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EDITORIAL DECLERATION

Dear authors and readers,

First of all, we would like to thank you for being our travel companion by writing, evaluating, and reading us about this broadcasting life we started six years ago. With these thoughts, we are especially thankful for researchers and academicians honoring with the articles, valuable scientists involved in editorial boards, and reviewers for their contributions to the evaluation processes through their opinions/ideas/contributions/criticisms. With this article, we wanted to inform you, our valuable stakeholders, about the development of The Black Sea Journal of Agriculture (BSJ Agri). The statistics of the BSJ Agri for the last six years are given below. Hope you will be with us in future issues.

Year	Articles	Cites	Cite Index*	CNA	CNC	CCI
2018	23	6	0.26	23	6	0.26
2019	36	19	0.53	59	25	0.42
2020	49	40	0.82	108	65	0.60
2021	23	79	3.43	131	144	1.10
2022	72	131	1.82	203	275	1.35
2023	108	179	1.66	311	454	1.46

CNA= cumulative number of articles, CNC= cumulative number of cite, CCI= cumulative cite

index

*according to Scholar Google

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- 2021: 13%
- 2022: 25%
- 2023: 8%

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Research Article
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OPTIMIZATION OF ULTRASOUND-ASSISTED PROTEIN EXTRACTION FROM WATERMELON SEEDS: TAGUCHI APPROACH

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Abstract: Nowadays, there has been a growing interest in finding alternative protein sources for both the food industry and nutritional purposes. Protein experts have recently focused on investigating watermelon seeds, which are not only a food processing waste but also contain high-quality proteins. Therefore, this study aimed to achieve maximum protein extraction from watermelon seeds using an ultrasound-assisted extraction process. The study investigated the effects of pH (A; 7—11), sonication temperature (B; 30—60 °C), and sonication time (C; 5—15 min) on protein recovery to develop a Taguchi model. Through optimization, the optimal conditions for maximum protein recovery (85.81%) within the range of process variables were found to be 11 pH, 45 °C sonication temperature, and 10 min sonication time ($A_3B_2C_2$). An analysis of variance (ANOVA) revealed that pH and sonication temperature significantly influenced the protein extraction process ($P<0.05$). The optimized extraction conditions resulted in a remarkable improvement (56.79%) in protein recovery compared to the initial process parameters ($A_1B_1C_1$). This study demonstrates the effectiveness of the proposed extraction model for obtaining proteins from high-protein seed sources.

Keywords: ANOVA, Protein recovery, Sonication, pH, Optimization

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1. Introduction

In recent years, consumers have become very interested in products derived from healthy and natural plant sources. Plant-based proteins are the most preferred protein sources due to their safety and the presence of antioxidant peptides that promote human health (Liu et al., 2017). On an industrial scale, there is a growing trend towards plant-derived proteins from agro-industrial wastes due to a number of challenges such as the increasing cost of animal proteins and concerns about food safety (Fatima et al., 2023). Moreover, this approach offers solutions to several critical issues such as environmental pollution, waste accumulation, and disposal expenses (Gadalkar and Rathod, 2020). Thus, by-products from fruit and vegetable processing waste possess the potential to serve as innovative, cost-effective, and non-traditional sources of protein (Firatligil-Durmus and Evranuz, 2010).

Watermelon is an iconic fruit of the summer months, typically grown in warm climate regions. While the juice and pulp of watermelon are utilized in human consumption and the production of non-alcoholic beverages such as juices and nectars, the peel and seeds of this fruit are generally considered waste materials. Notably, the seeds present a potential source of protein and oil. Due to their high arginine, glutamic acid, aspartic acid, and leucine content, the seed proteins are classified as high-quality proteins (Wani et al., 2006; Wani et al., 2011). Various solvents (i.e., hot water, NaOH, HCl, NaCl, and sodium bis(2- ethylhexyl) sulfosuccinate) and extraction techniques (conventional batch extraction, microwave, ultrasound-assisted extraction, mechanochemical-assisted extraction, enzyme-assisted extraction, and liquid-phase pulsed discharge) have been studied for protein extraction from watermelon seeds (WS) (Wani et al., 2006; Wani et al., 2008; Wani et al., 2011; Gadalkar and Rathod, 2020; Behere et al., 2021; Liu and Xi, 2021; Qin et al., 2021). However, these studies often rely on one-factor-at-a-time (OFAT) and response surface methodology approaches to optimize protein extraction from WS. The extraction of seed proteins poses a notable challenge in selecting a suitable strategy and optimization method. Conventional extraction techniques and OFAT optimization can be time-consuming and costly (Guldane and Dogan, 2022). Therefore, the use of ultrasonic-assisted extraction technology, which reduces costs and is an environmentally friendly and time-saving method, has gained prominence (Fatima et al., 2023). Sonication equipment generates sound energy with very high-frequency sound waves (> 16 kHz), which cannot be perceived by the human ear. This US energy causes compression and solubilization in tissues through the

cavitation effect, leading to the rapid removal of targeted components from biological structures (Biswas and Sit, 2020). Gadalkar and Rathod (2020) employed an ultrasound-assisted extraction for protein recovery from WS. They used the OFAT technique to optimize extraction conditions, including pH (7-12), mass-to-solvent ratio (1:20-1:60 (w/v)), temperature (30-60°C), ultrasound power (30-120 W), and frequency (25 and 40 kHz). However, this optimization method is time-consuming and may not consider the potential interaction between extraction parameters.

Taguchi method (TM) has found applications in a wide range of fields for optimizing processes and predicting experimental datasets (Dimou et al., 2009; Ayoubi-Feiz et al., 2019; Güldane and Doğan, 2020; Pathak et al., 2020; Kannan and Thangaraju, 2022). TM has proven to be particularly effective in addressing challenges within the engineering industry. TM is capable of optimizing process variables with a much smaller number of experiments compared to the traditional OFAT technique. It identifies optimum process conditions by utilizing the signal-to-noise ratio (SNR), aiming for a higher SNR value to achieve maximum efficiency. The main objective of Taguchi optimization is to minimize the influence of noise (N) factors that adversely affect the process (Güldane, 2023).

Research on the utilization of the TM for enhancing production processes in food applications has been limited. Furthermore, a notable research gap exists in the current literature regarding the optimization of the protein extraction process from plant seeds, particularly utilizing the TM approach. This study aims primarily to improve the recovery of protein from WS through ultrasound-assisted extraction, employing the TM. To achieve this goal, the study evaluates the impact of process parameters, including pH, sonication temperature, and sonication time, on protein recovery, considering SNR values obtained from Taguchi L9 (3^3) orthogonal experimental design.

2. Materials and Methods

2.1. Materials

The watermelons with no commercial value (i.e., damaged and/or left in the watermelon field after harvest), were obtained from a local producer in Pamukova, Sakarya (Türkiye). As soon as the watermelon arrived at the laboratory, the peels were removed using a knife. The remaining part was then cut into small pieces, crushed using a blender (Kenwood KM070, UK), and transferred to a beaker. Distilled water was added to the beaker in a 1:1 (w/w) ratio, thoroughly mixed, and allowed to stand for 10 min to allow the seeds to settle at the bottom. Subsequently, the top portion was removed, and the remaining kernels were collected. This process was repeated until no watermelon residue remained in the samples. Finally, the clean seeds were spread on filter paper on the laboratory bench and left to dry for 24 hours. The dried samples were then packed and stored under dry conditions at room temperature. The chemicals utilized in protein analysis were of analytical grade and purchased from Merck, (Germany).

2.2. Methods

2.2.1. Preparation of watermelon seeds for analysis

The method proposed by Wani et al. (2006) was employed with some modifications to prepare the WS samples for protein extraction. Initially, the watermelon seed oil was removed through the Soxhlet extraction procedure. Briefly, the WS was ground with a blender apparatus (Kenwood KM070, England). The resulting sample was then mixed with hexane in a ratio of 10:1 (v/w) and subjected to extraction in a Soxhlet extractor for 12 hours. Following this defatting process, the seeds were dried in an oven at 40 °C for 24 hours. After drying, the seeds were ground and pulverized. The resulting defatted seed powder was packed into polyethylene bags and stored at +4°C until analysis.

2.2.2. Protein extraction process

An ultrasound-assisted extraction technique was employed to extract proteins from defatted WS. The sonication process was carried out in an ultrasonic water bath (ÇALIŞKAN, İstanbul, Türkiye) with a constant frequency of 40 kHz. Briefly, 5 g of defatted seed powder was dispersed in 200 mL of distilled water. The pH was adjusted to 7, 9, or 11 using 0.1M NaOH. Subsequently, the samples were then subjected to sonication at different temperatures (30, 45, and 60 °C) for varying durations (5, 10, and 15 min). Following ultrasound treatment, the mixture was filtered using Whatman no:1 filter paper. The filtered solution was then centrifuged at 10000g for 15 min at +4 °C. The resulting supernatant was employed to determine the total nitrogen content using the Kjeldahl method. The protein recovery (PR) was calculated using Equation 1.

$$\text{PR}\ (\%) = \frac{\text{protein content in the extract}}{\text{protein content in defatted seed powder}} * 100 \qquad (1)$$

2.2.3. Experimental design and statistical analysis

The experimental design for protein extraction from WS was carried out using MINITAB 19.0 software. Unlike Design Expert software, MINITAB allows for the selection of process variables, thereby minimizing the influence of uncontrollable factors. This step is crucial in the optimization process (Pathak et al., 2020).

The extraction parameters and their corresponding levels are provided in Table 1. To optimize the process variables, an L9 (3^3) orthogonal matrix was employed, involving three factors and three levels, as shown in Table 2. To maximize protein recovery, the S/N ratio of the experimental results was assessed using Minitab software, applying the "larger the better" criteria (Equation 2).

$$\frac{S}{N} = -10log\left[1/R\sum_{f=1}^{R} 1/{y_i}^2\right] \qquad (2)$$

where R indicates data points and y_i refers to i^{th} data point value.

An analysis of variance (ANOVA) was performed on the Taguchi experimental test results to determine the statistical significance of the control parameters in the ultrasonic-assisted protein extraction process. The Fischer test (F-value) and the associated probability of the F-value (P-value) were utilized to assess the significance of the selected parameters in Taguchi methodology (Table 1 and 2).

3. Results and Discussion

3.1. Taguchi Optimization

Taguchi optimization technique evaluates the experimental results based on the SNR values (Taguchi, 1986). In the ultrasonic-assisted extraction of proteins from WS, a Taguchi L9 design matrix was employed to maximize the PR by selecting the "Larger the better" option. The mean experimental results and corresponding SNR values are presented in Table 2. The results show that the highest protein content was obtained when the process variables (pH, sonication temperature, and sonication time) were set at 11, 45 °C, and 5 min, respectively ($A_3B_2C_1$). In contrast, the lowest extraction efficiency was observed in Run 1 ($A_1B_1C_1$). Moreover, the influence of each extraction parameter on PR was investigated through average SNR values for the levels of the extraction parameters, as outlined in Table 3. Delta values, representing the difference between the maximum and minimum SNR values for the respective levels of each process variable, indicate their influence on the extraction process (Bose et al., 2013). Observations from Table 3 revealed that the alkalinity of the extraction medium had the highest delta value (Δ=2.46) and was the most influential process factor affecting the PR from WS, compared to the other two parameters. Following pH, sonication temperature (Δ= 0.96) and sonication time (Δ= 0.42) were identified as important factors affecting the extraction process.

Figure 1 illustrates the mean SNR graph for the protein extraction process. By considering the "Larger the better" criteria, the primary objective of the TM is maximize the SNR values of the control parameters. The data in Figure 1 suggests that the optimum process parameters for PR from WS were found to be a pH of 11, a sonication temperature of 45 °C, and a sonication time of 10 min ($A_3B_2C_2$). The results also revealed a positive relationship between an increase in pH and PR. Hence, there was an improvement of about 52% in PR when the pH of the extraction medium increased from 7 to 11 as shown in Table 2. A similar trend of improved PR with increasing pH was reported by Gadalkar and Rathod (2020), who attributed the improvement to the increased surface negative charge on proteins resulting from the dissociation of acidic groups with increasing alkalinity. In addition, sonication temperature was also a significant role in protein extraction.

Table 1. Extraction parameters and their levels in Taguchi optimization

Factor	Symbol	Level 1	Level 2	Level 3
pH	A	7	9	11
Sonication temperature (°C)	B	30	45	60
Sonication time (min)	C	5	10	15

Table 2. Taguchi L9 (3^3) design matrix, test results, and SNR values

Run	Factors			Protein recovery (%)	SNR (dB)
	A	B	C		
1	7	30	5	54.73	34.76
2	7	45	10	66.03	36.39
3	7	60	15	61.48	35.77
4	9	30	10	68.93	36.77
5	9	45	15	73.35	37.31
6	9	60	5	70.33	36.94
7	11	30	15	76.73	37.70
8	11	45	5	83.17	38.40
9	11	60	10	81.45	38.22
Mean (η_m)				70.69	

Table 3. Response table for SNRs (dB) of protein recovery (%)

Parameters	1	2	3	Delta (Δ)	Rank
A	35.64	37.01	38.11	2.46	1
B	37.37	37.37	37.13	0.96	2
C	37.13	37.13	36.93	0.42	3

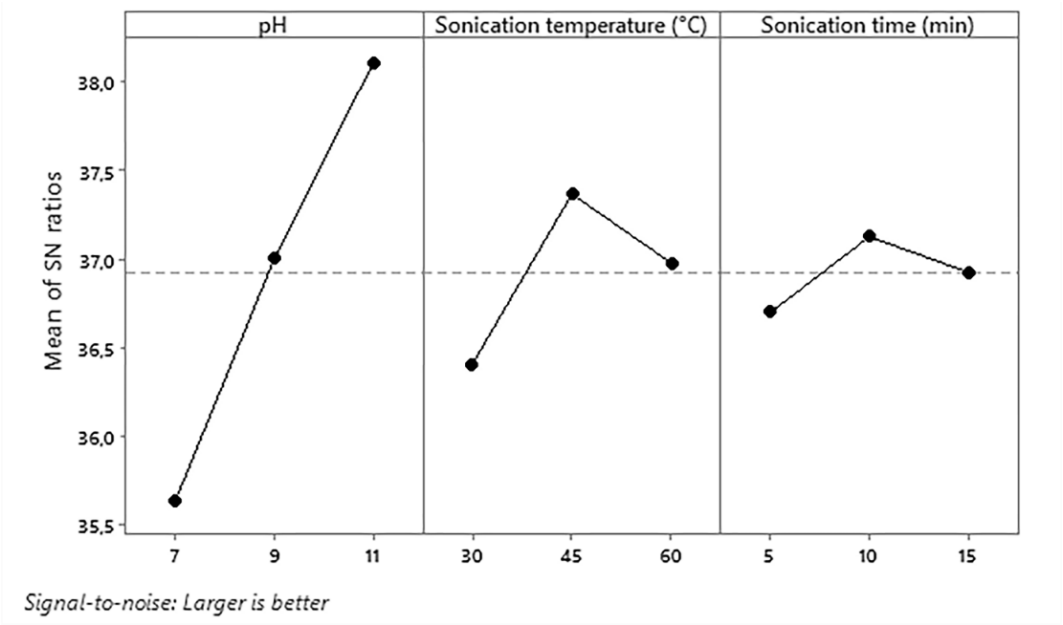

Figure 1. The S/N ratio response graph for total protein recovery.

The SNR of the PR value increased from 34.76 to 38.40 as the sonication temperature increased from 30 to 45 °C. However, a further increase in process temperature to 60 °C, led to a notable decrease in SNR of PR. This observation may be attributed to partial protein denaturation resulting from the combined effect of temperature and sonication. Wani et al. (2006) reported an optimal temperature of 40 °C for protein extraction from WS, which aligns well with our findings. Figure 1 also highlights the potentially detrimental impact of prolonged ultrasound treatment in the protein extraction process. Extended sonication may lead to increased interactions between denatured proteins and other components within the defatted seed structure.

The ANOVA results confirmed the significance of process factors in protein extraction from WS. As shown in Table 4, pH exhibited the highest F-value (178.69) and the lowest *P*-value (0.006), highlighting its role as the most influential process factor affecting PR. Following this, sonication temperature also played a vital role (F-value = 25.31; *P*-value = 0.038). Thus, the influence of these two process variables on the protein extraction process was found statistically significant within a 95% confidence interval. However, it was observed that sonication time had the least significant impact on PR (P<0.05). A similar observation regarding the insignificant effect of extraction time on PR from red pepper seeds was reported by Firatligil-Durmus and Evranuz (2010). These findings were further supported by percent contribution values, with pH having the highest contribution at 85.72%, followed by sonication temperature and sonication time contributing 12.14% and 1.66%, respectively. The percentage contribution of error was calculated to be 0.48, indicating that the impact of non-process parameters on PR is minimal.

3.2. Confirmation Experiments

Validation tests were performed to confirm the correlation between the observed and predicted response values for the PR process using optimal extraction parameters ($A_3B_2C_2$) and to identify whether an improvement was achieved compared to the initial process conditions ($A_1B_1C_1$). The predicted response value was calculated using Equation 3 with the optimal levels of process factors:

$$\eta_0 = \eta_m + \sum_{i=1}^{j} (\eta_i - \eta_m) \quad (3)$$

Table 4. ANOVA results for Taguchi optimization

Source	DF	SS_f	MS	F-Value	*P*-Value	Contribution (%)
pH	2	582.336	291.168	178.69	0.006*	85.72
Sonication temperature (°C)	2	82.480	41.240	25.31	0.038*	12.14
Sonication time (min)	2	11.282	5.641	3.46	0.224	1.66
Error	2	3.259	1.629			0.48
Total (SS_T)	8	679.357				

S= 1.27620, R-sq= 0.9952, R-sq(adj)= 0.9808 and R-sq(pred)= 0.9029, * significant (P<0.05).

Table 5. Results of confirmation experiments

	Initial process parameters	Prediction	Experiment
Factors	$A_1B_1C_1$	$A_3B_2C_2$	$A_3B_2C_2$
Protein recovery (%)	54.73 ± 0.02[b]	85.39 ± 0.12[a]	85.81 ± 0.14[a]
S/N ratio	34.36 ± 0.03[b]	38.76 ± 0.09[a]	38.80 ± 0.05[a]
Improvement (%)			56.79

Values are means ± standard deviation. a-b refers to the significant differences between the values in the same line (P<0.05).

where η_m was the overall average of the mean values or S/N ratio, η_i was the average value corresponding to optimal levels, and j was the number of experiments (Güldane, 2023).

The predicted and experimental test results for the optimal process conditions ($A_3B_2C_2$) and the results for initial process parameters ($A_1B_1C_1$) are presented in Table 5. The variation between the predicted and observed values for tPR was found to be within the range of a 95% confidence level. Furthermore, a substantial enhancement of 56.79% was achieved under the optimal extraction conditions compared to the initial process parameters.

4. Conclusion

This study aimed to optimize an ultrasound-assisted technique for extracting high yields of protein from defatted watermelon seeds, which are rich in high-quality proteins and have the potential to be utilized as valuable industrial waste. The Taguchi approach was employed to model protein recovery from watermelon seeds, using process variables such as pH, sonication temperature, and sonication time. The experiments were designed using a Taguchi L9 (3^3) orthogonal matrix. The Taguchi optimization process resulted in a remarkable increase (> 50%) in protein recovery rate compared to the initial process parameters. However, ANOVA analysis revealed that the pH variable had the most significant contribution to this improvement among other process variables. These results demonstrated that the Taguchi model can be effectively employed to optimize the process variables in protein extraction from fruit seeds.

Author Contributions

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

	M.G.
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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EVALUATION OF *Gossypium herbaceum* LEAF POWDER'S NUTRITIONAL COMPOSITION AND NUTRACEUTICAL PROPERTIES

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Abstract: The objective of this study is to identify the proximate composition, phytochemical profile, and anti-diabetic, anti-inflammatory and antioxidant properties of *Gossypium herbaceum* leaf powder (GLP). The fresh leaves of the *G. herbaceum* were collected, cleansed with fresh water, drained and allowed to dry in the shade, ground to GLP and analysed. The crude fibre (42.93%) and nitrogen-free extract (36.46 %) have a relatively high proportion in GLP; while ash (2.47%) has the lowest proportion. The GLP has relatively high phenol (219.20 mg/g) when compared to flavonoids (81.03 mg/g), tannins (69.56 mg/g), saponins (66.67 mg/g) and alkaloids (55.80 mg/g). The α-amylase inhibition and α-glucosidase inhibition of GLP were 48.45% and 30.68%, respectively. The percentage of albumin denaturation inhibition and anti-proteinase activity of GLP was 22.88% and 43.87%, respectively. The lipid peroxidation inhibition, vitamin C, Fe chelation and 2,2-diphenyl-1-picrylhydrazyl were 35.43%, 23.87%, 11.76% and 88.16%, respectively. GLP exhibits anti-inflammatory, anti-diabetic and antioxidant properties.

Keywords: *Gossypium herbaceum*, Phytogenic supplement, Anti-oxidant, Anti-diabetic, Anti-inflammatory

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1. Introduction

Because of an increased interest in using plants and phytochemicals to obtain additional health advantages beyond the essential nutritional value present in foods or feeds, researchers are looking at the chemical composition of botanicals (Oloruntola, 2022). As a result, there is an increase in the discovery, and production, of more plant-based food/feed supplements, additives, nutraceuticals, and similar items that can be termed functional foods or feed since they have extra physiological or health benefits beyond the basic nutritional value they offer (Nicoletti, 2012; Falowo et al., 2023).

The diversification of the active compounds caused by the intrinsic factors, such as the plant part used, the harvest season and the geographical origin, and extrinsic factors, such as the additive production technique forms the basis for several biological properties and activities possessed by the botanicals or their phytochemicals and their use as growth promoters, antioxidant, antimicrobial, anti-stress, and immunity booster in human and animal nutrition (Bahadoran et al., 2013; Ganguly, 2013; Valenzuela-Grijalva et al., 2017). As an illustration, antioxidant, antidiabetic and anti-inflammatory properties of phytogenic supplements such as *Dysphania ambrosioides* (L.) and *Crassocephalum crepidioides* leaf meal (Falowo et al., 2023), *Juglans regia* kernel meal (Oloruntola, 2022), *Justicia carnea* leaf powder (Oloruntola et al., 2022) and fig tree leaves (Osowe et al., 2021) were reported.

Gossypium herbaceum, a typical wild plant in Nigeria, is said to have some medicinal qualities. According to Larayetan et al. (2021), *G. herbaceum* leaf may offer novel plant-derived therapeutic compounds that are efficient in treating infectious disorders brought on by numerous drug-resistant bacteria and are a target in the management of oxidative stress. Comparing *G. herbaceum*

leaf meal to other phytogenic supplements from botanicals with significant nutraceutical value, however, there is not enough data on its nutritional and health benefits.

Recently, it was revealed that there was a need for ongoing and additional research or characterisation of the bioactive content profile of phytogenic supplements or phytochemicals (Oloruntola et al., 2022). As a result, the goal of this research is to determine the proximate and phytochemical compositions, anti-diabetics, anti-inflammatory, and antioxidant properties of *G. herbaceum* leaf meal.

2. Materials and Methods

2.1. *Gossypium herbaceum* Leaf Powder and Reagent

The fresh leaves of the *Gossypium herbaceum* were collected from a farm in Ado Ekiti, Nigeria. A Crop scientist from the Department of Agricultural Technology at The Federal Polytechnic in Ado Ekiti, Nigeria, validated the plant. After being thoroughly cleansed with fresh water, the samples were drained and allowed to dry in the shade for 14 days. Having been grounded into *Gossypium herbaceum* leaf powder (GLP), they were stored at 4°C until analysis. The parameters were examined in three copies. For each parameter, three iterations of analyses were performed on the GLP samples. All of the chemicals of the analytical reagent grade used for chemical analysis were purchased from Sigma-Aldrich.

2.2. *Gossypium herbaceum* Leaf Powder Proximate and Phytochemical Analysis

Using the AOAC method, GLP was assessed for moisture, crude fat, crude fibre, crude protein, ash, and nitrogen-free extract (AOAC, 2010). Oloruntola et al., (2022) reported the methods for determining alkaloids, saponins, flavonoids, tannins, and phenols.

2.3. *Gossypium herbaceum* Leaf Powder Antidiabetic properties

The procedures for determining the α-amylase inhibition (Wickramaratne et al., 2016) and α-glucosidase inhibitory activity (Dej-adisai and Pitakbut, 2015) of GLP were recently published by Oloruntola et al. (2022).

2.4. *Gossypium herbaceum* Leaf Powder anti-inflammatory properties

The methods for evaluating albumin denaturation inhibition (Osman et al., 2016) and antiproteinase activity (Rajesh et al., 2019) of GLP were recently published by Oloruntola et al. (2022).

2.5. *Gossypium herbaceum* Leaf Powder anti-oxidant activities

The procedures for determination of lipid peroxidation inhibition (Bajpai et al., 2015), ferrous chelation (Ebrahimzadeh et al., 2008) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Otles and Yalcin, 2012) were recently published by Oloruntola et al. (2022).

Using the Benderitter et al. (1998) method published by Oloruntola (2021), the vitamin C content of the GLP was determined. 270 mg copper sulphate ($CuSO_4$. $5H_2O$), 75 µl DNPH solution (i.e. 2 g dinitrophenyl hydrazine, and 230 mg thiourea in 100 ml of 5 ml/L H_2SO_4) was introduced to 500 µl extract mixture (300 µl of an adequate dilution of the extract with 100 µl 13.3% trichloroacetic acid and water). After that, the reaction mixture was incubated for three hours at 37°C before adding 0.5 ml of 65 percent H_2SO_4 (v/v) to the medium and measuring the absorbance at 520 nm with a UV spectrophotometer. The level of vitamin C in the GLP was then determined using ascorbic acid as a reference substance.

2.6. Statistical Analysis

Each assay was carried out three times, and the results' average mean was provided. To better comprehend the average mean, bar graphs were made in Excel.

3. Results

The proximate composition of GLP is shown in Figure 1. The crude fibre (42.93%) and nitrogen-free extract (36.46 %) have a relatively high proportion in GLP; while ash (2.47%) has the lowest proportion. The GLP has relatively high phenol (219.20 mg/g) when compared to flavonoids (81.03 mg/g), tannins (69.56 mg/g), saponins (66.67 mg/g) and alkaloids (55.80 mg/g) (Figure 2).

The anti-diabetics properties of *Gossypium herbaceum* leaf powder was depicted in Figure 3. The α-amylase inhibition and α-glucosidase inhibition of GLP were 48.45% and 30.68%, respectively.

Figure 4 shows the anti-inflammatory properties of *Gossypium herbaceum* leaf powder. The percentage of albumin denaturation inhibition and anti-proteinase activity of GLP was 22.88% and 43.87%, respectively. The anti-oxidant properties of *Gossypium herbaceum* leaf powder are shown in Figure 5. The lipid peroxidation inhibition, vitamin C, Fe chelation and DPPH were 35.43%, 23.87%, 11.76% and 88.16%, respectively.

4. Discussion

Quantifying the proximate amounts of a typical feed/food ingredient or supplement is essential to demonstrate its nutritional profile and determine the right amount to add to a compounded feed/food (Oloruntola, 2022). The relatively high crude fibre content of GLP could be of nutraceutical importance. For instance, dietary fibre is a component of plant matter that is resistant to enzymatic digestion and has a good impact on health because it has been linked to a reduction in the incidence of some diseases (Dhingra et al., 2012). High-fibre diets are advantageous because they increase faecal bulk, shorten intestinal transit time, lower cholesterol and glycaemic levels, trap substances that can be harmful to the human body (such as mutagenic and carcinogenic agents), and promote the growth of the intestinal flora, among other things (Heredia et al., 2002). Consequently, dietary fibre is being employed in a variety of functional meals, including baked goods, beverages, meat products, and drinks (Chau and Huang, 2003; Dhingra et al., 2012).

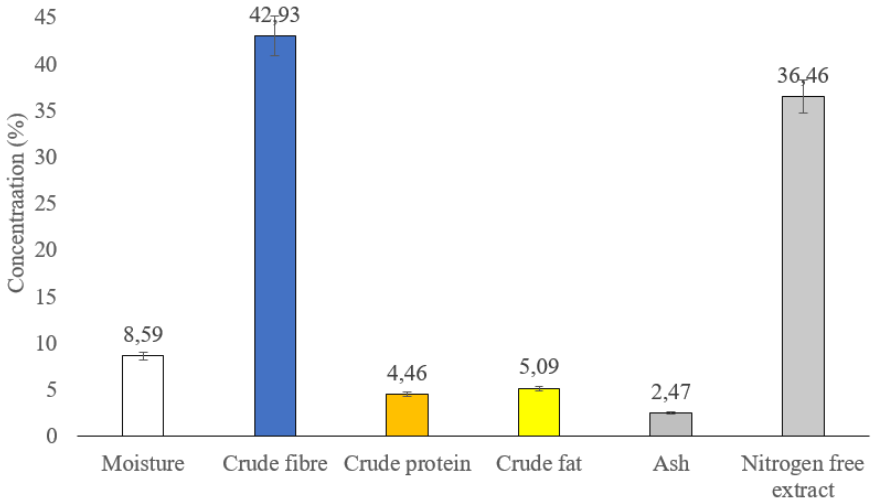

Figure 1. Proximate composition of *Gossypium herbaceum* leaf powder.

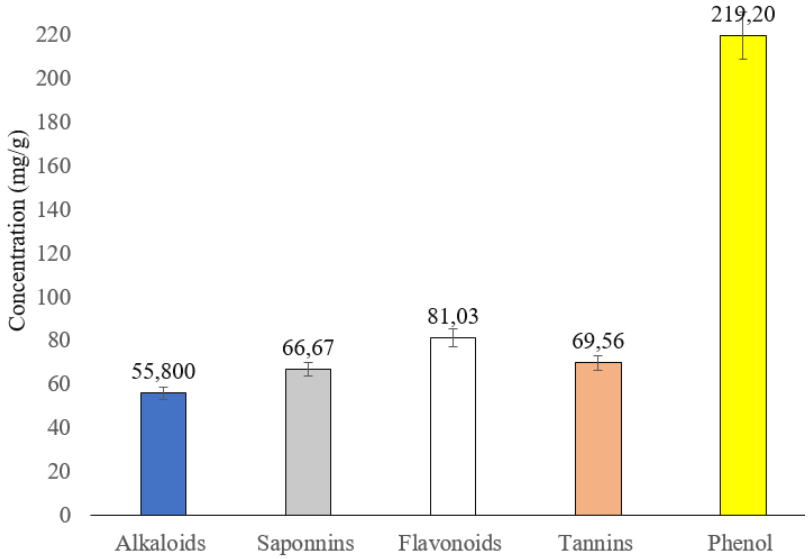

Figure 2. Phytochemical composition of *Gossypium herbaceum* leaf powder.

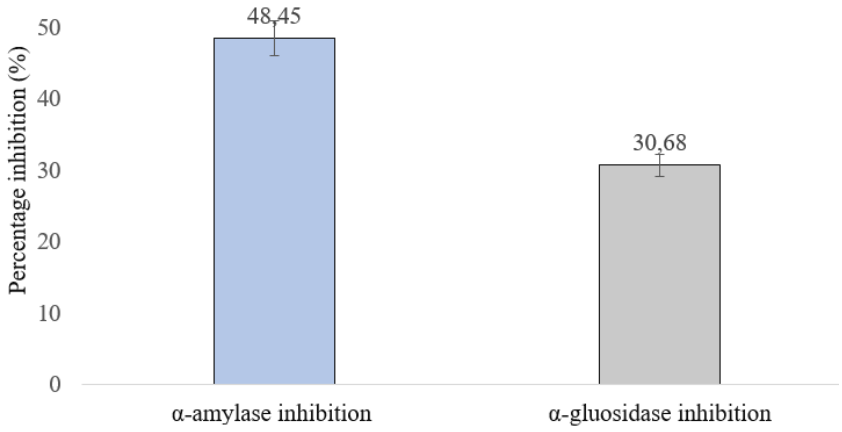

Figure 3. Antidiabetic properties of *Gossypium herbaceum* leaf powder.

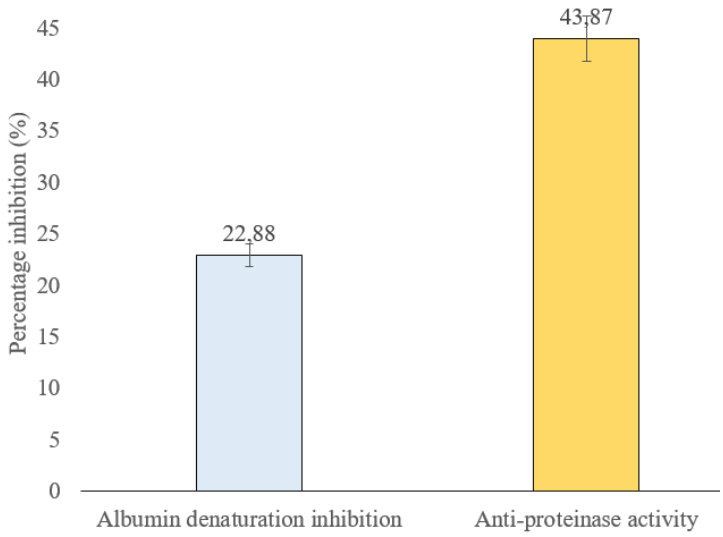

Figure 4. Anti-inflammatory properties of *Gossypium herbaceum* leaf powder.

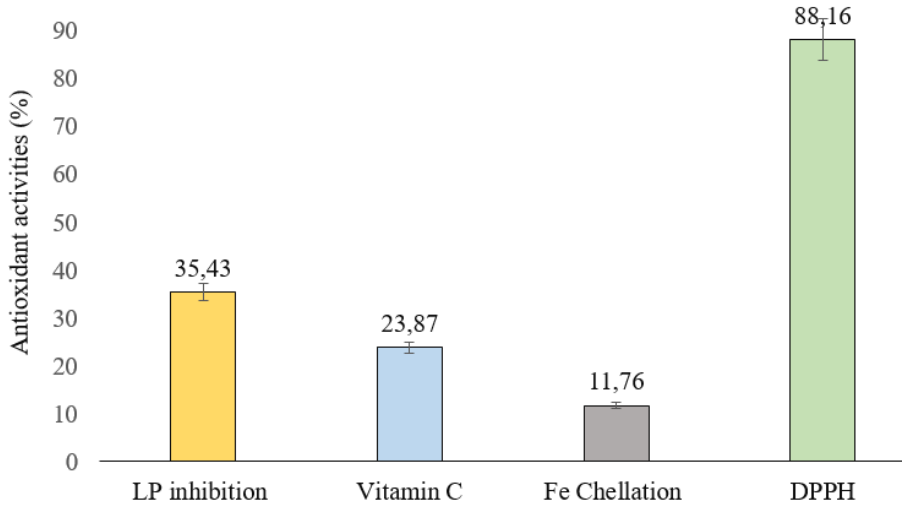

Figure 5. Anti-oxidant properties of *Gossypium herbaceum* leaf powder: LP= lipid peroxidation, DPPH= 2,2-diphenyl-1-picrylhydrazyl.

