egypt, Brinza in Israel, Teleme in Romania, and Queso Blanco in the United States (Bintsis and Papademas, 2002; Hayaloglu et al., 2002). Although it is produced in every region in Türkiye, the majority of the production is carried out in the Thrace, Marmara, Aegean and Central Anatolia regions (Üçüncü, 2013).

White Cheese can be produced from pasteurized or unpasteurized milk without the use of starter culture, mostly in small dairy farms (Karakuş and Alperden, 1995; Hayaloglu et al., 2002). However, while high quality raw milk is mostly used for drinking purposes, the raw milk used for cheese making is generally of low quality. Especially in businesses where traditional and local type cheese are produced, cleaning and hygiene rules are not properly followed, and thus, microorganism contamination is generally highly possible in such environments. As a result of failing to comply with hygiene requirements during milking, transportation and storage, many microorganisms can be transmitted to milk. The sale of cheeses produced using these milks without enough maturation poses a health risk to consumers due to the potential of pathogenic microorganisms to survive (Çelik and Uysal 2009; Yerlikaya, 2018).

The aim of this study is to detect some pathogens in the microbiota of traditionally produced White Cheese.

2. Materials and Methods

2.1. White Cheese Production and Sampling

The cheese variety that ranks first in terms of production and consumption amount in Türkiye is White Cheese. Production is generally carried out in small dairy farms by using unpasteurized milk without the use of starter culture (Üçüncü, 2013). The milk is mostly hand-processed by the cheese master. The milk that will be used to make cheese is put through the clarifier, heated to the proper temperature for fermentation, and then transferred to the fermentation tank. Rennet is added to the extent that it will coagulate in 150 minutes. The clot is divided into pieces of 2 cm^3 and allowed to rest for 5 to 10 minutes to release the whey. 100 kg of cheese milk are put under 20 kg of pressure until the output of whey stops. After the weights are removed, the cheese mass is cut into 7×7×7 cm^3 blocks and placed in 14% brine for two hours. Salted blocks are rested in tin cans in rows for 24 hours. In this way, boxes are packed with 17 kg of cheese in 4 days. Each layer is salted, then the cans are filled with brine (10g/100 ml) and sealed (Hayaloglu et al., 2002; Öner et al., 2006).

In this study, 10 White Cheese samples obtained from production and sales points in 4 different provinces of

Türkiye were used.

2.2. Isolation of Bacteria

10 grams of cheese samples were weighed into sterile stomacher bags and homogenized by adding 90 ml of sterile physiological saline water (PSW). Then, dilution was prepared from the samples, and 10^{-4} and 10^{-5} dilutions were planted on Tryptic Soy Agar (TSA) (MERCK) media by spread plate method, and the petri dishes were incubated aerobically at 37 °C for 48 hours. At the end of the incubation process, morphologically different colonies that developed in the solid medium were selected, taken into the Tryptic Soy Broth (TSB) (MERCK) medium, and incubated at 37 °C for 48 hours. At the end of this period, inoculation was made from TSB medium again to TSA medium with the drawing method and after incubation at the same temperature and time, single colonies were transferred to TSB and incubated again under the same conditions. Stock solutions of bacteria isolates were prepared using 40% glycerol to be used in subsequent analyses and stored at -80 °C.

2.3. Genomic DNA Isolation

Bacterial cells were collected by centrifugation at 7000xg for 10 minutes by taking 1 ml of the culture grown overnight in the liquid culture medium into an Eppendorf tube. After removing the supernatant in the tube, 450 µl of TE (Tris EDTA) buffer was added to the collected cells, and the cells were suspended in the buffer with a gentle mixing. 50 µl of 10% SDS (Sodium dodecyl sulfate) and 2 µl of Proteinase K were added to the suspension cells and incubated for one hour at 37 °C after being thoroughly vortexed. After the incubation, 0.5 ml of phenol:chloroform:isoamyl alcohol (25:24:1) mixture was added, the tubes were mixed thoroughly by turning them upside down and incubated for 5 minutes at room temperature. After the content was centrifuged at 7000xg for 10 minutes at 4 °C, the supernatant-like high-viscosity gel was collected using an automatic pipette and transferred to a new tube. The process was repeated once again with a mixture of phenol-chloroform-isoamyl alcohol, and the supernatant-like high viscosity gel formed was collected in a new tube. 50 µl of 5M sodium acetate was added to the content and mixed gently. It was gently mixed by inverting it after adding 1 ml of isopropanol until white strands of the precipitated DNA appeared. After the content was centrifuged at 3000xg for 10 minutes, the supernatant was removed, 0.5 ml of 70% ethanol was added to the obtained pellet, and after light mixing, and the content was centrifuged at 3000xg for 10 minutes. After removing the supernatant, the contents were kept at 37 °C for 5-10 minutes to remove the remaining ethanol, and then the DNA obtained was suspended by adding 100 µl of distilled water (İspirli, 2016).

2.4. Genotypic Characterization by RAPD-PCR

RAPD-PCR was carried out to perform preliminary discrimination from bacteria. PCR mixtures were prepared containing 5×PCR buffer for Taq polymerase (Promega), 2.5mM of dNTPs (Bioline), 1.5 U Taq polymerase (Promega) and 25 pMol of primer M13 (GAGGGTGGCGGTTCT). PCR was performed with the following program: 35 cycles of 94 °C for 1 min, 40 °C for 20 s, then final step of 72 °C for 2 min. The PCR products were separated with electrophoresis on 1.6% (w/v) agarose gels at 90 V for 1.5 h and band patterns were visualised (Yuvaşen et al., 2018).

2.5. PCR Amplification of the 16S rRNA Region

After RAPD-PCR, 16S PCR was applied for colonies that were thought to be genotypically different. Universal primers AMP F (5'-GAGAGTTTGATYCTGGCTCAG - 3') and AMP R (3'- AAGGAGGTGATCCARCCGCA - 5') were used to amplify the 16S rRNA gene of the strains. PCR mixes were prepared by adding 1µl template DNA, 4 µl $MgCl_2$, 0.4 µl dNTPs, 1.0 µl 20 mM AMP-F primer, 1.0 µl 20 mM AMP-R primer, 0.25 µl Taq polymerase and up to 50 µl of sterile H_2O. PCR program was performed as 1 cycle at 95 °C for 2 min, 25 cycles at 95 °C for 30 s, at 55 °C for 20 s, at 72 °C for 30 s and at 72 °C for 5 min final extension.

PCR products were run on a gel to control amplification, and amplicons were sent to Medsantek (Istanbul) for sequencing.

2.6. Nucleotide Access Numbers and Phylogenetic Analysis

The 16S sequences of 20 pathogenic bacteria identified in this study were deposited in Genbank with different nucleotide access numbers. Phylogenetic trees were constructed using MEGA4.

3. Results and Discussion

As a result of genotypic characterization of 80 bacteria isolated from artisanal Turkish White Cheese, 20 pathogen bacteria belonging to 8 different species were determined (Table 1).

Citrobacter braakii, Hafnia paralvei, Kosakonia sacchari were detected in one sample, *Raoultella ornithinolytica, Raoultella terrigena, Serratia liquefaciens, Serratia plymuthica* in two samples and *Klebsiella grimontii* in six samples (Table 1). *Klebsiella grimontii* was the prominent pathogen bacteria in White Cheese samples (Figure 1). 1 species in each of samples 6 and 9, 2 species in each of 2 and 8, 3 species in each of 3 and 7, and 5 species of potentially pathogenic bacteria were detected in sample number 1. No pathogenic bacteria were detected in samples 4, 5 and 10.

2 isolates each of *Citrobacter braakii*, *Raoultella ornithinolytica*, *Raoultella terrigena*, *Serratia liquefaciens*, *Serratia plymuthica*, 1 each of *Hafnia paralvei*, *Kosakonia sacchari* and 8 isolates of *Klebsiella grimontii* were identified. Figure 2 shows phylogenetic relationships of 16S rRNA genes of different pathogenic bacterial strains isolated from Turkish White Cheese. According to Figure 2, it is seen that *Klebsiella grimontii*, *Kosakonia sacchari*, *Citrobacter braakii*, *Raoultella terrigena*, *Raoultella ornithinolytica*, *Serratia liquefaciens* clustered together, *Hafnia paralvei* and *Serratia plymuthica* separated from them and formed a different group.

Table 1. Distribution of pathogenic bacterial strains detected in traditionally produced Turkish White Cheese samples (+and - represent the presence of each species within the corresponding sample).

Isolate code	GAN	Pathogenic bacteria	Codes of cheese samples									
			1	2	3	4	5	6	7	8	9	10
TS-1	OP985117	*Citrobacter braakii*	+	-	-	-	-	-	-	-	-	-
TS-3	OP985119	*Citrobacter braakii*	+	-	-	-	-	-	-	-	-	-
TS-7	OP985122	*Hafnia paralvei*	+	-	-	-	-	-	-	-	-	-
TS-4	OP985120	*Klebsiella grimontii*	+	-	-	-	-	-	-	-	-	-
TS-14	OP999616	*Klebsiella grimontii*	-	-	+	-	-	-	-	-	-	-
TS-31	0P985316	*Klebsiella grimontii*	-	-	-	-	-	-	+	-	-	-
TS-33	OP985319	*Klebsiella grimontii*	-	-	-	-	-	-	+	-	-	-
TS-34	OP985321	*Klebsiella grimontii*	-	-	-	-	-	-	-	+	-	-
TS-35	OP999353	*Klebsiella grimontii*	-	-	-	-	-	-	-	+	-	-
TS-52	OP999367	*Klebsiella grimontii*	-	-	-	-	-	+	-	-	-	-
TS-60	OP999613	*Klebsiella grimontii*	-	-	-	-	-	-	-	-	+	-
TS-2	OP985118	*Kosakonia sacchari*	+	-	-	-	-	-	-	-	-	-
TS-36	OP999354	*Raoultella ornithinolytica*	-	-	-	-	-	-	-	+	-	-
TS-54	OP999630	*Raoultella ornithinolytica*	-	-	-	-	-	-	+	-	-	-
TS-9	OP985123	*Raoultella terrigena*	-	+	-	-	-	-	-	-	-	-
TS-18	OP985300	*Raoultella terrigena*	-	-	+	-	-	-	-	-	-	-
TS-11	OP999365	*Serratia liquefaciens*	+	-	-	-	-	-	-	-	-	-
TS-56	OP999370	*Serratia liquefaciens*	-	-	-	-	-	-	+	-	-	-
TS-17	OP985318	*Serratia plymuthica*	-	-	+	-	-	-	-	-	-	-
TS-43	OP999366	*Serratia plymuthica*	-	+	-	-	-	-	-	-	-	-

