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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 seven 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	31	0.86	59	37	0.63
2020	49	51	1.04	108	88	0.81
2021	23	103	4.48	131	191	1.46
2022	72	150	2.08	203	341	1.68
2023	108	229	2.12	311	570	1.83
2024	103	284	2.76	414	854	2.06

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

*: according to Scholar Google

Rejection rate:

- 2020: 18%
- 2021: 13%
- 2022: 25%
- 2023: 08%
- 2024: 17%

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Research Article
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ECONOMIC EVALUATION OF BROILER DIETS SUPPLEMENTED WITH EITHER SELECTED HERBS OR THEIR ASSOCIATED ESSENTIAL OILS

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Abstract: In recent years, there has been a growing interest in incorporating phytogenic feed additives (PFAs) into broiler chicken diets as potential alternatives to traditional growth-promoting additives. This study evaluated the economics of individually incorporating either six different dried herbs or their essential oils into broiler diets: chamomile, rosemary, lavender, oregano, thyme, and St. John's wort. A total of 390 day-old male broiler chicks (Ross 308) were randomly divided into 13 groups of 30 chicks with three replicates (10 chicks/replicate). One group received a basal diet (control group), while the others received a basal diet supplemented with 2% of each dried herb (E1-E6 groups) or 0.02% of their essential oils (E7-E12 groups) for 39 days. The parameters measured were feed intake, body weight gain, feed conversion ratio, feed costs, economic efficiency and European Broiler Index (EBI). The results showed better economic efficiency with 2% dried St. John's wort herb, as well as 0.02% St. John's wort, rosemary, thyme or lavender essential oils compared to the other treatments ($P<0.05$), but not compared to the control group ($P>0.05$). Unsatisfactory results were observed with dry lavender herb and essential oils of chamomile or oregano, which resulted in a significant decrease in net income and economic efficiency due to higher feed costs per kilogram live weight ($P<0.05$). EBI values were not significantly increased in any of the treated groups compared to the control group ($P>0.05$). These results suggest that while certain PFAs can improve economic efficiency, their overall effect is variable and some may not outperform traditional growth promoters.

Keywords: Broiler performance, Phytogenic feed additives, Herbs, Essential oils, Economic efficiency

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1. Introduction

The global poultry industry is continuously searching for sustainable and economically viable strategies to enhance the efficiency of broiler production. Over the years, synthetic products, including antibiotic growth promoters, have been extensively used in broiler production to improve growth rate, feed efficiency, prevent diseases, and reduce mortality (Abd El-Hack et al., 2022; Mohamed and Hassan, 2023). However, increasing concerns about the emergence of antibiotic-resistant pathogenic bacteria, as well as antibiotic residues in poultry products, have led to the exploration of alternative, safe, and cost-effective additives that can maintain or even improve production performance without compromising the birds' health, the quality of poultry products, human health, and the environment (Alagawany et al., 2021).

Phytogenic feed additives (PFAs), derived from plant sources with bioactive compounds like aromatic and medicinal plants, their extracts, or essential oils, have gained attention due to their numerous biological and beneficial properties (Puvaĉa et al., 2015; Hassan and Awad, 2017; Giannenas et al., 2018; ; Kadhim, 2018; Singh et al., 2018; Jin et al., 2020). Previous studies indicate that the inclusion of PFAs in diets significantly impact various aspects of broiler performance. It has been reported to stimulate appetite and feed intake, promote the release of digestive enzymes, enhance nutrient utilization, improve gut morphology, modulate the immune system, as well as enhance resilience to heat stress (Omar et al., 2016; Galli et. al., 2020; El-Ashram and Abdelhafez, 2020; Moustafa et al., 2020; Alagawany et al., 2021; Ayalew et al., 2022; Phillips et al., 2023; Señas-Cuesta et al., 2023; Mahasneh et al., 2024). Some essential oils (EOs), such as oregano and thyme, have demonstrated potential in reducing the incidence of common poultry diseases, including coccidiosis and necrotic enteritis (Adhikari et al., 2020). Taken together, these findings suggest a range of potential benefits for maximizing the genetic potential of chickens and reducing mortality, thus increasing profitability (Puvaĉa et al., 2022). Additionally, some additives enhance nitrogen absorption, control excreta odor, and reduce ammonia concentration, thereby decreasing nitrogen

excretion into the environment (Chowdhury et al., 2018). Moreover, phytogenic feed additives have shown potential in improving meat quality (fatty acid profile, flavor and shelf-life) which can positively impact consumer preference and marketability (Giannenas et al., 2013; Galli et. al., 2020; Mohamed and Hassan, 2023).