The nitrogen-free extract (NFE), the second-highest proximate component of GLP and purportedly representing the soluble carbohydrates of the GLP, is similarly beneficial nutritionally because, by implication, GLP could be useful as a source of energy for animals and humans (Bach Knudsen et al., 2013). The crude fibre (42.93%) and NFE (36.46%) of GLP in this study are at variance with the 16.95% and 49.58%, respectively reported for *Anacardium occidentale* leaf (Oloruntola 2021).

The identification of botanicals' phytochemical components gives scientific support for their use as dietary supplements or foods with medicinal properties (Muhammad et al., 2014, Falowo et al., 2023). The amount of GLP's detectable alkaloids (55.80 mg/g), flavonoids (81.03 mg/g), and phenol (219.20 mg/g) in this investigation further reveal the potential antioxidant function it might have when used as a supplement or ingredient. The significant antioxidant activity of alkaloids generated from natural sources indicates that these bioactive substances inspired by natural products may have a huge positive impact on both human health and the food processing industry (Atpadkar et al., 2023); while almost all flavonoids (a class of organic compounds with varying phenolic structures) have antioxidant properties and the most effective flavonoids for defending the body against reactive oxygen species, according to reports, are flavones and catechins (Panche et al., 2016). In addition, plant phenolics are regarded as an essential dietary component and have numerous health advantages in addition to their powerful antioxidant activity (Kumar and Goel, 2019).

The saponins and tannins found in GLP may also be useful in nutraceuticals. The health-promoting saponins may influence the immune system in ways that assist the body fight cancer, lower cholesterol, and even reduce blood glucose response. Inhibiting dental cavities and platelet aggregation, treating hypercalciuria, and serving as an antidote for acute lead poisoning are all possible uses for a high-saponin diet (Shi et al., 2004). Since

saponins are essential to both human and animal nutrition and are found in a wide variety of plants and plant-based products, many foods high in saponins are recommended as dietary supplements to individuals with diabetes and other health challenges (Sharma et al., 2023). The anticarcinogenic, antimutagenic and antimicrobial properties of tannins were also reported (Chung et al., 1998). Since moderate levels of tannin may have positive impacts on ruminant performance, health, and environmental sustainability (Adejoro et al., 2020), tannin extract used as a dietary supplement in ruminant nutrition was being promoted (Yanza et al., 2021).

Finding alternative anti-diabetic medications, especially those made from plants or herbs, is necessary due to the development of resistance and negative effects with prolonged use of synthetic diabetes medications (Alam et al., 2022). It is possible to regulate postprandial hyperglycemia and lower the risk of developing diabetes by inhibiting the activities of α-amylase and α-glucosidase (Poovitha and Parani, 2016). Therefore, the α-amylase inhibition and α-glucosidase inhibition activities recorded for GLP in this study suggest its use as a supplement could retard the digestion of carbohydrates and consequently reduce the postprandial glucose level, particularly in diabetic patients (Zhang et al., 2015a; Poovitha and Parani, 2016). This result is similar to the antidiabetic activities of mistletoe leaves from moringa and kola nut trees being recently reported (Oloruntola and Ayodele, 2022).

Since ancient times, many herbal substances have been utilised with little risk of negative effects in blocking inflammatory pathways (Maroon et al., 2020). The percentage albumin denaturation and anti-proteinase activity of GLP unveil its anti-inflammatory properties and also qualifies it as a potential phytogenic or natural anti-inflammatory feed or food supplement. These findings backed up the plant's historic use for several painful and inflammatory illnesses (Dharmadeva et al., 2018). For instance, the anti-inflammatory activity of *Ficus racemose* L (Dharmadeva et al., 2018), and *Justicia carnea* leaf powder was reported (Oloruntola et al., 2022). The biologically active components (flavonoids, tannins, phenolic compounds, and phytosterols) may work individually or in combination to provide analgesic and anti-inflammatory effects (Dharmadeva et al., 2018).

The levels of lipid peroxidation inhibition, vitamin C, Fe chelation and DPPH recorded in this study show that GLP could serve as an antioxidant phytogenic feed or food supplement. Antioxidant phytochemicals have anti-cancer, anti-inflammatory, anti-obesity, anti-diabetes, and anti-ageing properties (Zhang et al., 2015a,b). In addition, important antioxidant components found in some botanicals have the exceptional ability to treat oxidative stress-related degenerative diseases with little damage (Ozata et al., 2002) and previously, lipid oxidation inhibition capacity (Burri et al., 2020), vitamin C (Traber and Stevens, 2011), and Fe chelation property (Sudan et al., 2014) of botanicals were reported.

5. Conclusion

These findings indicated that GLP could be a source of dietary fibre and energy, and that it has anti-diabetic, anti-inflammatory, and antioxidant properties. GLP is advised for use as a dietary supplement in feeding trials with an animal model.

Author Contributions

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

	A.D.O.	S.O.A.	V.O.A.	O.A.J.	B.A.F.	C.O.O.	F.S.O.
C	40	30			30		
D	20	20	20	20		20	
S	100						
DCP	20	20	20		20		20
DAI	20	20	20		20	20	
L	15	15	15	15	15	15	10
W	30	20	15	20			15
CR	15	15	15	25	15	15	
SR	30	20	15		20	15	

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.

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 PHENOTYPIC AND GENOTYPIC FACTORS ON SOME YIELD TRAITS IN HOLSTEIN FRIESIAN COWS WITH HIGH MILK YIELD REARED IN TÜRKİYE

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Abstract: This study was carried out to determine the effects and phenotypic relationships of some environmental factors (first calving age, calving year, and calving season) on Lactation period, dry period, milk yield and Milkability traits. The material of the study was the lactation records of 1079 Holstein cows raised in a private dairy farm. As a result of analyses, the values of 305-DMY yield (305 DMY), the lactation period (LP), dry period (DP), time to reach peak yield (Tmax), peak yield (Ymax), average daily milk yield (ADMY), total lactation milk yield (ATMY) and age at first calving (AFC) were determined as 9926.3±178.1 kg, 318.1±1.4 days, 60.05±0.9 days, 95.2±2.1 days, 42.3±0.3 kg, 32.2±0.3 kg, 10248.7±94.1, and 26.7±0.2 months, respectively. The study found the average milk flow rate (MFR) and the average milking time (MT) as 2.0±0.0 kg min^{-1} and 360.9±4.7 seconds, respectively. In addition, estimates of the 305-day mature equivalent milk yield (ME 305-d) and MFR were also found to be 0.41±0.24 and 0.51±0.30, respectively. As a result, this dairy farm can be recommended as an example to breeders who have just started their dairy farm in Türkiye and countries with similar environmental conditions and are looking for a model.

Keywords: Calving, Heritability, Holstein Friesian, Milkability, Milk yield

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1. Introduction

The Holstein Friesian cattle breed has been taken to many countries of the world as a breeding breed due to its high yield and adaptation characteristics (Kaygısız et al., 2017). Breeders prefer Holstein Friesian cattle due to their high milk yield, good fattening performance, and their easy adaptation to the environment where they are taken to (Koçak et al., 2007; URL, 2016). The Holstein Friesian breed is a breed developed under good care, feeding and cool climatic conditions. Therefore, they best demonstrate their yield ability in cool-climate plain areas where abundant forage can be produced. In areas with hot climates, the level of yield falls under poor care and feeding conditions (URL, 2016).

Dairy cattle breeding, it is mainly aimed to increase milk yield. To achieve the desired success in the breeding study to be applied, it is necessary to determine the relationship between milk yield characteristics. Milk yield characteristics are properties that affect each other in selection. The relationships between these characteristics need to be calculated accurately and reliably. This will directly affect selection success in herd management (Genç and Soysal, 2018). The main economic goal of dairy cattle prodution is to obtain high levels of quality milk (Koçak et al., 2007; Erdem and Okuyucu, 2020). Obtaining a high milk level from a cow depends on the continuity of fertility. In an ideal herd, it is aimed to take one calf per year from each cow. For this, it is necessary to keep some parameters (the dry period is 60 days, the first insemination age is 450-500 days, the service period is 80 days, and the calving interval is 365 days) within certain limits. The age at which female cattle raised as a breeder gives their first calf is known as the first calving age (FCA). For a profitable breeding, this period is expected to be between 24-26 months in culture breeds (Beavers and Doormaal, 2015). Many studies have shown that optimal FCA is ≤ 24 months (Shamay et al., 2005; Stevenson et al., 2008).

The optimal age at first calving in Holstein cows is considered to be 24 months. Today, many cows appear to have their first calves at the age of 22-23 months (Ettema and Santos, 2004; Mohd Nor et al., 2013; Heinrichs et al., 2017). However, Hutchison et al. (2017) reported that cows calving at the age of 24 months gave higher milk in the first lactation and had shorter long-term milk yields than those calving at the age of 21 and 22 months. There is no negative effect on milk yield and body health of Holstein cows at 22-24 months of age (Nilforooshan and

Edriss, 2004).

The effect of the calving season on milk production is due to factors such as heat stress and photoperiod in addition to the diversity of food sources. Studies have shown that milk production increases in cows calving in autumn, whereas it decreases in cows calving in spring (Barash et al., 1996; Coulon and Pe´rochon, 2000; Dahl and Petitclerc, 2003).

In some studies, it has been observed that calving year has a significant effect on all milk yield characteristics such as 305 DMY, dry period, lactation period (Çilek and Tekin, 2005; M'hamdi et al., 2012). It has been reported that the differences observed in nutrition, care-management factors between calving years have a significant effect on milk yield (Çobanoğlu and Kul, 2019).

Dairy cows need a certain dry period between two lactations to give a regular and sufficient amount of milk in the second and later lactations. This period is associated with dairy cows' milk yield, milk composition, reproductive performance, calves' birth weight, survival rate, and growth performance. Most dairy farms have a dry period of 51-60 days. (Grummer and Rastani, 2004; Collier et al., 2012; Hossein-Zadeh and Mohit, 2013; Rahbar et al., 2016; Kıyıcı et al., 2020).

One of the characteristics of economic importance in dairy cattle is milk ability, which is defined as the ease of milking of dairy cows (Meyer and Burnside, 1987; Gray et al., 2011). The most common of the Milkability characteristics are MFR, MT, and maximum MFR (Güler et al., 2009). The Milkability characteristic is also used in the selection criterion of animals (Bruckmaier et al., 1995), in the monitoring of animal breeding and breast health (Duda, 1995; Naumann et al., 1998), and in the development of milking machines and the regulation of parameters for their use (Rasmussen, 1993).

In a study conducted in the Eastern Cape and Gauteng Provinces of South Africa, the means for milking time (MT), mean milk flow (AMF), maximum milk flow (MMF) and somatic cell score (SCS) were 5.20 min, 1.91 kgmin^{-1}, 2.99 kg min^{-1}and 2, respectively (Tshilate et al., 2020).

Managing and evaluating of milkability helps the effective use of the labor force. Also, it is a functional characteristic that should be considered in addition to characteristics such as calving ease, fertility, feed conversion, and health (Gäde et al., 2006), and its main indicators are MFR, MT, and the highest milk flow rate (HMFR) (Güler et al., 2009).

For imported breeds, the most important adaptation criteria are calf and/or cow life force. For this reason, in dairy cattle enterprises, live calves should be obtained once a year and work should be carried out to reduce calf losses as much as possible (Karakaş, 2002). It has been determined that breeders in different regions also adopt the Holstein Friesian breed brought to Türkiye, but there are significant problems in terms of care, feeding and housing (Kaygısız et al., 2017). Therefore, the need arises to investigate adaptation abilities in culture breed cattle populations imported from different countries. To date, most of the adaptation studies related to cattle of the culture breed have been conducted in public enterprises. No extensive research has been conducted on the yield level of the breed under breeder conditions. Unlike previous studies, this study was conducted in breeder conditions.

The purpose of this study was to determine the current milk yield and Milkability characteristics (milk flow rate, the milking time) of the Holstein Friesian breed grown in a private enterprise in Balikesir province, to determine the effects of phenotypic, genetic and environmental factors on these characteristics, and to contribute to breeding and selection studies in the light of scientific studies.

2. Materials and Methods

The research material was the lactation records between 2013 and 2016 of 1079 Holstein cows raised in a private dairy farm. These yield records and pedigree records are kept in the herdbook system by the Turkish Cattle Breeders Association.

In the study, average daily milk yield (ADMY), total lactation milk yield (TMY), Adjusted milk yield for 305 days (305-DMY), mature age equivalent of cow expresses milk yield adjusted for 305 days (ME-305-d milk yield :ME 305-d) lactation period (LP), dry period (DP), first calving age (CA), Milkability traits are milking time (MT) measured in minutes, average milk flow rate (AMF) measured in kilogram per minutes were examined.

Milkability is the rate at which milk is completely drawn from a cow's udder, which measures the cow's ability to let down milk and to be completely milked. Generally, the Milkability of a cow is expressed as a function of milking speed and milking time, measured by either manual scoring or using specially designed instruments. The most used Milkability traits are milking time (MT), measured in minutes, and average milk flow rate (AMF) measured in kilogram per minute and also measured in kilograms per minutes. These provide valuable information about the efficiency and capability of milk release (Tshilate et al., 2020).

To determine effects of calving age (CA), calving season, and calving year on milk yield, and Milkability characteristics, the Variance Analysis Method was used with the help of the SAS program (Orhan et al., 2004). Duncan test was used to compare subgroups. The mathematical equation used to determine the effect of environmental factors is given below (Equation 1).

$$Yijkl=\mu+ai+cj+dk+eijkl \quad (1)$$

where;

Y_{ijklm} refers to observation value related to the examined characteristic of l cow at i calving year, j calving season, and k calving age.

μ: Population mean,

a_i: The impact amount of i calving year (2013, 2014, 2015, 2016),

c_j: The impact amount of j calving season (winter, spring, summer, autumn),
d_k: Impact amount of k calving age (20-24, 25-30, 31-36, 37-45), and
e_{ijkl}: Term of the random error.
Variance elements and heritability levels belonging to milk yield and milk flow rate were estimated with a computer program by using the Restricted Maximum Likelihood (REML) technique (Multiple Trait Derivate Free Restricted Maximum Likelihood MTDFREML) (Boldman et al., 1993).

3. Results

The effect of calving year, calving season and calving age on lactation duration was insignificant (P>0.05). Whereas the effect of calving year on 305-DMY yield was not statistically significant (P>0.05), effects of calving season and calving age were found to be significant (P<0.01 and P<0.05, respectively). While the effect of calving year on dry period was determined as significant (P<0.05), effects of calving season and calving age were not significant (Table 1).
The least squares mean and significance levels of factors affecting ADMY, TMY and AMY are given in Table (2).

Table 1. Least squares mean, standard errors, significance, and multiple comparison test results of LP, 305-DMY and DP by calving year, calving season, calving age and Duncan test

Factors	N	LP (day) $\overline{X}\pm s_{\bar{x}}$	N	305-DMY (kg) $\overline{X}\pm s_{\bar{x}}$	N	DP (day) $\overline{X}\pm s_{\bar{x}}$
Calving Year		ns		ns		*
2013	82	319.89±3.12	82	10106.71±178.11^{b}	82	63.24±1.21^{b}
2014	87	319.31±3.03	87	9956.14±167.84^{ab}	73	58.00±1.48^{ab}
2015	128	317.07±2.08	128	9914.79±126.16 ^{ab}	9	55.67±2.75^{a}
2016	36	315.03±4.21	36	9487.28±212.08^{a}	-	-
Calving Season		ns		**		ns
Winter	94	320.14±2.77^{ab}	94	10081.65±160.84^{b}	47	61.15±2.05
Spring	57	311.79±3.10^{a}	57	9506.14±185.72^{a}	21	64.57±4.06
Summer	75	321.15±3.31^{b}	75	9546.00±181.08^{a}	43	58.86±1.20
Autumn	107	317.63±2.43^{ab}	107	10281.25±130.01^{b}	53	59.62±1.19
First Calving Age (Mo)		ns		*		ns
20-24	108	318.65±2.59	108	9571.34±137.44	34	62.15±1.86
25-30	177	318.52±1.97	177	10121.25±107.12	97	58.77±0.91
31-36	34	314.91±3.91	34	10179.09±265.03	23	64.96±4.36
37-45	14	317.00±7.54	14	9593.79±611.07	10	61.30±3.20

ns= non-significant (P>0.05), *= significant at the level of P<0.05, **= significant at the level of P<0.01, LP= lactation period (day), 305-DMY= 305-days milk yield (kg), DP= dry period (day), Mo= months, ab= the difference between averages (mean) indicated by different letters in the same column is significant.

Table 2. Least squares mean and standard errors of ADMY, TMY and AMY by calving year, calving season, calving age and Duncan test

Factors	N	ADMY (kg) $\overline{X}\pm s_{\bar{x}}$	N	TMY (kg) $\overline{X}\pm s_{\bar{x}}$	N	AMY (kg) $\overline{X}\pm s_{\bar{x}}$
Calving Year		ns		ns		ns
2013	82	32.75±0.57^{b}	82	10464.86±201.45^{b}	82	10761.06±181.85^{b}
2014	87	32.32±0.54^{ab}	87	10330.79±203.58^{b}	87	11021.48±178.99^{ab}
2015	128	32.22±0.42 ^{ab}	128	10204.85±138.56^{ab}	128	11387.60±148.74^{b}
2016	36	30.88±0.68^{a}	36	9713.29±238.52^{a}	36	11490.50±286.58^{a}
Calving Season		**		*		ns
Winter	94	32.60±0.53^{b}	94	10417.01±177.30^{bc}	94	11460.54±148.80^{ab}
Spring	57	31.10±0.60^{a}	57	9696.14±208.37^{a}	57	11083.52±125.17^{b}
Summer	75	31.01±0.59^{a}	75	9960.67±212.07^{ab}	75	11037.97±311.24^{b}
Autumn	107	33.37±0.41^{b}	107	10596.89±156.04^{c}	107	9838.21±571.30^{a}
First Calving Age (Mo)		*		*		**
20-24	108	31.10±0.45	108	9889.45±154.09	108	11460.54±148.80^{ab}
25-30	177	32.86±0.34	177	10463.08±126.08	177	11083.52±125.17^{b}
31-36	34	33.11±0.85	34	10420.57±289.85	34	11037.97±311.24^{b}
37-45	14	30.99±1.93	14	9890.80±702.25	14	9838.21±571.30^{a}

ns= non-significant (P>0.05), *= significant at the level of P<0.05, **= significant at the level of P<0.01, ADMY= average daily milk yield (kg), TMY= total lactation milk yield (kg), AMY= ME-305-d milk yield (kg), Mo= months, ab= the difference between averages (mean) indicated by different letters in the same column is significant.

Least squares mean and significance levels of the average daily milk yield, Total lactation milk yield (TMY) and ME-305-d milk yield (ME 305-d) are given in Table 2. According to this, whereas the average daily milk yield was not affected by calving year, it was affected significantly by calving season (P<0.01) and calving age (P<0.05) (Table 2).

The least squares mean for the time to reach peak day, peak day milk yield, milk flow rate, and milking time by calving year, calving season, and calving age are given in Table 3.

Whereas the average peak-day milk yield and milk flow rate were not affected by calving year, time to reach peak day and milking time were affected significantly by calving year (P<0.05). While milk flow rate and milking time were not affected by calving season, time to reach peak day, and peak-day milk yield were affected. Whereas time to reach peak day, milk flow rate, and milking time were not affected by first calving age, peak-day milk yield was affected (P<0.05) (Table 3).

In this context, phenotypic correlations between milk yield and Milkability of Holstein Friesian cattle were examined in this study, and summarized in Table 4.

Table 3. Least squares mean, standard errors and significance levels of the time to reach peak day, peak-day milk yield, milk flow rate, and milking time by calving year, calving season, and calving age

Factors	N	T_{max}(day) $\overline{X}\pm\mathbf{S}_{\bar{x}}$	N	Y_{max}(kg) $\overline{X}\pm\mathbf{S}_{\bar{x}}$	N	MFR $\overline{X}\pm\mathbf{S}_{\bar{x}}$	N	MT $\overline{X}\pm\mathbf{S}_{\bar{x}}$
Calving Year		*		ns		ns		*
2013	82	102.84±4.82^{b}	82	42.09±0.57	82	2.08±0.06	82	337.02±10.73^{a}
2014	87	100.01±4.16^{b}	87	42.67±0.62	87	1.94±0.05	87	369.94±9.73^{b}
2015	128	90.03±3.01^{ab}	128	42.20±0.53	128	1.95±0.03	128	367.22±6.23^{b}
2016	36	84.33±4.28^{a}	36	41.87±0.88	36	1.96±0.06	36	371.11±15.23^{b}
Calving Season		*		**		ns		ns
Winter	94	99.26±3.50^{b}	94	42.98±0.65^{b}	94	1.94±0.04^{ab}	94	374.82±7.51
Spring	57	80.79±4.64^{a}	57	42.53±0.74^{b}	57	1.99±0.06^{ab}	57	361.53±10.89
Summer	75	97.83±5.56^{b}	75	40.10±0.60^{a}	75	1.90±0.06^{a}	75	347.81±12.64
Autumn	107	97.40±3.07^{b}	107	43.00±0.48^{b}	107	2.06±0.04^{b}	107	357.56±7.68
First Calving Age (Mo)		ns		**		ns		ns
20-24	108	91.79±3.65	108	40.44±0.53^{a}	108	2.01±0.04	108	349.81±7.35
25-30	177	98.37±2.80	177	43.15±0.39^{ab}	177	1.99±0.03	177	363.58±6.52
31-36	34	90.71±7.13	34	43.49±0.93^{b}	34	1.87±0.09	34	373.97±15.97
37-45	14	91.86±7.60	14	42.03±2.46^{ab}	14	1.79±0.18	14	381.14±32.84

ns= non-significant (P>0.05), *= significant at the level of P<0.05, **= significant at the level of P<0.01, T_{max}= time to reach peak day (day), Y_{max}= peak-day milk yield (kg), MFR= milk flow rate, MT= milking time, Mo= months, ab= the difference between averages (means) indicated by different letters in the same column is significant.

Table 4. Phenotypic correlations between milk yield, fertility, and milkability characteristics of the Holstein Friesian cattle

	305-DMY	AMY	CA	ADMY	LP	TMY	T_{max}	T_{max}	MFR	MT	DP
AMY	0.986**	1									
CA	0.122*	0.160**	1								
ADMY	-0.056	-0.057	-0.091	1							
LP	-0.043	-0.058	-0.060	-0.043	1						
TMY	-0.070	-0.080	-0.112*	0.869**	0.451**	1					
T_{max}	-0.042	-0.073	-0.171**	0.126*	0.227**	0.228**	1				
Y_{max}	-0.001	-0.001	-0.012	0.890**	-0.009	0.790**	0.010	1			
MFR	-0.095	-0.095	-0.092	0.313**	0.058	0.306**	-0.075	0.358**	1		
MT	-0.003	0.006	0.126*	0.161**	-0.063	0.113*	0.089	0.137*	-0.383**	1	
DP	-0.083	-0.088	-0.232**	-0.149	0.008	-0.114	0.116	-0.181*	-0.128	-0.036	1
AFC	-0.176**	-0.189**	-0.095	0.046	-0.046	0.026	-0.027	0.123*	-0.107	0.080	0.059

*= significant at the level of P<0.05, **= significant at the level of P<0.01, 305 DMY= 305-days milk yield (kg), AMY= ME-305-d milk yield (kg), CA= first calving age, ADMY= average daily milk yield (kg), LP= lactation period (day), TMY= total lactation milk yield (kg), T_{max}= time to reach peak day (day), Y_{max}= peak-day milk yield (kg), MFR= milk flow rate ($kgdk^{-1}$), MT= milking time (dk), DP= dry period (day), AFC= age at first calving (day), between the characteristics, r<0.5 indicates to weak, 0.5<r<0.7 indicates to moderate, and 0.7<r indicates to high.

Table 5. Variance elements and heritability estimates of AMY and MFR

Parameters	σ^2_a	σ^2_e	σ^2_p	σ^2_{a1a2}	h^2
ME-305-d milk yield (AMY)	116.264	169.665	285.929	-	0.41±0.24
Milk Flow Rate (MFR)	0.10536	0.10192	0.20728	-	0.51±0.30
Genetic Correlation of AMY x MFR	-	-	-	0.18258	0.52±0.35

MFR= milk flow rate, AMY= ME-305-d milk yield.

Positive and significant correlations were identified between 305DMY and ME 305-D; ME 305-D and CA; CA and Tmax; ADMY and TMY, Ymax, MFR and MT; LP and TMY and Tmax; TMY and Tmax, Ymax, and MFR; Ymax and MFR and MT (P<0.01). On the other hand, there was a negative and significant relationship between 305 DMY and AFC, between ME 305-D and ACF, and between CA and DP (P<0.01) (Table 4). It was determined that there was a positive and significant correlation between CA and MT; ADMY and Tmax; TMY and MT; Ymax and MT and AFC (P<0.05). In addition, there was a negative and significant correlation between CA and TMY and between Ymax and DP (P<0.05) (Table 4).

Estimations related to variance elements and heritability levels for ME-305-d milk yield and milk flow rate values are presented in Table 5.

In this study, heritability estimates calculated by using REML technique for ME-305-d milk yield and milk flow rate were found to be 0.41±0.24 and 0.51±0.30, respectively.

Among milk yield characteristics, heritability levels of ME-305-d milk yield and Milk flow rate were found to be high. For this reason, it seems possible for them to progress through selection. Therefore, to increase yield at the herd level, cows and their calves with the desired characteristics should be kept in breeding.

4. Discussion

The mean lactation period examined in terms of milk yield characteristics was found as 318.1±1.4 days (Table 1). This value was 13 days longer than the standard lactation period. This might be because the enterprise's care, management and feeding conditions had not changed depending on the years and seasons. Compared to other studies, the obtained lactation period was shorter than values determined by Boğokşayan and Bakır (2013) (343 days), Sahin and Ulutas (2012) (326 days), and Genc and Soysal (2018) (364 days), while it was longer than values found by Toghiani (2012) (279 days) and Hossein-Zadeh (2012) (292 days). On the other hand, it showed a similarity with the value (319 days) determined by Sahin and Ulutas (2011). This value was higher than values obtained by some studies conducted on Holstein Friesian cattle herds, such as Toghiani (2012) (6564 kg), Sahin and Ulutas (2012) (6606 kg), Zavadilová and Zink (2013) (5870 kg), Tiezzi et al. (2013) (9760 kg), Boğokşayan ve Bakır (2013) (5673 kg), EHRC (2020) (6785 kg), Genc and Soysal (2018) (6010±3.48 kg) and Karaağaç and Genç (2019) (7350.5±30.70 kg).

In this study, the mean dry period was identified as 60.5±0.9 days and this value was within the limits generally considered ideal in dairy cattle breeding (Table 1). Compared to the findings of other researchers, this value was lower than values found by Genç and Soysal (2018) (61.8±0.1 days) and Sahin and Ulutas (2011) (85 days). It was determined that the values obtained for DP were close to the ideal duration. This can be interpreted as that herd care, supervision, and management were done well.

When Table 2 was examined, the average daily milk yield in Holstein Friesian cattle was determined as 32.23±0.27 kg and this value was higher than values determined by Akkas and Sahin (2008) (17.4 kg), Bayril and Yılmaz (2010) (25.8 kg) and Yildirim et al. (2018) (24.91±0.2 kg). Total lactation milk yield (10248.64±94.08 kg) obtained in this study was higher than values found as 4998.58±1.63 kg and 7160.6±33.0 kg by Duru and Tuncel (2004) and Özkök and Uğur (2007), respectively. ME-305-d milk yield (11148.79±92.59 kg), on the other hand, was found to be higher than the value (7882.4 kg) obtained in the study conducted by Bayril and Yilmaz (2010).

This study determined the mean time to reach peak and peak-day milk yields as 95.2±2.1 days and 42.3±0.3 kg, respectively. For both characteristics, these values were found much higher than values (52.2±3.3 days and 21.5±06 kg) obtained Holstein Friesian cattle by Yılmaz and Kaygısız (2000) and values (26.1±1.1 days and 15.4±0.7 kg) obtained Zavot cattle by Yüksel (2019). This study determined that the milk flow rate was 2.0±0.0 kg/min and milking time was 360.9±4.7 seconds (Table 4). In terms of the milk flow rate, value of the study was higher than the value (1.049±0.019 kgmin^{-1}) that Güler et al. (2009) found in Holstein Friesian cattle and value (0.972±0.013 kg min^{-1}) that Aydin et al. (2008) found in Brown Swiss cattle. In terms of milking time, the value found in this study was also higher than the values found in same studies (5.83±0.07 min and 5.46+0.05 min, respectively).

This study determined the heritability of ME-305-d milk yield as 0.41±0.24. This heritability value was higher than the values reported in Holstein Friesian breed cattle (0.26±0.07) by Sarar and Tapki (2017), in Jersey breed cattle (0.30±0.10) by Missanjo et al. (2013), in Holstein Friesian cattle at the first lactation (0.28±0.05) by Bohlouli et al. (2015), and in Holstein Friesian cattle (0.325±0.222) by Güngör and Zulkadir (2020). Milk flow rates based on objective measurements of milk meters have higher heritability values between 0.27 and 0.54 kgmin^{-1} (Ilahi and Kadarmideen, 2004; Gray et al., 2011). In this study, milk flow rate heritability was determined

as 0.51±0.30. This value was higher than the value obtained as 0.48 by Wethal and Heringstad (2019) in Norwegian Red cattle.

5. Conclusion

This study is important in determining Holstein Friesian cows' adaptation ability to Turkish conditions. Because Holstein Friesian cattle are imported to Türkiye from USA and EU countries and are grown in a wide area in Türkiye.

In this study, according to the lactation performance data of Holstein Friesian cows, it is understood that the farm has a professional herd management working for high milk production. It is thought that this success achieved by the farm in high milk yield is due to the exemplary level of general competencies such as herd management, care and feeding.

This dairy farm can be recommended as an example to breeders who have just started their dairy farm in Türkiye and countries with similar environmental conditions and are looking for a model. Although the number of studies on 305-d milk yield is sufficient for the Holstein Friesian breed in Türkiye, there are not enough studies on adaptation ability and milking ability.

Author Contributions

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

	O.Ş.	İ.Y.	A.K.
C	50	50	
D	50	50	
S			100
DCP	100		
DAI		50	50
L	50	50	
W	50	50	
CR			100
SR	50	50	
PM	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.

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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EFFECT OF GENOTYPE AND HOLDING DURATION ON SOME POST-HATCH TRAITS OF DAY-OLD BROILER PURE LINE CHICKS

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Abstract: In this study, it was aimed to investigate the effects of varying holding durations on some post-hatching characteristics of broiler pure line chicks with different selection backgrounds. Fifty d-old chicks from each of 3 dam (A1, A2, A3) and 1 sire (B1) ANADOLU-T broiler pure lines were used. Ten chicks of each genotype were treated with holding durations of 0, 12, 24, 36 and 48-h after hatching. At the end of each holding duration, individual chick weight (g), weight loss (g, %), chick length (mm), wing feather length (mm), rectal temperature (°C), yolk sac weight and percentage (g, %) and yolk-free body mass (g) were determined. Hatching egg weights were similar in A1 (60.9 g), A2 (60.9 g) and B1 (61.1 g) lines, but higher than A3 (59.2 g) (P<0.001). Chick weights were significantly different between genotypes both at hatch and at the each holding duration (P<0.01) and B1 line chicks were the heaviest, A3 the lightest. Absolute and relative mean weight loss occurred in the A3, B1, A1 and A2 lines as 3.7 g and 8.6%, 3.6 g and 8.8%, 3.0 g and 7.3% and 3.1 g and 7.2%, respectively (P<0.01). While the chick length increased linearly as the holding duration progressed in the B1 and A2 lines, it decreased after the 12-h holding period in the A1 line chicks (Interaction effect, P=0.026). The A1 (11.1 mm) and A3 (9.8 mm) line chicks had significantly shorter wing feather lengths (P<0.001) than A2 (15.4 mm) and B1 (15.1 mm) chicks. Rectal temperature values were lower in the A1 line than the others (P<0.01). Genotype x holding duration interaction on yolk sac weight and percentage was significant (P<0.05). A3 chicks with the highest yolk sac weight (6.2 g) and percentage (15.2%) at hatch had higher yellow sac absorption than other genotypes during the 48-h holding. Yolk-free body mass was the highest in B1 (36.7 g) and lowest in A3 chicks (34.5 g) (P<0.001). In conclusion, chick weight, chick length and yolk-free body mass were greatly influenced by egg weight. Regardless of the genotype, the extended holding durations at hatch resulted in deterioration in the general chick characteristics. Further studies are needed to reveal embryonic development and early post-hatch chick characteristics that are likely altered by different selection strategies for each pure line.

Keywords: Broiler, Pure line, Selection, Hatching, Holding duration, Chick quality

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1. Introduction

In recent years, chicken meat production has reached the highest levels as a result of the increasing demand for animal protein sources. Worldwide chicken meat production has increased more than 10 times since 1961 (FAO, 2023). In addition to the higher number of chick production, the improvement in the meat production efficiency contributed significantly to the increase in meat production. While 85-90% of the productivity increase was attributed to genetic modification, the remaining 10-15% was contributed by hatchery practices management, feeding and other factors (Zuidhof et al., 2014).

The commercial expansion of the broiler industry after the 1950s has been associated with the development of large-scale hatcheries and large industrial incubators. However, as a result of this, the distance between hatcheries and farms has also increased (Bergoug et al., 2013). Current EU legislation (Council of the European Union, 2005) states that broiler chicks can be deprived of feed or water for a maximum of 24-h after hatching, which is based on the fact that the chick's metabolic reserves stored in the yolk sac can last up to 72-h (EFSA Animal Health and Welfare Panel (AHAW), 2011). Dehydration is an important problem in d-old chicks that are transported for a long time (Fairchild et al., 2006). Chick mortality is mostly seen in the first week of the rearing period and occurs due to poor adaptation to post-hatching management, handling and housing conditions (Bayliss and Hinton, 1990; Heier et al., 2002; Sarıca et al., 2022). In previous studies, it was stated that weight loss increased due to the increase in holding time, and this was caused by dehydration and yolk sac absorption (Tona et al., 2003; Peebles et al., 2004; Almeida et al., 2006; El Sabry et al., 2013; Erensoy et al., 2020). As the holding duration increases after hatching, chick length first increases and then decreases (Yalçın et al., 2013),

and chick length is positively correlated with body weight at slaughter (Molenaar et al., 2008).