GAN= genbank accession number

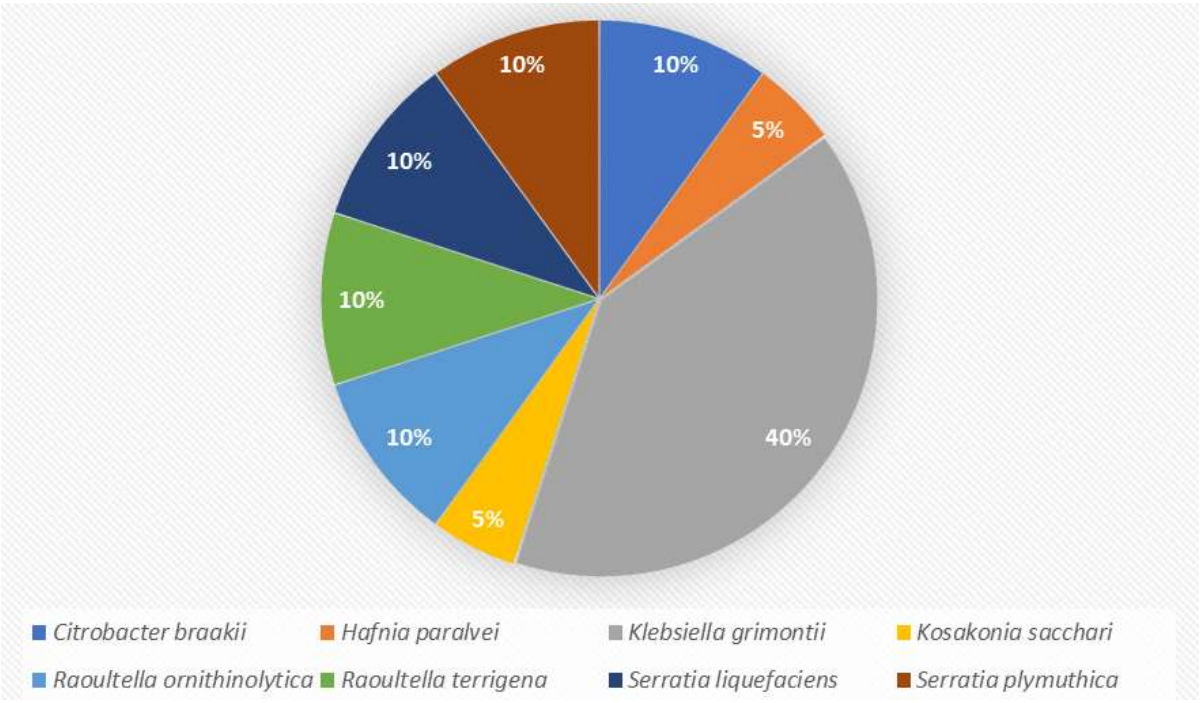

Figure 1. Proportional distribution of pathogenic bacteria in the Turkish White Cheese.

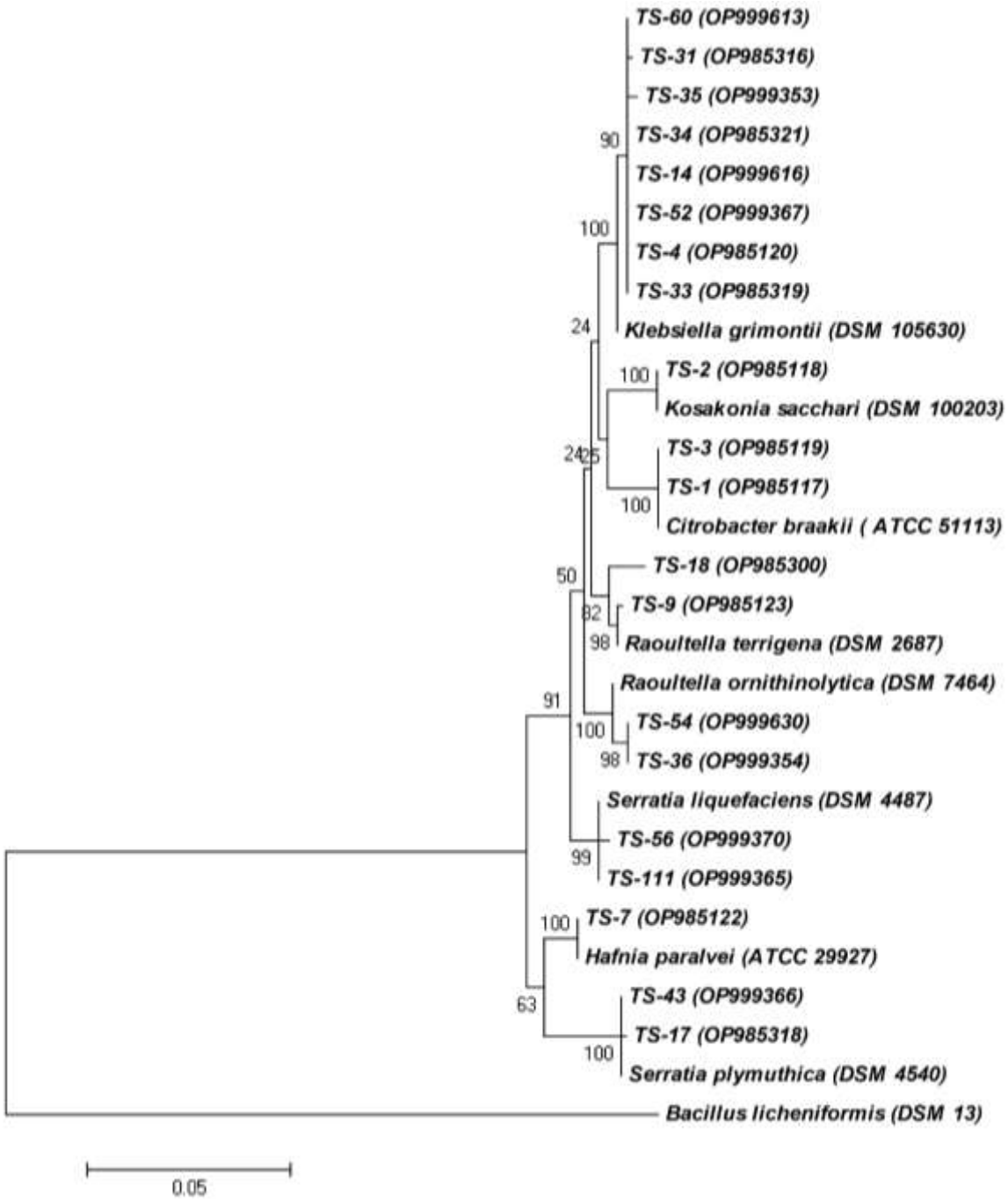

Figure 2. Dendrogram showing multiple sequence alignment of 16S rRNA gene sequences of pathogenic bacteria isolated from Turkish White Cheese.

Citrobacter braakii is a common species in nature and is a pathogen primarily found in the human urinary tract (Trivedi et al., 2015; Castellanos-Rozo et al., 2021). Gupta et al. (2003) reported that Citrobacter spp. are increasingly observed in infections in immunocompromised individuals. *C. braakii* was detected in artisanal Italian ewe's cheese by Chaves-López et al. (2006); Paipa Cheese by Castellanos-Rozo et al. (2021); May Bryndza Cheese, a traditional Slovak cheese produced from unpasteurized sheep's milk, by Pangallo et al. (2014); Bryndza Cheese by Kačániová et al. (2020). In a study examining the distribution of Enterobacteriaceae and some pathogenic microorganisms in unbranded White Cheese samples sold in Ankara, 1.16% of *C. braakii* was detected (Uraz et al., 2009). This species is considered to be among the species responsible for early swelling in soft and semi-hard sheep milk cheeses made from raw milk (Tabla et al., 2016).

Putrescine is one of the most common Biogenic amines (BAs) in foods. An excessive presence in foods can cause food poisoning due to increased toxic effects of other BAs and reduced food quality. This amine is potentially carcinogenic and *C. Braakii* was shown by Wunderlichová et al. (2014) among the microorganisms that form Putrescine in foods. Chaves-López et al. (2006) conducted a study on pasteurized skim milk and reported that *C.*

braakii that was isolated from artisanal Italian ewe's cheese produced biogenic amines such as Ethylamine, Tryptamine, Phenylethylamine, Putrescine, Cadaverine, Spermidine, Spermine, especially histamine.

Hafnia paralvei was another species detected in the White Cheese samples. Hafnia, a gram-negative enterobacterium genus, is frequently found in raw milk and raw milk cheeses. Although most gram-negative bacteria in cheese are known to cause spoilage and off-flavor, Hafnia species such as *H. alvei* and *H. paralvei* can improve cheese flavor by producing sulfur aromatic compounds (Yoon et al., 2016).

Characterization of autochthonal *Hafnia* spp. *strains isolated* from *Spanish soft raw* ewe's *milk PDO cheeses* for use as co-cultures resulted in the determination of *H. paralvei* as the dominant species among 17 different species (Merchán et al., 2022). *Hafnia paralvei* was among the species identified by Castellanos-Rozo et al. (2021) in cheeses produced by formal and informal micro-enterprises in Paipa, Colombia. The report stated that, for Paipa cheese, the focus should be on improving the hygienic quality of the milk from which it is made.

In a study on the safety evaluation of Gram-negative bacteria associated with traditional French cheeses, it was stated that 4 species, including *H. paralvei*, were highly toxic to larvae, suggesting the presence of potential lethal factors in these strains. To the best of our knowledge, no foodborne poisoning or outbreak has been reported for any GNB (gram negative bacteria) of the genera/species related to the strains tested so far. The role of multiple dynamic interactions between cheese microbiota and GIT (gastrointestinal tract) barriers may be key factors explaining the safe consumption of the corresponding cheeses (Imran et al., 2019).

Klebsiella grimontii was the most prevalent species in the White Cheese samples examined in the present study. It was detected in six samples. Eight of the 20 isolates identified were *K. grimontii*. *K. grimontii* is a newly described species of the genus Klebsiella in the family Enterobacteriaceae. The genus Klebsiella contains important human and animal pathogens and is widely observed in the environment and animals (Passet and Brisse, 2018). *K. grimontii* is associated with human infections such as bacteremia and soft tissue infection and has been found in France, Germany, and South Africa (Liu et al., 2018). In the present studies, no study was found in which this species was detected in cheese or dairy products.