The economic efficiency of diets containing these supplements is crucial in determining their viability and practicality for broiler production. Therefore, the objective of this study is to evaluate the economics of incorporating either six herbs or their essential oils as phytogenic feed additives in broiler chicken diets. The findings of this research will contribute to the existing knowledge on alternative feed additives and provide valuable information to poultry producers, feed manufacturers, and other stakeholders helping them make informed decisions regarding the incorporation of phytogenic feed additives in broiler chicken diets.

2. Materials and Methods

The present study was conducted at the Poultry farm of the Agricultural Institute, Stara Zagora. A total of 390 day-old Ross 308 male broiler chicks, with an initial body weight of 49.78±0.2 g, were randomly assigned to thirteen treatment groups. Each group, comprising 30 chicks, was further divided into three replicates (10 chicks/replicate). Subsequently, each replicate was allocated to a floor pen equipped with one feeder and drinker. Additionally, a continuous lighting program was implemented. The temperature was initially set at 33 °C during the first week and gradually decreased by 3 °C per week until stabilizing at 21 °C in the fourth week.

The dietary treatments included a control group fed basal diets without supplementation and twelve experimental groups. The experimental groups received basal diets supplemented with either 2% herbs (groups E1-E6) or 0.02% of their essential oils (groups E7-E12). The following herbs were used separately in dried powder form: chamomile (*Matricaria chamomilla*), rosemary (*Rosmarinus officinalis*), lavender (*Lavandula angustifolia*), oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), and St. John's wort (*Hypericum perforatum*). The essential oils were obtained from commercial companies (Nature Energies LTD; ALTEYA ORGANICS LTD, Bulgaria).

Chicks were fed a starter diet (1-10 days of age), a grower diet (11-28 days of age) and a finisher diet (29-39 days of age). The diets were formulated to meet the nutritional recommendations of the National Research Council (NRC, 1994). The composition and calculated nutritional value are shown in Table 1. Feed (in mash form) and water were provided ad libitum to the chicks throughout the experiment.

Table 1. Ingredients and calculated nutrient composition of starter (1-10 d), grower (11-28) and finisher (29-39 d) diets

Ingredients, %	Starter (1-10 days)	Grower (11-28 days)	Finisher (29-39 days)
Soybean meal	35.00	31.00	25.00
Wheat	30.00	29.69	35.87
Maize	21.22	25.00	24.00
DDGS	5.00	5.00	5.00
Sunflower oil	5.00	6.00	7.00
Dicalcium phosphate	2.05	1.80	1.65
Limestone	0.60	0.50	0.55
Salt	0.20	0.20	0.20
Premix*	0.20	0.20	0.20
Lysine	0.25	0.17	0.12
Methionine	0.18	0.14	0.11
Salgard	0.20	0.20	0.20
Optizim	0.10	0.10	0.10
Calculated nutrient composition, %			
Metabolizable energy, kcal/kg	2912.80	3042.19	3111.17
Crude protein	22.47	21.01	19.02
Ether extract	7.03	8.10	9.08
Crude fiber	3.99	3.77	3.48
Calcium	1.03	0.90	0.85
Available phosphorus	0.50	0.45	0.43
Lysine	1.44	1.25	1.05
Methionine	0.50	0.45	0.40
Methionine + cysteine	0.85	0.77	0.68

*Composition/kg of premix: Vit. A= 6 000 000 IU, Vit D3= 2 500 000 IU, Vit. E= 45 000 mg, Vit B1= 2 000 mg, Vit B2= 4 500 mg, Vit B6= 2 500 mg, Pantothenic acid= 10 000 mg, Biotin= 125 mg, Vit. K3= 2 000 mg, Folic acid= 1 100 mg, Nicotinic acid= 32 500 mg, Vit. B12= 10 mg, Selenium= 150 mg, Manganese= 60 000 mg, Iron= 12 500 mg, Zinc= 45 000 mg, Copper= 7 500 mg, Iodine= 500 mg

Performance parameters, including body weight and the feed consumed, were measured at the end of the starter, grower, and finisher periods. Then body weight gain, feed intake, and feed conversion ratio were calculated for specified periods, as well as for the entire fattening period of the chickens. Additionally, mortality rates were also recorded daily.