When compared with their hybrids, broiler pure lines used as breeding material are more sensitive to environmental factors. Therefore, starting the rearing period by providing healthy chicks will be an important first step for the next production stages. In this study, it was aimed to investigate the effects of varying holding durations on some post-hatching characteristics of broiler pure line chicks with different selection backgrounds.

2. Materials and Methods

The study material, broiler pure line chicks, was obtained by incubation of eggs obtained from Eskişehir Transitional Zone Agricultural Research Institute at Ondokuz Mayis University Agricultural Faculty Research and Application Farm in January, 2019. In this study, 50 d-old chicks from each of 3 dam (A1: slow-feathering, A2: fast-feathering, A3: slow-feathering) and 1 sire (B1: fast feathering) of ANADOLU-T pure lines were used. The A1 and A3 lines are selected for reproduction, while A2 and B1 are for fast-growth, developmental and feed efficiency characteristics over generations.

Before incubation, 100 eggs from each genotype were individually weighed. Incubation of the eggs was carried out at 37.7 °C and 60% relative humidity during the first 18 d, and 37.4 °C and 70% between 18-21 d. The incubation period was terminated at the 510-h of incubation and 50 chicks from each pure line genotype were kept in the same tray in the hatching machine during the holding duration. Ten chicks of each genotype were treated with holding durations of 0, 12, 24, 36 and 48-h. All chicks were individually numbered at hatch and their initial weight (g) was recorded. At the end of each holding duration, individual chick weight (g), weight loss (g) and percentage (% of first hatch weight), chick length (mm, from beak to tip of middle finger), wing feather length (mm, from the 4th primary wing feather), rectal temperature (°C, 2 cm into the cloaca for 30 sec) (Noubandiguim et al., 2021), yolk sac weight (g) and percentage (%, relative to chick weight) and yolk-free body mass (g) were determined. At each holding duration, ten individually numbered chicks were killed by cervical dislocation and the residual yolk sac weight and yolk-free body mass (chick weight - yolk sac weight) were determined (Özlü et al., 2018 and 2020).

All statistical analyses were performed with SPSS version 21.0 (SPSS Inc., Chicago, IL, USA) using the GLM procedure. Differences between levels of main effects (genotype and holding duration) and their interactions were tested for significance (at $P<0.05$) using Tukey HSD's test for multiple comparisons.

3. Results

The effects of genotype and holding duration on some post-hatch traits of d-old broiler pure line chicks are given in Table 1. Hatching egg weights were similar in A1 (60.9 g), A2 (60.9 g) and B1 (61.1 g) lines, but higher than A3 (59.2 g) ($P<0.001$). Chick weights were significantly different between genotypes both at hatch and at each holding duration ($P<0.01$) and B1 line chicks were the heaviest, A3 the lightest. Chick weight at hatch (40.1 g) and 12-h holding duration (40.5 g) was higher than that at 24, 36 and 48-h ($P<0.01$). Absolute and relative weight loss occurred in the A3, B1, A1 and A2 lines as 3.7 g and 8.6%, 3.6 g and 8.8%, 3.0 g and 7.3% and 3.1 g and 7.2%, respectively ($P<0.01$). As the holding duration progressed, the weight loss also increased and the 12, 24, 36 and 48-h holding durations caused weight loss of 4.4%, 6.8%, 8.3% and 12.4%, respectively. While the chick length increased linearly as the holding duration progressed in the B1 and A2 lines, it decreased after the 12-h holding period in the A1 line chicks (Figure 1; Interaction effect, $P=0.026$). The A1 (11.1 mm) and A3 (9.8 mm) line chicks had significantly shorter wing feather lengths ($P<0.001$) than A2 (15.4 mm) and B1 (15.1 mm) chicks. It was determined that the wing feather length increased as the holding duration increased, and the length, which was 9.6 mm at the 0 h, reached 15.5 mm at 48-h holding time ($P<0.001$). Rectal temperature values were lower in the A1 line than the others ($P<0.01$). The rectal temperature, which was 37.1 °C after hatching, was highest at 12-h (38.3 °C) and 36-h (38.2 °C), and lowest at 48-h (36.2 °C) ($P<0.001$). Genotype × holding duration interaction on yolk sac weight (Figure 2) and percentage (Figure 3) was found significant ($P<0.05$). A3 chicks with the highest yolk sac weight (6.2 g) and percentage (15.2%) at hatch had higher yellow sac absorption than other genotypes during the 48-h holding period. Yolk-free body mass was the highest in B1 (36.7 g) and lowest in A3 chicks (34.5 g) ($P<0.001$). Yolk-free body mass, which was 35.1 g at the hatch, increased to 36.8 g at the end of the 12-h holding duration, and decreased with the advancing holding, reaching the lowest at 34.9 g at the 48-h ($P<0.01$).

4. Discussion

Broiler pure lines are breeding material with elite characteristics and more importance is attributed to them than their descendants (grand-parents, parents and hybrids) produced in other stages of production. Because the yield increases in their commercial hybrids are due to the genetic progress achieved at pure line level (Erensoy and Sarıca, 2022). Therefore, a good physiological start of the pure line chicks in the post-hatch period would have provided significant advantages for the later life.

The heaviest hatching weight of B1 chicks and the lightest of A3 chicks seem to be largely related to egg weight, in line with Iqbal et al. (2017) and Nowaczewski et al. (2022), because B1 eggs were the heaviest, while A3's were the lightest. In our study, heavier and taller chicks hatched from large eggs, consistent with Nangsuay et al. (2011).

Table 1. The effects of genotype and holding duration on some post-hatch traits of day-old pure line chicks

Genotype	Holding duration (h)	Chick weight (g) [1]	Chick weight (g) [2]	Weight loss (g)	Weight loss (%)	Chick length (cm)	Wing feather length (mm)	Rectal temperature (°C)	Yolk sac weight (g)	Yolk sac percentage (%)	Yolk-free body mass (g)
	0	39.1	39.1	-	-	19.0	9.1	37.1	4.5	11.4	34.6
	12	42.1	40.8	1.3	3.1	19.2	10.3	38.1	3.8	8.9	37.1
A1	24	40.7	38.1	2.6	6.4	18.8	11.2	37.0	3.5	9.2	34.6
	36	41.6	38.4	3.2	7.7	18.9	12.2	37.9	2.9	7.6	35.5
	48	41.9	36.8	5.1	12.1	19.0	12.5	35.4	2.5	6.7	34.3
	0	39.4	39.4	-	-	18.9	11.1	37.0	4.5	11.5	34.9
	12	43.2	41.6	1.7	3.8	19.0	11.8	38.6	3.4	8.1	38.1
A2	24	41.7	39.4	2.3	5.4	19.1	16.8	37.5	2.2	5.6	37.2
	36	42.1	38.6	3.5	8.3	19.2	18.6	38.0	2.1	5.4	36.5
	48	41.2	36.4	4.8	11.5	19.4	18.7	36.4	1.5	4.1	35.0
	0	40.3	40.3	-	-	18.4	7.3	36.6	6.2	15.2	34.1
	12	40.2	38.2	1.9	4.8	18.4	8.2	38.6	3.5	9.1	34.8
A3	24	41.8	38.4	3.4	7.9	18.8	9.3	38.4	3.1	7.9	35.3
	36	40.6	36.9	3.8	9.2	18.7	11.7	38.6	2.5	6.6	34.4
	48	41.1	35.6	5.4	13.2	19.0	12.6	36.3	1.7	4.8	34.0
	0	41.6	41.6	-	-	18.8	10.8	37.6	4.8	11.5	36.8
	12	43.9	41.3	2.5	5.8	18.9	13.2	38.1	4.3	10.3	37.1
B1	24	42.3	39.1	3.2	7.6	19.1	14.7	38.2	2.7	6.8	36.5
	36	42.4	38.9	3.5	8.1	19.5	18.5	38.5	2.1	5.5	36.8
	48	43.5	37.9	5.6	13.0	19.9	18.2	36.6	1.7	4.4	36.3
SEM		0.206	0.207	0.133	0.309	0.397	0.330	0.092	0.111	0.258	0.173
						Main effects					
Genotype		0.003	0.003	0.010	0.009	0.000	<0.001	0.002	0.007	<0.001	<0.001
A1		41.1^{b}	38.6^{bc}	3.0^{b}	7.3^{ab}	19.0^{b}	11.1^{b}	37.1^{b}	3.4^{a}	8.8^{a}	35.2^{bc}
A2		41.5^{b}	39.1^{ab}	3.1^{ab}	7.2^{b}	19.1^{ab}	15.4^{a}	37.5^{a}	2.8^{b}	7.0^{b}	36.3^{ab}
A3		40.8^{b}	37.9^{c}	3.6^{ab}	8.8^{a}	18.7^{c}	9.8^{b}	37.7^{a}	3.4^{a}	8.7^{a}	34.5^{c}
B1		42.8^{a}	39.8^{a}	3.7^{a}	8.6^{ab}	19.2^{a}	15.1^{a}	37.8^{a}	3.1^{ab}	7.7^{b}	36.7^{a}
Holding duration		0.006	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002
0 h		40.1^{b}	40.1^{a}	-	-	18.8^{c}	9.6^{c}	37.1^{c}	5.0^{a}	12.4^{a}	35.1^{b}
12 h		42.3^{a}	40.5^{a}	1.9^{c}	4.4^{d}	18.9^{bc}	10.9^{c}	38.3^{a}	3.7^{b}	9.1^{b}	36.8^{a}
24 h		41.6^{a}	38.8^{b}	2.9^{b}	6.8^{c}	18.9^{bc}	13.0^{b}	37.8^{b}	2.9^{c}	7.4^{c}	35.9^{ab}
36 h		41.7^{a}	38.2^{b}	3.5^{b}	8.3^{b}	19.1^{b}	15.3^{a}	38.2^{a}	2.4^{c}	6.3^{d}	35.8^{ab}
48 h		41.9^{a}	36.7^{c}	5.2^{a}	12.4^{a}	19.3^{a}	15.5^{a}	36.2^{d}	1.8^{d}	5.0^{e}	34.9^{b}
Interaction		0.633	0.450	0.867	0.850	0.026	0.210	0.140	0.018	0.007	0.436

1= chick weight at hatch (g), 2= chick weight at the end of the respective holding period (g), Means shown with different letters in the same column differ significantly from each other according to the Tukey HSD test. SEM: Standard error of the mean.

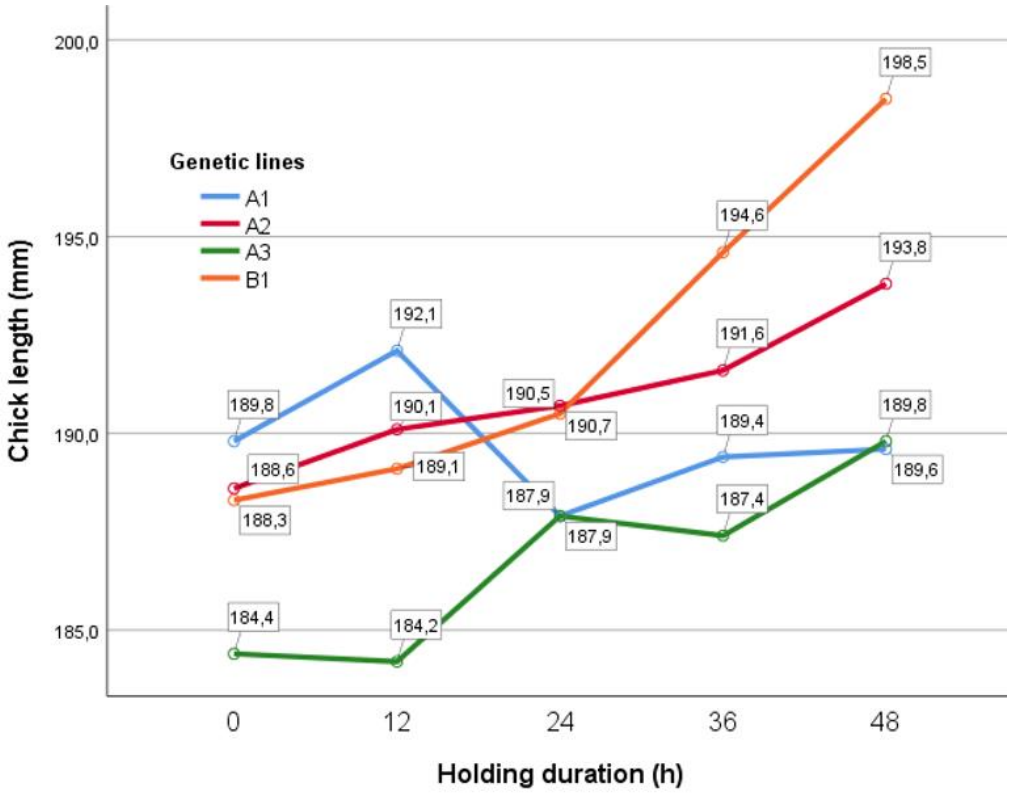

Figure 1. The effects of genotype and holding duration on chick length (mm).

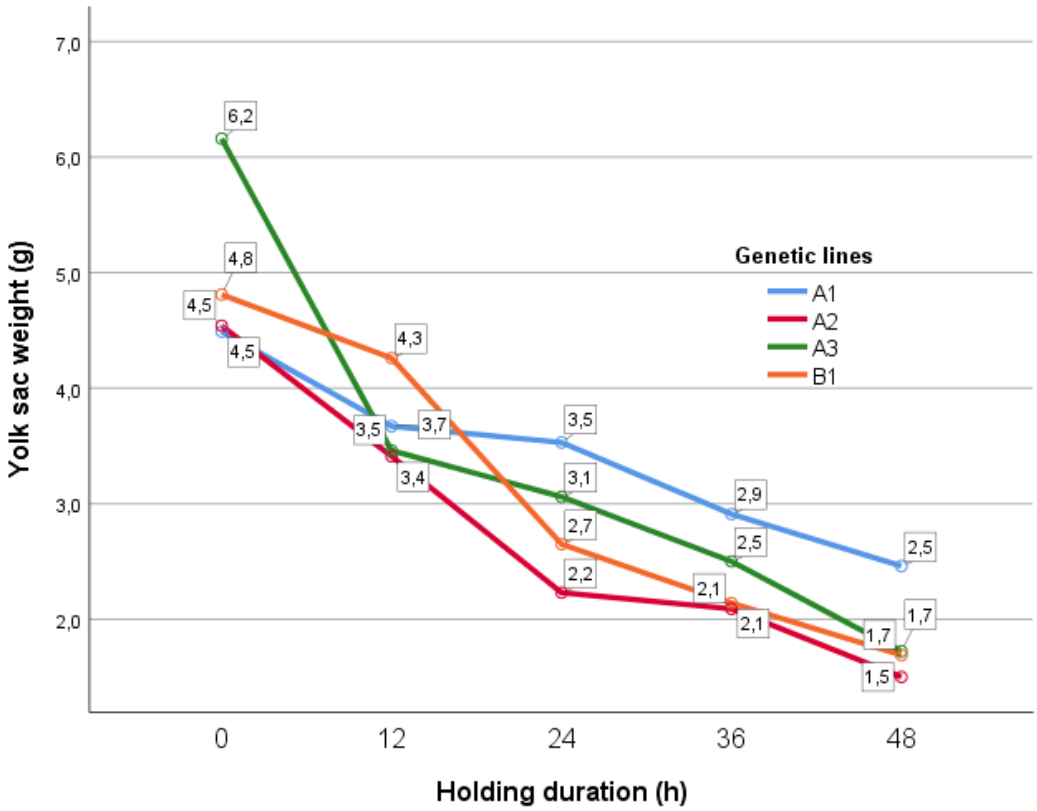

Figure 2. The effects of genotype and holding duration on yolk sac weight (g).

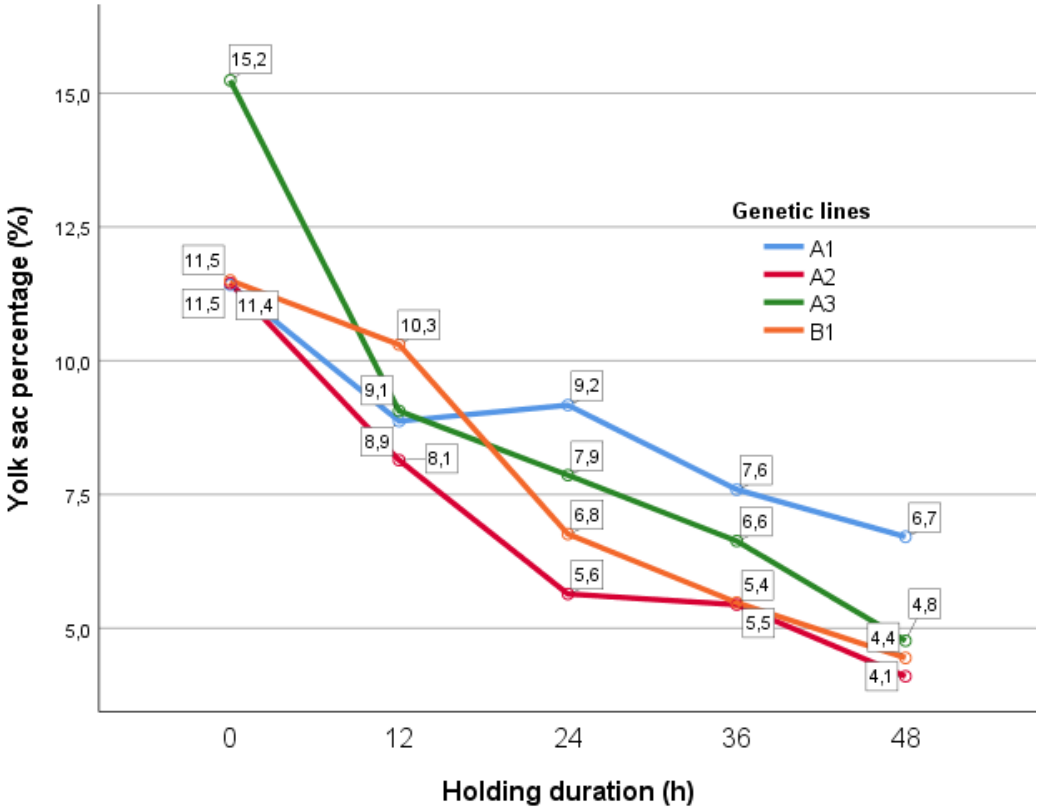

Figure 3. The effects of genotype and holding duration on yolk sac percentage (%).

This supports that chick weight and length, and yolk-free-body mass are largely determined by egg size. Although broiler chicks hatch in ~21-d on average, a 24-48-h period, called the hatching window, passes between the first and last hatched chicks (Tong et al., 2015). In addition to this, the long duration of hatchery procedures (sex separation, coding, weighing, vaccination, transportation, etc.) applied to pure line chicks after hatching causes the holding period to be longer (Erensoy et al., 2020). Although chicks can be deprived of feed and water for up to 72-h (Willemsen et al., 2010), lack of access to feed and water after hatching leads to weight loss due to dehydration and yolk sac absorption (Tona et al., 2003; Tong et al., 2015; Erensoy et al., 2020; Özlü et al., 2022). Similarly, the advancing holding duration increased weight loss in chicks in our study, regardless of genotype. Both relative and absolute weight loss were higher in A3 and B1 line chicks than in the others. However, this situation was surprising for A3 and this could probably be related to the earlier use of yolk sac reserves due to earlier hatch than others. Because the residual yolk sac, which was 6.2 g (15.2%) at the hatch, decreased to 1.7 g (4.8%) at the end of the 48-h holding period supports this hypothesis. Otherwise, less resources would be sufficient for the maintenance of the low yolk-free-body mass of A3 chicks (Nangsuay et al., 2011; Özlü et al., 2020). For the B1 line, more yolk absorption and thus weight loss could be expected in order to maintain the higher yolk-free body mass. In addition, prolonged holding times adversely affect post-hatch performance in terms of growth, immune system, stimulation of digestive enzymes and organ development (Gonzales et al., 2003; Willemsen et al., 2010). For this reason, prolonged holding durations after hatching is an important risk factor, especially for chicks that hatch earlier. In this case, we may assume that A3 chicks are subjected to longer holding durations, which makes it difficult to get a good start for post-hatch life.

Rectal temperature is a factor that affects post-hatch quality and early performance of chicks, and was between 39.4-40.5 °C in ROSS-308 hybrids (Aviagen, 2021; Özlü et al., 2022), and 37.1-37.8 °C in ANADOLU-T pure lines at d-old age (Noubandiguim et al., 2021). While our study results were similar to Noubandiguim et al. (2021), because we used the same pure line material, they were lower than ROSS-308 hybrids, which could probably be attributed to differences in metabolic heat production, possibly due to the genetics of pure line chicks. This is because selection for growth rate after hatching is likely to have altered embryonic metabolism and heat production by affecting the developmental pattern of the embryo (óDea et al., 2004; Janke, 2004). A recent study showed that ROSS-308 chicks were superior to ANADOLU-T pure lines in terms of growth characteristics (Erensoy and Sarıca, 2023). This may partly explain the elevated body temperature associated with the relatively higher metabolic rate of ROSS-308 hybrids. The lower rectal temperature of A1 line chicks than the others may also indicate lower energy requirements for post-hatch maintenance (Piestun et al., 2015). On the other hand, the higher absolute and percentage of residual yolk sac in A1 and A3 chicks

indicates that these genetic lines may require less energy for metabolic functions. Noubandiguim et al. (2021) reported that the A2 and B1 lines were fast-feathering, and this was also confirmed in our study. We speculate that these lines need more resources to support faster feather development in the early post-hatch (holding) period, which causes body reserves (yolk sac) to be depleted faster. In addition, the selection of A2 and B1 for higher body weight compared to A1 and A3 may have increased the metabolic rate (óDea et al., 2004), which may have contributed to the greater use of the yolk sac.

5. Conclusion

In conclusion, chick weight, chick length and yolk-free body mass are greatly affected by egg weight. Regardless of genotype, advancing holding duration causes deterioration in general chick traits. In addition, genetic lines responded to the same holding durations with varying levels of yolk sac absorption. Therefore, further studies are needed to reveal embryonic development and early post-hatch chick characteristics that are likely altered by different selection targets for each pure line. In this way, it seems possible to start the post-hatch period with better quality chicks by determining the optimum hatching management and procedures for each genetic line.

Author Contributions

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

	K.E.	M.S.	N.K.
C	100		
D	100		
S	50	50	
DCP	100		
DAI	100		
L	100		
W	40	30	30
CR	40	40	20
SR	40	40	20
PM	10	90	
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 experimental procedures were approved by the Local Animal Care and Ethics Committee of Ondokuz Mayıs University (protocol code: 2017/31, date: June 30, 2017).

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ANALYZING THE NEXUS BETWEEN PERSONAL VALUES AND CONSUMPTION VALUES OF CONSUMERS' PREFERENCE FOR FRESH FISH

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Abstract: This study aimed to investigate the nexus between personal values and consumption values towards consumers' preferences for fresh fish. A cross-sectional data of 300 respondents was selected using a multi-stage random sampling procedure. A well-structured questionnaire and personal interview were used to collect data from the respondents. Factor analysis and canonical correlation analysis were conducted to achieve the study's objectives. Findings showed that females (67.5%) were the majority of respondents, household size ranged between 4-6 persons, and many (76.7%) were married. The mean age was 41 years, and 83.3% were formally educated. The relationship between personal values and consumption values was positive and statistically significant at the 1% level. The study revealed that variables like benevolence, security and self-direction were strongly correlated with emotional and functional values of the first canonical covariates. The study concludes that high benevolence, security and self-direction evoke high emotional and functional values when consumers buy fresh fish. Based on these findings, the study recommends that fresh fish marketers should pay more attention to the price-quality relationship, the performance and content of the product and the quality of packaging, as most respondents place more value on what they consume.

Keywords: Consumption values, Personal values, Fresh fish, Canonical correlation analysis

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1. Introduction

In Nigeria, the push for a balanced diet and a reduction in carbohydrate consumption has increased protein consumption (Ojianwuna et al., 2021). Plant-based proteins are the preferred source of protein (Pojic et al., 2018). Unfortunately, the typical Nigerian diet is not very high in protein from vegetable sources. Hence, most Nigerians rely on protein from animal sources. Fish is a recommended source of animal protein for both young and older people since it is simply tasty and has minimal saturated fat compared to red meat (Colombo et al., 2021). There is a fish variety that would suit consumers' needs, regardless of their socio-economic status, race, culture, religious beliefs, or gender (Oleschuk et al., 2019).

Nigeria's demand for fish has increased due to the rising price of alternative animal protein and the burden of an expanding population (Tuninetti et al., 2022). Fish is mostly consumed in fresh and frozen forms due to the rising demand for fish products and is distributed to consumers through the marketing system. Marketing aims to ensure that customers receive the goods they desire in the ideal shape (form utility), supplied in the ideal location (place utility), at the ideal price (possession utility), and at the ideal time (time utility), to delight the customer fully.

The specific preferences of various consumers are represented by their pleasure with the products they have purchased (Justin and Jyoti, 2012). There have been many theories and models to explain why people choose the things they do, but as of now, there is yet one approach that can explain why people make the purchases they do. Some factors, such as globalization, the rise in the variety and number of products and brands available on the market, and the rapid improvement in information and communication abilities, have clearly influenced consumer expectations and wants, which continue to be not only physical, but also psychological in nature as well. Due to Nigeria's rising demand for fish, it is crucial for marketing experts to be sensitive to consumers' psychological demands in order to advertise fish successfully. Personal values are a noteworthy element that may be used to examine consumers' psychological demands. The factors affecting what is essential in people's life are personal values (Ertosun and Adiguzel, 2018). Consumption values are a modern theory that explains why consumers select a certain brand or product (Cabrera and Williams, 2012).

According to the consumption values model, consumers pay attention to functional, social, emotional, conditional, and epistemic values in product attributes (Khan and Mohsin, 2017).

Previous studies on fish demand and marketing has mostly focused on consumer attitudes and how they affect decisions on kinds, form, and value (De Medeiros et al., 2016). On the other hand, customer behavior and attitude towards choices significantly impact market expansion. Consumers typically express their sentiments based on known facts and customs. These perspectives are challenging because they affect the choice of a certain product as well as its quantity and quality. Some researchers have investigated the relationship between personal values and consumption values before now. For example,we reviewed that Candan and Yıldırım (2013), Gleim and Lawson (2014), Gonçalves et al. (2016), and Sreen et al. (2018), applied it to green products consumption; Latter et al. (2010) and Candan and Yildirim (2013) deployed the approach to study brand loyalty and consumer satisfaction. Zaidi et al. (2019) and Pankaj and Anand (2022) used the approach to investigate green purchase intention while Shende (2014), Altaf et al. (2017), and Zubair (2017) used the approach to study automobile purchases.

To the best of our knowledge, we found no study at present in the study area that applied same approach adopted in this study to consumers' preferences for fresh fish. The current study further differs from previous ones by location, population and nature of data collected. We, therefore, feel it would be crucial to bridge the knowledge gap by adding to the existing literature, the relationship between personal and consumption values of fresh fish using canonical correlation analysis.

The main purpose of the study is to examine the relationship between the consumption values of fresh fish and the personal values of consumers who purchase it. We were aware that the scope of this study may be limited by the area of coverage and availability of fund, meaning that this does not represent the entire country. However, the outcome of our study will still provide the necessary information for producers, distributors, marketers, consumers, and researchers on fish business.

2. Theoretical Consideration

2.1. Consumption Values

The ease and speed with which consumers can now obtain a wide range of products is a result of globalization. This theory also covers a wide range of product categories, including industrial commodities, services, and goods for physical and non-physical consumption. Consumption values can be used to explain the reasoning and motive behind buying the majority of goods and services (Han and Hwan, 2015). Sheth (1991a) used the consumption values theory of Newman and Gross (1991) in various studies to explain consumer behavior (Han and Hwan, 2015). The three pillars of "price, quality, and value" dominate customers' rational purchasing decisions and product choices.

2.1.1. Social value

Social value is defined as the benefit that is perceived and obtained in relation to one social group or several social groups (Sheth et al., 1991). According to demographic, socioeconomic, and cultural (ethnic) groupings, the social benefit acquired may be favorably or adversely correlated (Garc'aGo'meza et al., 2012). Social class, symbolic value, conspicuous consumption, reference groups, and opinion leadership are all words used to study social values. Work, education, and income status are typically used to categorize people into different social classes. In addition, according to Zehir et al. (2011), social classes can be classified according to prestige, status, adopted values, etc.

2.1.2. Emotional value

The benefit derived from an emotional or sensual situation is known as emotional value. The responses customers have toward a product are related to its value (Woisetschläger et al., 2011). Emotional values that influence consumer preferences can be positive or negative. For example, "loyalty, nostalgia, excitement" might be positive, while "fear, anger, and guilt" can be negative.

2.1.3. Epistemic value

Sheth (1991a) defined epistemic value as the advantage that satisfies the want and need for innovation as well as the curiosity that is recognized or acquired from the product. When the purchasing tendencies of customers are examined, it is found that these consumers are exploratory and seek out diversity (Chiu et al., 2013). The primary driving force behind the actions of customers who seek diversity is "innovativeness."

2.1.4. Conditional value

The benefit that results from a certain condition that the individual making a preference comes across and is experienced at that particular time is what is meant by conditional value. In an unanticipated circumstance, this additional benefit materializes as a factor that raises functional or social value.

2.1.5. Functional value

According to Sheth (1991a), the term "functional" can be used to describe the benefit felt or attained from a situation's functional, pragmatic, and physical performance. Functional value is determined by taking into account a product's performance, reliability, soundness, and pricing (Garc'a Go'meza et al., 2012).

2.2. Personal Values

Based on Schwartz‘s (1992) theory of human value, values can be categorized as: the content of values and the structure of values. The content of a value is its source of motivation, and the structure of a value is the relationship between the values. Schwartz’s theory is based upon 57 single values, which can be abstracted into 10 value types encompassing similar motivations and similar content. The 10 value types included in the theory are: Universalism, Benevolence, Success, Tradition, Security, Power, Achievement, Hedonism,

Stimulation, and self-direction. Schwartz found 45 of the 57 values in his theory to be universal in nature; that is, they have similar meanings across all cultures. They may, however, be more or less prized in different cultures (Schwartz, 1992). The list of 57 values was also found to be a comprehensive list of all values across cultures.

2.2.1. Power

The power values are social status, prestige, dominating over other people, and controlling other people and sources (social power, authority, welfare, and image). Neuman (1986) found that there is no association between accumulation of personal wealth and social recognition and the energy conservation construct. Shaw et al. (2005) found the power values to be of little relevance to ethical consumers.

2.2.2. Achievement

The individual achievement a person attains in the frame of social standards can be assessed through the following variables: success, ability, bossy and passion.

2.2.3. Hedonism

This personal value dimension describes people's pleasure, joys (such as pleasure, satisfaction, and enjoyable life).

2.2.4. Stimulation

The stimulation values are related to people's excitement, innovations and challenges in life (a brave and rich life, an exciting life).

2.2.5. Self-direction

The self-direction values include Freedom, Creativity, Independence, Choosing own goals, Curious, and Self-respect. Empirically, Shaw et al. (2005) found the values of curiosity, freedom, independent, and self-respect to be related. This value is expected to have positive relationship with the consumption of fresh fish.

2.2.6. Universalism

Universalism value are values that are related to being tolerant and respective, welfare for all human beings (open-mindedness, wisdom, social justice, equality, world peace, protecting the environment, welfare for the whole world)

2.2.7. Traditionalism

The traditional value has to do with people having respect for others' ideas, commitments, and and acceptance of the customs and ideas that traditional culture and religion provide, living in accordance with a culture or religion. This value is expected to have a positive relationship with the consumeption of fresh fish.

2.2.8. Benevolence

The benevolence value are maintaining and developing people's welfare, maintaining interpersonal relations useful, honest, forgiveness, loyal and responsible. Available literature shows that there is an association between the value mature love and energy conservation (Neuman, 1986). Similarly, Shaw et al. (2005) found positive relationship between the Helpful and Honest values and ethical consumption. This value is expected to have positive relationship with the consumption of fresh fish.

2.2.9. Conformity

Restraint from behaviours that are harmful to others, limiting expectations (i.e kindness, obedient, self-discipline, respect for parents and old, honoring elders)

2.2.10. Security

The security values include the following: family security, national security, social order, clean, reciprocation of favors, sense of belonging, and healthy. The motivations of safety, harmony, and stability underpin these values. Security's goal is to prevail over the uncertainty that arises with the self, relationships, and/or society (Schwarz, 1992, 1994).

3. Methods

3.1. Data Collection

The study was conducted in Akure Metropolis, the capital of Ondo State. A multi-stage random sampling procedure was used to select respondents for the study. In the first stage, purposive sampling was used to choose Akure town in Ondo State because of its urban nature, the extent of development and the presence of ultra-modern markets and the availability of fresh fish sales. In the second stage, six markets were randomly selected. The third stage involves the random selection of fifty respondents from each market, making a total of three hundred respondents analyzed in this study.

Primary data were collected using a structured questionnaire administered through an interview method. The questionnaire covered data on the socio-economic characteristics of the consumers buying fresh and other related information. Schwartz's Values List (SVS) was adopted to assess respondents' personal values. The SVS presents two lists of value items. The first contains 20 items that describe potentially desirable end-states in noun form; the second includes 22 items that describe potentially desirable ways of acting in adjective form. Each item expresses an aspect of the motivational goal of one value. Respondents were asked to rate the importance of each value item "as a guiding principle in MY life" on a 7-point scale labeled 7 (of supreme importance), 6 (very important), 5, 4 (unlabeled), 3 (important), 2, 1 (unlabeled), 0 (not important), -1 (opposed to my values), 6. To determine respondents' consumption values, the consumption values (functional, social, conditional, epistemic, and emotional) scale developed by Sheth et al. (1991) was adapted and modified to study consumer preference for fresh fish.

First, a focus group study was conducted to form consumption values related to fresh fish. Twenty (20) people participated in the focus group discussion. In the consumption value scale, respondents expressed for each value dimension, their degree of agreement with statements given to them by using 5 point liker scale: I don't agree at all (1), I don't agree (2), I am not sure (3), I agree (4), and I strongly agree (5).

3.2. Data Estimation and Model Specification

The study was analyzed by using both the descriptive and inferential statistics. Descriptive statistics was

employed to summarize the socio-economic characteristics of the consumers. Factor analysis was used to eliminate the insignificant and uninformative variables in the personal and consumption value scales. In contrast, the relationship between personal and consumption values was assessed through a canonical correlation analysis using a Stata version 14 (Startcorp, 2015).