Another pathogenic bacterium detected in cheese samples was *Kosakonia sacchari*. *K. sacchari* was isolated from the surface sterilized stem of sugarcane cultivar in China in 1994 (Chen et al., 2014). It was isolated from surface-sterilized stem of a sweet potato by Shinjo et al. (2016). Abd-Elhafeez et al. (2018) reported that this strain leads to soft rot in potatoes. In the current studies, no study was found in which this species was detected in cheese.

Raoultella ornithinolytica was another species detected in two White Cheese samples. *R. ornithinolytica* has been described by a number of researchers as a pathogenic species living in aquatic environments (Seng et al., 2016; Papadakis et al. 2021). This bacterium was detected in Montasio Cheese by Maifreni et al. (2013), in Bryndza Cheese by Kačániová et al. (2021a), in Parenica Cheese by Kačániová et al. (2021b), in Brazilian Minas Frescal Cheese by Teider et al. (2019), in soy cheese by Djogbe et al. (2019).

Raoultella terrigena was detected in two cheese samples. Two of the 20 isolates belonged to this species. *Raoultella terrigena* (*Klebsiella terrigena*) was previously classified in the genus Klebsiella (Podschun et al., 2000). This species is an opportunistic pathogen that is rare in nature and has a high mortality rate (up to 44%) (Lekhniuk et al., 2021).

R. terrigena was detected in raw milk by Kongo et al. (2008), in Bryndza Cheese by Pangallo et al. (2014), in the microbiota of Istrian Cheese by Fuka et al. (2010)

Serratia liquefaciens was detected in two cheese samples. *S. liquefaciens* is a psychrotrophic and highly mobile microorganism commonly found in water, soil, vegetation, and more specifically in dairy and raw milk. *S. liquefaciens* is a pathogenic microorganism that can cause infections in humans, especially in immunocompromised hosts. Pink color defects on the surface of some cheeses have been associated with this bacterium by some researchers (Martelli et al., 2020)

S. liquefaciens was the dominant species detected in the microflora of Spanish farmhouse goat's milk cheeses and Picante Cheese (a hard, very salty and spicy traditional cheese produced in Portugal from a mixture of goat's and sheep's milk) (Freitas et al., 1996; Martín-Platero et al., 2009).

Another species detected in the White Cheese samples was *Serratia plymuthica. S. plymuthica* is found in soil and has also been isolated from different types of food. Elshaer (2019) stated that *S. plymuthica* is rarely isolated from clinical specimens, but it should be considered as a serious multi-drug resistant pathogen, especially in immunocompromised patients. Hleba et al. (2021) reported that the isolates obtained from milk and dairy products samples were resistant to three antibiotics (Tetracyclines, Chloramphenicol, Ampicillin).

In the study where Uraz et al. (2009) examined the distribution of Enterobacteriaceae and some pathogenic microorganisms in unbranded White Cheese samples sold in Ankara, *Serratia plymuthica* was found to be 1.16%.

4. Conclusion

In conclusion, eight different pathogenic bacteria were isolated from the microbiota of traditionally produced Turkish White Cheese. Traditionally produced White Cheese may pose health risks to consumers. Therefore, it is crucial to reduce microbiological risk by taking certain measures such as making production out of pasteurized milk using starter culture, adhering more strictly to hygiene rules throughout the production, and conducting a proper maturation process.

Author Contributions

The percentage of the author contributions is present below. The author reviewed and approved final version of the manuscript.

	E.M.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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DISTRIBUTION OF BIRTHS OF BAFRA SHEEP REARED IN THE MEDITERRANEAN REGION DURING THE DAY

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Abstract: In this study, the distribution of birth data during the day (24 hours) of 651 lambs born in 2019-2022 of Bafra sheep reared in a private sheep farm in Kızıllı village of Antalya province was investigated. In the study, the effects on the distribution of births during the day and the characteristics of lambs such as birth type, sex, difficult and normal births, and dam's lactation number were investigated. The 24 hours were divided into four equal periods such as 06:01-12:00 = 1, 12:01-18:00 = 2, 18:01-00:00 = 3, and 00:01-06:00 = 4 to evaluate the distribution within the day. $\chi 2$ (Chi-square) test analysis was used to determine whether the characteristics such as, birth type, sex, difficult and normal births, and dam's lactation number affected the distribution of births during the day. As a result of the analysis, the distribution of a total of 651 lamb births during the day was observed as 260 heads (39.9%) at 06:01-12:00, 199 heads (30.6%) at 12:01-18:00, 109 heads (16.7%) at 18:01- 00:00, and 83 heads (12.7%) at 00:01-06:00, respectively. In the first and second time periods between 06:01 and 18:00, 70.5% (459 heads) of lamb births occurred. The ratio and number of lambs in sex distribution were determined as 45.8% male (298 head) and 54.2% female (353 head), respectively. The number and rate of lamb births of ewes in the first lactation comprised 366 heads and 56.2%, respectively. According to the $\chi 2$ test of the time periods of the births, the effect of the lamb's birth type ($P<0.05$), difficult and normal births ($P<0.05$) and dam lactation number ($P<0.01$), were statistically significant while the effect on the lamb's sex was found to be insignificant. As a result, considering the distribution of births during the day, it was seen that they were concentrated during the daytime hours. Being more careful in herd management during these hours will increase the profitability of Bafra sheep, which is one of the sheep breeds with high fertility.

Keywords: Bafra sheep, Distribution of births, Birth type, Dystocia

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1. Introduction

Sheep breeding is an important livestock activity in Türkiye due to reasons such as meadow-pasture conditions, climate and geographical structure, providing employment, reversing migration from village to city, fertility, suitability for socio-economic structure and being an alternative to beef (Güngör and Akçapınar, 2013; Aytekin et al., 2015). Domestic breeds constitute the majority of the sheep population reared in Türkiye. One of the methods to increase fertility in sheep breeding is to increase the genetic value of sheep or to improve the genotype (Sönmez et al., 2009). For this purpose, as a result of the back crossing of the Chios breed (sire line) bred in the coastal areas of the Aegean region and the Karayaka breed (dam line) in the Black Sea coastal regions, a new type called Bafra sheep, which is 75% Chios and 25% Karayaka in terms of milk and reproduction, has been obtained. Breed registration of the Bafra breed has also been made, and its breeding is becoming widespread in many regions of Türkiye due to its adaptability (Sönmez et al., 2009; Güngör and Akçapınar, 2013).

Another way to increase productivity is to provide better environmental conditions. Knowing the animal behavior makes it possible to optimize the most suitable environmental conditions. Animal behaviors; they are the physical reactions against certain stimuli with the effect of genotype and environment (Metin and Kaliber, 2011). Knowing animal behaviors enables understanding an animal's distinctive features and how it reacts to the environment (Özçalık, 2010). It is also important to optimize both productivity and animal welfare. Behavior is an important criterion in the evaluation of animal welfare and is an indicator of the quality of life perceived by the animal (Bracke and Hopster, 2006). Achieving the desired welfare levels of animals may increase with its reflection on the yields to be obtained from animals (Akbaş, 2013). Thus, considering the reactions of the animals, the most efficient methods that can result in problem-free animal breeding can be determined (Zülkadir and Karabacak, 2013). In addition, recognizing the behavior of sheep will provide some important advantages for animal breeding, care, feeding and herd management. Also, taking reproductive behavior into account will lead to an increase in fertility in animal production. Increasing the fertility of the offspring depends on the provision of suitable conditions as soon as possible after birth and the successful first hours of

the offspring's life depending on the relationship between the born offspring and the dam. Because the period from the beginning of the birth up to a few hours is a period during which there is intense stress for the dam and the lamb and various complications can occur, and the bond established between the dam and the lamb with the beginning of the birth affects the survival power of the lamb (Konyalı et al., 2004; Öztürk, 2012). It is important for breeders to know when the births take place or in what time period, to have a strong bond with the dam, to increase the viability of the born offspring, to provide a suitable environment for birth, and to implement the breeding systems in ideal criteria for animal welfare in herd management.

This study aims to examine the birth distribution of Bafra sheep with multiple births reared in Antalya province, reveal the practices that can contribute to herd management by using this distribution, and present this information to the breeders.

2. Materials and Methods

2.1. Materials

The animal material of the study consisted of the birth records of 651 lambs born in 2019-2022 in Altepe Bafra Sheep Breeder (ABSB) Farm located in Antalya province Kızıllı village located at 37° 6' 46.3860'' North and 30° 42' 50.2776'' East coordinates. No experimental animals were used in this study, and the records kept in the enterprise were taken into consideration.

2.2. Method

Reproduction management in the ABSB enterprise is in the form of three lambing in two years, and additional feeding is applied in order to ensure pregnancy and increase the viability of the lambs. In this context, 1 kg/day alfalfa hay and 500 g/day barley grain are given per animal. The application continues for four weeks until the mating and 4-4.5 weeks after the mating. After mating of sheep, for about 2-2.5 months, per animal is fed with only 1 kg/day alfalfa hay. According to the results of the pregnancy examination performed with ultrasound after 2.5 months, the pregnant animals are given 1 kg/head/day of dried alfalfa grass for the first month, and ryegrass dried grass instead of alfalfa hay and in addition to this, 600 g of concentrate starting from 200 g barley is given for one and a half month before the birth. Half of the concentrated feed is given as grain barley in the morning and the remaining half is given as concentrate feed in the afternoon. After giving birth and suckling their lambs, the ewes are fed with 1 kg/head/day alfalfa hay and 1 kg milk feed for two months. At the end of two months, the lambs are weaned and their dams are taken out to dry in preparation for the next mating season. 1 kg/head/day of barley straw and alfalfa are given to the dried animals. Sheep showing signs of giving birth are taken to the individual delivery compartment that has been prepared beforehand in the sheepfold (Figure 1). During the lambing period, the herd housed in the sheepfold is observed hourly and 24/7 (day/hour) by camera throughout the day until the birth is completed. Earrings of the born lambs were prepared; data on date of birth, time of birth, type of birth and sex were recorded. To evaluate the data on the distribution of births during the day, a day was divided into 4 equal time periods of six hours each. The time periods are set as 06:01-12:00=1, 12:01-18:00 =2, 18:01-00:00=3 and 00:01-06:00=4. In the birth data of Bafra sheep; the birth distributions of the characteristics such as birth type (single=1, twin=2, multiples=3, quadruplets=4, quintuplets=5), sex of lambs (male, female), veterinary-assisted birth; difficult birth (1), normal birth without any intervention (2) and the number of lactation (1, 2, 3, 4) were evaluated according to the time periods during the day.