Input-output analysis was used to assess the economic efficiency of the experimental diets, assuming other costs remained constant, as suggested by Hassan and Awad (2017), as follows:

1) Total feed cost = feed intake per bird x cost of 1 kg diet
2) Feed cost/kg weight gain = feed conversion x cost of 1 kg diet
3) Net revenue/kg gain = revenue/kg gain – feed cost/kg gain
4) Economic efficiency = net revenue/feed cost per kg gain.

Input costs data were collected by calculating the prices of feed ingredients available on the market at the time of the experiment. The additional costs of the tested dried herbs and essential oils were included in the feed price.

In the economic assessment, the total feed cost, feed cost per kg of weight gain for each feed period, as well as for the entire experimental period were taken into account. Total revenue per bird sell was also considered assuming 4.80 BGN/kg live body weight constant for all treatment birds.

The economic efficiency of growth was determined through the calculations of European Broiler Index (EBI) based on the following formula given in Equation 1 (Marcu et al., 2013):

$$EBI = \frac{\text{Viability (\%) x ADG (g/chick/day)}}{\text{FCR (kg feed/kg gain) x 10}} * 100 \qquad (1)$$

where, ADG= average daily gain; FCR= feed conversion ratio.

2.1. Statistical Analysis

The data were analyzed using General Linear Model procedure of SPSS (version 19.0). Means were compared using Duncan's Multiple Range Test, with the level of significance set at P<0.05. The replicate pens served as experimental units for all parameters.

3. Results

Table 2 presents the economics of cost in relation to the supplementation of dried herbs in broiler diets during the starter, grower, and finisher periods. The inclusion of the tested herbs in the basal diet resulted in an increase in the price of the dietary mixtures for all three phases, ranging from 0.10 to 0.40 BGN/kg. However, no significant differences in feed consumption and feed conversion were found during these periods (P>0.05). As a result, most of the groups given herbal diets had higher total feed costs and feed costs per kilogram of gain compared to the control group (P<0.05), except for those receiving rosemary (E2), thyme (E5) or St. John's wort herb (E6) during the starter and grower phases, which were comparable to the control birds (P>0.05). On the other hand, the starter diet containing lavender herb (E3) resulted in the highest total feed cost (34.48%) and feed cost per kilogram of gain (45.45%), a trend that continued in subsequent periods (P<0.05), followed by the group fed a diet supplemented with chamomile (E1) during both the grower and finisher periods compared to the control group.

Table 2. Cost effectiveness of broiler diets supplemented with 2% dried herbs

Parameters	Groups							SEM	*P*-value
	C	E_1	E_2	E_3	E_4	E_5	E_6		
Starter period (1-10 d)									
Feed price, BGN/kg	0.93	1.17	1.12	1.31	1.07	1.03	1.03	-	-
Feed intake, kg/bird	0.32	0.29	0.28	0.30	0.33	0.30	0.30	0.01	0.177
Total feed cost, BGN	0.29^{b}	0.34^{abc}	0.32^{bc}	0.39^{a}	0.36^{ac}	0.31^{bc}	0.31^{bc}	0.02	0.004
Feed conversion, kg/kg	1.78	1.71	1.94	1.83	1.85	1.89	1.86	0.09	0.672
Feed cost/kg gain, BGN	1.65^{c}	2.00^{b}	2.18^{ab}	2.40^{a}	1.99^{b}	1.94^{bc}	1.91^{bc}	0.10	0.004
Grower period (11-28 d)									
Feed price, BGN/kg	0.90	1.14	1.09	1.29	1.05	1.00	1.00	-	-
Feed intake, kg	1.57	1.56	1.47	1.58	1.58	1.64	1.48	0.07	0.610
Total feed cost, BGN	1.42^{c}	1.78^{b}	1.61^{bc}	2.03^{a}	1.66^{bc}	1.64^{bc}	1.48^{c}	0.08	0.002
Feed conversion , kg/kg	1.70	1.56	1.83	1.58	1.59	1.70	1.63	0.08	0.304
Feed cost/kg gain, BGN	1.53^{b}	1.78^{ab}	2.00^{a}	2.03^{a}	1.66^{b}	1.70^{b}	1.62^{b}	0.09	0.011
Finisher period (29-39 days)									
Feed price, BGN/kg	0.86	1.11	1.06	1.26	1.02	0.97	0.97	-	-
Feed intake, kg	1.10	1.05	0.88	0.97	1.06	1.08	0.95	0.05	0.080
Total feed cost, BGN	0.95^{b}	1.16^{a}	0.94^{b}	1.21^{a}	1.08^{ab}	1.04^{ab}	0.92^{b}	0.06	0.011
Feed conversion, kg/kg	2.04	2.06	1.74	2.12	2.19	2.14	1.71	0.13	0.114
Feed cost/kg gain, BGN	1.77^{b}	2.29^{ad}	1.86^{bc}	2.66^{a}	2.22^{cd}	2.07^{bcd}	1.65^{b}	0.13	0.001