3.2.1. Factor analysis

The factor analysis model expressed as (Equation 1):

$$X = \mu + L\,F + e \quad (1)$$

where X is the p x 1 vector of measurements, μ is the p x 1 vector of means, L is a p × m matrix of loadings, F is a m × 1 vector of common factors, and e is a p × 1 vector of residuals. Here, p represents the number of measurements on a subject or item and m represents the number of common factors. F and e are assumed to be independent and the individual F's are independent of each other. The mean of F and e are 0, Cov(F) = I, the identity matrix, and Cov(e) = Ψ, a diagonal matrix. The assumptions about independence of the F's make this an orthogonal factor model.

3.2.2. Canonical correlation analysis (CCA)

The study used canonical correlation analysis to estimate the relationship between personal values and consumption values and determine the level of their associations. Canonical correlation analysis is used when the objective is to measure the relationships between two sets of variables. Canonical correlation is appropriate in the same situations where multiple regression would be but where there are multiple intercorrelated outcome variables. Canonical is the statistical term for analyzing latent variables that are not directly observed that represent multiple variables that are directly observed. CCA is found to be commonly used in social and behavioral sciences and has been used by many researchers (Prera, et al. 2014; Andleeb, 2016; Suchanek and Kralova, 2018). Several advantages are associated with CCA over others statistical tools.

3.3. Research Model

The left hand side of Figure 1 represents the subscale of the criterion variables, while the right hand side of the model represents the subscale of the predictor variables. The variables are factors affecting individual scale in the model. We formulate that there is positive correlation between consumption value and personal values that derive consumer preference behavior for fresh fish purchases. In other words, a high personal value is predicted to invoke high consumption value for consumer preference for fresh fish.

3.4. Research Hypothesis

H_0: The consumption value of fresh fish has no association with the personal values of consumer preference behavior.

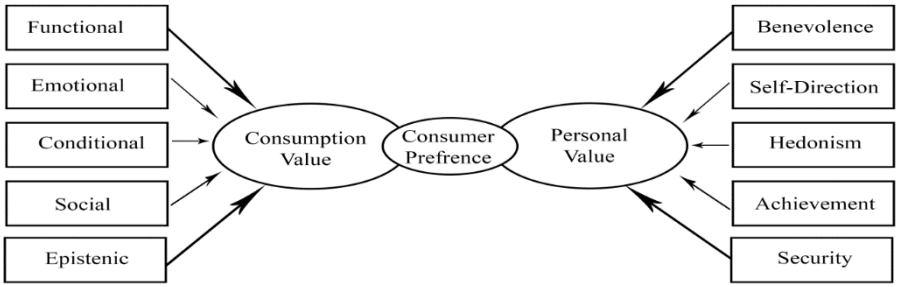

Figure 1. Research model for the relationship between personal and consumption value.

4. Results and Discussion

4.1. Socio-Economic Characteristics of the Respondents

The socio-economic characteristics of the respondents included sex, age, and marital status, level of education, monthly income, and household size.

The result in Table 1 showed that 67.5% of the participants were female, while 32.5% were male, indicating that women are more involved in the purchase of fresh fish for their homes than men, most (76.7%) of them were married, 19.2% was single, and 2.5% were widows and widowers while the remaining 1.7% were separated, implying that married people buy/patronize markets to buy fresh fish in the study area. The mean age of sample members was 41 years, indicating that they are still fairly young.

Individual may make informed decisions about what kind of foods they eat and how to take care of their health with the help of education. Therefore, it is impossible to overstate the importance of education in helping consumers choose one product over another (Sagynbekova et al., 2021). The distribution of respondents by educational level is displayed in Table 1. According to the table, the majority of the survey participants (83.3%) had received formal education; this suggests that they are literate, which is predicted to impact their consumption preferences and the importance they place on consuming fresh fish. The study's findings also showed that 42.5% of interviewees' had finished higher education as their highest degree of schooling. This implies that respondents had a high educational background which should influence their personal values and also consumption values of fresh fish.

The household size of sampled member as presented in table 1 showed that majority (78.3%) had a household

size ranging from 4 to 6. The distribution of the respondents by primary occupation, showed that less than half (31.7%) of them were traders, 30.0% were civil servants and 22.5% were into farming as their primary occupation. The monthly income of the respondents is also captured on Table 1. This table showed that the highest (56.7%) income level is less or equal to ₦50,000 and the lowest (0.8%) income level among the respondents is greater or equal to ₦762,500. The mean level of income of the respondents was ₦73,165.35. This implies that most of the respondents' income level revolved around ₦73,165.35. It can also be deduced from this result that most of the respondents in the study area are low income earners, which is attested to by the fact that most of them are traders and this is expected to influence their consumption preference, personal values and consumption values of fresh fish. The percentage of an individual's monthly income they spend on food is to some extent important in determining the personal value of that individual (Sanjuan-Lopez and Resano- Ezcaray, 2020).

The respondents spent about ₦20,000 monthly on food which accounted for 42.5%, while a monthly income of about 56.7% was generated. Looking at the mean income level (₦73,165.35) of the respondents in the study area in relation to their mean monthly expenditure (₦28,500.00) on food, it can be deduced that they spend about one-third of their monthly income on the consumption of food and this is expected to have a positive influence on their personal values in the consumption of fresh fish in the study area.

The position/rank a person occupies in their occupation or community can determine to a reasonable extent the choice of preference of his/her consumption (Carmen, 2016). This could be the reason why a person will prefer a commodity over and above another commodity with similar attributes. The rank/position of the respondent in the study area is also presented in Table 2. This finding showed that 34.2% of the respondents own of their businesses. This is understandable as most of the respondents are traders as can be seen on Table 1. This result also implies that decisions to buy or not to buy a commodity will not be difficult and can be greatly influenced by the psychological needs which the personal values of that individual can measure.

4.2. Factor Analysis Related to Personal Values of Respondents

Personal values are the constituents defining what is important in a person's life (Steiner and Tuljapurkar, 2012). Each individual has a lot of values different from each other. Personal values define the personal affinities and upset all kinds of peoples' choices and preferences (Paetz, 2021). Personal values are shaped by variables, such as security, reputation and upholding the position in the society. Individuals use these values to reach certain goals (Schwartz, 2016).

To measure the role of personal values on consumers' preference for fresh fish, the study employed seven dimensional scales that consist of 42 variables to achieve the goal. Before the application of factor analysis, a KMO was conducted and a score of 0.692 was obtained. The Barlett Sphericity Test obtained was statistically significant at 1% (0.861, $P<0.01$) (Table 2). This values indicate that, factor analysis is suitable for the study data. Similarly, the reliability of the scale was also checked by conducting a Cronbach Alpha Parameter test. The value obtained was 0.693 (Table 2). This value was approximated to 70%, which implied that the personal values scale used for this study is reliable (Candan and Yildirim, 2013). All the outcomes of the tests conducted justified the use of factor analysis on the data.

After these tests, factor analysis was run to eliminate the insignificant and much less informative variables that are in the personal values scale. As seen in Table 2, five (5) fundamental factors were extracted in the personal values scale for this study, based on the criteria that they feature on the first factor loading (Factor 1) of the factor analysis.

Table 1. Summary of socio-economic characteristics (n =300)

Characteristics	Categories	Percentage (%)
Gender	Male	32.5
	Female	67.5
Marital status	Married	76.7
Age	≤ 30	75.8
Level of education	Formal	83.3
	No Formal	16.7
	Completed Tertiary Education	42.5
Household Size	4-6	78.3
Primary Occupation	Trading	31.7
	Civil Servants	30.0
Monthly income from primary occupation	≤ 50,000	56.7
Monthly expenditure	≤ 20,000	42.5
Rank/Position in Primary occupation	Proprietorship	34.2
	Senior Officer	28.3

These are: self-direction (creativity, being curious, living an independent life, freedom), hedonism (being self-indulgent), achievement (caoble, influential, intelligent, best, respectful), benevolence (responding to favour, being helpful, having true friendship, being honest, always forgiving, having responsibility, maturity in loving, being loyal, having meaning in life, being moderate, society problems), and security (family security, social order, being clean, living healthy, reciprocation of favour, having a sense of belonging and national security) in sequence. Two factors (stimulation and tradition) were eliminated because factor loads related to the variables could not be gathered under the first factor loading (Table 2).

4.3. Factor Analysis on Consumption Value for Fresh Fish

Factor analysis was conducted on the consumption value scale to trim down its multidimensional variables. The KMO score (0.7360) was above the threshold of 0.70 as displayed in Table 3. This value showed that the consumption value scale has an acceptable validity. The Cronbach Alpha parameter test was found to be 0.9469, signifying that the consumption value scale is realible and factor analysis can be performed on consumption value scale. The result provides evidence proving that our data and instrument were sufficient to predict the relationship between personal and consumption values for fresh fish

Table 3 reveals that the results of the factor analysis on consumption value were measured by five dimensions, which consist of 27 variables. Six of the variables were eliminated due to insignificant information in them. From the table, about 58.6% variance in the functional value scales was explained. All Cronbach Alpha scores obtained for each subscale were reasonably high.

Table 2. Factors related to personal values

Variable	Factor Loading	Variance (%)	Eigenvalue	Cronbach's Alpha
SELF-DIRECTION		0.4302	3.4	0.889
Creativity	0.7092			
Being Curious	0.6379			
Living an independent life	0.648			
Freedom	0.6255			
HEDONISM		0.7617	4.7	0.7855
Being pleasurable	0.8474			
Live delighted	0.9579			
Being enjoyable	0.8059			
ACHIEVEMENT		0.9247	5.201	0.9381
Capable	0.941			
Influential	0.9487			
Intelligent	0.99			
Best	0.968			
Respectful	0.9595			
BENEVOLENCE		0.8522	9.373	0.9831
Respond to favour	0.9072			
Being helpful	0.9526			
Having true friendship	0.7932			
Being honest	0.9529			
Always forgiving	0.9691			
Having responsibility	0.9472			
Maturity in loving	0.9464			
Being loyal	0.9304			
Having meaning in life	0.938			
Being moderate	0.9664			
Society problems	0.8336			
SECURITY		0.8461	4.949	0.9585
Family security	0.9411			
Security order	0.9155			
Being clean	0.9205			
Having a sense of belonging	0.9248			
National security	0.8968			
Total variance (%)		0.7422		
KMO		0.692		
Cronbach Alpha		0.6939		

Table 3. Factors analysis of consumption values

Variable	Factor loading	Variance%	Eigenvalue	Cronbach's Alpha
FUNCTIONAL VALUE				
I buy fish due to low cholesterol	0.8005	0.7282	4.105	0.8902
I buy fish because the price is reasonable	0.9034			
Numerous minerals in Fresh fish attract me to buy it.	0.8299			
I buy fresh fish because of it is body builder	0.9308			
I buy fish because it contains omega-3	0.7933			
EMOTIONAL VALUE		0.4857	3.6092	0.6047
The joy of taking fish has no bound	0.8179			
I feel highly esteemed when I choose to buy fresh fish	0.6312			
Buying fresh fish makes me to feel like morally right	0.6306			
Buying fresh fish makes me to feel like eating the best food	0.6753			
I don't entertain any fear when I consume fresh fsh	0.7125			
CONDITIONAL VALUE		0.5247	2.6658	0.7764
I choose to buy fresh fish when meat price is too high	0.8121			
I buy fresh fish because I hate red meat	0.6563			
I buy fresh fish because of its availability in the market	0.7593			
People consume fresh fish because of its health appeal	0.6574			
SOCIAL VALUE		0.5401	1.3612	0.6808
Consumption of fresh fish will improve my status	0.649			
Consumption of fresh fish will be perceived as contribution to society	0.8174			
Consumption of fresh fish will help me to be environmentally conscious	0.7286			
EPISTEMIC VALUE		0.72243	3.3675	0.8935
Fresh fish is relatively abundant in my area	0.7904			
Free from pollutnts	0.8584			
Method of fish culturing in my area makes me perceives it is environmentally safe for consumption	0.965			
Ease to prepare by me	0.7726			
Total variance (%)		0.60		
KMO		0.7360		
Cronbach Alpha		0.9469		

Table 4. Results of canonical correlation analysis

Function	Correlation coefficient (R_c)	Canonical Root (R^2)	Wilks' Lambda	F	P-value
1	0.536	0.287296	0.515	3.178	0.000
2	0.445	0.198025	0.723	2.36	0.002
3	0.296	0.087616	0.901	1.313	0.230
4	0.105	0.011025	0.988	0.346	0.847
5	0.035	0.001225	0.999	0.137	0.712

4.5. Canonical Correlation Analysis

The overall model fit for the relationship between personal values and consumption values of fresh fish is presented in Table 4. The table shows the canonical function, correlation coefficient, R-squared, eigenvalue and wilks' statistic. The results showed that the overall model was significant at the 1% level, with a Wilks's λ=0.52 (F=3.18, P<0.01). Therefore, the effect size (r^2) of the full model was 0.48 which indicates that the full model explained a moderate proportion of the dependent variables. In the table, only canonical functions 1 and 2 were found to be statistically significant at the 1% level. This result means the null hypothesis that the canonical correlation for a function equals zero is rejected. Consequently, we can also say that these two functions could explain the relationship between the personal values and consumption values for consumer preference. However, the first canonical root explained the larger part of the relationship set and was found statistically significant at the 1% level. Then, it is only the first part that will be interpreted. The correlation between the first pair, personal values and consumption values, was 0.536 while the second canonical function was 0.445.

4.5.1. The relationship between personal values and consumption values

Table 5 shows the orthogonal rotated output of standardized canonical covariates and canonical loadings. Our interpretation of the results is based on the level of significance of the model fitted, magnitude of canonical loadings and percentage of redundancy index. Although there is no general rule as regard the selection of the size of coefficient that are interpretable compared to factor analysis, but based on previous studies, the present study also reported only canonical variates whose absolute value is greater than 0.20 as cut-off point (Prera et al. 2014). The results in Tables 5 and 6 are presented according to Dattalo (2014) for easy interpretation.

The personal values stand as the predictors while the consumption values are criteria here. The first two columns represent the coefficients and canonical loadings for personal values. The results showed that the redundancy index was about 0.08, suggesting that about 8% of the variance in the consumption values was explained by the variables in the personal values. From the second column of the first canonical variates, it shows that security (r=0.9806) was more associated with personal values than benevolence (r = 0.6935) and self-direction (r=0.4441). The canonical variates for achievement (r=0.1398) and hedonism (r=0.1881) seem to have less influence on the consumer preference for fresh fish. In the same manner, the functional and emotional values are major determinants in the consumption of fresh fish. Hence, this result indicates that higher security of the consumers, benevolence and self-dirrection were associated with higher functional values (0.9690) and emotional values (0.6700). The finding in this study corroborates the findings of Candan and Yıldırım (2013) on the analysis of the relationship between personal value and consumption value on green product buyers. This result indicates that people pay more attention to the physical appearance of the fish, to find out whether it is good for consumption, taste and benefits it will give to their bodies. The results show that fish consumers are proud of eating fish and wishing others to enjoy the same treatment. People who attach more importance to benevolence, security and self-direction, pay more attention to the emotional values in the demand for fresh fish. The canonical loadings of the first canonical function are more important in explaining the consumer behavior for fresh fish consumption since more than half (54%) of the total variance was explained by the first canonical function compared to the second function.

Table 5. Unrotated standardized coefficient and canonical loadings for set 1 and set 2

Variables	First Canonical Variate		Second Canonical Variate	
Set 1-Personal values	Coefficient	Loading	Coefficient	Loading
Benevolence	0.8292	0.8577	-0.2344	0.2446
Security	0.4571	0.7781	0.3676	0.3299
Self-Direction	-0.433	0.1936	-0.101	0.2382
Achievement	-0.2529	-0.0698	0.9706	0.9697
Hedoanism	-0.0117	0.0553	0.0419	0.4533
Percent variance	9.62		6.7	
Redundancy	0.08		0.05	
Set 2- Consumption values	Coefficient	Loading	Coefficient	Loading
Emotional value	0.9305	0.7303	-0.9494	-0.3185
Functional value	0.2895	0.6841	1.0048	0.4067
Conditional value	-0.5284	-0.1486	-0.4808	-0.3402
Epstemic value	-0.4013	-0.1362	0.3185	0.1135
Social value	0.1593	-0.0672	0.3234	0.2785
Percent variance	23.09		9.42	
Redundancy	0.060		0.02	
Canonical correlation (R_C)	0.536			

Table 6. Rotated standardized coefficient and canonical loading for set 1 and set 2

Variable	First Canonical Variate		Second Canonical Variate	
IV = Personal Values	Coefficient	Loading	Coefficient	Loading
Benevolence	-0.0135	0.6935	0.0451	0.2451
Security	1.0443	0.9806	-0.5906	0.0738
Self-Direction	0.0579	0.4441	1.3376	0.866
Achievement	0.0616	0.1398	0.006	0.1275
Hedonism	-0.2606	0.1881	-0.2504	0.5057
DV= Consumption Values	Coefficient	Loading	Coefficient	Loading
Emotional value	0.1659	0.6700	-0.0641	0.3598
Functional value	0.9307	0.9690	0.0091	0.2108
Conditional value	0.0113	0.1914	0.0217	0.4414
Epstemic value	-0.2868	0.0619	1.192	0.9296
Social value	0.0266	0.0976	-0.4487	0.2150

DV= dependent variables, IV= independent variables.

5. Conclusion

The study concluded that the respondents in the study area have free opinions and place much value on actions that would bring about creativity, excitement, and innovations to achieve pleasure and individual joys, thereby maintaining and developing their families' welfare, interpersonal relations, security, harmony, and stability in the society. The results showed that there is a positive significant correlation between personal value and consumption value.

Hence, the hypothesis that the correlation between personal value and consumption value is equal to zero was rejected. The results concluded that respondents are loyal and consistent with what they believe will give them joy and contribute to their welfare. Respondents also are desirous of social security for all in the society. It is also worth to know that respondents in the study area are proud of their achievement such that the higher the respondent's position, the more s/he prefers fresh fish. Based on the findings of this study, the following recommendations are hereby put forward for improving as well as sustaining the marketing of fresh fish in the study area to ensure that consumers get the product they desire in the right form (form utility), in the right place (place utility), at the right price (possession utility), and at the right time (time utility) to fully satisfy the consumer.

Fresh fish marketers in the study area can increase their profits by targeting the packaging of fish in such a way as to excite consumers and increase their demand for their produce. Fresh fish marketers should pay more attention to the price-quality relationship, product's performance and content, and packaging quality, as most respondents place more value on maintaining and developing their families' welfare.

Author Contributions

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

	O.E.M.	S.B.J.	F.A.
C	100		
D	70	20	10
S	60	35	5
DCP	30	60	10
DAI	35	50	15
L	20	30	50
W	45	35	20
CR	30	50	20
SR	20	70	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.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Permissions were obtained from the Rufus Giwa Owo Ethics Committee (protocol code: 2021/27 and date: Februrary 12, 2021).

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SOME PHYSICAL, CHEMICAL, SENSORY PROPERTIES, MINERAL SUBSTANCES, AND HEAVY METAL CONTENTS OF MOLDY CHEESE PRODUCED IN BAYBURT AND THE SURROUNDING AREA

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Abstract: Moldy cheese produced in Bayburt and its surroundings is a mature cheese with a distinctive flavor that can be prepared in three ways. It is made from only Civil cheese, only cottage cheese (çökelek) or mixing the shredded Civil cheese with cottage cheese made from moderately fatty, or non-fat milk, pressing it into appropriate containers, draining the water, and allowing the cheese to organically mold. It is produced traditionally, and there are no production standards. This study was carried out to identify some of the physical, chemical, and sensory properties, mineral substances, and heavy metal contents of moldy cheeses produced and consumed in the region. In the cheese samples (24 pieces), the average dry matter (DM) rate was 51.26%, ash rate was 5.68%, salt rate was 6.21%, fat rate was 4.81%, acidity level was 0.96% and pH value was 5.79, L color value was 74.94, a color value was 0.94, b color value was 8.24. In sensory analyses, the samples scored an average of 5.94 points for color and appearance, 6.10 points in moldiness, 6.11 points for texture, 5.72 points for odor, 6.03 points for taste, 6.30 points for saltiness, and 6.01 points for general acceptability. 29 elements were examined to determine mineral substances and heavy metal concentrations. These findings are important in terms of revealing some general characteristics and heavy metal content of moldy cheese produced in the region.

Keywords: Moldy cheese, Chemical properties, Color values, Mineral substances, Heavy metal content

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1. Introduction

Moldy cheese produced in Bayburt and its region is also produced and consumed in many provinces of Türkiye (Arslaner and Salık, 2020). Moldy Civil Cheese, also known as Göğermiş Cheese, produced in Erzurum, was granted Geographical Indication registration (origin) in 2010, and its production area is limited to the territory defined by the province of Erzurum and its districts (Anonymous, 2010). This cheese is a sort of mature cheese with a unique flavor, obtained by shredding Civil cheese plainly or with curd cheese (Lor), pressing it into plastic bins suitable for food packaging, draining the water, and letting the cheese to molding naturally (Anonymous, 2010).

In the production of moldy cheese produced in Bayburt and its districts, curd cheese is not used, it is made with only Civil cheese or only cottage cheese or by mixing Civil cheese and cottage cheese made from fat and skim milk. The raw materials used in production, production methods, and storage conditions differ by region, and this affects the physical, chemical, microbiological, and sensory properties of the cheese (Arslaner and Salık, 2020).

While milk and dairy products are sources of a variety of minerals, they may occasionally contain pollution-causing chemicals such as heavy metals in varying amounts. In parallel with technological advancements, environmental pollution has significantly increased as a result of numerous industrial activities and increasing road traffic. These heavy metals, which are ingested by animals grazing or feeding in enclosed spaces, pose a risk to the animal's health and can also pass into milk as a result of excretion of the mammary gland (Capcarova et al., 2017). Due to the aforementioned issue or contamination from the metal surfaces of the tools and equipment used during processing, cheese may contain various heavy metals (Tarakci and Kucukoner, 2008; Moreno-Rojas et al., 2010). The risk of metals in the composition of the tools and equipment used in the production of acidic foods such as cheese to dissolve and pass into the product may be easier when compared to other foods (Özlü et al., 2012). Small concentrations of heavy metals are necessary for maintaining health, but larger quantities can be toxic or dangerous (Jaishankar et al., 2014). Because heavy metal levels in cheese have a direct impact on public health, it is desirable to both

control and collect data on these levels.

In this study, the aim was to determine some physical and chemical properties, mineral composition, and heavy metal contents of moldy cheese samples, which are traditionally produced and widely consumed in Bayburt Province.

2. Materials and Methods

2.1. Supply of Cheese Samples

24 moldy cheese samples used in the study were obtained from different sales points operating in Bayburt and its districts. Cheese samples were put into 500 g sterile sample bags and taken to the laboratory through cold chain and kept in the refrigerator at 4 °C until analysis.

2.2. Physical, Chemical, and Sensory Analyses

Total dry matter, ash, acidity (lactic acid%), salt, and fat analyses in moldy cheese samples were performed according to Kurt et al. (1996). pH values were determined by using a combined electrode digital pH-meter (WTW 340-1 brand), while color determination was carried out using a Minolta Colorimeter (CR-200 Minolta Colorimeter, Osaka, Japan) according to Sert et al. (2010). Color measurements of cheese samples were performed on three different sites directly on the sample surface. Sensory analyses were carried out by 10 panelists. In the sensory analysis scorecard, 9-8 means very good, 7-6 refers to good; 5-4-3 refers to average, and 2-1 means bad.

2.3. Determining Mineral Concentrations and Heavy Metal Contents

The mineral composition of cheese samples was determined as per the method suggested by Ataro et al. (2008) with a slight modification. First, the samples were dried in a microwave oven at 70 °C until their dry matter contents reached a stable weight. A sample of approximately 0.5 g of the dried samples was weighed into Teflon containers, and 10 ml of nitric acid (65%)-perchloric acid (70-72%) solution mixed in the ratio of 8:2 by volume was added. After the samples were heated in a microwave oven (Milestone, Ethos Easy) at 200 °C, they were washed with ultrapure water and then put in volumetric containers, and the volume was completed to 25 ml. Following this process, the samples were filtered using a 0.45 µm filter. Then, the mineral matter content of the samples was determined using an Inductively Coupled Plasma Mass Spectrometry (Agilent 7800 ICP-MS).

2.4. Statistical Analyses

The data obtained were analyzed statistically via the SPSS 22.0 package program (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Physical, Chemical and Sensory Analyses

The results of physical and chemical analyses performed on moldy cheese samples are given in Table 1. According to the Geographical Indication Registration Certificate for Moldy Civil Cheese (Göğermiş Cheese), this cheese has dry matter content between 45.00 and 65.00%, a milk fat content between 1.00 and 4.00%, a salt content between 3.00 and 7.00%, a lactic acid acidity percentage between 0.55 and 1.70, and a pH range between 5.1 and 5.6. It can be noted that, excluding fat values, the average values of moldy cheeses produced in Bayburt are compatible with these values. The fat values in our samples are higher, and the reason for the higher fat content in our samples is likely the use of full-fat milk cottage cheese in addition to Civil cheese during production.

Arslan (2020) reported the average salt, pH and acidity values in 27 Moldy Civil Cheese samples collected from Erzurum and its vicinity as 7.34%, 5.25, and 0.48%, respectively. The average salt values of these cheese samples are higher than the values found in our study, and the reason for this difference is thought to be the lack of a standard production technique. Although the pH values were comparable to our findings, the acidity values were determined to be lower. The high salt content of the Moldy Civil Cheeses produced in Erzurum may have prevented the increase in acidity by slowing down the activities of the microbial flora.

Table 1. Results of some physical and chemical analyses performed on moldy cheese samples

	n	Min.	Max.	Mean (x ± Sx)
DM (%)	24	43.68	59.89	51.26±4.87
Ash (%)	24	3.15	8.57	5.68±1.37
Salt (%)	24	3.79	10.29	6.21±1.53
Fat (%)	24	0.80	19.00	4.81±4.68
Acidity	24	0.55	1.43	0.96±0.22
pH	24	4.76	7.38	5.79±0.51
L	24	49.17	87.99	74.94±8.53
a	24	-1.24	3.13	0.94±1.27
b	24	2.00	14.06	8.24±2.86

Cakmakci et al. (2014) produced four different types of Moldy Civil Cheese and allowed them to ripen for 180 days. Throughout the ripening process, chemical and microbiological parameters were measured periodically, and the changes were noted. At the end of the storage period, the total dry matter ratio of the cheese samples was found as 46.97%, fat content as 1.72%, acidity as 0.72%, pH as 6.48, salt content as 7.21%, and ash content as 7.41%. When these results were compared to the average values we obtained, it was seen that the DM, fat and acidity values were lower, while the pH, salt, and ash rates were higher. It is thought that the high salt content of the moldy cheeses produced in Erzurum, the use of curd in production, the different ripening periods of the cheeses or the use of different animal milk are the sources of these differences in composition.

L values found in the color analyses performed on the cheese samples ranged from 49.17 to 87.99; a values from -1.24 to 3.13, and b values from 2.00 to 14.06. There is no study in the literature that has examined and

reported the color values of the moldy cheese produced in Bayburt and the nearby regions.

Table 2 showed the results of sensory analyses conducted on moldy cheese samples. Cheese samples were rated between 3.10-7.10 for color and appearance; 2.80-7.90 for moldiness; 3.60-7.10 for texture; 2.80-7.40 for odor; 3.40-7.40 for taste; and 3.70-7.30 for general acceptability. When sensory analysis results are evaluated along with the relevant physical, chemical, and color analysis results, the sample with 77.88; -0.28; 8.01 L, a, b color values respectively was the most favored sample by the panelists in terms of color and appearance, while the sample with 5.61% salt content was the most favored by the panelists in terms of salt content.

Table 2. Results of sensory analyses performed on moldy cheese samples

	n	Min.	Max.	Mean (x ± Sx)
Color and Appearance	24	3.10	7.10	5.94±0.91
Moldiness	24	2.80	7.90	6.10±1.30
Texture	24	3.60	7.10	6.11±0.90
Odor	24	2.80	7.40	5.72±1.01
Taste	24	3.40	7.40	6.03±0.96
Saltiness	24	5.30	8.00	6.30±0.65
General Acceptability	24	3.70	7.30	6.01±0.90

3.2. Mineral Concentration and Heavy Metal Contents Analysis

Table 3 shows the mineral and heavy metal analysis results of cheese samples. Accordingly, the average Na content of moldy cheese samples is 42094.84±13905.46 mg/kg dry weight. This value is similar to the Na values (14225.25±127.39-91688.18±46.01mg/kg dry weight) determined by Arslaner and Salık (2020) in Civil cheese samples obtained from Bayburt. Many researchers reported that the high Na levels detected in cheese samples are caused by salt content, and the Na level rises as the salt level increases (Demirci, 1988; Sağun et al., 2005; Öksüztepe et al., 2013). Özlü et al. (2012) reported that the amount of Na in ripened Kashar cheese was higher than that of fresh Kashar cheese and stated that this may be due to a proportional increase in moisture loss as the cheese ripens.

K and P were the second and third most often found elements in cheese samples, with average concentrations of 7624.34±1245.68 and 1825.81±657.93 mg/kg dry weight, respectively. These values are within the range of the values determined by Arslaner and Salık (2020) (6403.39±13.04-11397.51±196.79, 755.38±9.05-2056.00±52.36 mg/kg dry weight). P is one of the elements that can be found in the structure of proteins, and thus protein-rich milk and dairy products such as cheese are among the foods rich in phosphorus (Uribarri and Calvo, 2003). Milk and dairy products are an important source of K. In a study conducted in Poland, milk and dairy products were reported to come in fourth place (11.9%) as a source of potassium. This value was 9.6% in the USA, 11.3% in France, and 14.1%–15.5% in Australia (Górska-Warsewicz et al., 2019).

The average Ca and Mg concentrations in cheese samples were determined as 1654.96±560.21, and 296.55±75.60 mg/kg dry weight, respectively. Arslaner and Salık (2020) calculated the amounts of Ca as 772.89±4.24 - 2146.25±11.21 and Mg as 332.56±2.15-1919.05±22.53 µg/kg dry weight. In the cheese-making process, paracasein micelles combine in the presence of Ca ions to generate cheese curd (Metin, 2005). Therefore, cheese is a good source of (especially bioavailable) calcium (Walther et al., 2008; Fox et al, 2017). Calcium content in hard or semi-hard cheeses is 6-11 $g.kg^{-1}$. In softer varieties of cheese, this rate is lower. Hard or semi-hard cheese delivers half or third of the daily necessary calcium intake of 1200 mg in one serving (50 g) (Walther et al., 2008). Compared to cheeses curdled with acid, cheeses curdled with rennin have increased calcium content (Demirci, 1990). In addition to calcium, milk is a rich source of Mg (Walther et al., 2008).

The average Si and Zn contents of the cheese samples were determined as 72.92±27.69 and 46.98±16.08 mg/kg dry weight, respectively. When compared to the values found by Arslaner and Salık (2020), the amount of Si was found to be within the range reported by them (TE-132243.58±88.68 mg/kg dry weight), while the amount of Zn was found to be lower (49949.43±84.82-94558.05±80.33 µg/kg dry weight). Zn is one of the most important essential trace minerals in human nutrition, and its deficiency is a worldwide nutritional problem. The Recommended Dietary Allowance for Zn in the US is 15 mg/day (Gascho, 2001; Gulbas and Saldamli, 2005; Snyder, Matichenkov and Datnoff, 2016).

The average concentrations of Al, Fe, and Sr were found to be 22.54±19.85, 15.66±8.05, and 8.11±3.37 mg/kg dry weight, respectively, in the cheese samples. These values were found to be similar to the values determined by Arslaner and Salık (2020) (TE-35487.79±45.51, TE-31113.0±86.56, 4810.50±10.79-36405.24±280.34 µg/kg dry weight). Al is a non-essential metal that humans are frequently exposed to (Soni et al., 2001). The majority of Al in the diet results from the use of food additives (such as baking soda, coloring agents, anti-caking agents, acidifying agents, stabilizers, thickening agents, bleaching agents, and emulsifiers) during processing, in particular. Al levels in processed cheese wrapped in aluminum foil have been found to be significantly higher than in cheese packaged in glass containers (AI-Ashmawy, 2011).

Table 3. Mineral substance and heavy metal contents of moldy cheese samples (mg/kg dry weight)

Minerals and heavy metals		n	Min.	Max.	Mean (x ± Sx)
Na	Sodium	24	18794.61	73068.09	42094.84±13905.46
P	Phosphorus	24	5627.27	10220.34	7624.34±1245.68
K	Potassium	24	1119.54	3989.18	1825.81±657.93
Ca	Calcium	24	910.93	3000.17	1654.96±560.21
Mg	Magnesium	24	206.90	452.18	296.55±75.60
Si	Silicium	24	31.82	133.45	72.92±27.69
Zn	Zinc	24	28.50	91.01	46.98±16.08
Al	Aluminum	24	ND	71.55	22.54±19.85
Fe	Iron	24	4.17	35.62	15.66±8.05
Sr	Strontium	24	3.61	14.70	8.11±3.37
Ti	Titanium	24	0.55	114.63	8.03±25.31
Sn	Stannum	24	2.80	30.96	7.18±6.28
Cu	Copper	24	0.80	12.27	2.16±2.59
Ba	Barium	24	0.67	4.43	1.69±0.88
B	Boron	24	ND	8.88	0.86±2.03
Mn	Manganese	24	0.35	1.45	0.72±0.25
Se	Selenium	24	0.09	0.44	0.23±0.10
Pb	Lead	24	0.05	1.66	0.22±0.34
Mo	Molybdenum	24	0.04	0.29	0.15±0.06
Ni	Nickel	24	ND	0.46	0.12±0.13
Cr	Chromium	24	0.03	0.27	0.09±0.05
V	Vanadium	24	ND	0.04	0.01±0.01
In	Indium	24	ND	0.28	0.06±0.09
Ag	Silver	24	ND	0.06	0.01±0.01
Co	Cobalt	24	ND	0.03	0.01±0.01
Ga	Gallium	24	ND	0.02	0.01±0.01
Cd	Cadmium	24	ND	0.01	ND
As	Arsenic	24	ND	0.01	ND
Hg	Mercury	24	ND	ND	ND

ND= not detected

Dairy products are a great source of calcium and protein, but they are poor in iron (Zhang and Mahoney, 1989; Jalili, 2016). As a result, numerous studies have been carried out on iron fortification in dairy products, and it has been found that enriching cheese with 80 $mg.kg^{-1}$ microencapsulated iron and 150 $mg.kg^{-1}$ L-ascorbic acid is possible without causing any unpleasant flavors (Jalili, 2016). According to the most recent research, Sr, one of the trace elements frequently found in the earth's crust, is harmful to humans, plants, and animals (Anke et al., 2008).