Figure 1. Individual birth sections in Altepe Bafra Sheep Breeder Farm.

2.3. Statistical Analysis

Whether there is a dependency between the time period and other traits was checked with the help of the chi-square test. The "chisquare" package of the R software was used for all analyses (R Core Team, 2020).

3. Results and Discussion

3.1. Distribution of Births during the Day

The distribution of births according to the 24-hour period of the day is given in Table 1.

According to Table 1, while the most intense (39.9%) lamb birth occurred between 06:01-12:00 hours, which is the 1st time period, the least lamb birth (12.7%) occurred in the 4th time period, between hours 00:01-06:00. Of the birth of 651 lambs, 459 (70.5%) occurred between 06:01-12:00 and 12:01-18:00 in the 1st and 2nd time periods, while 192 heads (29.5%) were born in the 3rd and 4th time periods between the hours of 18:01-00:00 and 00:01-06 00. Öztürk (2012) reported that births in Akkaraman sheep were the most intense (31.2%) between 16:00 and 22:00, and the lowest (15.6%) between 22:00 and 04:00.

Table 1. Distribution of birth during the day

Time Period	n	%
06:01-12:00 (1)	260	39.9
12:01-18:00 (2)	199	30.6
18:01-00:00 (3)	109	16.7
00:01-06:00 (4)	83	12.7
Total	651	100

Karabacak and Zülkadir (2014) stated that 54% of births occurred between 04.01-16.00 and 46.07% between 16.01-04.00 in Anatolian Merinos. Büyüktekin et al. (2015) stated that 53.10% of births occurred between 04:00- 16:00, and 46.90% occurred between 10:00-04:00 in Akkaraman sheep. The same researchers also reported that the distribution of lamb births during the daytime was 56.95% between 06:00-18:00, and 43.5% in the night time between 18:00-06:00. Karabacak et al. (2015) reported that 47.30% (238 heads) of births occurred between 05:01-17:00 during the day and 52.70% (265 heads) at 17:01-05:00 at night in 503 Anatolian Merinos. Ramirez et al., (1995) determined in their study on 90 Murciano-Granadina goats that over 90% of births were between 06.00-18.00 hours and 9% between 18.00-06.00 hours. As reported by Konyalı et al. (2004), 88.1% of births in 32 heads of Turkish Saanen goats occurred between 06.00-18.00, 4.8% and 7.1% of them occurred between 18.00-24.00 and 24.00-06.00, respectively. Erduran and Yaman (2014) reported that the birth rate of 189 Saanen goats was 78.2% and 21.8% between 04:01-16:00 and 16:01-04:00, respectively. The results of the present study are consistent with some of the above-mentioned research results while they are inconsistent with others. Probably the most important reason for this is the high twin or multiple births seen in Bafra sheep. Besides; this distribution may change according to the noise pollution of the enterprise. The sheep may prefer to give birth at quieter times of the day, or perhaps the presence of the workers, their movements or the sounds they make may have created an environment of trust (no threat of predators etc.). Because in this study, the births were mostly from the hours when the working hours started at 06.01 until 18.00. In addition, it may be due to the fact that other studies were carried out in different breeds, under different care and feeding conditions, in different regions, with changes in herd management and different mating methods.

3.2. Distribution of Births during the Day According to Birth Types

The results of the distribution of births during the day by birth type are presented in Table 2.

Table 2 shows that the highest number of births (260 births) occurred between 06.01-12.00 hours. In this interval, twin births were the highest with 42.3%, followed by triplets, singletons, quadruplets and quintuplets, respectively. In general, the order of the number of births in other time periods was observed in twin, triplet, quadruplet, singleton and quintuple births. In the study, the number and rate of lambs in the distribution of multiple births were 568 heads and 87.2%, respectively. As can be seen in Table 2, the number and proportions of twin lambs were the highest with 272 heads and 41.8%, the number and rates of lambs of triplets, quadruplets and singletons were 183 heads and 28.1%, 88 and 13.5% and 83 heads 12.7%, respectively. Quintuplet lambs showed the lowest values. The total rate of lambs with twins (41.8%) and triplets (28.1%) was 69.9% according to the time periods of the day. In addition, quintuple births were never seen between 00:01-06:00 in the time frame of births. The differences observed in the distribution of births during the day showed significant differences according to the birth type ($P<0.05$). Erduran and Yaman (2014) reported that the distribution of births during the day in Saanen Goats was between 10.01-16.00 at the peak time in both single (58.16%) and twin (44.51%) births. Births that occurred between 04.01-10.00 were in the second rank. This was followed by the 16.01-22.00 time frame, while the lowest birth density was between 22.01-04.00. Öztürk (2012) stated that the distribution of births according to singleton and twin birth types in Akkaraman sheep was highest in the range of 16.00-22.00 with 30.6% and 37.5%, respectively, and the lowest in the range of 22.00-04.00 (16.2% for singles and 8.3% for twins). Karabacak et al. (2012) determined that in the distribution of births during the day in Akkaraman sheep, single births were highest with 30.1% between 10.01-16.00, while the lowest between 16.01-22.00, and twin births were the highest (38.5%) in the time period of 16.01-22.00, and the lowest (15.4%) between 22.01-04.00 and 04.01-10.00. The similarities or differences in the literature reports may also be due to the yield aspects of the animal, such as the type of birth that shows differences between species and breeds.

3.3. Distribution of Births during the Day by Lamb Sex

The distribution of births during the day by lamb sex is given in Table 3.

According to the sex of male lambs in Table 3, 40.27% of total 298 male lambs were born between 06.01 and 12.00, and 32.21% were between 12.01 and 18.00, while 72.48% of male lambs were born between 06.01-18.00 in these two-time intervals. 27.52% of the remaining male lambs were born between 18.01-06.00. On the other hand, 39.66% of 353 female lambs were born between 06.01-12.00 and 29.18% between 12.01-18.00. The sum of these two time periods is 68.84%. Of female lambs, 21.16% were born between 18.01-06.00. This result coincides with the birth times of male lambs. According to the χ^2 test, the distribution of births during the day did not differ according to the sex of the lamb. In a study of Karabacak et al. (2012) on Akkaraman sheep, they found that out of total 119 lambs, the distribution of male and female lambs was 57.9% between 04.01-16.00, while the total share of both sexes was 42% between 16:01-04:00.

Zülkadir and Karabacak (2013) reported that the most intense births were in the 23.01-05.00 time period, and male and female lamb birth rates in Akkaraman sheep were 38.04% and 47.06%, respectively, and male and female lamb birth rates in Awassi sheep was 45% and 34.55%. The same researchers found the lowest birth rates in Akkaraman male lambs between 17.01-23.00 as 16.30%, and in female lambs in the range of 11.01-17.00 as 10.29%, and in Awassi breeds the lowest birth rate in the same time period in male and female lambs, between 17.01- 23.00, as %10.00 and 14.55% respectively. Büyüktekin et al. (2015) reported that in the distribution of Akkaraman sheep during the day, 51.25% were male (1069 heads) out of 2086 lambs, while 48.75% were female (1017 heads). The same researchers stated that the highest birth rate between 06:00-18:00 hours was 28.19% male lambs and 28.76% female lambs. Karabacak et al. (2015) found that the distribution of births during the day in Anatolian merino sheep was most intense between 23.01-05.00 hours, with 29.04% and 35.06% in male (97 heads) and female (122) lambs, respectively, and the lowest between 11.01-17.00 hours with male and female lamb numbers and rates with 72 (21.56%) and 67 (19.25%) lambs, respectively.

3.4. Distribution of Births during the Day According to Difficult and Normal Births

The distribution of deliveries during the day according to difficult and normal deliveries is given in Table 4.

According to Table 4, 26.1% of 651 births were difficult delivery. While 65.88% of these difficult deliveries were most intense between 06.01-18.00, it was the lowest with 10% in the 00.01-06.00 range. When we look at normal deliveries, 72.14% of them are in the range of 06.01-18.00, as in difficult births. 27.86% of normal deliveries were realized between 18.01-06.00. The rates of difficult and normal births in total cases were 26.1% and 73.9%, respectively. According to the results of the χ^2 test, the variation in the distribution of births during the day compared to difficult and normal births was statistically significant ($P<0.05$). There is no literature on classifying and evaluating the distribution of births during the day according to difficult or normal births. However, regarding difficult births, Konyalı et al. (2004) reported that 64% of the births were twins in their study on goats and 63% of these births were completed without assistance. According to Ali, (2011), the causes of difficult birth in sheep and goats can be maternal and fetal origin (multiple birth, lamb/kid size, fetal development disorder, anomalies, etc.).

Table 2. Distribution of births during the day according to birth types

Birth Type	Time Period				Total
	06:01-12:00 n (%)	12:01-18:00 n (%)	18:01-00:00 n (%)	00:01-06:00 n (%)	n (%)
Single	46 (17.7)	17 (8.5)	10 (9.2)	10 (12.0)	83 (12.7)
Twin	110 (42.3)	88 (44.2)	48 (44.0)	26 (31.3)	272 (41.8)
Triplet	66 (25.4)	60 (30.2)	26 (23.9)	31 (37.3)	183 (28.1)
Quadruplets	28 (10.8)	24 (12.1)	20 (18.3)	16 (19.3)	88 (13.5)
Quintuplets	10 (3.8)	10 (5.0)	5 (4.6)	0 (0)	25 (3.8)
Total	260 (39.9)	199 (30.6)	109 (16.7)	83 (12.7)	651 (100.0)
	χ^2= 25.437		DF= 12	P= 0.013	

n= number of animals, χ^2= Chi-square result, DF= degree of freedom, P= significance level.

Table 3. Distribution of births during the day according to sex types of lambs

Sex	Time Period				Total
	06:01-12:00 n (%)	12:01-18:00 n (%)	18:01-00:00 n (%)	00:01-06:00 n (%)	n (%)
Male	120 (46.2)	96 (48.2)	47 (43.1)	35 (42.2)	298 (45.8)
Female	140 (53.8)	103 (51.8)	62 (56.9)	48 (57.8)	353 (54.2)
Total	260 (39.9)	199 (30.6)	109 (16.7)	83 (12.7)	651 (100.0)
	χ^2= 1.247		DF= 3	P= 0.742	

n= number of animals, χ^2= Chi-square result, DF= degree of freedom, P= significance level.