$^{a-d}$ -Means in the same row with different superscripts are significantly different P<0.05, C = Control group, E1 = 2% *Matricaria*, E2 = 2% *Rosmarinus officinalis*, E3 = 2% *Lavandula*, E4 = 2% *Origanum vulgare*, E5 = 2% *Thymus*, E6 = 2% *Hypericum perforatum*.

Data on the economic efficiency of feeding different experimental diets over a period of 39 days, influenced by dried herbs, are presented in Table 3. There were no significant (P>0.05) differences in total feed consumption and feed efficiency between the groups. However, the total feed cost showed a significant (P<0.05) increase of 36% in the diet supplemented with lavender herb (E3), followed by the diets supplemented with either chamomile herb (E1) by 23.5% or oregano herb (E4) by 16 % compared to the control group. This indicates that the choice of herb supplementation in the diets had a notable impact on the overall feed cost. In terms of feed cost per kilogram of gain, data analysis revealed the most significant (P<0.05) increase of 39 % in the lavender supplemented group (E3). In contrast, the St. John's wort supplemented group demonstrated the lowest value (10-26%) compared to the other treated groups (P<0.05), which were comparable to those of the control group (P>0.05). The economic efficiency values observed in the study were not influenced by the specific properties of each herb, including its potential effects on feed consumption and broiler growth, but rather by the different market prices of the herbs used.

According to the input-output analysis (Table 3), the economic efficiency (EE) values varied among the treatments, with the highest value of 1.96 observed for broilers fed the control diet, followed by 1.88 for chicks fed diets supplemented with St. John's wort, while the lowest value of 1.13 was recorded for the lavender supplementation (P<0.05).

The cost economics related to dietary supplementation of essential oils (EOs) in broiler diets during the starter, grower, and finisher periods are shown in Table 4. The inclusion of essential oils in broiler diets during the starter period had a significant effect on all the parameters studied (P<0.05). Although feed intake was reduced in the groups supplemented with rosemary (E8) or lavender (E9) essential oil (P<0.05), no significant difference in feed utilization was observed compared to the control group (P>0.05).

Table. 3. Economic efficiency of broiler diets supplemented with 2% dried herbs

Parameters	Groups							SEM	*P*-value
	C	E_1	E_2	E_3	E_4	E_5	E_6		
Average feed price, BGN/kg	0.90	1.14	1.09	1.29	1.05	1.00	1.00	-	-
Feed intake, kg	2.99	2.90	2.63	2.84	2.98	3.02	2.73	0.09	0.069
Total feed cost, BGN	2.68^{d}	3.31^{b}	2.88^{cd}	3.65^{a}	3.11^{bc}	3.01^{bcd}	2.72^{d}	0.10	0.000
Feed conversion, kg/kg	1.80	1.75	1.81	1.75	1.79	1.85	1.67	0.05	0.251
Feed cost / kg gain, BGN	1.62^{c}	2.00^{b}	1.97^{b}	2.25^{a}	1.87^{b}	1.85^{b}	1.67^{c}	0.05	0.000
Net revenue/ kg gain, BGN	3.18^{a}	2.80^{b}	2.83^{b}	2.55^{c}	2.93^{b}	2.95^{b}	3.14^{a}	0.05	0.000
Economic efficiency (EE)	1.96^{a}	1.40^{b}	1.44^{b}	1.13^{c}	1.57^{b}	1.59^{b}	1.88^{a}	0.07	0.000
Relative EE	100^{a}	71.43^{b}	73.47^{b}	57.65^{c}	80.10^{b}	81.12^{b}	95.92^{a}	3.31	0.000