In cheese samples, the average concentrations of Ti, Sn, and Cu were found to be 8.03±25.31, 7.18±6.28, and 2.16±2.59 mg/kg dry weight, respectively. In Codex (1998), the Temporary Tolerable Intake amount is expressed as 14 mg/kg/week for Sn and 0.5 mg/kg/day for Cu. İşleyici et al. (2017) found the average amount of Sn in Divle Tulum Cheese samples as 0.01 mg/kg, and Öztürk, Kaptan and Şimşek (2012) determined it as 0.0366 ppm in Kashar cheese. Arslaner and Salık (2020) determined the Cu rate in Civil cheese as TE-891.60±3.01 µg/kg dry weight. It is thought that the differences in the results are due to the equipment used in cheese making being made of different materials or the mineral composition of the milk (Yüzbaşı et al., 2003; Lucas et al., 2006; Stergiadiset al., 2021).

The average concentrations of Ba, B, and Mn in cheese samples were determined as 1.69±0.88, 0.86±2.03, 0.72±0.25 mg/kg dry weight, respectively. Arslaner and Salık (2020) determined Ba and Mn rates as 1226.05±2.32-4951.59±37.55 and 34.15±0.42-1077.14±38.41 µg/kg dry weight, respectively. The results we obtained are similar to the current study.

Simsek et al. (2003) determined the average B ratios of White Cheese, Tulum Cheese and Urfa cheese as 0.61, 1.84 and 1.12 mg/kg, respectively. Our results are similar to these values. B is a dynamic trace element that can affect the metabolism or utilization of many other substances involved in life processes, and the lowest value given for the safe intake range is 1.0 mg B/day (Berger and Truog, 1939).

Kodrik et al. (2011) examined the impact of road traffic on the heavy metal content of cow milk and cheese and they found that there was no significant difference in the Mn concentrations between cheese samples made from milk obtained from locations near and far from highways. Se, Pb, Mo ratios of cheese samples was determined as 0.23±0.10, 0.22±0.34, 0.15±0.06 mg/kg dry weight, respectively. Arslaner and Salık (2020) determined Se and Pb values as TE-723.57±7.00 and TE-181.81±5.19

µg/kg dry weight. Our findings appear to be consistent with this study. Selenium is a trace mineral essential for human life. The Recommended Dietary Allowance for Se in the US is 50 µg/day (Gulbas and Saldamli, 2005).

The Temporary Tolerable Intake amount for Pb was listed as 25 mcg/kg bw per week in Codex (1998). Pb has reportedly been linked to numerous child fatalities in Zambia and Nigeria. Additionally, Pb is associated with a decline in intellectual performance and cognitive development (Dai, et al., 2023). Molybdenum is a cofactor (molybdopterin) that stimulates oxidation and reduction reactions for some enzymes and is an essential trace element. The US Institute of Medicine (IOM, 2001) reported the average daily molybdenum intake for adults as 22 µg/day (EFSA, 2013).

The concentrations of Ni, Cr, and V in cheese samples were determined as 0.12±0.13, 0.09±0.05, and 0.09±0.01 mg/kg dry weight. Arslaner and Salık (2020) determined Cr values as TE-1203.44±62.83 µg/kg dry weight. In the study by Orak, Altun, and Ercag (2005), the Ni concentration of White Cheese samples was found to be 1.057±0.209 (0.654-1.518) µg/g, and it was noted that this value was greater than the values in the literature. Different equipment made from nickel alloy is used in the dairy industry. According to Orak, Altun, and Ercag (2005), the low quality of these alloys and the salt used in brine and other pollution sources may cause an increase in the Ni content in cheese.

As a metal present everywhere in nature, Cr influences the insulin function of the body, which in turn impacts how carbohydrates and proteins are metabolized. The typical daily consumption of Cr ranges from 30 to 200 g, and small amounts of Cr are present in milk and dairy products. The Turkish Food Codex Contaminants Regulation does not place any restrictions on the quantity of Cr that can be found in foods (Kahvecioğlu et al., 2003; Orak, Altun and Ercag, 2005).

According to Kodrik et al. (2011), the quantity of V in milk increased in locations with heavy traffic compared to rural areas, but the amount of V in cheese remained the same. There is no significant evidence to establish that V is an essential component of the human diet (Imtiaz et al., 2015).

The average concentrations of In, Ag, and Co were found to be 0.06±0.09, 8.51±0.01, and 7.00±0.01 mg/kg dry weight, respectively, in cheese samples. These rates were calculated by Arslaner and Salık (2020) to be TE-7040.92±10.30, TE-376.63±2.80 and TE-28.64±0.35 µg/kg, respectively.

The maximum Ga, Cd and As rates in cheese samples were determined as 0.02, 0.01, and 0.01 mg/kg dry weight, respectively. It was observed that Hg levels were below the detectable limit in all cheese samples. Arslaner and Salık (2020) determined the Cd rate as TE-979.69±1.41 µg/kg dry weight and the As rate as TE-TE. Heavy metals such as Cd, As and Hg have no physiological function for living organisms. These metals are characterized by bioaccumulation and biomagnification properties and reach the human body through the ingestion of contaminated food and water, leading to various toxicological consequences (Dai, et al., 2023).

5. Conclusion

Moldy cheese is one of the most produced and consumed cheese types in Bayburt and its region. It is produced traditionally, and there are no production standards. Production techniques may differ from similar cheeses produced in the region. This study is important in terms of providing information about various physical, chemical and sensory properties, heavy metal and mineral composition of moldy cheeses produced in Bayburt and its region, and can serve as a source for future studies. The results we obtained can contribute to the development of a standard production with a quality appreciated by consumers.

Author Contributions

The percentage of the author contributions is presented below. The author reviewed and approved the 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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Research Article
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DGAT1 GENE POLYMORPHISM IN MORKARAMAN AND TUSHIN

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Abstract: This study aims to investigate the polymorphism of the Diacylglycerol acyltransferase1 (DGAT1) gene locus in 105 Morkaraman and 65 Tushin lambs to determine the distribution of genotype and allele frequencies of lambs in terms of related genes. DGAT1/Alu1 gene polymorphism was defined by using the PCR-RFLP method in the DNAs isolated from hair samples taken from Morkaraman and Tushin lambs used in this study. PCR- RFLP products were run in an electrophoresis medium and the results were visualized on an ultraviolet (UV) transluminator. When the population was examined in terms of allele frequencies, it was defined that the C allele and the T allele were 0.72% and 0.28% for the Morkaraman, and 0.71% and 0.29% for Tushin, respectively. The CC, CT, and TT genotype frequencies of the DGAT1 gene in the population were found to be 53.3%, 38.1%, and 8.6% for the Morkaraman and 50.8%, 40.0%, and 9.2% for the Tushin, respectively. In the Hardy-Weinberg genetic equilibrium test, it was observed that the distribution of genotype frequencies was in balance (P>0.05) in the population. It has been defined that the genotype and allele frequencies determined in terms of DGAT1 gene polymorphism may be found to be sufficient to reveal the genotype diversity of the breeds. The genotype and allele frequencies determined in terms of DGAT1 gene polymorphism were sufficient to reveal the genotype diversity of the breed, the sheep with CC genotype are economically advantageous in the herd, and therefore DGAT1 gene can be used for marker-assisted selection (MAS).

Keywords: Polymorphism, DGAT1 gene, PCR-RFLP method, Sheep

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1. Introduction

The most valuable product in human nutrition is products of animal origin. Animal proteins obtained from these products, which have a great contribution to the healthy and balanced diet of humans, can't be replaced by a different agricultural product (Gürer and Ören, 2013). Animal products are very important in the adequate and balanced nutrition of humans. In a healthy diet, nutrients such as carbohydrates, proteins and fats, which are the basic needs of the body, should be consumed in proportion. 40% of the amount of protein consumed by humans should be of animal origin (Turhan et al., 2010).

Today, the level of awareness of the need for animal products is taken into account as a development indicator of countries. The reason for this is that animal protein foods such as meat, milk and eggs are of great importance in human nutrition. In parallel with the economic and social development in developing countries, it is seen that the consumption amounts of animal products are increasing day by day with the change in consumption patterns (Yılmaz and Yılmaz, 2012).

The two basic methods to increase productivity in sheep breeding are to provide good environmental conditions for sheep and to increase their genetic value, in other words, to improve the genotype. Improving the genotype is important in animal breeding because it is permanent and continuous (Sönmez et al., 2009).

Quantitative genetic studies conducted on sheep show that some genetic factors are effective in the birth weight of lambs. It is reported that the heritability of lamb birth weight in various sheep breeds is between 0.15 and 0.24 (Safari, et al., 2005). The application of genomic selection in sheep is a successful approach to improve the studied trait since the heritability of the yield traits of these animals has been determined in the studies on this subject. Therefore, the application of genomic selection is considered a successful approach for improving the studied traits. For example, despite the small impact on livestock breeding, some candidate genes are reported to help improve some polygenic traits, such as growth, to help accurately predict the genetic value of different livestock species, including sheep (Dekkers, 2004; Ranjbari et al., 2012).

Thanks to the molecular genetic technologies developed in farm animals, markers, which are markers used at the molecular level to define the genetic structure that affects yield, provided an advantage for the determination, identification and conservation programs of populations that can be used as genetic resources (Öner et al., 2011). It has been stated in the studies that the rate of genetic progress in livestock with MAS can increase by around

15-30% (Bal and Akyüz, 2014). The genetic progress aimed to be achieved in the desired breed with MAS is faster than classical selection methods (Reis et al., 2001).

Molecular genetics techniques allow the identification of relationships between yield traits and diversity in the Quantitative Trait (QTL), and the identification of genetic variation at various loci. Selection aims to estimate the genetic value of the animal with greater accuracy and thus increase the genetic gain resulting from the selection. It has been reported in studies that variations in genes that affect physiological events related to the phenotype may be effective on quantitative variations in the related phenotype (Tambasco et al., 2003).

Phenotypic traits and polymorphisms in marker genes are also used in the characterization of races. In addition, genetic polymorphisms in candidate genes have been an important research topic in genetic selection and specifying evolutionary relationships between different races. One of these genes is the Diacylglycerol acyltransferase1 (DGAT1) gene (Bal and Akyüz, 2014).

DGAT1 is a microsomal enzyme involved in the synthesis of triglycerides in adipocytes (Winter et al., 2002). DGAT1 also plays a fundamental role in intestinal fat absorption, lipoprotein assembly, regulation of plasma triacylglycerol concentrations, fat storage in adipocytes, energy metabolism in muscles, and milk production, including mammalian oocytes. It catalyzes the terminal and only stable step in the synthesis of triacylglycerol by using diacylglycerol and fatty acyl-coenzyme A as substrates. Acyl CoA catalyzes the terminal and only stable step in the synthesis of triacylglycerol using diacylglycerol and fatty acyl-coenzyme A as substrates. (Cases et al., 1998). The DGAT1 gene encoding this enzyme is found in many tissues. However, it is predominantly found in adipose tissue and the small intestine (Buhman et al., 2002). Studies have shown that there is a relationship between DGAT1 gene and fat accumulation in sheep and cattle carcasses. The DGAT1 gene is a putative alternative gene for milk fat content in sheep (Curi et al., 2011; Mohammadi et al. 2013). However, studies investigating the relationship between SNPs in the DGAT1 gene and mutton productivity are scarce. In one of the studies conducted with the Mogan Iranian sheep breed, it was reported that there is a relation between the polymorphism in exon 7 of the DGAT1 gene and the carcass weight (Noshahr and Rafat, 2014). DGAT1 is a candidate gene due to its important role in fat metabolism, milk fat content and carcass characteristics in dairy sheep and goats (Sadeghi et al., 2020).

Morkaraman breed is generally raised in many provinces of Türkiye, especially in Erzurum, Kars, Ağrı, Muş and Van provinces located in the Eastern Anatolia Region. In the environmental conditions of the regions where it is grown, the race has characteristics such as being well-adapted, walking long distances, resistance, and high viability. Morkaraman comes after Akkaraman sheep breed in terms of breeding density in Türkiye. The average birth weight of lambs was 3-4 kg, average live weight of lambs weaned in 90 days was 20 kg. While the average mature live weight is between 50 and 60 kg in rams, it was between 40 and 60 kg in sheep. Morkaraman lambs were found to have an average daily live weight gain of 200 gr, an average hot carcass weight of 21 kg and an average hot carcass yield of 49% under the current pasture conditions in the region (Köprücü, 1975; Geliyi and İlaslan, 1978; Ulusan and Aksoy, 1996; Macit and Aksoy, 1996; Esenbuğa et al., 1998).

Tushin sheep is a breed that is mostly bred in northeastern Türkiye in Kars (Çıldır district), Ardahan and Iğdır provinces. This breed is usually small in size, the body fleece is bright and white. It is known as a breed that makes good use of pastures because it can be cultivated in regions with mountainous, high altitude and rough terrain conditions. The average birth weight of lambs in this breed is 3.7 kg, 18-month live weight is between 45 and 50 kg, daily live weight gain is 190 g and carcass weight is around 20 kg (Anonymous, 2009).

This study aims to investigate the presence of polymorphism in terms of DGAT1 gene locus using PCR-RFLP methods in Morkaraman and Tushin sheep raised on the mentioned farm and to reveal the distribution of genotypes and allele frequencies of sheep in terms of this gene locus.

2. Materials and Methods

2.1. Materials

The hair samples were taken from 105 Morkaraman breed and 65 Tushin breed sheep and genomic DNA was obtained from them, which were raised at Atatürk University, Food and Livestock Application and Research Center, Sheep Breeding Branch. Birth weights were taken within the first 24 hours of birth and ear-number earings were attached to the ears of each animal. Birth and weaning weights were measured with a 100 g precision scale. Depending on the pasture conditions, the average age of the lambs on the day they went out to graze was calculated as 49 days for Morkaraman and 60 days for Tushin.

Hair samples were taken from the sheep and taken into 10 ml Eppendorf tubes, the label numbers were recorded on the tubes, and the samples were transported to the Genetics Laboratory of the Department of Animal Science, Faculty of Agriculture, Atatürk University, with sample carrying bags containing.

2.2. Methods

Genomic DNA isolation was conducted using a commercial DNA isolation kit (Purgene DNA kit, Gentra Systems, Minnesota). The qualitative and quantitative controls of the obtained DNAs were determined by using the NanoDrop ND-1000 (NanoDrop Technologies Inch.) spectrophotometric methods.

In the PCR, the 309 bp DNA region was amplified using primers F: 5'-GCA TGT TCC GCC TCC TGG-3' and R: 5'-GGA GTC CAA CAC CCC TGA-3'. For PCR, 3 µl of genomic DNA samples were taken into 0.2ml tubes, and 3.75 µl of

10x Buffer, 1 µl of Primer R, 1 µl of Primer F, 1 µl of MgCl2, 0.5 µl of DNTPS, 2.4 and 20 µl of the 12.5 µl dH2O mixture was added and centrifuged. Afterwards, the tubes were placed in the PCR device and the PCR program was applied. The PCR program was set to 35 cycles with an initial denaturation at 96 °C for 5 minutes, denaturation at 96ºC for 50 seconds, bonding at 58ºC for 50 seconds, elongation at 72 °C for 1 minute, and final elongation at 72 °C for 5 minutes.

10 µl of each amplified DGAT1 PCR product was taken and placed in 0.2 ml sterile Eppendorf tubes, and 5 µl of Alu1 enzyme, 5 µl of Buffer R and 2.4 µl of Buffer Tango were added. Then, it was centrifuged by covering it with approximately 5-10 µl of mineral oil. Incubation was carried out at 37 °C for 12 hours. The incubated products were carried out on a 2% agarose gel at 45 volts for 90 minutes and electrophoresis was applied. After the electrophoresis, the gel was taken and examined under UV light for genotyping.

2.3. Statistics Analyses

Whether the DGAT1 genotype frequencies are in Hardy-Weinberg equilibrium was investigated by the Chi-square test. A correlation study was conducted using the birth and weaning weight records of Morkaraman (105) and Tushin (65) sheep raised at Atatürk University Food and Livestock Application and Research Center Farm. The data obtained from the records of these breeds were analyzed for variance separately for each breed, and the SPSS 20.0 statistical package program was used for these analyses.

3. Results and Discussion

This section may be divided into subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

3.1. Observation of PCR Results

Each of the DNA samples obtained from Morkaraman and Tushin sheep hairs was PCR performed and run on a 1% agarose gel and DNA bands were obtained. The agarose gel image of the PCR products under UV light is shown in Figure 1.

3.2. PCR-RFLP Results

DNA samples obtained from Morkaraman and Tushin breed sheep were amplified in PCR device and cut with Alu1 Restriction Endonuclease enzyme and DGAT1 gene polymorphic regions were determined. In theory; It gives bands of CC: 309 bp, CT: 309/272/37 bp and TT: 272/37 bp in length. An exemplary agarose gel image of the PCR-RFLP results under UV light is presented in Figure 2.

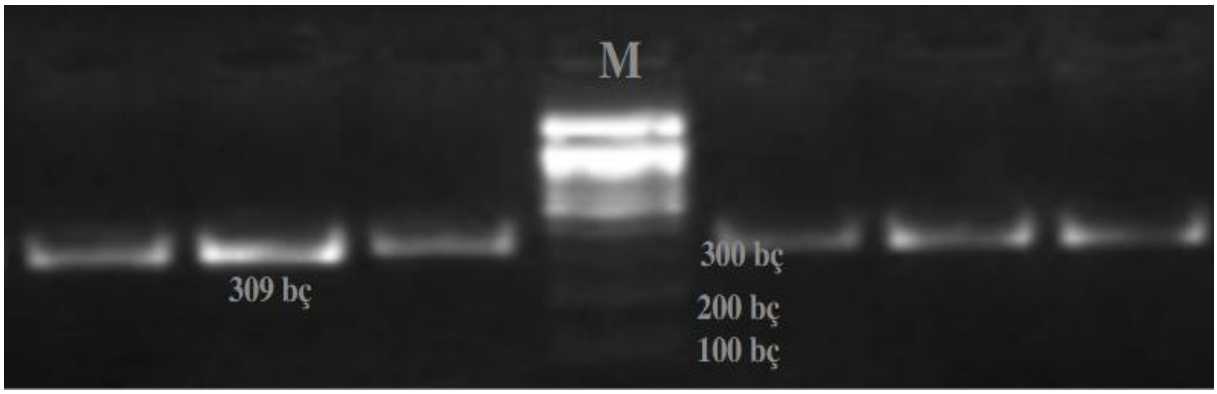

Figure 1. DGAT1 gene PCR products treated with ethidium bromide 1.5% agarose gel DNA band views (M: Marker, Other lines amplification products: 309 bp).

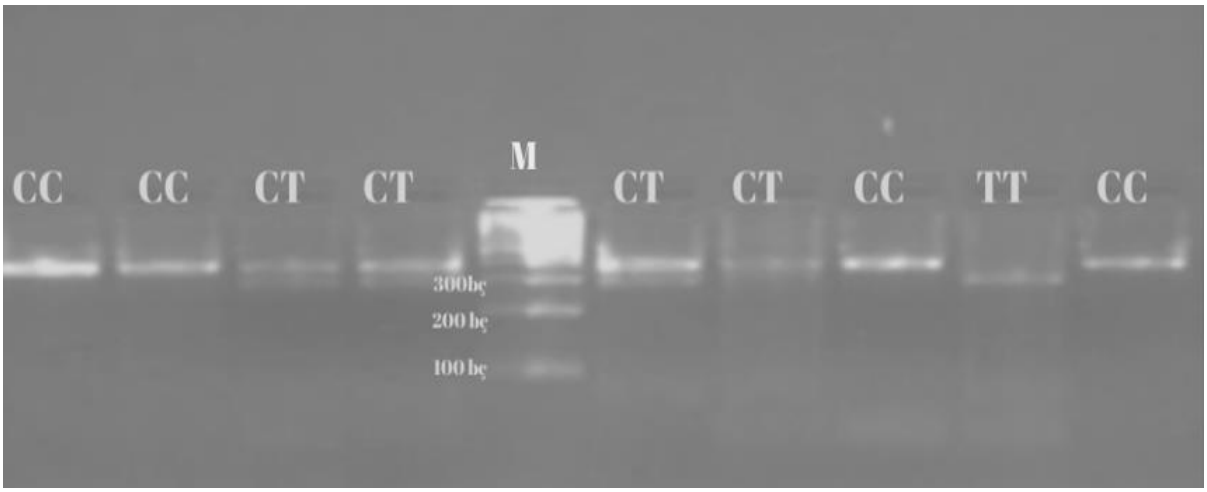

Figure 2. Electrophoretogram of *Alu*I digested PCR product generated by amplification of genomic DNA, using DGAT-specific primers. Lane: M: 100-bp DNA ladder; Lanes 1.5: CT, Lines 2.4: CC, Line 3: TT.

3.3. Genotype Frequencies and Genetic Equilibrium Test Results

According to the Hardy-Weinberg genetic equilibrium test, the distribution of genotype frequencies belonging to Morkaraman and Tushin sheep was found to be in equilibrium (P>0.05) in the distribution of genotype frequencies belonging to 105-head Morkaraman and 65-head Tushin sheep.

The Hardy-Weinberg genetic equilibrium test results of the breed DGAT1 gene Alu-1 polymorphism are presented in Table 1.

In the study, 3 different genotypes belonging to the Alu1 enzyme cutting region on the DGAT1 gene polymorphic region were defined as TT, TC and CC. Detected genotypes and allele gene frequencies are presented in Table 2.

When the population was examined in terms of allele frequencies, it was determined that the C allele was 76 (72%) and the T allele was 29 (28%) in Morkaraman sheep, while the C allele was 46 (71%) and the T allele was 19 (29%) in the Tushin sheep breed (Table 2). It was observed that the C allele was at a higher frequency than the other allele in both breeds.

Animals with 56 CC genotypes, 40 CT genotypes and 9 TT genotypes were determined in the Morkaraman sheep breed, 33 CC genotypes, 26 CT genotypes and 6 TT genotypes were determined in Tushin sheep breed. The CC, CT and TT genotype frequencies were obtained as 53.3%, 38.1% and 8.6% in Morkaraman sheep, 50.8%, 40% and 9.2% in Tushin sheep, respectively. It was observed that the CC genotype frequencies were the highest in the population in both breeds, while the TT genotypes had the lowest frequency.

Unlike this study, the result that the DGAT1 gene is polymorphic with two genotypes, CC and CT, obtained from other similar studies on DGAT1 gene with Barki, Rahmani and Osseimi (Mahrous et al., 2015), Deccani, Mandya and Ganjam (Kumar et al., 2016), Akkaraman (Bayram et al., 2019), Barki, Najdi and Harri (Altwaty et al., 2020) sheep breeds was determined differently from this study, and when the frequencies of these genotypes were taken into consideration, it was determined that the frequency of the CC genotype had the highest value compared to the frequencies of other genotypes in both breeds in this study as in other studies.

Among the previous studies on the DGAT1 gene with different sheep breeds, Barki, Rahmani and Osseimi sheep breeds (Mahrous et al., 2015), Deccani, Mandya and Ganjam sheep breeds (Kumar et al., 2016), Akkaraman sheep breed (Bayram et al., 2019), Barki, Najdi and Harri sheep breeds (Altwaty et al., 2020) were found to be polymorphic with two genotypes, CC and CT, unlike this study, and when the frequencies of these genotypes were considered, it was observed that the frequency of the CC genotype had the highest value, which was consistent with the results of the study. Among the studies conducted with other sheep breeds, Tan, Ganjia, Oula and Qiaoke sheep breeds (Yang et al., 2011), Moghani sheep breed (Noshahr and Rafat, 2014), Mehraban sheep breed (Sajad et al., 2014), Turcana breed (Tăbăran et al., 2014), Jaisalmeri, Muzzafarnagri, Nali, Nellore and Magra sheep breeds (Kumar et al., 2016), Malpura sheep breed (Meena et al., 2016), Jaisalmeri, Muzaffarnagri, Nali, Nellore and Magra sheep breeds (Kumar et al., 2016), Lori sheep breed (Nanekarani et al., 2016), Egyptian Barki sheep breed (Abousliman et al., 2020), and Awassi sheep breed (Bayraktar and Shoshin, 2021) were found to be polymorphic with three genotypes (CC, CT and TT), which was consistent with this study. At the same time, in these studies indicating DGAT1 gene polymorphism, it was reported that the CC genotype was at a higher frequency than the CT and TT genotypes, and it was found to be similar to the findings of this study.

The C allele gene frequency was found to be higher than the T allele gene frequency in both breeds. This result is consistent with the results published by Yang et al., 2011; Ala Noshahr and Rafat, 2014; Tăbăran et al., 2014; Mahrous et al., 2015; Meena et al., 2016; Kumar et al., 2016; Nanekarani et al., 2016; Bayram et al., 2019; Altwaty et al., 2020; Abousoliman et al., 2020; Bayraktar and Shoshin, 2021. However, in some other studies (Xu et al., 2008; Mohammadi et al., 2013; Noshahr and Rafat, 2014; Özmen and Kul, 2016), contrary to this study, T allele gene frequency was found to be higher than the C allele gene frequency.

Table 1. Genotype frequencies and Hardy-Weinberg genetic equilibrium test results

	N	Observed			Expected			χ^2	P
		CC (%)	CT (%)	TT (%)	CC (%)	CT (%)	TT (%)		
Morkaraman	105	56 (53.3)	40 (38.1)	9 (8.6)	55	42	8	0.234	0.629
Tushin	65	33 (50.8)	26 (40.0)	6 (9.2)	32.5	26.9	5.6	0.072	0.789

Table 2. DGAT1 Allele gene frequencies of Morkaraman and Tushin

Allel Gene Frequency (%)	Morkaraman		Tushin	
	C	T	C	T
	76(%72)	29(%28)	46(%71)	19(%29)

5. Conclusion

Thanks to molecular techniques such as PCR and RFLP based on DNA, provide the opportunity to determine genotypes more easily and accurately at very early ages, regardless of gender. These techniques are used as a tool for the early identification of animals and the association between genotypes and performance traits. In addition to polymorphism studies, studies on associating polymorphism with various performance characteristics should also be conducted. It is thought that working with different breeds and larger populations of these breeds in demonstrating the usability of the polymorphism determined by these studies in animal breeding may reveal new possibilities in the definition and development of animal husbandry in Türkiye.

Author Contributions

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

	İ.A.	S.K.
C	50	50
D	40	60
S	30	70
DCP	80	20
DAI	20	80
L	80	20
W	20	80
CR	20	80
SR		100
PM	20	80
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 experimental procedures were approved by the Ethics Committee of the Faculty of Agriculture of Atatürk University, (protocol code: 2023/05 and date: May 15, 2023).

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NUTRIENT CONTENT, ANTIOXIDANT CAPACITY, AND FATTY ACIDS PROFILE OF CHERRY LAUREL (*Laurocerasus officinalis* Roemer) UNSHELLED KERNEL TO BE USED IN POULTRY NUTRITION

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Abstract: This study aims to assess the total phenolic and ascorbic acid contents, antioxidant capacity, and fatty acid profile, as well as nutrient content estimation of the cherry laurel (*Laurocerasus officinalis* Roemer) unshelled kernel (CLUK) that is considered to have the potential to improve product quality and general health in poultry nutrition. The CLUK blend obtained from fruit collected to represent cherry laurel produced in Türkiye was dried, unshelled, and ground to pass through a 1-mm sieve. This CLUK blend was analyzed according to the relevant method of each parameter to describe assessment results. The crude protein, ether extract, neutral detergent fiber, and acid detergent fiber contents of the CLUK blend were recorded to be 28.94, 34.55, 26.25, and 36.70%, respectively. The ferric reducing antioxidant power (FRAP), the radical-scavenging potencies such as DPPH (2.2-diphenyl-1-picrylhydrazyl), and ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid) were 139.84, 11.79, and 8.00 µg trolox equivalents mg^{-1}, respectively. A total phenolic of 3.31 mg gallic acid equivalent g^{-1} and ascorbic acid of 1.57% contents was determined for the CLUK blend. The primary fatty acids for the CLUK blend were identified as oleic (66.61%), linoleic (15.61%), and palmitic (11.78%). These results reveal that the studied CLUK blend has the potential for quality, healthy, and eco-friendly poultry production.

Keywords: Hard stone fruit kernel, Radical-scavenging potency, Phenolic content, Fatty acid profile, Antioxidative potential, Feed ingredient and additive

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1. Introduction

Products that undergo industrialisation processes (such as sorting/cleaning, processing, cooking, and packaging) have a great biodiversity and produce non-traditional wastes (leaves, peels, pulp, kernels, etc.) that can be used in animal nutrition (Kathirvelan et al., 2015; Gungor and Erener, 2020). Recently, because fruit residues, including shelled or unshelled kernels, are inexpensive, readily available, and include bioactive molecules, several studies have shifted to such residues as sources of antioxidants (Ibrahim et al., 2017; Jideani et al., 2021). Indeed, stone fruit kernels such as candlenut (Rohaida, 2014), grape (Karabacak et al., 2015), apricot (Orhangazi, 2017), date (Tareen et al., 2017), sour cherry (Gungor and Erener, 2020), and mango (Beriso et al., 2022) kernels have attracted great interest due to their potential for quality, healthy, cost-effective, and eco-friendly poultry production. All these studies have suggested that fruit processing by-products are effective and less expensive natural sources of bioactive compounds that exhibit significant antioxidant and synbiotic properties (Ibrahim et al., 2017; Jideani et al., 2021).

Cherry laurel (*Laurocerasus officinalis* Roemer) belongs to the Rosaceae family and is naturally found in the eastern regions of the Black Sea, Taurus Mountains, Northern and Eastern Marmara (Yildiz et al., 2014). Karabegović et al. (2014) noted that it is native to Asia Minor, Serbia, Bulgaria, Western Europe, Caucasia, Iran, and some Mediterranean countries. It is commonly consumed in fresh form and processed into jam, marmalade, fruit juice, tea, canned, dried, or pickled forms (Islam et al., 2020; Munekata et al., 2022). Kernels released during fresh consumption or food processing of cherry laurel fruits (Ayla et al., 2019) can also considered among the kernels mentioned above (Barasoglu, 2022). However, these kernels, including the Taflan kernel, are sometimes misjudged as well as judged as effective remedies (Munekata et al., 2022) because of the adverse effects of their anti-nutritional factors on humans (Kovačević et al., 2020) and poultry (Hasted et al., 2021).

In several previous investigations, several attempts have been made to evaluate the bioactive compounds, the antioxidant potential and phenolic profile, as well as nutrients of cherry laurel fruit (Kolayli et al., 2003;

Yaylaci-Karahalil and Sahin, 2011; Beyhan et al., 2018; Islam et al., 2020). Such as, there has been increasing awareness about how much the unshelled kernel from Cherry laurel fruit (CLUK) contains the total phenolic and ascorbic acid contents, antioxidant capacity, and fatty acid profile, as well as its nutrients (Barasoglu, 2022). In this context, the study reported herein aims to assess the total phenolic compounds as gallic acid equivalent (GAE) and ascorbic acid contents, antioxidant capacity [ferric reducing antioxidant power, FRAP and radical-scavenging potencies such as DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid)] as trolox equivalent (TE), and fatty acid profile, as well as nutrient content estimation of the CLUK, a plant-based agriculture waste, and, thus, it is functional feed ingredient/additive capacity for poultry sectors.

2. Materials and Methods

Cherry laurel fruits were harvested in August 2021 from the districts of Taflan in Samsun province, Devrek in Zonguldak province, and Adapazarı in Sakarya province of Türkiye. Subsequently, the fruits were packed in an insulated package and transported under a proper cold chain to the Department of Animal Science Laboratory at the Faculty of Agriculture, Eskişehir Osmangazi University. After removing the seeds from the fruits, they were dried in an oven at 50 °C for four days. Then, the peel was removed and ground to a particle size of 1 mm (Figure 1). Thus, the CLUK samples prepared for analysis were a blend that represented cherry laurel produced in Türkiye.

The dry matter (DM, method 930.15), ash (method 942.05), and crude protein (CP, method 976.05) analyses of the CLUK blend were analysed using approved methods (AOAC, 2006). The ether extract (EE) content was determined using an automatic fat extraction (ANKOMXT15 Extractor) system (Seenger et al., 2008). Fibre content, including acid detergent fiber (ADF) and neutral detergent fiber (NDF), was determined following the literature (Van Soest et al. 1991) using the ANKOM A200/220 Fiber Analyzer (ANKOM Technology Corp., Fairport, NY, USA).

To assess total phenolic compounds, an ethanol and distilled water mixture (80:20 v/v) containing 0.1% HCl was acidified and utilized to extract phenolic compounds, followed by determination of total phenolic content using the Folin-Ciocalteu test (Singleton et al., 1999). To accomplish this, 1000 µl of sample extract was mixed with 500 µl of Folin-Ciocalteu and 250 µl of 20% sodium carbonate, and the final volume was adjusted to 10 ml with distilled water. The mixtures obtained were kept in a dark environment at room temperature for 30 minutes, and their absorbances were measured at 760 nm. Using gallic acid, a standard curve was obtained, and the results were computed as mg GAE g^{-1} dry weight.

Antioxidant activity was assessed using three different *in vitro* antioxidant assays. The results for all three tests were expressed in µg TE mg^{-1} dry weight. DPPH free radical scavenging activity was conducted following the method outlined by Brand Williams et al. (1995), with some minor adaptations. Specifically, 100 µl of the sample extract was transferred to a test tube and treated with 2900 µl of a 0.1 mM DPPH solution. The mixtures were agitated and incubated at 30 ºC for 30 minutes. Subsequently, the absorbance measurements were taken against the control at 517 nm. The free radical scavenging activity of ABTS was carried out according to the protocol of Re et al. (1999). Initially, a solution of ABTS stock (7 mM) containing potassium persulfate (2.45 mM) was left in the dark for 12 h to facilitate radical cation formation and produce the ABTS test solution. The ABTS test solution was diluted with ethanol until the absorbance reached 0.700±0.02 at 734 nm.

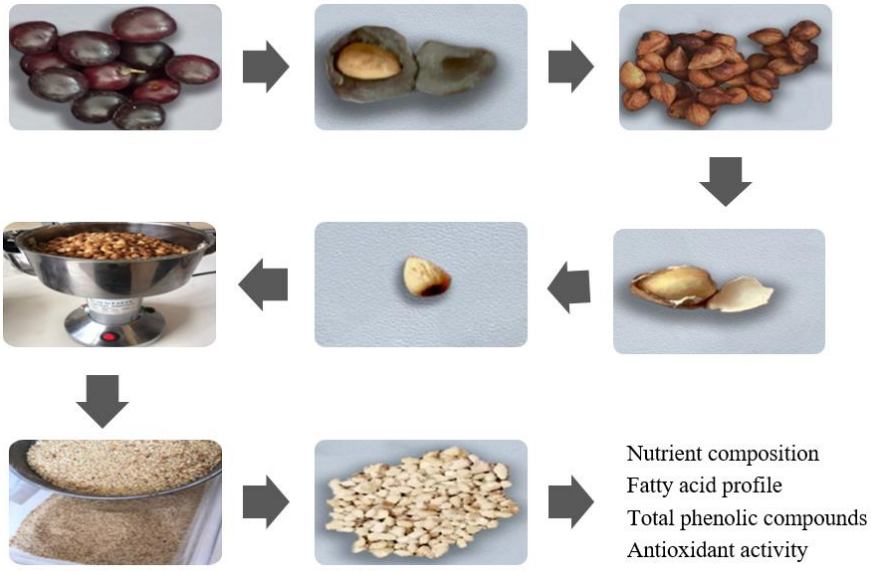

Figure 1. Flowchart summarising the steps involved in the preparation of cherry laurel (*Laurocerasus officinalis* Roemer) unshelled kernel for analyses.