Table 4. Distribution of deliveries during the day according to difficult and normal deliveries lambs

Birth	Time Period				Total
	06:01-12:00 n (%)	12:01-18:00 n (%)	18:01-00:00 n (%)	00:01-06:00 n (%)	n (%)
Difficult	54 (20.8)	58 (29.1)	41 (37.6)	17 (20.5)	170 (26.1)
Normal	206 (79.2)	141 (70.9)	68 (62.4)	66 (79.5)	481 (73.9)
Total	260 (39.9)	199 (30.6)	109 (16.7)	83 (12.7)	651 (100.0)
	χ^2= 13.634		DF= 3	P= 0.003	

n= number of animals, χ^2= Chi-square result, DF= degree of freedom, P= significance level.

Normal and difficult birth rates were 55.9% and 44.1% in the Awassi breed (161 heads), respectively, and 73.7% and 26.3% in the Naidi breed (19 heads). The maternal and fetal birth rate in the 1st lactation was reported as 81.1% and 18.9%, respectively, and as 43.2% and 56.8%, respectively, in multiple (2nd lactation and later) lactation. Kuru et al. (2016) reported that the incidence of difficult delivery in sheep and goats varied between 3-5% on average, and the most common cause of congenital disabilities was fetal congenital disabilities with a rate of 50-60%. They stated that 35 of them were due to obstructions in the birth canal in sheep and 20% of them were due to the pelvic diameter disproportion of the fetus and the maternal in goats. Mostefaı et al. (2019) stated that while the rate of fetal causes in difficult birth is 75%, it is common in 16% of maternal causes, and it constitutes 9% of both fetal and maternal causes. According to Jacobson et al. (2020), risk factors for dystocia in lambs include malpresentation, sickness, or congenital abnormalities, as well as fetopelvic disproportion, uterine inertia, the difficulty of the cervix to fully dilate, and malpresentation. High (fat) or low liveweight ewes, as well as tiny first parity ewes, all enhance the risk of dystocia. Inadequate levels of muscle glycogen, pregnancy toxicity, mineral imbalances causing hypocalcaemia, and a lack of antioxidant nutrients are all possible contributors. For this reason, sheep with single and multiple pregnancies must be fed differently.

The current study's finding of a difficult birth rate of 26.1% may be related to the high rate of multiple births in Bafra sheep, as well as the literature findings noted above.

3.5. Distribution of Births within the Time of Day According to Lactation Number

The distribution of births within the time of day according to lactation number is shown in Table 5.

In Table 5, the highest number of births occurred in the sheep in the first lactation. While 33.9% of the sheep in the first lactation gave birth between 06.01-12.00, the lowest birth rate during this lactation was 16.7% in the range of 01.01-06.00. However, the lowest birth rate (4.6%) was seen in the 4th lactation. In the fourth lactation, the deliveries were highest at 43.3% between 18.01-00.00 and lowest at 3.3% between 00.01-06.00.

As can be seen from Table 5, sheep with lactation number 1 accounted for the highest proportion of 366 lambs at 56.2%, while the least lamb birth occurred with 30 lambs with 4.6% in lactation number 4. In the time period of the day, 260 (39.9%) lambs were born between 06:01-12:00 at the most, and 83 (12.7%) lambs were born at the least between 00:01-06:00. Lamb birth of sheep with lactation number 1 and 2 was 88.8% with 578 lambs. According to the $\chi 2$ test, it was found significant that the distribution of deliveries during the day differed according to the lactation number ($P<0.01$).

In the literature, few sources (Karabacak et al., 2012; Zülkadir and Karabacak, 2013; Karabacak and Zülkadir, 2014; Karabacak et al., 2015) have been found in the domestic sheep breeds regarding the distribution of births during the day according to the lactation number and maternal age was considered as factor instead of lactation number. However, Karabacak et al. (2012) observed Akkaraman sheep in all time periods of the day; 26.9% (32 heads) of sheep giving birth for the first time, 34.5% (41 heads) in the 3rd lactation, 21.9% (26 heads) in the 4th lactation, 10.9% (13 heads) in the 5th lactation and 5.8% (7 heads) in the 6th lactation. They found that the highest number of births in the time periods between 04.01-10.00 was with 43.75% (14 heads) in the 1st lactation and 36.58% (15 heads) in the 3rd lactation between 10.01-16.00. Zülkadir and Karabacak (2013) reported the number and rates of births in Akkaraman breed at 23.01-05.00, 05.01-11.00, 11.01-17.00 and 17.01-23.00 time periods, respectively, as 67 (41.88%), 38 (23.75%), 26 (16.25%) and 29 (18.13%), and in the Awassi breed as 37 (38.95%), 25 (26.32%), 21 (22.11%) and 12 (12.63%). The same researchers stated that the highest number of births in Akkaraman breed occurred in sheep in the 3rd lactation and they occurred at the rates of 37.31%, 28.95%, 42.31% and 37.93%, respectively, at 23.01-05.00, 05.01-11.00, 11.01-17.00 and 17.01-23.00 hours. In the Awassi breed, they reported 57.14% in the 1st lactation between 11.01-17.00 hours, and those in the 6th lactation and above in the 23.01-05.00, 05.01-11.00 and 17.01- 23.00 time periods, respectively, as 24.32%, 24.00% and 33.33%. Karabacak and Zülkadir (2014) stated that the highest number of births occurred between 04:01-10:00 (27.18%) when considering the distribution of births during the day according to the lactation number in the Anatolian Merino breed.

Table 5. Distribution of births within the time of day according to lactation number

Lactation number	Time Period				Total
	06:01-12:00 n (%)	12:01-18:00 n (%)	18:01-00:00 n (%)	00:01-06:00 n (%)	n (%)
1	124 (33.9)	118 (32.2)	63 (17.2)	61 (16.7)	366 (56.2)
2	106 (50.0)	66 (31.1)	32 (15.1)	8 (3.8)	212 (32.6)
3	19 (44.2)	10 (23.3)	1 (2.3)	13 (30.2)	43 (6.6)
4	11 (36.7)	5 (16.7)	13 (43.3)	1 (3.3)	30 (4.6)
Total	260 (39.9)	199 (30.6)	109 (16.7)	83 (12.7)	651 (100.0)
χ^2= 60.614		DF= 9	P= 0.000		

n= number of animals, χ^2= Chi-square result, DF= degree of freedom, P= significance level.

The same researchers reported the highest birth rates of those in the 2nd, 3rd and 4th lactations in the same time period as 28.88%, 31.78% and 29.26%, respectively. Karabacak et al. (2015) found that the highest birth rate was 30.82% between 23.01-05.00 hours, and the highest birth rate (35.61%) was observed in 3 years old of dams. According to the results of the current study, when all lactations are taken into account, it can be stated that 56.2% of the sheep in the first lactation had the highest birth rate, and 33.8% (124/366) of them occurred in the range of 06.01-12.00 and 32.2% in the 12.01-18.00 time period. In other words, 66% of the sheep in the first lactation gave birth during working hours (06.01-12.00 and 12.01-18.00). The birth rate of sheep with two lactation numbers was in the second rank (32.6%). The total share of sheep in the first and second lactation is 88.8%. Accordingly, the sheep's birth in the first and second lactation should be followed more carefully in herd management. In both lactations, a very important part of the births occurred between 06.01-18.00, which can be considered working hours.

4. Conclusion

The distribution of a total of 651 lambs of sheep in the Altepe Bafra Sheep Breeder Farm during the day; 260 lambs were born in the 1st time periods (06:01-12:00), 199 lambs in the 2nd time periods (12:01-18:00), 109 lambs in the 3rd time (18:01-00:00) and 83 lambs were born at the 4th time periods (00:01-06:00). According to the 1st and 2nd time periods, 70.5% of the births took place during the working hours of the enterprise, and the other 29.5% occurred outside of working hours from evening to morning. One factor affecting the enterprise's profitability is fertility, and the first thing that comes to mind when it comes to the sheep farm is lamb delivery. Sucking the dam and lamb as soon as possible after birth and their communication with each other will increase the survival power of the lambs. Increasing the viability of born lambs is possible with proper herd management. Because lamb losses are one of the factors affecting enterprise profitability, minimizing the losses is an important issue. In addition, lamb yield means meat yield. Therefore, the fact that 29.5% of birth distributions occur outside of working hours from evening to morning is a considerable level that cannot be underestimated. In herd management, measures such as knowing the distribution of births during the day, controlling the animals during the peak times of birth, ensuring adequate workforce, monitoring individual care and feeding, employment of labor specific to the birth season outside of working hours will contribute to the prevention of lamb losses and increase the lamb's viability.

Author Contributions

The percentage of the author contribution is present below. The author reviewed and approved final version of the manuscript.

	Ö.Ş.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Data collection and animal husbandry procedures were carried out in compliance with Law No. 5996's Article 9's rules for animal welfare. This work did not involve the use of animal for laboratory studies. There is no violation of animal right. No approval from research ethics committee was required to accomplish the goals of this study. However, the use of all the animals was with the consent of the participating farmer.

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Research Article
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SOME BIOLOGICAL FEATURES OF TENCH (*Tinca tinca*, L., 1758) INHABITING SIDDIKLI DAM

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Abstract: The length–weight relationship, length–length relationship and the condition factor values of Tench, *Tinca tinca* (L., 1758) inhabiting the Sıddıklı Dam was described in this study. A total of 102 specimens captured between September 2015 and August 2016 were used to conduct this study. For each individual, the total length (TL), fork length (FL) and standard length (SL) as well as body weight were measured. Sex composition was determined as 49.02 % female, 43.14 % male and %7.84 undetermined sexes. The overall sex ratio showed no significant difference from the expected value of 1:1. Total length and weight of all specimens ranged from 5.9 to 38.6 cm and from 2.4 to 783.24 g, respectively. The length-weight relationships of females and males were W = 0.0068*TL3.232 (R^2 = 0.991) and W = 0.0084*TL3.157 (R^2 = 0.997), respectively. Weight increased positive allometrically with the total length for both sexes (b> 3, P< 0.001). The mean condition factor values of female and male were 1.305 and 1.330, respectively. This study brought the first data about the species in the Sıddıklı Dam to the literature.