$^{a-d}$ -Means in the same row with different superscripts are significantly different P<0.05, C = Control Group, E1 = 2% *Matricaria chamomilla*, E2 = 2% *Rosmarinus officinalis*, E3 = 2% *Lavandula angustifolia*, E4 = 2% *Origanum vulgare*, E5 = 2% *Thymus vulgaris,* E6 = 2% *Hypericum perforatum*.

Table 4. Cost effectiveness of broiler diets supplemented with 0.02% essential oils

Parameters	Groups							SEM	*P*-value
	C	E_7	E_8	E_9	E_{10}	E_{11}	E_{12}		
Starter 1-10 d									
Feed price, BGN/kg	0.93	1.41	0.97	0.97	1.37	1.12	0.97	-	-
Feed intake, kg	0.32^{a}	0.32^{a}	0.27^{bc}	0.25^{c}	0.28^{abc}	0.31^{ab}	0.30^{ab}	0.01	0.010
Total feed cost, BGN	0.29^{d}	0.45^{a}	0.26^{de}	0.24^{e}	0.39^{b}	0.34^{c}	0.29^{d}	0.01	0.000
Feed conversion, kg/kg	1.78^{ab}	2.03^{a}	1.59^{b}	1.63^{b}	1.63^{b}	1.85^{ab}	1.76^{ab}	0.09	0.048
Feed cost/kg gain, BGN	1.65^{c}	2.85^{a}	1.55^{c}	1.58^{c}	2.13^{b}	2.06^{b}	1.71^{c}	0.10	0.000
Grower 11-28 d									
Feed price, BGN/kg	0.90	1.38	0.95	0.95	1.35	1.09	0.95	-	-
Feed intake, kg	1.57	1.55	1.61	1.67	1.63	1.39	1.46	0.10	0.443
Total feed cost, BGN	1.42^{b}	2.14^{a}	1.53^{b}	1.59^{b}	2.19^{a}	1.52^{b}	1.38^{b}	0.11	0.000
Feed conversion , kg/kg	1.70	1.64	1.60	1.65	1.61	1.46	1.50	0.10	0.600
Feed cost/kg gain, BGN	1.53^{b}	2.26^{a}	1.52^{b}	1.57^{b}	2.17^{a}	1.60^{b}	1.42^{b}	0.11	0.000
Finisher 29-39 d									
Feed price, BGN/kg	0.86	1.35	0.91	0.91	1.31	1.05	0.91	-	-
Feed intake, kg	1.10	1.00	1.03	1.03	1.19	1.05	1.10	0.06	0.412
Total feed cost, BGN	0.95^{c}	1.35^{b}	0.94^{c}	0.91^{c}	1.55^{a}	1.10^{c}	1.00^{c}	0.06	0.000
Feed conversion, kg/kg	2.04	1.86	1.77	1.93	2.31	1.95	2.18	0.20	0.530
Feed cost/kg gain, BGN	1.77^{c}	2.50^{ab}	1.61^{c}	1.76^{c}	3.02^{a}	2.06^{bc}	1.98^{bc}	0.22	0.005

$^{a-d}$ -Means in the same row with different superscripts are significantly different P<0.05, C = Control group , E7 = 0.02% *Matricaria chamomilla* oil, E8 = 0.02% *Rosmarinus officinalis* oil, E9 = 0.02% *Lavandula angustifolia* oil, E10 = 0.02*%, Origanum vulgare* oil, E11 = 0.02% *Thymus vulgaris* oil, E12 = 0.02% *Hypericum perforatum* oil.