The sample extract (50 µl) and the ABTS test solution (2950 µl) were diluted accordingly and mixed in a test tube. Following a 6-minute incubation period, absorbance readings were carried out at 734 nm. FRAP analysis was conducted using the method outlined by Benzie and Strain (1996) with some adjustments. The FRAP reagent was prepared by combining solutions of 2,4,6-Tris(2-pyridyl)-s-triazine (10 mmol/L), $FeCl_3.6H_2O$ (20 mmol/L), and acetate buffer (0.3 mol/L, pH 3.6) in the appropriate ratios (1:1:10). The absorbance of a mixture containing 100 µl of sample extract, 900 µl of distilled water, and 2000 µl of FRAP reagent was measured using a spectrophotometer at a wavelength of 593 nm. The measurement was taken after a 4-minute incubation period at 37 °C.

To determine the ascorbic acid content, the spectrophotometric method, based on decolorizing the 2,6 dichlorophenol indophenol through ascorbic acid. The excess dye extracted with xylene was measured using a spectrophotometer set at a wavelength of 500 nm. The ascorbic acid content was then computed utilizing a calibration curve that was established with ascorbic acid solutions of varying concentrations (0-25 mg L^{-1}), according to Cemeroglu (2010).

The fatty acid profile was analyzed, as reported by Folch et al. (1957), utilizing the Supelco™-2380 capillary column with helium gas as carrier gas with a flow rate of 1ml/min. Chromatograms of all substances leaving the gas chromatograph column were obtained using the GC device. These were then compared with the chromatograms acquired from fatty acid standards to make qualitative and quantitative determinations (by TS EN 14214 or ISO 5508).

The measurements were undertaken in duplicates and outlined as the average value of the individual measurements.

3. Results

The crude protein, ether extract, neutral detergent fiber, and acid detergent fiber contents of the CLUK blend were recorded to be 28.94, 34.55, 26.25, and 36.70%, respectively (Table 1). The FRAP, DPPH, and ABTS were 139.84, 11.79, and 8.00 µg trolox equivalents mg^{-1}, respectively (Table 2). A total phenolic of 3.31 mg gallic acid equivalent g^{-1} and ascorbic acid of 1.57% contents was determined for the CLUK blend. As shown in Table 3, the primary fatty acids for the CLUK blend were identified as oleic (66.61%), linoleic (15.61%), and palmitic (11.78%).

Table 1. Nutrient content of cherry laurel (*Laurocerasus officinalis* Roemer) unshelled kernel

Nutrient	%
Dry matter	95.22
Ash	3.60
Crude protein	28.94
Ether extract	34.55
Acid detergent fibre	26.25
Neutral detergent fibre	36.70

Table 2. Total phenolic content, antioxidant activity, and ascorbic acid of cherry laurel (*Laurocerasus officinalis* Roemer) unshelled kernel

Bioactive component	Quantity
Total phenolic content, mg GAE/g^{-1}	3.31
Antioxidant activity, µg TE/mg^{-1}	
DPPH radical scavenging	11.79
ABTS radical scavenging	8.00
Ferric reducing antioxidant power (FRAP)	139.84
Ascorbic acid, %	1.57

DPPH= 2,2-diphenyl-1-picrylhydrazyl; ABTS= 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); FRAP= fluoride-resistant acid phosphatase.

Table 3. Fatty acids profile of cherry laurel (*Laurocerasus officinalis* Roemer) unshelled kernel

Fatty acid (FA)	g/100 g FA
Palmitic acid (C16:0)	11.78
Palmitoleic acid (C16:1)	2.60
Heptadecenoic acid (C17:1)	0.09
Stearic acid (C18:0)	2.54
Oleic acid (C18:1)	66.61
Linoleic acid (C18:2)	15.61
Alpha-linolenic acid (C18:3, omega-3)	nd
Arachidic acid (C20:0)	0.48
Eicosenoic acid (C20:1)	0.19
Eicosatrienoic acid (C20:3, omega-3)	nd
Linolenic acid (C20:3, omega-6)	0.06
Lignoceric acid (C24:0)	0.05
Saturated fatty acids	14.85
Monounsaturated fatty acids	69.49
Polyunsaturated fatty acids	15.67

nd= not detected.

4. Discussion

One of the feeding strategies for sustainable animal production is the utilisation of alternative resources and wastes. For this purpose, scientific research focuses on the waste products generated in crop production and discusses the usability of these natural products in animal nutrition. The nutrient and phytochemical composition of fruit and agricultural waste is crucial for animal health and overall performance. Bioactive and therapeutic compounds have properties that significantly enhance the health of livestock and poultry. Phytochemicals' antimicrobial and/or antioxidant

properties are vital in combatting pathogenic microorganisms, fungal growth, inflammation prevention, and health-threatening oxidative free radicals. Consequently, they contribute to the rapid growth and development of the livestock and poultry industry (Patel et al., 2017). While the CLUK blend is not a commonly used plant source as a feed additive (Barasoglu, 2022), our current results suggest that the CLUK blend can positively impact poultry's general health status, growth, and laying performance (Dhama et al., 2014). The metabolites within the CLUK blend can inhibit harmful agents in farm and poultry animals, thus halting their growth and development. Additionally, the nutrients contained within the CLUK blend can hold importance in metabolic reactions and physiological transformations within the animal body.

Choudhary et al. (2023) stated that mango kernel contains 53.34-76.81% carbohydrate, 5.20-10.48% protein, 9.84-18.0% fat, and 0.26-10.60% crude fiber. The researchers concluded that mango kernel, a significant source of phytochemicals and nutrients, may be used as a maize substitute in rations. Beyene et al. (2019) reported that mango kernels can replace maize entirely in laying hen diets, resulting in improved economic returns without impacting egg yield or quality parameters. Moreover, Beriso et al. (2022) reported that incorporating up to 15% boiled mango kernel in Hubbard broiler diets can improve production performance and feed conversion parameters without adverse effects. Our results on nutrient content indicate the potential of the CLUK blend as a feedstuff is controversial for poultry nutrition due to the high ADF and NDF percentages in the CLUK blend. Fiber components naturally found in poultry diets directly affect intestinal morphology, organ growth, nutrient utilisation, and microflora modulation to varying degrees (Tejeda and Kim, 2021). The effects of each fiber component need to be determined chemically and physiologically. A high fiber content and/or the presence of antinutritional substances in the kernel can reduce the digestibility of feed. Furthermore, it should be noted that the fiber values identified in our study were obtained from the kernel after removing the shell of the cherry laurel kernel. Fruit kernels may contain antinutritional elements, which could harm poultry (Rad et al., 2015). Therefore, it is necessary to establish whether the CLUK blend has toxic or antinutritional properties. It has been reported that date kernel has a negative impact on the performance parameters of broiler chickens, resulting in a decrease in live weight when included at levels of 10% and 20% (Masoudi et al., 2011; Kheiri and Nasr, 2013). Arbouche et al. (2012) showed that adding apricot kernel to the diet above the level of 6% adversely affected the growth performance of broiler chickens due to the antinutritional factors present in its kernels. However, there are measures to reduce these antinutritional factors, effectively eliminating the negative effects (Jazi et al., 2017). Hence, *in vivo* studies are necessary to determine and use antinutritional factors of CLUK blend at varying doses. In addition, the production and consumption amounts of the cherry laurel fruit in our country still need to be clarified. As such, regrettably, its use as a raw material cannot be recommended. After clarifying the abovementioned issues, it is possible to reduce the cost by using it as feed raw material at low doses in small enterprises regionally.

Fruit and vegetable waste contains numerous antioxidative phytochemicals, such as vitamins C and E, carotenoids, polyphenols, and other bioactive compounds (González-Aguilar et al., 2008). Phenolic compounds-comprising a hydroxyl group attached to a benzene ring-are generally responsible for the odor, color, and flavour of plants. Cherry laurel is abundant in antioxidant substances, including phenolics such as chlorogenic acid, phenolic acids, anthocyanins, and vanillic acid, as well as ascorbic acid (Ayaz et al., 1997; Kolayli et al., 2003; Yaylaci-Karahalil and Sahin, 2011). Engin (2007) reported that the leaves, fruits, and seeds of cherry laurel exhibit antioxidant activity, suggesting their potential use as natural antioxidants in the food, cosmetics, and pharmaceutical industries. Islam et al. (2020) found the total phenolic contents of the fruit of 7 different cherry laurel genotypes between 2.76-8.30 mg GAE g^{-1} and FRAP and DPPH values between 4.96-25.37 mmol TE g^{-1} and 1.07-12.19 mmol TE g^{-1}, respectively. Based on the total phenolic content, antioxidant activity, and vitamin C results obtained in this study, we can conclude that CLUK blend demonstrates antioxidant activity and thus, can serve as a suitable feed additive for poultry diets. In fact, various studies assess fruit kernels as feed additives due to their high concentration of antioxidative phytochemicals. Karabacak et al. (2015) stated that the co-administration of the grape kernel with ionophoric antibiotics effectively reduced the adverse effects of these antibiotics. These effects of the grape kernel could be ascribed to the ability of substances with high antioxidant capacity to scavenge radicals and their regulatory impact on the balance between oxidants and antioxidants. Gungor and Erener (2020) noted that cherry kernels, which have anticarcinogenic, anti-inflammatory, antidiabetic, antioxidant, and antimicrobial properties, can be a potential feed additive that improves growth performance when used in broiler diets at a rate of 1%.

In the present study, the EE content and fatty acid profile results suggested that while the CLUK blend contributes to meeting the energy needs of laying hens, it can enrich the fatty acid profile of the egg. In laying hens, we have shown that the level of unsaturated fatty acids in eggs can be enriched by manipulating the diet, with the fatty acid profile of the yolk being highly dependent on the fatty acid profile of the diet (Kop-Bozbay et al., 2021). Similarly, in broiler chickens, Rohaida (2014) suggested that candlenut kernels, which are rich in alpha-linolenic and linoleic acids, can be a source of omega-3 fatty acids and that dietary supplementation can enrich the fatty acid content of chicken meat. It is desirable for

consumers and producers to increase the content of unsaturated fatty acids in foods of animal origin. In light of the information described, it is concluded that the CLUK blend can improve meat and egg quality and contribute to consumer health by modifying the fatty acid profile.

5. Conclusion

These results reveal that the studied CLUK blend has the potential for quality, healthy, and eco-friendly poultry production. Therefore, the possibilities of using CLUK blend as an alternative feed additive, as well as a feedstuff in poultry nutrition are worth investigating due to its high fiber content, total phenolic matter, antioxidant capacity, and fatty acid profile. More extensive research is needed to determine the effects of using CLUK blend as a feed additive on the reproductive, health, and yield characteristics of poultry and to demonstrate its benefits.

Author Contributions

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

	E.B.	C.K.B.
C	50	50
D		100
S		100
DCP	50	50
DAI	100	
L	50	50
W	50	50
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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MORPHOLOGICAL AND GENETIC DIVERSITY OF *Schoenoplectiella mucronata* (L.) J. JUNG & H. K. CHOI (RICEFIELD BULRUSH) IN RICE

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Abstract: Since the beginning of rice cultivation, weed control has been a problem in Türkiye as well as in many other countries. Rice has both an important cultural plant and limited production for Türkiye. There are significant yield losses due to weeds and therefore weed control has an important place in rice agriculture. Species belonging to the genus *Scirpus* in rice production areas have recently become an important problem in rice cultivation areas of Türkiye as well as in rice cultivation areas of many other countries. In order to determine the morphological and genetic diversity of *Scirpus mucronata*, which is a problem in rice cultivation areas in Türkiye, 62 populations collected from the rice production areas of the Marmara and Black Sea Regions were evaluated over 8 ISSR primers and 12 morphological parameters. In the ISSR study, observed and expected heterozygosity levels ranged from 0.192 to 0.970 and from 0.136 to 0.566, respectively. In the morphological and molecular analyses performed, differences were detected in some quantitative characters between the examined populations. While morphological similarities were found between the populations grown in different regions that could not be ignored, genetic diversity was found to be higher. Morphological and genetic relationships between populations were not found to be related to geographic distance. In the context of the results, it is important to focus on field management practices such as cultural methods, as well as good control of rice seed traffic and herbicide use. It should not be forgotten that these measures are important in terms of integrated weed management strategies.

Keywords: *Scirpus mucronata*, ISSR-PCR, Morphological diversity, Genetic difference

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1. Introduction

Rice, widely cultivated in tropical and temperate climate regions, is the only cereal crop grown in water, utilizing dissolved oxygen for its development. To meet the demand by the year 2050, rice yield needs to be increased by 50% worldwide. Looking at the rice production situation in our country, it is observed that 95% of the production is concentrated in the Marmara and Black Sea regions. The remaining 5% of production takes place in the Mediterranean and Southeastern Anatolia regions. When comparing yield per unit area, Türkiye exceeds the world average (FAO, 2020). However, despite this, the cultivated area is not sufficient to meet domestic consumption, leading to the annual import of around 200 thousand tons of rice.

Weeds pose one of the most challenging factors in rice cultivation areas, and if not addressed, they can cause more than 40% crop loss depending on cultivation systems, rice varieties, weed species, and their density (Busconi et al., 2012; Chauhan and Abugho, 2013). Due to the completely aquatic nature of rice production systems, we observe that a limited but significant number of weed species have adapted to this system. In countries with intensive rice cultivation areas, such as those in Asia, America, and Europe, weed species belonging to the genera *Echinochloa, Cyperus,* and *Scirpus* are identified as significant problems, posing challenges for their control (Talbert and Burgos, 2007; Mennan and Kaya-Altop, 2012). Similar to many developed countries, in Türkiye, weed control in rice cultivation heavily relies on herbicides due to high labor costs and a shortage of qualified personnel. Within the *Scirpus* genus, *Schoenoplectiella mucronata* (L.) J. Jung & H. K. Choi (Syn. *Scirpus mucronatus*) and *Scirpus maritimus* L. are two significant problematic species in Türkiye. The control of these weed species predominantly involves the use of ALS inhibitor herbicides.

The *Scirpus* genus comprises numerous aquatics, grass-like species commonly known as bulrush. With approximately 35 species, it is predominantly found in

temperate regions of the Northern Hemisphere, displaying the highest diversity in North America (12 species), followed by China (11 species) and Europe (5 species) (Liang and Tucker, 2010). These plants are frequently cultivated to prevent soil erosion and offer habitats for waterfowl and other wildlife. Typically characterized by clusters of small, brown spikelets, the Scirpus genus can be identified through specific features. The rachilla, a diminutive axis of a spikelet, bears the florets. The achene, a small, dry, indehiscent one-seeded fruit, lacks a typical vascular tissue supply, usually having only a single trace. The culm, referring to the stem of any plant, plays a crucial role in these species. Additionally, the spikelet, a small or secondary spike with a varying number of reduced flowers, is subtended by one or two scale-like bracts. Bristles, resembling stiff, hair-like structures, and filaments, slender stalks supporting and holding pollen sacs (anthers), are integral components of these plants (Moore, 2014).

Ricefield Bulrush, is a keystone wetland plant species commonly found in rice and comprises a diverse group of wetland plants exhibiting remarkable adaptability to different ecological niches (Ge et al., 2023).

With the advancement of molecular techniques in recent years, studies on the genetic diversity of weed populations have opened up new avenues in weed science (Huang et al., 2023). Genetic conservation efforts should prioritize preserving distinct genetic populations of *S. mucronata* to maintain genetic diversity, which may be critical for the species' adaptability to changing environmental conditions and serves as a valuable resource for understanding their evolutionary history. The use of molecular markers in detecting genetic diversity allows for the determination of not only genetic variation but also the degree of relatedness and morphological developments in both annual and perennial weed species (Mengistu et al., 2004). The broad morphological and genetic diversity exhibited by the species not only complicates control but also accelerates the formation of herbicide-resistant biotypes (Yabuno, 2001; Michishita and Yamaguchi, 2003). Species with high genetic diversity possess the ability to adapt, reproduce, and compete more successfully under changing environmental conditions over time and space (Birader, 2023). Genetic diversity studies have taken on a fundamental mission in determining how herbicides and environmental influences affect the dynamics of species, acquiring knowledge in combating significant weed species, and elucidating the reasons behind the variations that species may exhibit in response to herbicides (Sterling et al., 2004). The primary factor leading to high herbicide resistance in different rice fields has been identified as seeds carrying resistance genes. In this context, preventing seed production and dissemination in resistant biotypes constitute a fundamental objective in weed control strategies (Merotto et al., 2009).

It has been emphasized that spontaneous hybrid species can occur in species belonging to the Cyperaceae family, and this phenomenon is associated with polyploidy and asexual reproduction. It has also been highlighted that to explain this phenomenon, it is essential to evaluate both morphological and molecular differences together (Arriola and Ellstrand, 1996).

A variety of molecular methods are employed in calculating genetic diversity within weed populations. Inter simple sequence repeats (ISSR) typing offers significant advantages due to its relative simplicity, cost-effectiveness, rapid results, and the ability to analyze a large number of samples swiftly. ISSR markers have yielded promising results in obtaining data related to taxonomic relationships and genetic differences among weed species (Tayyar et al., 2003; Al Salameen et al., 2020). However, limited genetic diversity studies have been conducted on *S. mucronata.*

Understanding the genetic and morphological diversity within the genus *Scirpus* is crucial for its conservation within rice ecosystems, shedding light on its ecological adaptations and improving management strategies (Smith et al., 2020). This article delves into the intricate interplay between genetic and morphological variability in *S. mucronata*, a significant concern in Türkiye rice cultivation areas, aiming to investigate its impact on the existing resistance phenomenon. The observed high level of genetic diversity not only serves as a triggering factor in herbicide resistance but also contributes to the enhanced adaptability of resistant populations (Dixon et al., 2020). Despite the ecological importance of *Scirpus* species, the genetic and morphological diversity within the genus remains relatively understudied, emphasizing the need for further research in this area. In addition to genetic analyses, morphological traits, such as leaf architecture, stem morphology, and reproductive structures, play a pivotal role in comprehending the adaptive strategies of *Scirpus* species. Morphometric studies have been instrumental in elucidating phenotypic variability within and between populations (Thomson et al., 2000).

This study aims to fill this knowledge gap by providing an overview of the current state of research on the genetic and morphological aspects of *Scirpus* species and to provide guidance for a comprehensive interpretation to facilitate the determination of integrated management strategies against this species in Türkiye's rice fields.

2. Materials and Methods

2.1. Plant Material

A total of 62 populations were studied to determine the morphological and genetic diversity of *Schoenoplectiella mucronata* (Fam: Cyperaceae). The seed samples of the populations were collected from rice cultivation areas in the Marmara and Black Sea regions, including the provinces of Samsun, Balıkesir, Bursa, Edirne, Kastamonu, Kırklareli, Sinop, Tekirdağ, and Çorum during the 2016 vegetation period (Table 1).

Table 1. Geographic locations of the populations used to determine morphological and genetic diversity.

Number	Origin		Population	Coordinate	
1	Edirne	Havsa	EDI-S1	41° 25. 705'N	26° 48. 914'E
2			EDI-S2	41° 29. 246'N	26° 48. 811'E
3		İpsala	EDI-S3	40° 51. 748'N	26° 20. 546'E
4			EDI-S4	40° 52. 868'N	26° 23. 020'E
5			EDI-S5	40° 55. 921'N	26° 24. 520'E
6			EDI-S6	40° 56. 004'N	26° 24. 869'E
7			EDI-S7	40° 53. 652'N	26° 21. 898'E
8		Keşan	EDI-S8	40° 53. 353'N	26° 21. 493'E
9			EDI-S10	40° 53. 390'N	26° 21. 121'E
10			EDI-S11	40° 50. 381'N	26° 17. 704'E
11			EDI-S12	40° 44. 591'N	26° 25. 653'E
12			EDI-S13	40° 46. 678'N	26° 41. 873'E
13			EDI-S14	41° 05. 458'N	26° 22. 215'E
14		Meriç	EDI-S15	41° 06. 386'N	26° 20. 595'E
15			EDI-S16	41° 06. 426'N	26° 20. 542'E
16			EDI-S17	41° 03. 192'N	26° 21. 810'E
17		Merkez	EDI-S18	41° 30. 844'N	26° 36. 642'E
18			EDI-S19	41° 29. 712'N	26° 37. 067'E
19	Samsun	Alaçam	SAM–S1	41° 37. 400'N	35° 43. 456'E
20			SAM–S2	41° 38. 824'N	35° 49. 332'E
21		Bafra	SAM–S3	41° 42. 043'N	35° 55. 014'E
22			SAM–S4	41° 43. 412'N	35° 57. 281'E
23		Çarşamba	SAM–S5	41° 16. 568'N	36° 44. 104'E
24			SAM–S6	41° 12. 494'N	36° 36. 012'E
25		Ondokuz Mayıs	SAM–S7	41° 32. 075'N	36° 03. 828'E
26		Terme	SAM–S8	41° 13. 500'N	36° 58. 096'E
27			SAM-S9	41° 11. 305'N	36° 59. 033'E
28		Yakakent	SAM–S10	41° 37. 656'N	35° 33. 829'E
29	Kırklareli	Babaeski	KIR-S1	41° 20. 940'N	27° 07. 340'E
30			KIR-S2	41° 21. 425'N	27° 04. 110'E
31		Pehlivanköy	KIR-S3	41° 22. 044'N	26° 52. 956'E
32	Kastamonu	Hanönü	KAS-S1	41° 37. 248'N	34° 28. 703'E
33			KAS-S2	40° 56. 368'N	33° 52. 502'E
34		Tosya	KAS-S3	41° 02. 654'N	34° 11. 373'E
35			KAS-S4	41° 03. 730'N	34° 12. 300'E
36	Tekirdağ	Hayrabolu	TEK-S1	41° 03. 275'N	27° 03. 625'E
37			TEK-S2	41° 03. 229'N	27° 03. 672'E
38		Malkara	TEK-S3	40° 56. 830'N	27° 01. 020'E
39	Bursa	Merkez	BUR-S1	40° 10. 356'N	28° 11. 256'E
40			BUR-S2	40° 11. 873'N	28° 11. 337'E
41			BUR-S3	40° 11. 758'N	28° 11. 300'E
42	Sinop	Saraydüzü	SIN-S1	41° 23. 532'N	34° 56. 981'E
43		Boyabat	SIN-S2	41° 37. 290'N	34° 36. 730'E
44			SIN-S3	41° 32. 955'N	34° 42. 959'E
45		Durağan	SIN-S4	41° 26. 722'N	34° 54. 735'E
46			SIN-S5	41° 25. 954'N	34° 56. 650'E
47	Balıkesir	Gönen	BAL-S1	40° 07. 161'N	27° 43. 387'E
48			BAL-S2	40° 07. 056'N	27° 42. 101'E
49		Manyas	BAL-S3	40° 04. 680'N	28° 02. 410'E
50			BAL-S4	40° 04. 987'N	28° 02. 578'E
51			BAL-S5	40° 04. 993'N	28° 02. 581'E
52			BAL-S6	40° 06. 140 'N	28° 08. 241'E
53	Çorum	Kargı	ÇOR-S1	41° 06. 098 'N	34° 24. 910'E
54			ÇOR-S2	41° 04. 986'N	34° 26. 134'E
55			ÇOR-S3	41° 07. 123'N	34° 25. 272'E
56		Osmancık	ÇOR-S4	40° 58. 821'N	34° 55. 776'E
57			ÇOR-S5	40° 57. 726'N	34° 50. 011'E
58			ÇOR-S6	40° 56. 319'N	34° 51. 357'E
59		Bayat	ÇOR-S7	40° 31. 376'N	34° 20. 545'E
60		Dodurga	ÇOR-S8	40° 49. 609'N	34° 51. 519'E
61		İskilip	ÇOR-S9	40° 36. 055'N	34° 28. 523'E
62		Laçin	ÇOR-S10	40° 49. 602'N	34° 51. 529'E

2.2. Morphological Studies

Seeds of *S. mucronata* from each population were sown in plastic pots (diameter 20 cm; height 25 cm) and cultivated in a screen house. The pots were filled with rice soil, and the experiments were set up with five replicates according to a randomized block trial design. Fertilizer used in the regions and sufficient water were provided to the pots. In the cultivation process, the biological stages of the plants from seed to seed were monitored through daily observations, and morphological parameter data were recorded. The examined parameters included plant height (cm), first cotyledon time (day), second cotyledon time (day), leaf number (per plant), rays number (per spike), spike length (cm), spikelet lenght (cm), rays lenght (cm per spike), inflorescence number (per bract), total

inflorescence number (per plant), fresh weight (g) and dry biomass (g) (Tayyar et al., 2003; Więcław et al., 2021). After harvesting, the plants were dried at 70°C for 3 days for dry biomass measurement.

2.3. Genetic Studies

For DNA extraction, seeds of the 62 populations of *S. mucronata* were germinated in Petri dishes and then transferred to pots, where they were grown until the 4-6 leaf stage under controlled conditions at 30°C with a 12/12-hour lighting period in a greenhouse. Genomic DNAs from leaf samples were extracted using the DNeasy DNA extraction kit (Qiagen, Qiagen GmbH, Hilden, Germany) according to the kit protocol. The isolated DNA concentration and relative purity were checked using a Nanodrop ND-1000 (Thermo Scientific) and adjusted to 25 µL-1 (Danquah et al., 2002; Ruiz-Santaella et al., 2006; Kaya Altop and Mennan, 2011).

In the ISSR-PCR Analysis, 11 primers were tested, and 8 of them were successfully used for *S. mucronata* samples (Table 2). The primers were synthesized by Genox company (Ankara, Türkiye). The PCR reaction was prepared with a total volume of 25 µL, including 50 ng genomic DNA, 0.2 mM oligonucleotide primer, 1.5 mM $MgCl_2$, 0.4 mM dNTP, 0.2 units Taq DNA Polymerase, 1X PCR buffer, and 11 µL sdH_2O, and placed in a PCR device (Rotor-Gene Q 5plex HRM, Qiagen). The reaction conditions were set as follows: initial denaturation at 95°C for 1 min, followed by 45 cycles of denaturation at 95°C for 1 min, annealing at 72°C for 2 min, and extension at 72°C for 5 min.

After PCR, the resulting DNA fragments were subjected to agarose gel electrophoresis (Biorad), using a 2% agarose gel. A 1 Kb DNA marker (New England Biolabs) was used as a reference, and photographs of the DNA bands on the gel were taken using a gel imaging device. The evaluation of bands relied on whether the bands were visible or not on the gel after electrophoresis. In this study, optimal PCR conditions were established by repeating amplifications several times, and conditions that provided stable band profiles for each primer were selected. Monomorphic and polymorphic bands in the gels were identified to obtain statistical analysis.

2.4. Statistical Analyses

Different methods were used for the statistical analysis of morphological and molecular findings. The data obtained from morphological studies were subjected to hierarchical cluster analysis using IBM SPSS Statistics 20 (for Windows) statistical package program. Duncan's multiple comparison test was applied to the parameter values, and a dendrogram created using hierarchical clustering method. Euclidean distances were calculated across landraces and a distance matrix was produced. Moreover, a principal component analysis (PCA) plot was constructed from the combined morphological parameters (Kaya Altop and Mennan, 2011; Moore, 2014).

For molecular studies, band sizes were entered as present (1) or absent (0) and values were calculated using observed and expected heterozygosities, using the NTSYS package program. Band matrices were thus created for use in subsequent stages. In the final step, dendrograms of the varieties were drawn using the SAHN (Sequential, Agglomerative, Hierarchical, and Nested Clustering) clustering subprogram and the UPGMA algorithm based on similarity matrices according to Jaccard (Jaccard, 1908; Nei, 1972; Nei and Li, 1979; Mujeeb et al., 2017). The genetic similarity matrix is based on Euclidean distances. The Principal Component Analysis (PCA) is relatively objective and provides a reasonable indication of relationships, it was used to confirm the similarity of the grouping obtained with the UPGMA dendrogram (Więcław et al., 2021).

Table 2. Information about the primers used in ISSR application

Locus	Repeat motif	Primer sequence (5′–3′)	Ta (°C)
SM2	$(GA)_{14}$	GTCTCACGAGAGAGAGAGAGA	54.5
		GCTTGTTCGGAGTAGGTGTG	
SM4	$(GA)_{15}$	TACTGCAGAGAGAGAGAGAG	53
		GCGAAAGTAGAGGAGATAA	
SM5	$(CT)_{15}$	GGGGCGCCTCTCTCTCTCTC	54.5
		AGGCTCCAACAATCCAGTAA	
SM6	$(AG)_{15}$	CGGCTTGCCTTTGGTTTCAT	57
		GGGGGGGCTCTCTCTCTCTC	
SM7	$(CT)_{15}$	TTGACAGCTCTCTCTCTCT	50
		GAATCTTTGAGCGTTTAGT	
SM11	$(CT)_{10}$	TAATGGATGGAGCAGAGACAG	54.5
		CGCAGTGGAGTCGGAGA	
SM12	$(AC)_6(AT)_4\ (GC)_3$	ATTTTTCTTTCTCCACACACTCT	54.5
		CGCTCGCTCGTCGCTAAA	

Ta= annealing temperature, GenBank Accession numbers= EU121661- EU121669 (Zhou et al., 2009).

3. Results

3.1. Morphological Studies

It has been observed that plants from 62 *S. mucronata* populations collected from different geographic locations exhibit an average height of 80.48 cm, with the tallest plant belonging to the Edirne-Keşan (EDI-S13) population (126.03 cm) and the shortest plant belonging to the Samsun-Terme (SAM-S8) population (64.45 cm). The population Edirne-Ipsala (EDI-S5) takes the longest time, up to 14 days, to develop its first cotyledon leaf, while the population Samsun-Alaçam (SAM-S1) has the earliest development times for both the first and second cotyledon leaves, with 7.10 and 6.98 days, respectively.

Even within the same province, the number of leaves varies significantly, with the EDI-S16 population having the least number of leaves per plant (17.86) and the EDI-S4 population having the highest number of leaves per plant (44.06). The second cotyledon leaf in the EDI-S4 population emerges approximately 19 days later than in other populations, making it the latest to develop. The BAL-S1 biotype from Balıkesir-Gönen location achieved the highest values in terms of rays number (2.61 per spike), fresh weight (31.99 g), and dry weight (8.69 g) parameters, while the EDI-S17 biotype from Edirne-Meriç location reached the highest values in spikelet and rays height (32.31 mm and 16.45 mm) parameters. Regarding the parameters of inflorescence number, total inflorescence number, and spike height, the highest values were obtained from the EDI-S19 (21.08 per bract) population in Edirne and the SAM-S5 (175.92 per bract) and SAM-S10 (84.51 mm) populations in Samsun, which are the two most important provinces for rice farming in Türkiye. EDI-S12 from Edirne-Keşan showed the lowest values in terms of total inflorescence number and dry weight parameters, while SAM-S3 from Samsun-Bafra locations had the lowest values for spikelet height, rays height, and inflorescence number (data not shown).

The Table 3 illustrates the PC components obtained from the principal component analysis (PCA) results based on the morphological characteristics of *S. mucronata* populations. Three PC components, representing a total variation of 80.74%, were obtained among the total 12 features examined. The first PC component, which accounts for 54.44% of the total variation among the morphological characteristics, is primarily influenced by the feature dry weight (0.95 g), contributing the most to its explanation, while plant height (0.19 cm) has the least impact. The second PC component, representing 14.80% of the variation, is positively related to total inflorescence number (0.69 per bract). Both PC2 and PC3, which account for 11.50% of the total variation, are significantly influenced by plant height and spike length. It was observed that total inflorescence number, plant height and spike length have limited associations with other parameters (see Figure 1).

When the entire set of morphological parameter data from the populations was subjected to hierarchical clustering analysis, the dendrogram in Figure 2 was generated based on similarity levels. According to this analysis, two main groups have formed at a taxonomic distance of 25%. The first group consists of two subgroups, with the KAS-S2 population standing out as distinctive within this division. The second main group is represented solely by the SAM-S8 population. High similarity regions among the other populations were not significantly affected by geographical differences. No geographical isolations were observed.

3.2. Genetic Studies

In the genetic analysis, 17 primers were tested, of which 8 consistently produced amplified ISSR fragments. These ISSR primers generated a total of 32 alleles, with an average allele count of 4. The highest number of alleles was observed in loci SM2 and SM7. The ISSR primers produced band profiles with lengths ranging from 76 bp (SM7) to 258 bp (SM1). When examining the findings obtained from the 8 primers used, it was observed that HO (observed heterozygosity) values ranged from 0.192 to 0.970, with the highest value obtained from the SM12-SM1 primers. On the other hand, HE (expected heterozygosity) values ranged from 0.136 to 0.566, primarily derived from the SM12-SM7 primers.

Table 3. Correlation matrix of morphological parameters

Parameter	PH	FCT	SCT	LN	RN	SH	STH	RH	IN	TIN	FW	DW
PH	1.00											
FCT	0.09	1.00										
SCT	0.07	0.92**	1.00									
LN	0.12*	0.41**	0.45**	1.00								
RN	0.01	0.84**	0.84**	0.41**	1.00							
SH	0.50**	0.12*	0.13*	0.17**	0.07	1.00						
STH	0.23**	0.67**	0.69**	0.34**	0.67**	0.48**	1.00					
RH	0.16**	0.79**	0.80**	0.44**	0.89**	0.35**	0.81**	1.00				
IN	0.10	0.67**	0.68**	0.37**	0.74**	0.23**	0.60**	0.74**	1.00			
TIN	0.18**	0.10	0.13*	0.50**	0.02	0.20**	0.04	0.14*	0.34**	1.00		
FW	0.19**	0.44**	0.48**	0.69**	0.51**	0.31**	0.42**	0.58**	0.51**	0.57**	1.00	
DW	0.08	0.78**	0.79**	0.59**	0.94**	0.18**	0.66**	0.90**	0.75**	0.25**	0.73**	1.00

PH= plant height (cm), FACT= first cotyledon time (day), SCT= second cotyledon time (day), LN= leaf number (per plant), RN= rays number (per spike), SH= spike length (mm), STH= spikelet height (mm), RH= rays height (mm/per spike), IN= inflorescence number (per bract), TIN= total inflorescence number (per plant), FW= fresh weight(g), DW= dry weight (g)., *P≤0.05, **P≤0.01.