Keywords: Length-weight relationship, Condition factor, *Tinca tinca*, Sıddıklı Dam

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1. Introduction

Tench (*Tinca tinca* (L., 1758)) is a freshwater fish of Tincidae family included Cypriniformes group (Kottelat and Freyhof, 2007). It is really hard to determine the original distribution model in continental Europe as stocking operations were done in European scale during centuries. However, it has been assumed as a native species in most of Europe. There are no natural distribution areas in Ireland, Scandinavia, Western and Southern Greece, and the Eastern Adriatic basin. It naturally spreads in regions from east of Asia to the Western Yenisey drainage south of 60° North latitudes. It has also been reported in North and South Africa, Tasmania, Australia, New Zealand, India, North America, Chile and many other regions (Kottelat and Freyhof, 2007). Besides it has been reported in North and South Africa, Tasmania, Australia, New Zeeland, India, North America, Chile and many other areas (Kottelat and Freyhof, 2007).

Tench fish deploying initially in Black Sea coasts and also Thrace, Central Anatolia and Mediterranean basin in our country has an economic significance (Geldiay and Balık, 2007). The species quite resisted to lower oxygen levels is important ecologically. They typically live in shallow lakes having intense plant cover (vegetation) and in still water. They generally spend winter as bogged down. They spawn (lay) in areas in fresh water having intense plant cover. They can live up to age 20. The Tench reaching sexual maturity between the ages 2-6 carries out reproductive activity between May and September. Detritus, adults of the species feeding with herbal and zoological materials adopt the feeding strategy mostly on molluscs (Kottelat and Freyhof, 2007). Length-weight relationship is a commonly used parameter in fishing management and giving information about biology of fish population (Bolger and Connoly, 1989). This parameter that bases mathematical relationship between length and weight of the fish can change during lifecycles of the species (Le Cren, 1951). Condition factor can be used to indicate the biological condition of a fish and even to determine sub-populations of a species (Wootton, 1998; Jones, 2002). It is also the reflection of relationship of fish welfare with biotic and abiotic environment (Keyombe et al., 2017). These parameters are needed to analyze the data is necessary for species protection biology and to make healthy comparison with other populations.

There aren't any researches about this species in Sıddıklı Dam. This study is conducted for the purpose of not only to expand the scientific knowledge about the species but also to be a source to other researches. In this study it is aimed to determine *Tinca tinca*'s length-weight relationship and condition factor.

2. Materials and Methods

2.1. Study Area

Sıddıklı Dam (Figure 1) located in nearby Sıddıklı Küçükboğaz Village in 40 km west of Kırşehir was built for watering purposes. The surface area of Sıddıklı Dam is 1.65 km². Thanks to the dam, 4945 ha of agricultural land is irrigated. In addition, fishing activities are carried out economically in the Sıddıklı Dam (Anonymous, 2008).

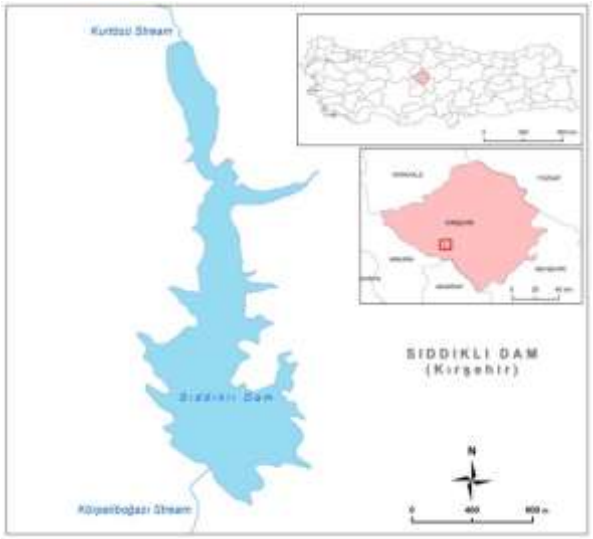

Figure 1. The location of Sıddıklı Dam.

2.2. Sampling

Fish samples were collected monthly from different parts of the Sıddıklı Dam using gill nets (25×25, 30×30, 35×35, 40×40, 45×45, 50×50, 55×55, 60×60, 65×65, 70×70, 75×75 and 80×80 knot to knot) between September 2015 and August 2016. A total of 102 *Tinca tinca* samples were obtained during the study.

The total, fork and standard lengths of each fish were measured with an accuracy of 0.1 cm and their weights were weighed using a digital scale with an accuracy of 0.01 g. Sex determination was made by macroscopic examination of the gonads.

2.3. Statistical Analysis

Whether there was a deviation from the expected value (1:1) in the sex ratio was checked with the chi-square (x^2) test. The following formula (equation 1) is used for length-weight relationship.

$$W = a \times L^b \quad (1)$$

In this formula, W is body weight (g), L is total length (cm), a is the intersection point and b is the slope (Bagenal and Tesch, 1978). The b coefficient obtained from the length-weight relationship was used to determine the growth type of the fish. Whether fish growth was isometric (b = 3) or allometric (b > 3, b < 3) was estimated by Student's t-test (Zar, 1999). Fulton's condition factor was calculated using the formula (equation 2) below (Ricker, 1975).

$$K = 100 \times \frac{W}{L^3} \quad (2)$$

In addition, relationships between TL vs FL, FL vs SL, and SL vs TL were determined separately according to females, males, and overall samples.

3. Results

Of the total amount of the 102 Tench obtained, 50 (49.02%) were female, 44 (43.14%) were male, and 8 (7.84%) were unidentified sex. The female: male ratio was determined as 1.00:0.88. It was determined that this ratio did not show a significant deviation from the expected 1:1 ratio (x^2=0.383, P>0.05). The total length of the examined fish varied between 5.9 - 38.6 cm. The highest number of specimens were in the length groups of 10.0-14.9 cm (56.7%) (Figure 2).

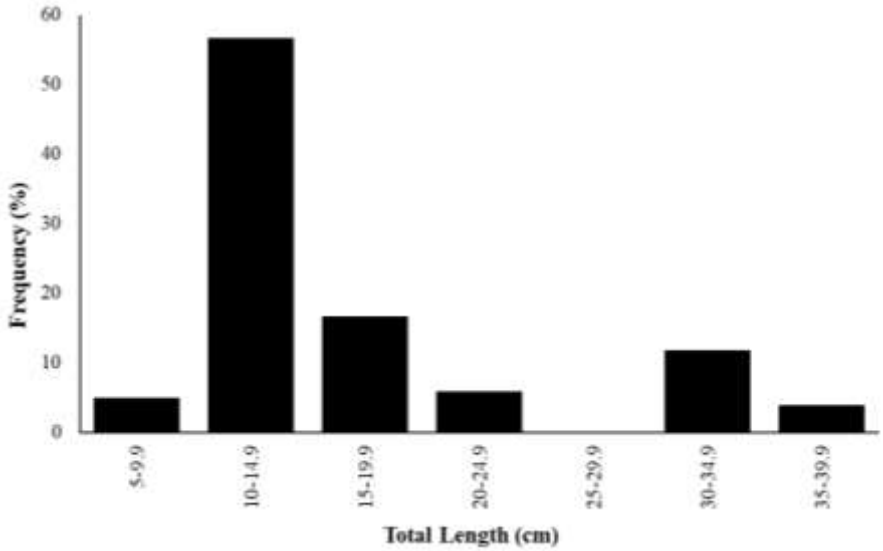

Figure 2. Length-frequency distribution of Tench inhabiting Sıddıklı Dam.

The weights of the fish ranged between 2.4-783.24 g. 76.5% of the samples weighed less than 100 g (Figure 3).

The b value of the length-weight relationship was determined as 3.232, 3.157 and 3.177 in females, males and all individuals, respectively. Very strong relationships were found in the length-weight relationship (Figure 4). According to the t-test results, the b value was different from 3 in females, males and all individuals (t-test, b>3, P<0.001). It can be stated that the species performed a positive allometric growth in the Sıddıklı Dam.

The average condition factor of *T. tinca* inhabiting Sıddıklı Dam was found to be 1.305 in female individuals, 1.330 in male individuals and 1.308 in all individuals. The difference between the average condition factor values of female and male individuals was not statistically significant (t-test, P> 0. 05).

The relationships between total, fork and standard length in *T. tinca* individuals are shown in Table 1. It was determined that there were strong linear relationships between different length types (R^2> 0.993, P< 0.001).

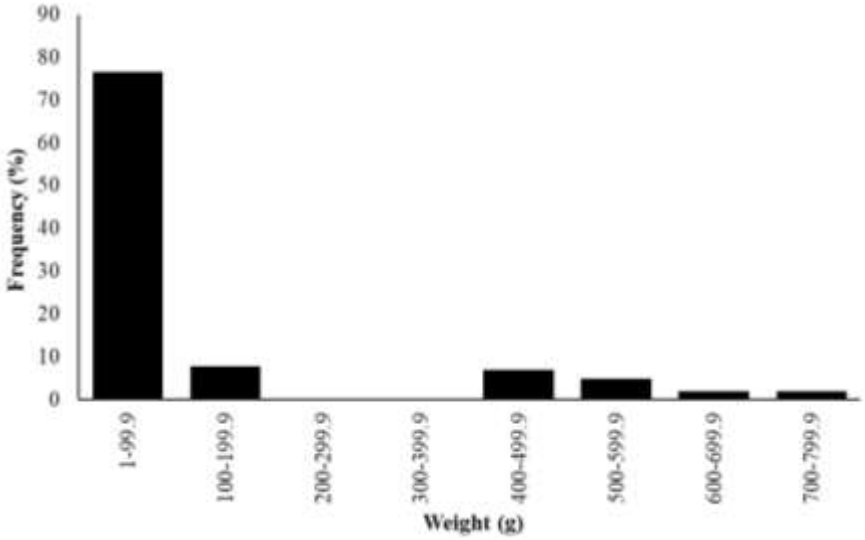

Figure 3. Weight-frequency distribution of Tench inhabiting Sıddıklı Dam.

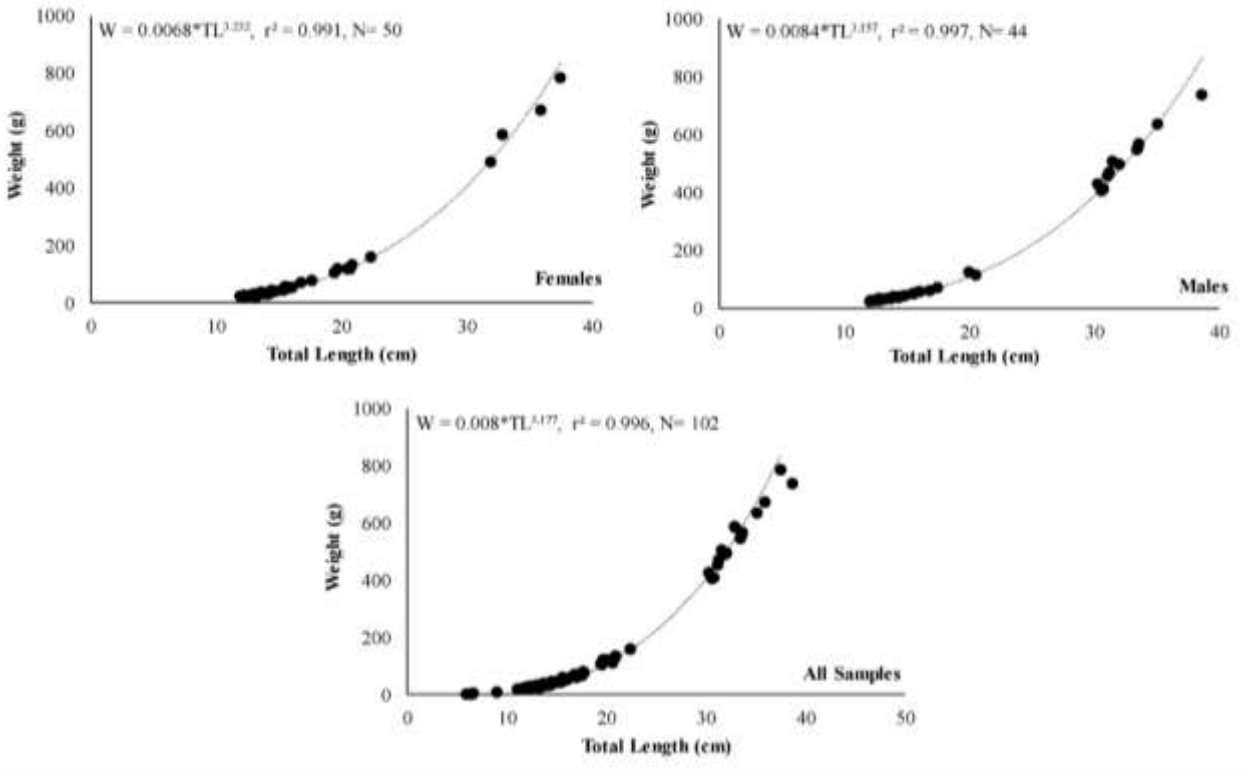

Figure 4. Length-weight relationship of Tench inhabiting Sıddıklı Dam.

Table 1. Length-length relationship parameters of Tench inhabiting Sıddıklı Dam.

Sex	N	Equation	a	b	R^2
Female	50	TL= a + bFL	0.1977	1.0289	0.998
		TL= a + bSL	0.352	1.1863	0.997
		FL= a + bSL	0.177	1.1509	0.995
Male	44	TL= a + bFL	0.1730	1.0289	0.999
		TL= a + bSL	0.445	1.1804	0.997
		FL= a + bSL	0.271	1.1468	0.996
All Samples*	102	TL= a + bFL	0.1865	1.0288	0.993
		TL= a + bSL	0.3322	1.1866	0.997
		FL= a + bSL	0.1540	1.1525	0.996

*Including non-sexed samples, N= sample size, a, b and R^2: the parameters of length-weight relationships.

4. Discussion

In this study, some biological characteristics of *T. tinca* species inhabiting Sıddıklı Dam were determined. The sex ratio (Female/Male) of the strain did not differ from the expected 1:1 ratio. Although the proportion of males is found to be higher in some studies (Ergüden and Göksu, 2010), the expected proportional equality has been reported in the literature in general. (Altındağ et al., 1998; Altındağ et al., 2002).

Considering the length distribution of the species, the minimum length value reported in the existing literature was obtained in this study. In addition, it may be an indication that the size distribution is in a very wide range and that the findings obtained in this way reflect the various biological periods of the fish. Possible reasons for the differences in length distributions in the literature (Table 2) are sampling times, hunting methods, size types used and differences in growth according to habitats. In order to make healthier comparisons, age-length data should be obtained.

Table 2: Length-weight relationship parameters and average condition factor values for tench reported from different studies.

Reference	Habitat	Sex	Length Distrubition (cm)	b	R^2	Growth Type	K
Altındağ et al., 1998	Kesikköprü Dam Lake	F+M	15.4-41.4	3.17	0.97	I	1.95
Altındağ et al., 2002	Bayındır Dam Lake	F+M	15.2-34.7	3.17	-	-	1.55
Balık et al., 2009	Beyşehir Lake	F+M	9.0-37.0	2.99	0.99	I	1.51
Şanlı Benzer et al., 2009	Hirfanlı Dam Lake	F+M	17.9-34.2	2.93	-	-	1.09
Ergüden and Göksu, 2009	Seyhan Dam Lake	F+M	11.0-26.5	2.78	0.97	-	-
Ergüden and Göksu, 2010	Seyhan Dam Lake	F+M	12.0-29.0	2.51	0.98	A (-)	1.58
Yılmaz et al., 2010	Hirfanlı Dam Lake	F+M	13.9-30.0	3.15	0.97	A (+)	1.65
Moradinasab et al., 2012	Anzali Wetlands	F+M	15.0-26.5	2.53	0.90	A (-)	1.60
Kırankaya et al., 2014	Hirfanlı Dam Lake	F+M	13.8-33.9	3.10	0.99	A (+)	1.58
Saylar et al., 2014	Hirfanlı Dam Lake	F+M	20.5-34.5	2.41	0.84	-	1.49
Saylar et al., 2018	Asartepe Dam Lake	F+M	19.0-42.9	3.05	-	A (+)	1.68
This Study	Sıddıklı Dam	F+M	5.9-38.6	3.17	0.99	A (+)	1.30

I= isometric, A (+)= positive allometric, A (-)= negative allometric, F= female, M= male, K= Fulton's condition factor, b and R^2: the parameters of length-weight relationship.

In this study, strong relationships were determined between the length and weight of the species. a and b values were obtained from this relationship. The b value indicates the shape of the fish according to the conditions it is in. Although it is known that the b value varies between 2.5 and 3.5 in many fish species (Erkoyuncu,

1995), it has also been stated that it varies between 2 and 4 (Tesch, 1971). The b value of Tench was determined as 3.177 in this study. When we look at the literature, it is seen that the b value of the species is generally above 3 and exhibits allometric growth. The species generally exhibited positive allometric growth in the Sıddıklı Dam and surrounding habitats. On the other hand, in some habitats, the b value was found to be less than 3 and showed negative allometry or isometric growth (Table 2). These differences may have been caused by the number of samples, length-weight distributions, sampling time, size type used and ecological conditions of the environments. In addition, the length-weight relationship in fish varies depending on factors such as nutritional adequacy, feeding rate, gonad development and breeding period (Bagenal and Tesch, 1978).

According to the values of Fulton's condition factor, it is seen that the species cannot effectively benefit from the nutritive capacity of the Sıddıklı Dam. Having lower condition values compared to the literature may be an indicator of this (Table 2). A low condition factor may be related to predation pressure, competition for nutrients, or habitat capacity.

Length conversions are very useful for comparing the results of different studies with each other. In this study, conversions of total length-fork length, total length-standard length and fork length-standard length were determined. A high correlation coefficient has been obtained, and researchers will benefit from these data in future studies using different length types.

4. Conclusion

As a result, it can be said that the species has a positive allometric growth pattern in Sıddıklı Dam and its fatness level is lower compared to other habitats. In terms of the future of the species, its biology should be investigated in more detail and various measures should be taken in particular for the Sıddıklı Dam. In addition, the findings of this study will be a source for future studies.

Author Contributions

The percentage of the author contribution is present below. The author reviewed and approved final version of the manuscript.

	O.Y.	R.Y.
C	55	45
D	60	40
DCP	50	50
DAI	45	55
L	40	60
W	60	40
CR	50	50
SR	70	30

C=Concept, D= design, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. The laboratory procedures were approved by the Local Animal Care and Ethics Committee of Kırşehir Ahi Evran University (Approve number: 68429034/05, Approve date: June 15, 2015).

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Review
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AN OVERVIEW OF ADDING RHO-ASSOCIATED COILED-COIL KINASE AND KNOCKOUT SERUM REPLACEMENT WITH TREHALOSE TO A LOW GLYCEROL TRIS-BASED SEMEN EXTENDER

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Abstract: It is known that livestock animal semen is very sensitive to cold shock during freezing processes, and this sensitivity directly affects post-thaw sperm qualities which are progressive motility, mitochondrial membrane potential, sperm nuclear DNA integrity and in vitro spermatological parameters such as plasma membrane and acrosome integrity, and sperm fertility. In addition, with the sudden decrease in the total antioxidant level of the semen after thawing, the sperm cells are insufficient to tolerate their damage. Consequently, significant losses occur in sperm fertility. For this reason, researches on freezing the semen of livestock animals include semen processing; cryopreservation/cryogenic damage – thawing methods - sperm extenders, added antioxidants, the mechanisms of action and metabolic pathways of these antioxidants and physiological and metabolic parameters such as sperm fertility. It has been explained that low dose glycerol (trehalose added to increase the cryoprotectant effect) added to the extender in the freezing of livestock animal semen, knockout serum replacement (KSR) and Rho-associated coiled-coil kinase (ROCK), which are antioxidant additives, can increase the in vitro quality parameters of frozen thawed semen.

Keywords: Sperm, Extender, Glycerol, Trehalose, Antioxidant, KSR, ROCK

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1. Introduction

The membranes of spermatozoa are physiologically fluid mosaics. This membrane structure consists of two sequential phospholipid layers surrounded by glycolipids, glycoproteins and proteins (Wolf et al., 1984). Since these thermodynamic structures consist of 65-70% unsaturated fatty acids, sperm membranes are vulnerable to cryo-shock damage. In addition, irreversible phase changes occur during the freezing of semen (transition from a liquid phase to gel phase) (Khan et al., 2021; Keles et al., 2021). The crystallization that occurs in the membrane due to the temperature change, especially between 5 °C and -5 °C, damages the sperm cells during the freezing process, (Said et al., 2010). Moreover, these phase changes in the membrane cause changes in the amount and kinetics of the enzymes in the membrane, resulting in a decrease in sperm viability and plasma membrane integrity (Gürler et al., 2016). Antioxidant and oxidant balance during and after sperm freezing is vital for sperm survival and function. Antioxidant structures protect cells against sperm dysfunction and developing peroxidative damage (Alvarez et al., 1983; Bilodeau et al., 2000,

Bilodeau et al., 2001, Arslan et al., 2019). As a result of the deterioration of the stabilization of the sperm membrane, the antioxidant - oxidant balance of the cells is disrupted and oxidative stress damages occur (Watson, 1995; Watson, 2000; Gürler et al., 2016).