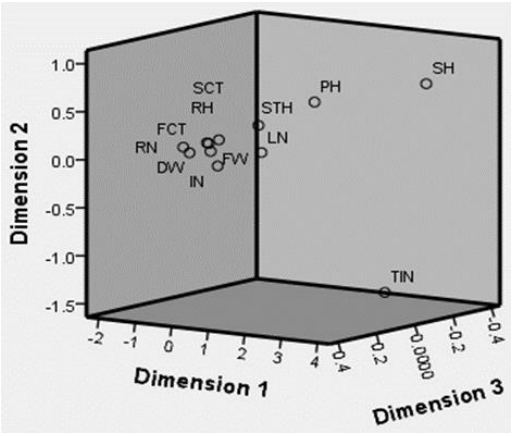

Figure 1. Morphological parameter relationship graph created according to the Euclidean distance model.

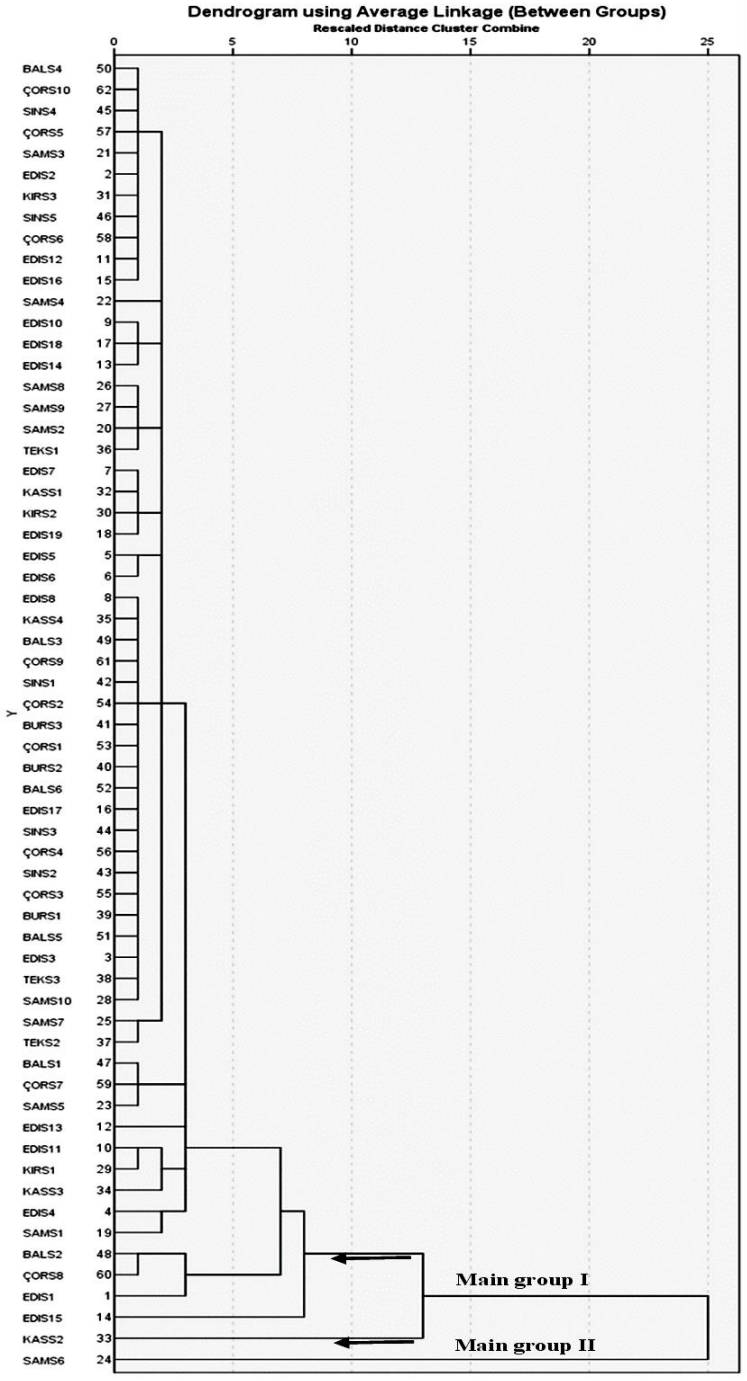

Figure 2. Phylogenetic dendrogram based on morphological characteristics.

The average HO value was determined as 0.501, and the average HE values were found to be 0.432 (Table 3).

The molecular data obtained from the ISSR analysis for *S. mucronata* populations were subjected to Principal Component Analysis (PCA) (Table 4 and 5). The PCA results indicated 9 PC axes explaining 87.41% of the variations and their corresponding factor groups. It was observed that the principal coordinates (PCA1-PCA9) were determined, and their ratios were closely related to each other. The first principal coordinate accounted for 39.85% of the variation, with significant contributions from the BUR-S3 and EDI-S9 populations. The second coordinate, explaining 24.78% of the variation, showed both positive and negative effects, with the BAL-S6 population contributing the most. Other coordinates (PCA3-PCA9) were influenced by various populations (data not shown).

In the hierarchical cluster analysis, where the presence or absence of band indices generated by polymorphic primers was considered, a dendrogram was created using the Average Linkage method. It was observed that populations analyzed at a genetic distance of 0.25 could be divided into two main groups (Figure 3). The populations were grouped into two main clusters based on the populations studied.

Table 4. Factor groups and PCoA axes of morphological parameters created by PCA (principal component analysis)

	PCA axes		
	1	2	3
Eigenvalues	6.53	1.78	1.38
Variance (%)	54.44	14.80	11.50
Cumulative (%)	54.44	69.24	80.74
		Factor groups	
Parametreler	PCA 1	PCA 2	PCA 3
PH	0.19	0.58	0.55
FCT	0.86	-0.29	0.04
SCT	0.88	-0.26	0.01
LN	0.61	0.39	-0.43
RN	0.90	-0.35	-0.02
SH	0.33	0.57	0.60
STH	0.79	-0.05	0.40
RH	0.93	-0.12	0.16
IN	0.82	-0.04	-0.03
TIN	0.31	0.69	-0.47
FW	0.72	0.43	-0.32
DW	0.95	-0.09	-0.14

PH= plant height (cm), FACT= first cotyledon time (day), SCT= second cotyledon time (day), LN= leaf number (per plant), RN= rays number (per spike), SH= spike length (mm), STH= spikelet height (mm), RH= rays height (mm/per spike), IN= inflorescence number (per bract), TIN= total inflorescence number (per plant), FW= fresh weight(g), DW= dry weight (g).

Table 5. Amplification results of Inter Simple Sequence Repeats (ISSR) analysis

Locus	Amplification			
	Size range (bp)	NA	*HO*	*HE*
SM1	254–258	4	0.970	0.566
SM2	113–151	5	0.642	0.493
SM4	242–311	3	0.723	0.495
SM5	135–150	4	0.483	0.593
SM6	149–183	4	0.346	0.269
SM7	76–97	5	0.323	0.594
SM11	232–322	4	0.330	0.310
SM12	112–143	3	0.192	0.136
Mean		4	0.501	0.432

NA= number of alleles, HO= observed heterozygosity, HE= expected heterozygosity.

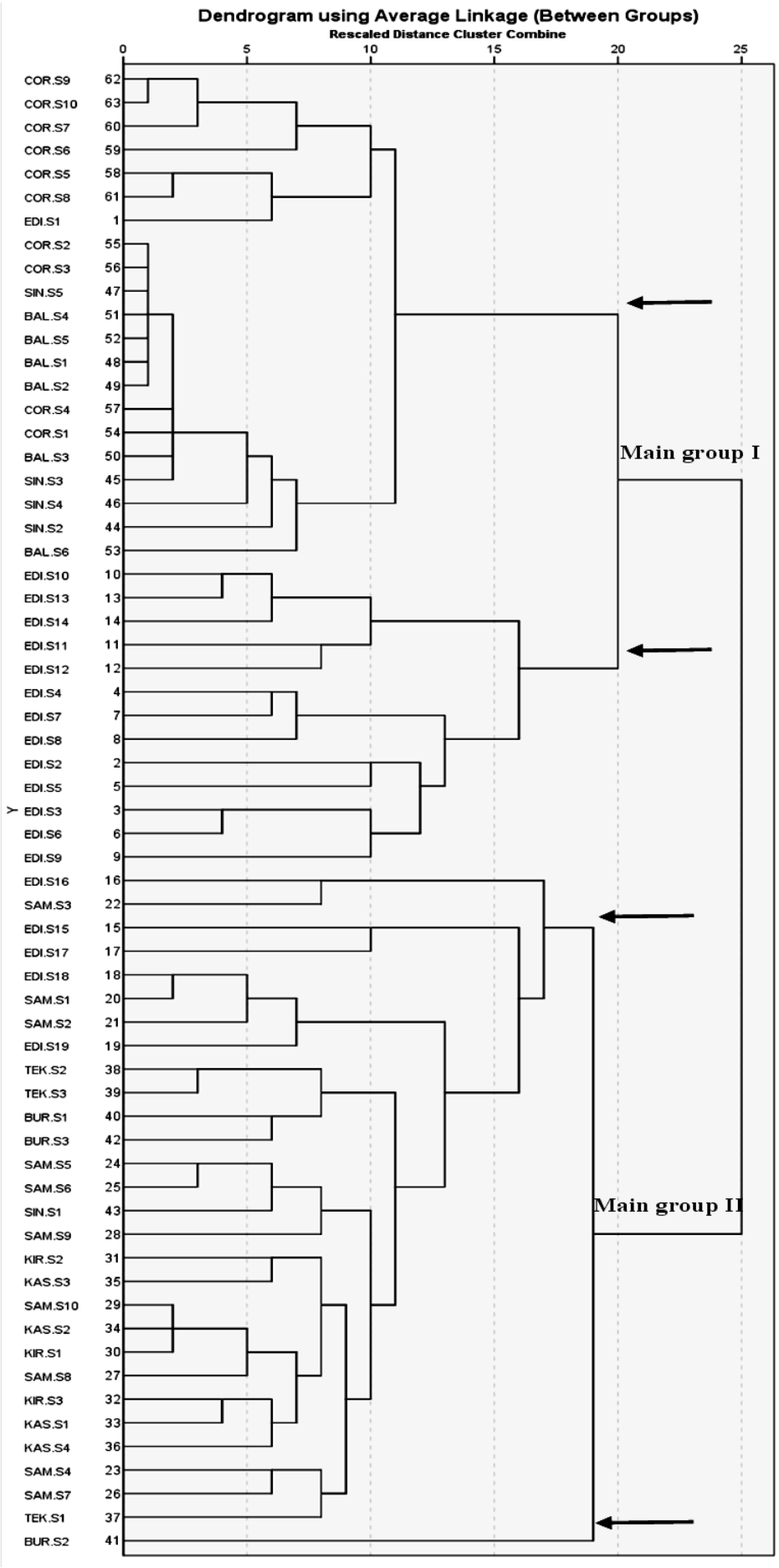

Figure 3. Phylogenetic dendrogram based on genetic characteristics.

According to the UPGMA analysis, the phylogenetic tree revealed a high level of genetic similarity among populations, particularly between COR-S9 (Çorum/İskilip), COR-S10 (Çorum/Laçin), COR-S2 (Çorum/Kargı), COR-S3 (Çorum/Kargı), SIN-S5 (Sinop/Duragan), BAL-S1 (Balıkesir-Gönen), BAL-S2 (Balıkesir-Gönen), BAL-S4 (Balıkesir-Manyas), and BAL-S5 (Balıkesir-Manyas) populations. The most distant genetic similarity was observed between BUR-S2 (Bursa/Merkez) and other population groups. The Jaccard genetic similarity coefficient ranged from 0.138 to 1.000, with an average genetic similarity of 54%. The results from the similarity index matrix were consistent with the dendrogram, highlighting the genetic distances among populations.

Notably, the population BAL-S6 exhibited low similarity, or in other words, genetic distance, with SAM-S3 (0.138) and EDI-S16 (0.171) populations. Furthermore, populations from Balıkesir province (BAL-S1-S2-S4-S5), Çorum province (ÇOR-S2-3), and Sinop province (SIN-S5) showed 100% genetic similarity. The same level of similarity was also observed between the populations' COR-S9 and COR-S10 (data not shown).

4. Discussion

The genetic and morphological diversity of *Scirpus mucronata* has been notably limited in the existing literature. In a study focusing on the morphological characteristics of *S. mucronata* populations, the obtained PC axes and corresponding factor groups were statistically evaluated. The findings, when assessing the PCA analysis of *Scirpus* species' morphological characterization based on morphological features, are supported by De Greef and Triest (1999)'s study. In their work, it was observed that the 2 PC axes explained 44% and 13% of the total variation, respectively. This highlights the relevance and applicability of PCA analysis in defining the morphological traits of *Scirpus* species.

In the molecular data obtained for *S. mucronata* populations, a total of 5 principal coordinates have been identified, with the proportions of genetic variation explained ranging between 2.18% and 42.7%. These results bear similarity to the component and variation findings reported by Danquah et al. (2002). The observed parallels suggest consistency in the genetic structure and variability patterns within *S. mucronata* populations, as indicated by both studies.

When compared to morphological studies, molecular investigations have yielded more detailed data. In the ISSR studies, eight different primers were employed. The utilization of numerous primers is known to be effective in genetic mapping (Vellend and Geber, 2005).

However, Millan et al. (1996) reported the use of 10 primers in roses, Abad et al. (1998) utilized 27 primers for *Cyperus esculentus* L., while *Ixora* varieties were studied with six primers. Schontz and Rether (1999) applied four primers in Italian ryegrass, Tasrif et al. (2004) and Juraimi et al. (2005) used four primers in *E. crus-galli* var. *crus-galli*, Tayyar et al. (2003) employed 16 primers for 35 populations, Merotto et al., (2010) utilized 75 SRAP markers for *Cyperus* spp., and Danquah et al., (2002) suggested that six AFLP primers and five microsatellite primers might be sufficient for detecting polymorphism in *E. crus-galli*.

Ren et al. (2005) suggested that if the variation among varieties is high, a limited number of primers may be sufficient. In the ISSR study conducted to assess the genetic diversity of the alien weed species *Xanthium italicum*, eight different primers were utilized across 10 distinct populations. The results indicated that the genetic variation among populations is likely primarily attributed to genetic drift and anthropogenic activities (Tang and Ma, 2020). In Türkiye, molecular studies on genotypes of *Polygonum cognatum* Meissn. have been carried out using 14 SSR and 23 RAPD primers (Önen et al., 2010). Additionally, successful applications of techniques were reported in wild poppy species belonging to the *Oxytona* section, using 12 SSR and 22 RAPD primers (Parmaksız et al., 2009).

All eight primers used in the study resulted in the formation of polymorphic bands. According to the available information, there is no existing study on the determination of genetic diversity in *S. mucronata* using ISSR markers.

While the genetic similarity rate varied between 1% and 98%, these findings are consistent with the results obtained by Samuelsson et al. (1997) through the examination of variation among *Vicia pisiformis* populations using morphological analyses and the RAPD marker system. Similarities were also observed with the results of Tayyar et al. (2003), who worked on *Cyperus* species. Furthermore, Budak et al. (2004) expressed confidence in the usability of a 57% genetic average obtained from their research using the RAPD method for result evaluation. The primers used in the study exhibited a high level of polymorphism, providing positive outcomes for the accurate assessment of the results.

In the molecular dendrogram, geographical isolations are more clearly visible compared to the morphological dendrogram. Although populations are grouped together morphologically, the wide geographic distribution can be attributed to the soil cultivation and transportation through harvest machinery in the rice fields where the samples were collected. Ecotypes showing high phenotypic similarity may not necessarily exhibit genetic similarity (Vellend and Geber, 2005) due to the potential existence of different gene pools. The assessment of genetic and morphological similarities can be accurately interpreted by examining phylogenetic data to evaluate the changing rates of gene flow over time and genes associated with variation that enables expression. Similar views have been expressed by Bromham et al. (2002). Genetic diversity is inversely proportional to the rate of gene flow, meaning that the higher the genetic diversity among populations, the lower the gene flow, or vice versa

(Merotto et al. 2010). While some studies have linked gene flow between populations to factors such as distance, cultivation systems, pollination characteristics, plant species, environmental conditions, and vectors (Levin and Kerster 1974), there are also studies suggesting that genetic relationships between populations are not necessarily associated with geographic distance (Merotto et al., 2009). In the study, the interpretation of UPGMA dendrogram information revealed that genetic relationships are not always associated with geographic distance, but clear geographic isolation exists among populations. When the data from these situations are evaluated in the triangle of genetic relationship, geographic location, and herbicide activity, it is found that these three concepts cannot be directly correlated. However, this indicates that resistant populations are rapidly spreading. In various previous studies, it has been suggested that herbicide resistance can spread through gene flow between populations with high genetic similarity (Rutledge et al., 2000; Stankiewicz et al., 2001; Tsuji et al., 2003; Merotto et al., 2010). Considering the high rate of self-pollination in *S. mucronata*, it is emphasized that gene flow may occur more through seed dispersal than pollen dispersal (Baker et al., 2007). As mentioned by Roy et al. (2000), uncertified seeds being transported and used from one region to another, coupled with the rapid adaptation of this invasive weed species to the region where it is planted, can lead to the formation of morphologically similar groups despite genetic differences. Cross-pollination, strong clonal growth, sexual reproduction, and human-mediated spread factors are effective in the formation of variation, a viewpoint supported by researchers (Tayyar et al., 2003; Ren et al., 2005).

In the conducted morphological and molecular analyses, differences were identified among the examined populations in terms of certain quantitative characters. While populations of *S. mucronata* from different regions exhibited significant morphological similarities, higher genetic diversity was observed. This indicates a high potential for gene flow. In conclusion, the joint evaluation of morphological and molecular studies revealed that variation among populations is primarily characterized by low morphological but high genetic diversity. This is attributed to adaptation to geographical areas, the transportation of seeds between regions by humans and tools, and the development of resistance by the weed, especially against herbicides commonly used in weed control methods. In this context, it is emphasized that the authorization, marketing, and supervision services in the seed sector should be conducted in accordance with standards to prevent the spread of *S. mucronata* seeds over both short and long distances. Continuous monitoring of adaptation processes and the integration of preventive measures into weed control strategies are crucial.

5. Conclusion

The results of this study emphasize the importance of considering both morphological and genetic characteristics when assessing the diversity and relationships among *S. mucronata* populations. The variations observed in plant height, leaf development, leaf numbers, and inflorescence-related parameters reflect the adaptability and response of these populations to their specific environments.

The genetic analysis, based on ISSR markers, provided valuable insights into the genetic diversity and relationships among the populations. The high level of genetic diversity observed in *S. mucronata* populations suggests their potential for adaptation and evolution in response to changing environmental conditions.

The findings from this study can serve as a basis for further research into the conservation and management of *S. mucronata* populations, especially in regions like Edirne and Samsun, where rice farming is of great importance. Understanding the genetic diversity and relationships among populations is crucial for the development of effective conservation strategies.

In conclusion, the combined evaluation of morphological and molecular studies indicates that there is low morphological but high genetic variation among populations. This is primarily attributed to adaptation to geographical areas, transportation of seeds between regions by humans and tools, and the development of resistance by the weed to herbicides commonly used in weed control strategies. In this context, it is crucial to implement seed sector authorization, marketing, and monitoring services in accordance with standards to prevent the spread of *S. mucronata* seeds both short and long distances. Continuous monitoring of adaptation processes and the integration of preventive measures with weed control strategies are essential considerations.

Author Contributions

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

	E.K.A.
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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EFFECT OF SALT STRESS ON MORPHOLOGICAL CHARACTERISTICS AND SECONDARY METABOLITES OF SOME FORAGE PEA CULTIVARS

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Abstract: Forage pea (*Pisum sativum* L.) is an annual legume forage crop grown in various regions of Türkiye. It is high in protein, carbohydrate, and digestible matter and contains minerals such as phosphorus, calcium, and vitamins A and D. Salinity stress is an important problem in the cultivation of forage peas. Salinity reduces the osmotic potential of soil solutes, making it difficult for the roots to absorb the water. This study aimed to determine some parameters of two registered forage pea cultivars at different concentrations of two salt types. The effects of these salts on the morphological characteristics and biochemical components of two different registered cultivars of pea, cv. Ateş and cv. Töre were investigated in the present study. The trials were conducted in pots and Na_2SO_4 and $CaCl_2$ were applied at concentrations of 0, 50, 100 and 150 mM. As a result of the trials, the morphological characteristics like fresh and dry weights and lengths of roots and shoots were investigated along with the biochemical properties like total antioxidant activity and total phenolic content. The study was performed in 2 replicates to determine the effect of different salt types and concentrations. The critical salt concentration values for the change in shoot and root fresh weight among morphological traits were determined as 100 and 150 mM for secondary metabolites. While the cv. Töre forage pea showed the highest salt resistance in shoot and root fresh weights in the presence of Na_2SO_4 the cv. Ateş forage pea showed the lowest salt resistance in the presence of $CaCl_2$. In terms of shoot and root dry weights, the cv. Töre forage pea showed the least resistance at 50 mM Na_2SO_4 concentration. As for plant length, the cv. Ateş forage pea cultivar showed the least resistance in shoot length at 150 mM $CaCl_2$ concentration, while it showed the highest resistance in root length at this value. The highest total antioxidant activity for the cv. Ateş forage pea and the highest total phenolic content for the cv. Töre forage pea were determined at 150 mM $CaCl_2$ concentration. The lowest total phenolic content value was estimated in the cv. Töre forage pea cultivar at 150 mM Na_2SO_4 salt concentration.

Keywords: Salt stress, Morphological properties, Total phenolic content, Radical scaving activity

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1. Introduction

Forage pea (*Pisum sativum* L.) is an annual legume fodder plant known as "*kulür*" or "*kürül*" in various regions of Türkiye. It is highly suitable for feeding all livestock. In European Union countries, it is an alternative to soybean due to its protein content (21-25%). In addition, it is suitable for consumption as livestock feed due to its high level of carbohydrates and digestible substances (86-87%) (Ouafi et al., 2016; Çaçan et al., 2018). Forage peas have the ability to bind nitrogen (5-15 kg ha^{-1}) from the air to the soil and thus the need for excess nitrogen fertiliser in agriculture is decreased. In addition to minerals such as phosphorus and calcium, it also contains vitamins A and D (Tekeli and Ateş, 2003; Uzun et al., 2012; Ouafi et al., 2016). Salinity is an important problem especially for forage peas (Arslan et al., 2013). It is the primary factor that will directly affect crop yield nowadays and in the future (Mohamed and Aly, 2008; Kang et al., 2010; Bu et al., 2015; Korkmaz and Durmaz, 2017). Currently, more than 6 per cent of the world's land area and 20 per cent of the world's irrigated land are affected by salinity. Salinity, even in well-watered soils, causes water deficit by reducing the osmotic potential of solutes in the soil, thus making it difficult for roots to draw water from the surrounding medium (Rhoades, 1988; Izadi et al., 2014). According to FAO's 2018 world soil salinity data, the soil salinity rate is increasing and it is thought that the salinity increase rate will reach up to 50% by 2050 (Kang et al., 2010; Tiryaki, 2018; Demirkol et al., 2019).

It was reported that Australia, China, Egypt, India, Iraq, Iraq, Mexico, Pakistan, Russia, and Syria as well as our country are facing salt stress (Rhoades, 1988). Approximately 1.5 million hectares of our country are under salinity stress. Of the 230 million hectares of irrigated land, 45 million hectares are affected by salt

stress. It is thought that a salt problem of this size will cause great economic losses in parallel with the loss in crop yield and quality (Bu et al., 2015; Dogru and Canavar, 2020). Salinity is naturally present in arid and semi-arid climate zones, and insufficient rainfall and high evaporation, poor drainage, improper agricultural practices and soil properties induce the salinity problem (Roy et al., 2014; Tiryaki, 2018; Zambi and Aşcı, 2020).

High salinity causes both ionic and osmotic stresses, leading to secondary stresses such as oxidative stress and nutritional disorders. Moreover, with increasing salt concentration in the soil, plant water uptake becomes difficult and plant growth slows down due to the deterioration of soil structure (Arslan et al., 2013; Bu et al., 2015).

There are also different plant mechanisms against salt stress such as osmotic effect, ion excretion and tissue tolerance. Osmotic effect decreases the water availability of plants as a result of increased salt concentration in the soil. The ion excretion mechanism reduces the accumulation of toxic salts in the leaves. Tissue tolerance is the growth retardation observed in plants in the face of salt stress (Arslan et al., 2013; Roy et al., 2014; Tiryaki, 2018).

Plants produce secondary metabolites as a defense mechanism against pathogens and insects. These metabolites can also be formed under different environmental stress conditions (e.g. salinity) and they contain the majority of plant antioxidants. Phenolic compounds are one of the secondary metabolites produced in plant tissues to scavenge free radicals and/or inhibit their production through hydroperoxide decomposition (Mohamed and Aly, 2008; Boughalleb et al., 2020). Salinity, one of the abiotic stress factors, increases the synthesis of phenolic compounds such as phenolic acids, flavonoids, proanthocyanins, and anthocyanins in plants (Tetiktabanlar et al. 2020). Phenolic compounds can chelate heavy metals with hydroxyl and carboxyl groups. They can prevent lipid peroxidation by capturing lipid alkoxy radicals (Michalak, 2006). Many studies have shown that peroxidase and polyphenol oxidase enzymes involved in the synthesis of phenolic compounds increase under biotic and abiotic stress conditions (Ruiz et al., 2003). Kıpçak et al. (2019) found that total phenolic content in green parts of bean cultivars treated with different concentrations of salt decreased significantly compared to control plants. In addition, reactive oxygen species are formed in plants under oxidative stress in saline conditions. These reactive oxygen species cause serious problems in plants such as inactivation of proteins and enzymes, injury to plant metabolism, change in the structure of photosynthetic components, and lipid peroxidation (Amirjani, 2010). Phenolic compounds neutralize these reactive oxygen species thanks to their antioxidant activities (Kıpçak et al., 2019).

This study aimed to evaluate the morphological and biochemical responses of two different Turkish forage pea cultivars to salinity stress induced by Na_2SO_4 and $CaCl_2$ salts during early growth.

2. Materials and Methods

2.1. Material

In this study, Töre and Ateş forage pea cultivars developed by Namık Kemal University constituted the material of the experiment. The study was carried out as a pot experiment at Bilecik Şeyh Edebali University, Faculty of Agriculture and Natural Sciences.

Soil picked up a depth of 0-30 cm was used as the growing medium. The 4 kg of soil was dried and passed through a 4 mm sieve before being filled into plastic pots measuring 8×8×9 cm in size and 4 cm in height. Twenty-five seeds of each genotype were sown in pots at a depth of 2.5 cm and watered with solutions. The pots were then placed in a greenhouse to germinate and grow.

2.2. Method

2.2.1. Salt concentrations of irrigation water

Four different concentrations of Na_2SO_4 and $CaCl_2$ (0, 50, 100 and 150 mM) solutions were used in the study.

2.2.2. Morphological measurements

The roots and shoots of the plants cultivated in pots were trimmed using a scalpel after the third week. The lengths of the roots and shoots were assessed using a caliper. Each root and shoot were weighed by analytical balance to determine their fresh weights. Then, each root and shoot were subjected to drying in an oven at 65°C for 48 hours, and their dry weights were determined gravimetrically.

2.2.3. Extraction of plant fractions

In the extraction step, 1-2 grams of dried sample were weighed and extracted with 30 mL of pure methanol for 1.5 hours in an ultrasonic bath (Bandelin Sonorex Digiplus). The samples were filtered through a silk sieve into an amber bottle and stored under refrigerated conditions until analysis.

2.2.4. Determination of total phenolic content with folin ciocalteu metod

The Folin-Ciocalteu method, a colorimetric method for total phenolic analysis, has been modified (Shahidi et al., 2001). First, 1 mL of the extracts was taken and mixed with 1 mL of Folin-Ciocalteu solution. Then, 3 mL of 20% Na_2CO_3 was added and the resulting mixture was vortexed and kept at 25 °C in the dark for 2 hours. Absorbance values were measured in a UV-spectrophotometer at 765 nm against pure methanol as a blind. Total phenolic contents were expressed in terms of gallic acid equivalent, (GAE) (standard curve equation: $y = 14.574x + 3.5652$, $R^2 = 0.9898$), mg of GAE g of extract. The experiment was repeated two times at each concentration.

2.2.5. Determination of DPPH radical scavenging activity

Free radical activities were determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH), which is a known free radical (Ayaz Seyhan, 2019). For the determination of DPPH radical scavenging activity, the concentration was

prepared by dissolving 0.004 mg DPPH in 100 mL methanol. Dilutions were prepared from the extracts in different concentrations from stock solution. For each sample, 4 mL of DPPH radical and 1 mL of extract solutions of different samples were added. After incubating in the dark for 30 minutes at room temperature, absorbance was measured at 517 nm (blind MeOH) on a spectrophotometer device. The calibration curve was expressed in mg mL^{-1} with the equation: y = 0.0539x -0.0168, R^2 = 0.9976. The DPPH stock solution was prepared with a concentration of 0.004 mg mL^{-1}. Calibration curve evaluation concentrations were selected between 0.0005 and 0.004 mg mL^{-1}.

2.2.6. Statistical analysis

The data obtained in the study were analyzes by Jump Statistical Analysis and the differences between the averages were determined with the Duncan Multiple Comparison Test. The correlations of the characteristics and path values were also calculated (Sokal and Rohlf, 1981).

3. Results

The shoot fresh weight decreased with increasing in salt concentration of irrigation water, irrespective of the salt type and the pea cultivar. Compared to the control group, the highest decrease was determined at 150 mM $CaCl_2$ concentration in both cv. Ateş and cv. Töre (Figure 1). As a result of the increase in $CaCl_2$ concentration, the shoot fresh weight in the control group was 0.55-0.59 g, while at 150 mM it was 0.35-0.41 g. With the increase in Na_2SO_4 in irrigation water, the shoot fresh weight in the control group was 0.50-0.57 g, while it was 0.39-0.45 g at 150 mM.

The root fresh weight decreased with increasing salt concentration regardless of salt type and forage pea cultivar. When compared with the control group, the highest decrease was determined in 150 mM $CaCl_2$ concentration in both Ateş and Töre forage pea cultivars, cv. Ateş and cv. Töre (Figure 2). As a result of the increase in $CaCl_2$ concentration, the root fresh weight in the control group was 0.08-0.09 g, while it was 0.040-0.045 g at 150 mM. As a result of the increase in $CaCI_2$ concentration, the highest decrease in root weight was observed in the cv. Töre. While the root fresh weight of the control group was 0.09 g, it was 0.04 g at 150 mM.

There was no significant change in shoot dry weight depending on salt type and concentration. Compared to the control group, both Ateş and Töre forage pea cultivars, cv. Ateş and cv. Töre showed a decrease at 150 mM concentrations (Figure 3). As a result of the increase in $CaCI_2$ concentration, shoot dry weight in the control group was 0.045-0.048 g, while it was 0.039-0.047 g at 150 mM. As a result of the increase in Na_2SO_4 concentration, the shoot dry weight of fever forage pea decreased the most. While the shoot dry weight of the control group was 0.045 g, it was 0.039 g at 150 mM.

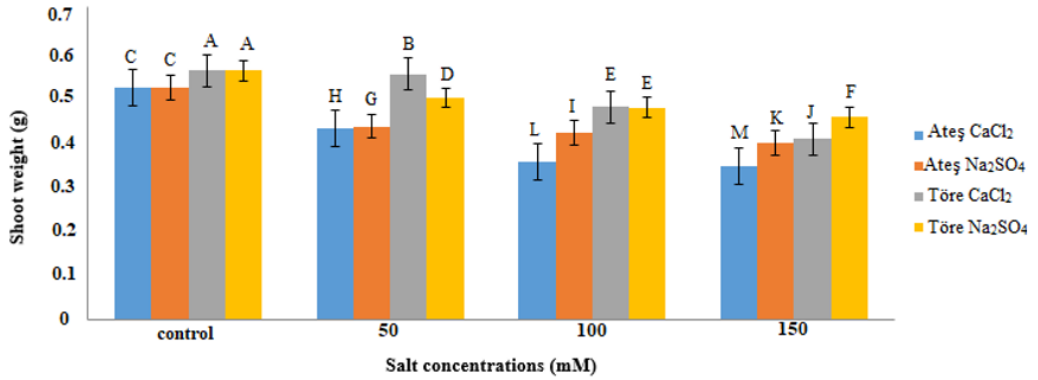

Figure 1. Change in shoot weight of Ateş and Töre forage pea varieties depending on $CaCl_2$ and Na_2SO_4 concentrations in irrigation water.

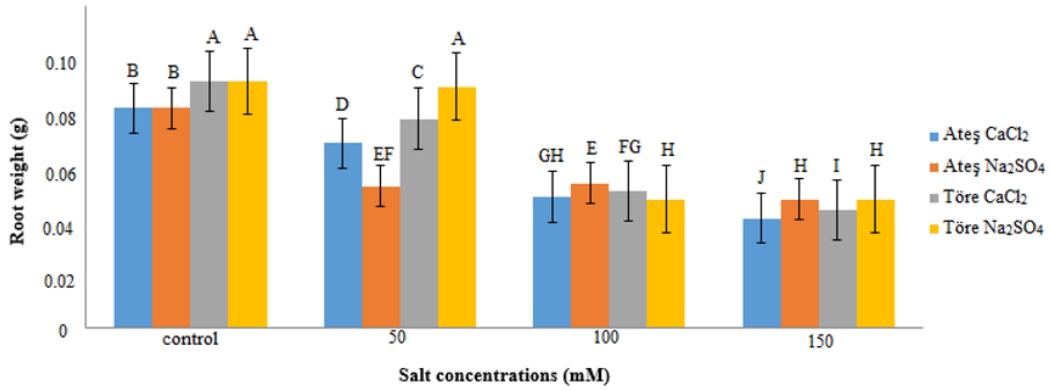

Figure 2. Change in root weight of Ateş and Töre forage pea varieties depending on $CaCl_2$ and Na_2SO_4 concentrations in irrigation water.

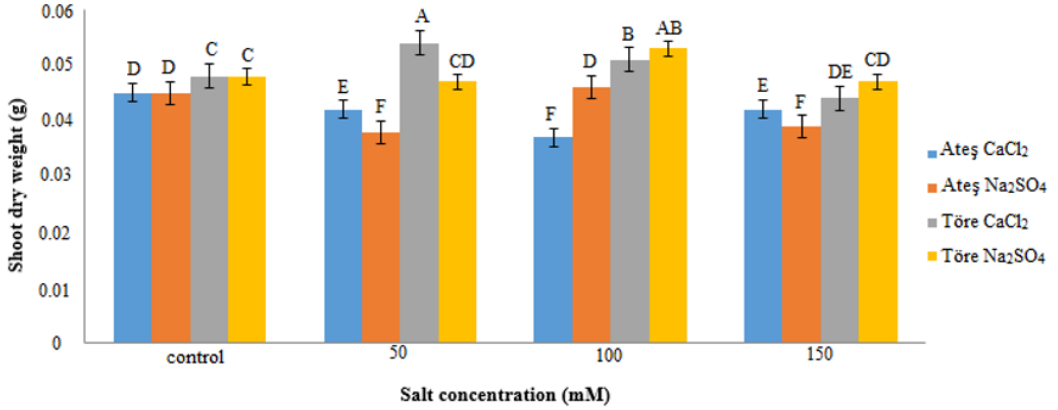

Figure 3. Change in shoot dry weight of Ateş and Töre forage pea varieties depending on $CaCl_2$ and Na_2SO_4 concentrations in irrigation water.

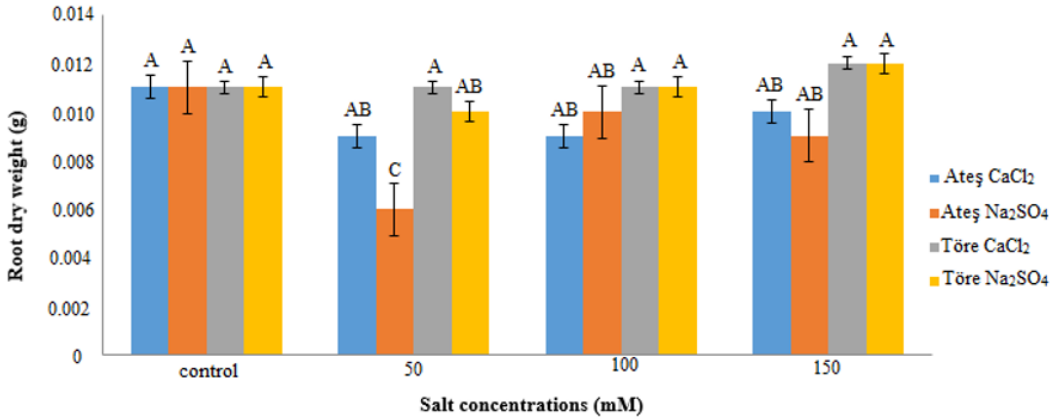

Figure 4. Change in root dry weight of Ateş and Töre forage pea varieties depending on $CaCl_2$ and Na_2SO_4 concentrations in irrigation water.

There was no significant change in root dry weight depending on salt type and concentration. Compared to the control group, both Ateş and Töre forage pea cultivars, cv. Ateş and cv. Töre showed a significant change at 150 mM (Figure 4). The highest decrease in root dry weight was estimated in 50 mM Na_2SO_4. While the shoot dry weight of the control group was 0.011 g, it was 0.006 g at 50 mM. When we compared the change in root dry weight with the control group, it was 0.011 g; while it was 0.010-0.012 g at 150 mM salt concentration.

Shoot length decreased with increasing in salt concentration regardless of salt and forage pea cultivar. This decrease was clearer in 100 mM $CaCl_2$ concentration. When compared with the control group, the highest decrease was determined at 150 mM $CaCl_2$ concentration in both Ateş and Töre forage pea cultivars (Figure 5). As a result of the increase in $CaCl_2$ salt concentration, the shoot length in the control group was 41-43 cm, while it was 29 cm at 150 mM. In Na_2SO_4 irrigation water, the shoot length in the control group was 40-43 cm, while it was 32-34 cm at 150 mM.

Root length generally decreased with increasing in salt concentration regardless of salt type and forage pea cultivar. When compared with the control group, the highest decrease was determined in 150 mM Na_2SO_4 concentration in both forage pea cultivars (Figure 6). As a result of the increase in Na_2SO_4 concentration, root length in the control group was 7.6-6.8 cm, while it was 4.2-4.8 cm at 150 mM. With respect to $CaCI_2$ in irrigation water, the root length of the control group was 6.8-7.5 cm, while it was 5.8-6.2 cm at 150 mM. Kayın et al. (2022), an increase in root length was estimated in cv. Töre at 100 mM Na_2SO_4 concentration.

When the total antioxidant activity of forage peas against salt stress were estimated, the total antioxidant activity increased with the increase in salt concentrations. When compared with the control group, the highest increase was determined in 150 mM $CaCl_2$ concentration in both forage pea varieties (Figure 7). As a result of the increase in $CaCl_2$ concentration, the total antioxidant activity in the control group was 0.08-0.09 mg mL^{-1}, while it was 0.16-0.18 mg mL^{-1} at 150 mM. As a result of the increase in Na_2SO_4 irrigation water concentration, while the total antioxidant activity in the control group was 0.08 mg mL^{-1}, it was determined as 0.14-0.16 mg mL^{-1} at 150 mM.

When the total antioxidant activity of forage peas against salt stress were determined, total phenolic content also increased with the increase in salt concentrations. When compared with the control group, the highest increase was determined in 150 mM $CaCl_2$ concentration in both cv. Ateş and cv. Töre (Figure 8).

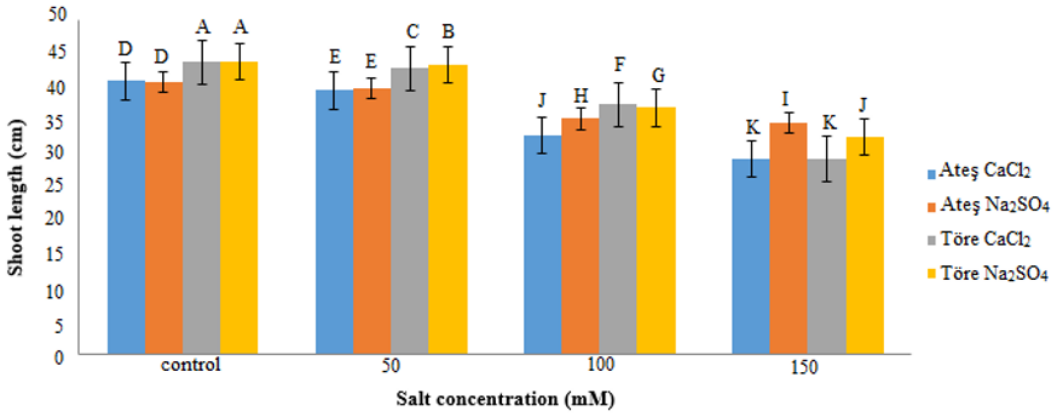

Figure 5. Change in shoot length of Ateş and Töre forage pea varieties depending on $CaCl_2$ and Na_2SO_4 concentrations in irrigation water.

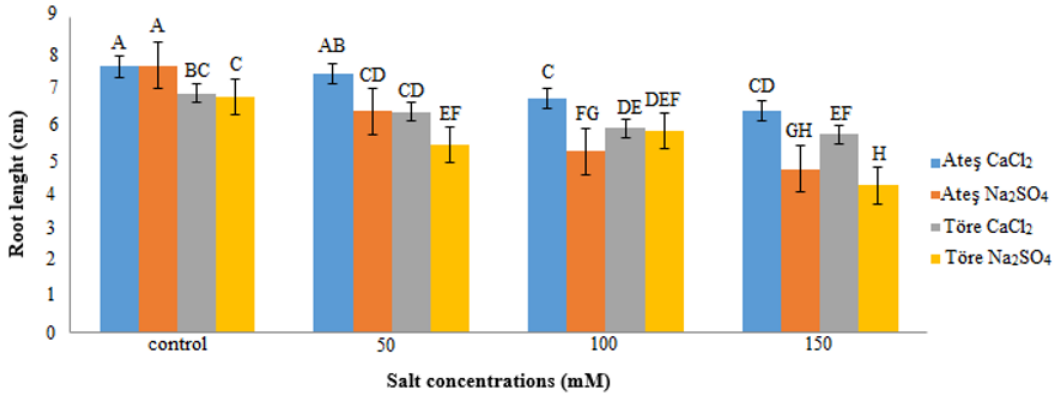

Figure 6. Change in root length of Ateş and Töre forage pea varieties depending on $CaCl_2$ and Na_2SO_4 concentrations in irrigation water.

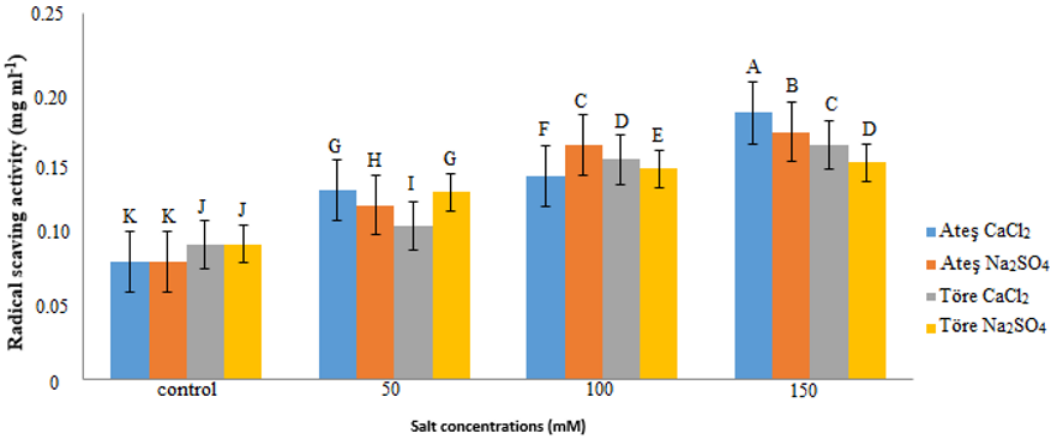

Figure 7. Change in total antioxidant activity of Ateş and Töre forage pea varieties depending on $CaCl_2$ and Na_2SO_4 at different concentrations.

In general, as a result of the increase in concentration, the total antioxidant activity in the control group was 0.001-0.03 mg GAE g^{-1}, while 0.002-0.005 mg GAE g^{-1} was determined at 150 mM. The highest increase in total phenolic content was observed when Töre forage peas were irrigated with 150 mM $CaCI_2$ solution.

In principal component analysis (PCA), the first component contains the most change, then the most variance is associated with the second component, and the last component has the least variance. PCA is a method often used to group cultivars, better interpret relationships, and determine the contribution of traits to total diversity. The percentage variance of each factor expresses the importance of that feature in interpreting general changes in the data.

In this analysis, two independent factors explained a total of 81.84% of the data changes. The first factor explained 51.45% of the total data variance and its eigenvalue was 4.12. This factor includes shoot fresh weight, root fresh weight, shoot length, and root length (Figure 9). The variance of the second factor is 30.39% and its eigenvalue is 2.43. Shoot dry weight, root dry weight, DPPH radical scavenging activity and Total Phenolic properties are the second factors.

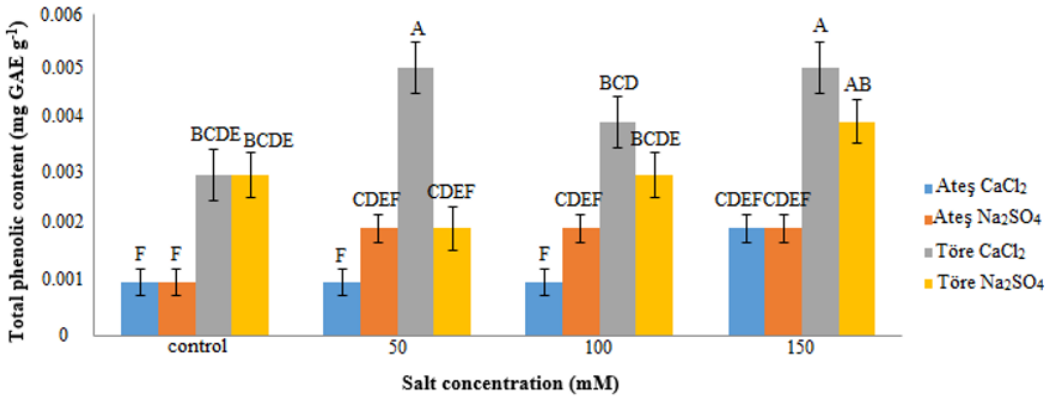

Figure 8. Change in total phenolic content of Ateş and Töre forage pea varieties depending on $CaCl_2$ and Na_2SO_4 at different concentrations.

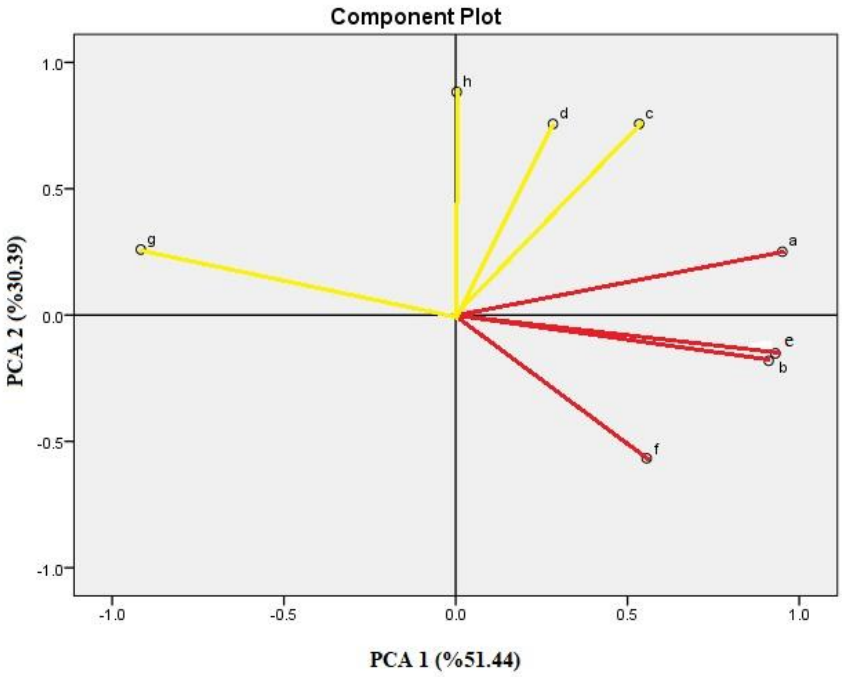

Figure 9. Distribution of investigated characters across the first two components based on PCA. a= shoot fresh weight, b= root fresh weight, c= shoot dry weight, d= root dry weight, e= shoot length, f= root length, g= total antioxidant activity, h= total phenolic content.

4. Discussion

In the study, a decrease in shoot fresh weight was observed as a function of salt concentration (Figure 1). Kayın et al. (2022) examined the effect of eight different NaCl concentrations on forage peas in their studies. They revealed that shoot fresh weight decreased in forage pea cultivars due to the increase in salt concentration. In addition, Avcı et al. (2018), Tsegay and Andargie (2018) and Demirkol et al. (2019) reported that the fresh weight of shoots decreased due to increasing salinity in pea cultivars. The effect of NaCl was evaluated in these studies. Similar results were observed when $CaCl_2$ and Na_2SO_4 were applied at increasing concentrations.

A significant decrease in root fresh weight was also observed, especially when $CaCl_2$ and Na_2SO_4 were applied at 100 mM concentration (Figure 2). Kayın et al. (2022) revealed that root fresh weight decreased in forage pea cultivars due to the increase in salt concentration.

There was no significant change in shoot dry weight depending on salt type and concentration (Figure 3). Kayın et al. (2022) observed fluctuations in dry weight of shoots at different salt concentrations. NaCl 200 mM NaCI concentration did not show a significant change compared to control conditions. The results obtained are consistent with other studies. Avcı et al. (2018), Tsegay and Andargie (2018) and Demirkol et al. (2019) reported that shoot dry weights decreased in pea cultivars due to increased salinity.

No significant change was observed in root dry weight compared to the control group (Figure 4). Kayın et al., (2022) also observed a decrease in root dry weight with increasing NaCl salt concentration. In addition, no change in root dry weight was observed in cv. Urunlu and cv. Nany up to 175 mM salt concentration. In other forage

pea cultivars, various fluctuations were observed in root dry weight values depending on salt concentration. Various fluctuations were also observed in our study (Figure 4).

Shoot length decreased with increasing salt concentration regardless of salt type and forage pea cultivar. This decrease was clearer in 100 mM $CaCI_2$ concentration (Figure 5). Kayın et al., (2022) observed a decrease in shoot length with increasing NaCl concentration in their studies. The highest decrease in shoot length was observed in the cv. Guifredo, cv. Kurtbey and cv. Özkaynak. They also determined the critical salt concentration as 150 mM NaCI.

Root length generally decreased with increasing salt concentration regardless of salt and forage pea cultivar. Compared to the control group, the highest decrease was determined at 150 mM Na_2SO_4 in both forage pea cultivars (Figure 6).

When the total antioxidant activity and total phenolic content of forage pea were estimated against salt stress, total phenolic contents and total antioxidant activity increased with the increase in salt concentrations (Figure 7 and Figure 8). Kara et al., (2019) revealed that the total phenolic content and total antioxidant activity of *Echinacea purpurea* L. increased under salt stress. Boughalleb et al., (2020) revealed that the total phenolic content and antioxidant activity of *Polygonum equisetiforme* plants under different salt concentrations increased especially up to 300 mM.

5. Conclusion

It was determined that the increase in the concentrations of $CaCl_2$ and Na_2SO_4 salts increased the negative effect on the morphological characteristics of different cultivars of both forage peas used in the research. On the other hand, an increase in total phenolic content and antioxidant activity values was observed in secondary properties. This is thought to be due to the increase in the defense mechanism of forage peas against salt stress. Investigation of bound phenolic substances (flavonoids, tannins, etc.) as well as free phenolic substances and the use of different forage pea cultivars will enable a more comprehensive evaluation of the results.

Author Contributions

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

	N.K.	A.A.B.	F.T.
C	100		
D	100		
S	50	25	25
DCP	80	20	
DAI	50		50
L	100		
W	60	20	20
CR	50	25	25
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.

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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Research Article
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EFFICACY OF *BEAUVERIA BASSIANA* (BALS.) VUILL. ISOLATES ON DRIED FRUIT MOTH (*PLODIA INTERPUNCTELLA* [LEPIDOPTERA: PYRALIDAE])

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Abstract: The dried fruit moth, *Plodia interpunctella* (Hübner, 1813) (Lepidoptera: Pyralidae) is one of the most important pests of both dried fruits and stored grains and products. One of the alternative control methods to chemicals in the control against this pest is the use of biological control methods. Entomopathogenic fungi (EPF) stand out because they do not have any negative effects on the environment, living organism and human health, other than the target pests. In this study, ET 10 and Bb 18 isolates of *Beauveria bassiana* (Bals.) Vuill. were applied to the 4th instar larvae of *P. interpunctella* under laboratory conditions and their effectiveness was determined. EPF isolates were sprayed to the larvae in plastic petri dishes at a concentration of $1x10^8$ conidia/ml. The experiments were carried out in a randomized plots experimental design with five replicates, with five 4th instar larvae in each petri dish. After the applications, the number of live larvae was recorded by counting the 1st, 3rd, 5th and 7th days and the % mortality rate was calculated. On the fifth day of the experiment, mortality rates of 92% were recorded for the ET 10 isolate of *B. bassiana* and 84% for the Bb 18 isolate. In the seventh day counts, 100% mortality rates were determined for both isolates of *B. bassiana*. As a result, it is concluded that *B. bassiana* may have a potential effect in the biological control of stored product pests.

Keywords: *Beauveria bassiana*, *Plodia interpunctella*, Mortality rate, Biological control, Stored product

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1. Introduction

Türkiye has an important place in the world production and export of cereals, pulses, oilseeds, hazelnuts, dried fruits and their products. Among these, walnut (*Juglans regia* L.) is a hard-shelled fruit species with high commercial value that is grown in temperate regions around the world for timber and nuts (Ajazi et al., 2014; Pollegioni et al., 2015). Walnut is one of the most important sources of energy with the protein, fats and minerals (Mir and Kottaiveeran, 2018).

The production of walnut, one of the high-yielding crops in horticultural, has become quite widespread in the world with increasing demand. While 3.3 million tons of shelled walnuts are produced in an area of 13 million decares in the world, Türkiye ranks 4th with approximately 287.000 tons of walnut production. While China (2.8 million da) and the USA (1.5 million da) rank first in the world walnut production area, Türkiye ranks 3rd with a production area of 1.4 million da (FAO, 2022).

Agricultural crops must be protected and stored with minimal losses in both the domestic and foreign markets, from the harvesting process to the consumption process. Biotic and/or abiotic stress factors affect the storage process of these crops. Biotic factors that cause product loss in stored crops include microorganisms, rodents, mites and insects. These pests cause direct and indirect damage by infecting stored crops (Kahraman, 2009). *Plodia interpunctella* (Hübner, 1813), the dried fruit moth, is a pyralid moth belonging to the order Lepidoptera, family Pyralidae. *P. interpunctella* is an economically important pest of stored crops worldwide (Sedlacek et al., 1996). *P. interpunctella* larvae feed and web intensively in the environments in which they feed, causing losses in crop quality due to the wastes and residues they produce (Yıldırım et al., 1997; Boxall, 2001). The most harmful crops include dried apricots, figs, raisins, hazelnuts, chestnuts, walnuts, pistachios, peanuts, almonds and carob, etc. As can be seen, *P. interpunctella* does not exhibit food selectivity. This makes dried fruit moth infestations inevitable in many food products. The aim of protecting stored crops against *P. interpunctella* is the integrated application of cultural, mechanical, chemical, physical, biotechnical, biological and quarantine measures. However, "fumigation", a chemical control method, is widely used against stored crop pests in the world and in our country (Fields and White, 2002; Işıkber et al., 2015). Methyl bromide (MeBr) and phosphine are primarily used in fumigation

applications. MeBr, which is widely used in the control against stored crop pests, is banned all over the world due to its ozone depleting effect. Due to the ban on the use of MeBr and its negative effect on the environment, the necessity of researching alternative methods to replace MeBr has become increasingly important. In this context, biological control stands out as the most basic method in terms of human-environmental health, agricultural and environmental sustainability, and ecological balance. The use of microbial insecticides with specific effects has an important place among alternative and safe methods in biological control (Pszczola, 1997; Demirezen, 2010).

Entomopathogenic fungi (EPF) are among the most common disease-causing pathogens in agricultural and forest pests (Mueller and Schmit, 2007). Unlike other pathogens, EPFs are used as important control agents in the stages when the insect is not feeding (last larva and pupa), since they infect the pest through the integument. Since insects that die from entomopathogenic fungal infection mostly fall into the soil from the plant they are on the soil environment creates an important fungal reserve and protects fungal spores from abiotic and biotic factors, allowing them to maintain their viability for a long time (Gök et al., 2018). *Beauveria* spp. are entomopathogenic fungi (EPF) that can be easily isolated and produced from almost all ecosystems (Rehner et al., 2011). Entomopathogenic fungi (EPF) have been used as microbial control agents for more than 100 years (Roberts, 1989). Nowadays, *Beauveria bassiana* (EPF) (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) is reported to have more than 700 hosts (Wraight et al., 2000). These hosts are contained in 521 genera, 149 families and 15 orders (Zimmermann, 2007). *B. bassiana* is an entomopathogenic fungus used in biological control against both harmful insects and plant pathogens and causes White Muscardine disease (Feng et al., 2004). *B. bassiana* produces the toxins Beauvericin and Bassianin, Bassianolide, Beauverolides, and Tennellin. These toxins kill the host by dissolving the tissues, degradation the cells, and re-germinating exiting the host body, forming the conidia and restoring the life cycle of the fungus (Sabbour, 2002). *B. bassiana* has been tested with success against various stored-product insect species in both laboratory and field trials (Lord, 2001; Padin et al., 2002; Akbar et al., 2004; Jyothi et al., 2014). Among the identified entomopathogenic fungi, *Beauveria bassiana*, *Lecanicillium* (*Verticillium*) *lecanii*, *Metarhizium anisopliae* var. *anisopliae* and *Paecilomyces farinosus* are used against dry fruit moth (Büda and Peciulyte, 2008). Faria and Wraight (2007) determined approximately 40% of the total mycoinsecticides were based on *Beauveria* spp., although many products are no longer available in the biopesticide market. Entomopathogenic fungi have important advantages such as being non-toxic to human and environmental health, having a wide host range, not developing resistance in hosts, being applied together with pesticides, being cheap, and easy to apply (Sinha et al., 2016).

The aim of this study is to determine the efficacy of ET 10 and Bb 18 isolates of *Beauveria bassiana* by applying ET 10 and Bb 18 isolates against the fourth instar larvae of *P. interpunctella* under laboratory conditions.

2. Materials and Methods

2.1. Fungi Cultures and Preparation of Spore Suspensions

Beauveria bassiana ET 10 isolate was isolated from *Sphenoptera antiqua* (Illiger, 1803) (Coleoptera: Buprestidae) in Erzurum province, Türkiye (Tozlu et al., 2017), and Bb 18 isolate was isolated from field soil in Düzce province, Türkiye (Erdoğan and Sağlan, 2023). *B. bassiana* isolates were grown on potato dextrose agar medium (PDA-39 g/l, Difco) in the dark at 25±1°C for 14 days and stored at +4°C in the refrigerator.

PDA medium was prepared and sterilized in autoclave at 121°C for 15 minutes. Approximately 25 ml of the PDA was poured into 90 mm petri dishes and allowed to cool and solidify. Mycelium plug (5 mm in diameter), taken from the leading growth edge of a 14-day-old culture of *B. bassiana* ET 10 and Bb 18 isolates grown on PDA, was placed in the center of a petri dish in a laminar flow cabinet. Parafilm-sealed petri dishes were incubated in the dark at 25±1°C for 14 days. The conidia were harvested by scraping the surface of 14 days old culture gently with inoculation needle. The conidia were suspended in distilled water containing 0.1% Tween80. The mixture was stirred by a magnetic shaker for 10 min. In order to calculate the spore density from the prepared suspension, a 10^{-2} dilution was made and counted with the help of Thoma slide under light microscope, and spore suspensions with a density of $1x10^8$ conidia/ml were prepared for each *B. bassiana* isolate (Fancelli et al., 2013).

2.2. Rearing of Tested Insect

Individuals of *P. interpunctella* were cultured in the climate cabinet (25±1°C temperature, 60±5% RH and dark conditions) in the Entomology laboratory within the Department of Organic Farming Business Management, Faculty of Applied Sciences, Pamukkale University. The nutrient medium consisted of a mixture of bran, corn flour, dry yeast, honey, milk powder and glycerin in a ratio of 2:1:0.25:0.25:0.50:0.25:0.25 respectively (Ozkan, 2006). The prepared food was taken into glass jars (1 liter) and the larvae of the pest were transferred onto the food. The mouth of the jars was covered with tulle to ensure air entry and prevent other pests from entering. In this way, the development of the pest in the nutrient medium was ensured and fourth instar larvae to be used in the experiment were obtained.

2.3. Bioassay Test

Fourth instar larvae of *P. interpunctella* were reared on their artificial diet at 25±1°C with a photoperiod of 14:10 (L:D) h, 60±5% RH in a climate cabinet. In the experiment, ET 10 and Bb 18 isolates of *B. bassiana* were applied to *P. interpunctella* larvae in plastic petri dishes

(90 mm) at a concentration of $1x10^8$ conidia/ml by spraying method. As a control, only pure water was sprayed on the pest. The experiments were carried out in a randomized plot design with five replications, with five fourth instar larvae in each petri dish. The number of live larvae was recorded by counting the 1st, 3rd, 5th and 7th days after the treatments and the % mortality rates were calculated.

2.4. Statistical Analysis

The data obtained in this study were subjected to analysis of variance (ANOVA) and the differences between the means were compared using Tukey's multiple comparison test at the P≤0.05 significance level (Tukey, 1949). Data analysis was performed using IBM® SPSS® Statistics software (Version 20.0, August 2011, SPSS Inc., Chicago, IL, USA) statistical package program. Additionally, LT_{50} values were determined by the Probit analysis program (Throne et al., 1995).

3. Results and Discussion

The mortality rates of *B.bassiana* ET 10 and Bb18 isolates to the fourth instar larvae of *P. interpunctella* by spraying method are given in Figure 1. In the experiment, except for the first counting day, % mortality rates increased depending on the treatment days. In the first day counts, mortality rate was not recorded. In the third day counts, a 40% mortality rate was recorded for the *B. bassiana* ET 10 and Bb 18 isolates. In the fifth day counts, a 92% mortality rate was recorded for the *B. bassiana* ET 10 isolate and an 84% mortality rate for the Bb 18 isolate. On the seventh day counts, a 100% mortality rate was recorded for both isolates applied. Considering the time-dependent mortality rates of *P. interpunctella* fourth instar larvae, the LT_{50} value, which indicates the time required for half of the *P. interpunctella* larvae to die, was calculated as 3.27 and 3.37 days for ET 10 and Bb 18 isolates, respectively (Figure 2 and 3).

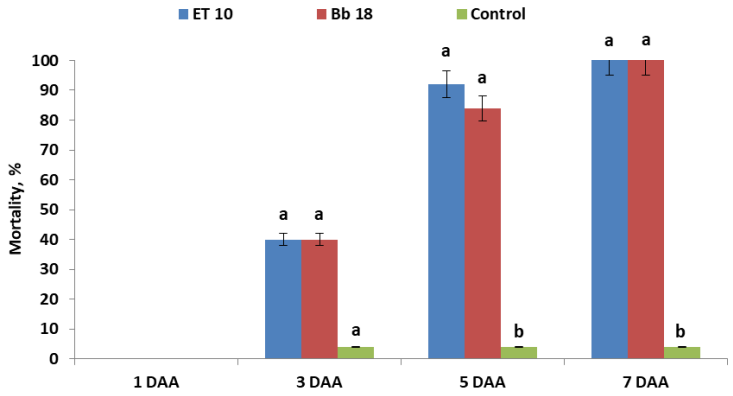

Figure 1. Percent mortality rates resulting from the application of *B. bassiana* ET 10 and Bb 18 isolates to *P. interpunctella* larvae. (The differences between the means (±standard error) of the columns indicated with different letters for each day are statistically significant (Tukey's HSD test P (Tukey's HSD test P<0.05)). DAA= days after application ($F3^{th}$=3.115, $df3^{th}$=2, 12, $P3^{th}$=0.081; $F5^{th}$=52.235, $df5^{th}$=2, 12, $P5^{th}$=0.000; $F7^{th}$=576.000, $df7^{th}$=2, 12, $P7^{th}$=0.000).

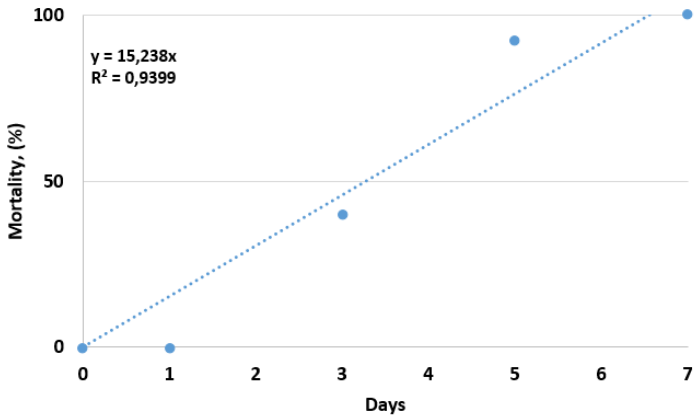

Figure 2. LT_{50} value obtained as a result of application of *B. bassiana* ET 10 isolate.

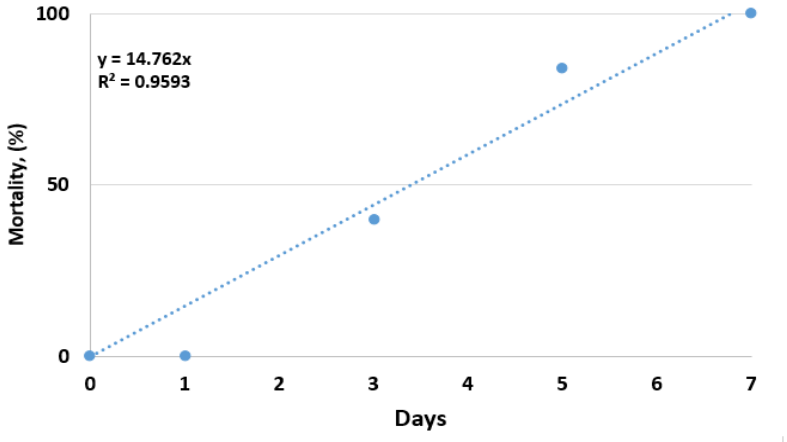

Figure 3. LT_{50} value obtained as a result of application of *B. bassiana* Bb 18 isolate.

According to literature searches, while there are studies on the effect of *B. bassiana* against storage pests in Türkiye no studies on the effect of *B. bassiana* against *P. interpunctella* have been found. There are few studies on this subject in the world. In a study by Büda and Peciulyte (2008) *B. bassiana*, *L. lecanii*, *M. anisopliae* var. *anisopliae* and *I. farinosa* entomopathogenic fungi (2.6×10^6 conidia/ml) were tested against late-stage larvae of the dried fruit moth, and the highest mortality rates for *I. farinosa* and *M. anisopliae* were recorded in the range of 35-40% at the end of the third day of the experiment. *B. bassiana* and *L. lecanii* were in the same group as the control. In another study, LC_{50} values were recorded as 138, 144 and 198, respectively, as a result of the application of *B. bassiana*, *M. anisopliae* and *I. fumosorose* isolates at 10^7 conidia/ml concentrations to the third instar larvae of *P. interpunctella* (Sabbour et al., 2012). Sedehi et al. (2014) found that there was a significant difference between the concentrations of *B. bassiana* spores on the eggs and larvae of *P. interpunctella* and determined the LC_{50} value as 7.4×10^7 conidia/ml for eggs and 6.6×10^6 conidia/ml for larvae. In a study similar to our results from the experiment, mortality rates were recorded as a result of applying *B. bassiana* (isolates TV and OZ1) and *M. anisopliae* (isolate CS1) isolates to the third instar larvae of *P. interpunctella* at a concentration of 1×10^8 conidia/ml. It has been reported that especially in these entomopathogenic fungal isolates, protease and lipase have a significant effect on the larvae (Golzan et al., 2023).

5. Conclusion

As a result of the study, ET 10 and Bb 18 isolates of *B. bassiana* isolated from different hosts showed that they could be effective in the control against storage pests, especially *P. interpunctella.* However, the control of storage pests depends not only on the characteristics of *B. bassiana* isolates, but also on biotic and abiotic factors. In addition, more detailed studies should be carried out under storage conditions in terms of the efficacy of promising isolates and pest management.

Author Contributions

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

	A.B.E.	O.E.	M.S.S.
C	50	50	
D	50	50	
S	50	50	
DCP	50		50
DAI	100		
L	50		50
W	50	50	
CR	50	50	
SR	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.

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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