2. The Usage of Antioxidants and Cryoprotectants

Antioxidant agents and cryoprotectants have recently started to be used in sperm extenders against the decrease in sperm quality parameters (motility, plasma membrane and acrosome integrity, acrosome reaction, vitality, DNA break, lipid peroxidation, total antioxidant capacity) (Maxwell and Watson 1996; Gadea et al., 2004; Bucak et al., 2007; Bucak and Uysal 2008; Amini et al., 2019; Arslan et al., 2019). Sperm cells are equilibrated with the cryoprotectants contained in the extender before the freezing process. Because they must be stabilized intracellularly. It has been observed that they are based on two bases when the mechanisms of action of cryoprotectants are examined; These additives reduce the number of ions in the environment and increase the amount of the unfrozen fraction. Furthermore, structural and molecular cryoprotectants have two basic properties. These have low molecular weight and create toxic effects only when they are added at high rates, respectively (Palasz and Mapletopt 1996). To reduce the toxicity of cryoprotectants; applications such as using low amounts in the extender, shortening the exposure time of sperm cells (incubation/equilibrium) and using non-permeable cryoprotectants should be done (Massip 2001). The cryoprotectants used in the extender as a preservative are biochemically divided into two main groups. They are divided into permeable and non-permeable. For those with permeable cryoprotectant properties; examples include glycerol, ethylene glycol, formamide, and dimethylsulfoxide (DMSO). Permeable ones show their effects by penetrating the cell membrane as they are permeable and 'colligative'. The working mechanism of cryoprotectants, which are in permeable structure, is as follows; they minimize the osmotic shrinkage caused by low temperatures, reduce the electrolyte density formed in the environment during the cryopreservation process, and regulate the dehydration that will occur during the freezing process and create protection in the protein structures of the sperm (Mcgann 1978; Leeuw et al., 1993; Holt, 2000a).

3. Use of Glycerol, Trehalose and Antioxidant Substances in Semen Extenders

Glycerol, which is widely used in sperm extenders and provides high success; It is a hydrophilic polyol compound. One of the main reasons that glycerol is successful in the freezing process, as the C/OH ratio is equal in biochemical structure. However, glycerol creates a toxic effect above a certain concentration in diluents. These effects are as follows; they cause osmotic stress and change the membrane bioenergy balance (Katkov et al., 1998; Woods et al., 2000; Alveranga et al., 2005). The toxic effect of glycerol is species-specific, it can irritate in the female genital tract when used in extenders of rabbit, fish and poultry semen. This irritation can have a contraceptive effect (preventing fertilization and pregnancy) in sperm fertilization. The glycerol used in the extender has threshold values for animal species. This threshold value is 4-5% in stallion sperm, 4-8% in ruminants, 5% in bucks, 3% in pigs and 1.75% in mice. Toxic effects can be seen when these threshold values are exceeded. Especially these toxic effects can change the membrane structure, protein and glycoprotein structures. In addition to these, decreases may occur in mitochondrial membrane potential due to the toxic effect, and serious declines may be observed in total and progressive motility. Also, these undesirable effects have been reported to cause irreversible damage to the acrosomal region of the sperm (Hammerstedt et al., 1990; Sinha et al., 1996; Katkov et al., 1998; Morrell and Hodge 1998; Holt, 2000b; Alvarenga et al., 2000). External cryoprotectants used in the extender try to reduce the peroxidative damage (lipid peroxidation) that occurs during freezing/thawing in the cell. Because these structures provide the cell membrane to gain a flexible structure. These external cryoprotectants cause increased membrane permeability to cations. If these structures are used in the diluent, a low rate of permeable cryoprotectant (glycerol, ethylene glycol, etc.) is used, and as a result of this process, the toxic effects of internal cryoprotectants will be minimized (Arav et al., 1993; Cabria et al., 2001). Another substance used as an external cryoprotectant in sperm extenders is trehalose. As a biochemical structure, it is a disaccharide compound and is formed by the bonding of two D-glucose molecules (Richards et al., 2002). The working mechanism of trehalose is not known exactly. However, they penetrate the sperm plasma membrane and expand the surface area by forming hydrogen bonds with the polar head groups of the phospholipids in this region during freezing/thawing. It is also thought that they act as a buffer and exert an osmotic protective effect and reduce the release of free oxygen radicals formed during freezing (Mcwilliams et al., 1995; Gao and Crister 2000; Aisen et al., 2005; Purdy 2006). Studies conducted in recent years have shown that the use of trehalose in mammalian sperm extenders and the end-of-solution quality parameters of sperm have increased. These are, respectively, increasing sperm motility, plasma membrane and acrosome integrity, protecting the total antioxidant capacity and reducing the lipid peroxidation that occurs (Aisen et al. 2000, 2002; Aboagla and Terada 2003; Bucak et al. 2007). Cryoprotective agents are known to have an indirect and direct effect on the fertility of frozen thawed semen. Due to the contraceptive effects of structures such as glycerol, it should be added to diluents in lower amounts and supplemented with trehalose to freeze sperm cells. Antioxidant additives must be added to the extender to achieve high sperm fertility (Hammerstedt

1993; Purdy 2006; Bucak et al., 2021). Since oxidative stress damage (superoxide, hydrogen peroxide, hydroxyl radicals, etc.) is thought to minimize and increase the total antioxidant capacity of semen due to lipid peroxidation that develops during freezing and thawing. However, the release of ROS products at the basal level is a positive effect on sperm fertility (for spermatozoa fusogenetics and necessary membrane lubrication). For this reason, it is not desired to eliminate ROS products in the environment and to increase the total antioxidant capacity excessively (Sies 1993). Classical extenders such as Tris-egg yolk, which are not commercial preparations and prepared by enterprises and laboratories, are also very sensitive to freezing damage due to the presence of traces and/or low antioxidants (Parrish et al., 1986). Antioxidant agents minimize cold shock damage, prevent intracellular crystal formation and have a protective effect against decrystallization and destabilization of the developing plasma membrane during solution. For this purpose, one of the additives with high antioxidant properties, which is extremely new today; it is thought to have a positive effect on the freezing of livestock animal sperm by adding Knockout Serum Replacement (KSR) and Rho-associated coiled coil kinases (ROCK). Also, these additives could increase motility values and mitochondrial membrane potential, reduce plasma membrane acrosome integrity damage, protect sperm viability and DNA integrity, suppress apoptosis and activate antioxidant response signalling pathways. For this, it is of great importance to use additional doses with an optimum effect so that antioxidants do not have a toxic effect on semen and adversely affect sperm fertility (Alvarez and Storey 1984; Aitken and Baker, 2004; Uysal et al., 2005; Bucak et al., 2007).

4. Knockout Serum Replacement

Knouckout Serum Replacement (KSR) is a rich source of antioxidants, vitamins, proteins, amino acids and trace elements. Recent studies have shown that it is a necessary serum substitute for mammalian cell survival, growth and development in vitro (Sato et al., 2011; Aoshima et al., 2013; Zhang et al., 2016, Taher-Mofrad et al., 2020). In addition, the use of KSR, which has an antioxidant effect, in the cryopreservation of different cell types has been reported and positive results have been obtained (Lee et al., 2014; Ha et al., 2016; Park et al., 2018). KSR provides activation of a protein structure, AKT (Protein Kinase B), as a working mechanism. AKT enables the glucose transport protein (GLUT-4) in the cell cytosol to move towards the plasma membrane and allows glucose, which is its main task here, to enter the cell (Ishii et al., 2015). In addition, KSR BIM (BCL 2-like protein 11) inhibits cytochrome C (electron-carrying structures for oxidative phosphorylation) originating from and secreted by mitochondria, positively affecting cell viability and preventing apoptosis (Ishii et al., 2015). Studies have shown that KSR stimulates the formation of BIM-resistant mitochondria and KSR has a direct and indirect effect on the energy pathways of the cell (Ishii et al., 2015). Adding 10% KSR to the extender had a positive effect on sperm acrosome and DNA integrity (Taher-Mofrad et al., 2020).

5. Rho-Associated Coiled-Coil Kinase (ROCK)

The Rho-associated coiled coil is a small G protein that mediates and/or transports intracellular signaling mechanisms. One of the effectors of this material is the Rho-associated helix-helix kinase (ROCK) (Ark et al., 2010; Karaşör et al., 2022). ROCKs have a multifunctional effect on the cell. It has numerous effects on cell cycle regulation, increased motility, regulation of actin dynamics that cause cell invasion, cell adhesion, migration, and inflammatory response (Ark et al., 2010; Schofield et al., 2012). ROCKs exert their primary inhibitory effects in the phagocytosis of apoptotic (programmed death of cells) cells because ROCKs act as complementary receptors in cell phagocytosis. Cells producing apoptosis need mature phagosomes to be cleared by fibroblasts and/or macrophages. This maturation pathway is the Rho-ROCK-ERM pathway (Tosello- Trampont et al., 2003; Erwig et al., 2006). The addition of 10, 20, and 40 μM ROCK to frozen-thawed cat sperm had a positive effect on sperm motility and membrane function.

In a study conducted on cats, the best sperm quality parameters were obtained with the addition of 10 μM ROCK (Tharasanit et al., 2020). Studies have shown that ROCKs contribute to increasing the in vitro fertilization ability of sperm cells (Tharasanit et al., 2020).

6. Conclusion

In the light of literature studies, successful freezing of sperms that are difficult to freeze will be achieved with future studies. In addition, it will be possible to benefit from reducing the number of spermatozoa used in insemination doses and benefiting from the breeders at the highest level.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	H.O.A.	E.K.	B.R.	D.A.A.	A.S.	O.B.	A.A.	M.R.	I.A.
C	20	10	10	10	10	10	10	10	10
D	20	10	10	10	10	10	10	10	10
S	20	10	10	10	10	10	10	10	10
L	20	10	10	10	10	10	10	10	10
W	20	10	10	10	10	10	10	10	10
CR	20	10	10	10	10	10	10	10	10
SR	20	10	10	10	10	10	10	10	10

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

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