However, there was an improvement in feed conversion compared to the chamomile oil treated group (P<0.05). The corresponding total feed cost was significantly lower for the diet supplemented with lavender oil (E9) compared to the other groups, which was attributed to reduced feed intake (P<0.05). However, there was a significant increase (P<0.05) in total feed cost in the chamomile oil diet (E7) due to the higher cost of chamomile oil. No significant difference (P>0.05) was observed between the control group and the rosemary (E8) or St. John's wort (E12) dietary supplement groups. Similar trends were observed for feed cost per kg gain. During the next two fattening periods, feed consumption and feed conversion ratio remained unaffected by the addition of essential oils (P>0.05). However, due to the higher prices of chamomile and oregano oil, total feed costs and feed costs per kg of gain were highest in these groups compared to the other groups (P<0.05). Specifically, they were between 30-35% higher during the grower phase and 30-41% higher during the finisher phase compared to the control group.

Table 5 shows the results of the economic efficiency of the dietary supplementation of essential oils in broiler diets. The total feed consumption of the treated groups was comparable to that of the control group (P>0.05). Throughout the experimental period, the broilers fed the diets supplemented with essential oils did not utilize the feed more efficiently than those in the control group (P>0.05). However, there was a highly significant difference between treatments in total feed cost, feed cost per kg gain, net revenue per kg gain and economic efficiency (P<0.001). It is evident that the inclusion of either chamomile or oregano oil in the diet increased the total feed cost by 47-55% and the feed cost per kg gain by 48-55% compared to the control group, resulting in the lowest economic and relative efficiency values, which were 0.98-1.00 and 50-51%, respectively. On the other hand, no significant (P>0.05) differences were found between the control group and the other supplemented groups.

The values for the economic efficiency of growth were obtained by calculating the European Broiler Index (EBI) and are shown in Figure 1(a,b). Typically, improvements in the European Broiler Index (EBI) result from better body weight gain, liveability and a lower feed conversion ratio. A high EBI value indicates good overall technical efficiency of the broiler operation and is desirable for optimal returns (Samarakoon and Samarasinghe, 2012). In our study, the European Broiler Index was not significantly affected by dietary supplementation with either dried herbs or their essential oils, with values ranging from 207.52 to 251.39 for dried herbs and 236.80 to 273.85 for essential oils, respectively. Based on our results, a trend can be observed that supplementing the birds' diet with either St. John's wort herb or rosemary essential oil was more economical than the other treatment groups. Contrary to our results, several authors have stated that the addition of dried herbs or essential oils to broiler diets may have a beneficial effect on EBI (Arczewska-Wlosek and Swiatkiewicz, 2012; Salama et al., 2023).

4. Discussion

In broiler rearing, feed is the major component of input costs, accounting for up to 70% of the total production costs (Shahin et al., 2020). Many efforts have been made to improve growth, enhance feed conversion, and reduce feed costs by supplementing broiler diets with phytogenic feed additives (PFAs), such as herbs and essential oils. Puvaĉa et al. (2020) noted that the cost per diet may increase depending on the specific natural additive used. While some studies have documented the economic benefits of PFAs, the results are not always consistent, suggesting that profitability may depend on factors such as feed prices and broiler's growth response to the additives. According to Mohamed and Hassan (2023), PFA supplementation has shown economic advantages. Similarly, Omar et al. (2016) found that diets containing herbal extracts proved more economical than control diets, potentially due to enhanced feed conversion efficiency or a reduction in the amount of feed required to produce a unit of meat. Other research has reported similar findings and documented that PFA supplementation in broiler diets led to improved economic efficiency, increasing returns and gross margins in broiler production systems (EL-Faham et al., 2014; Oleforuh-Okoleh et al., 2014; Salama et al., 2023).

Table 5. Economic efficiency of broiler diets supplemented with 0.02% essential oils

Parameters	Groups							SEM	*P*-value
	C	E_7	E_8	E_9	E_{10}	E_{11}	E_{12}		
Average feed cost, BGN/kg	0.90	1.38	0.95	0.95	1.34	1.09	0.94	-	-
Feed intake, kg	2.99	2.87	2.91	2.95	3.09	2.75	2.86	0.14	0.694
Total feed cost, BGN	2.68 ^{b}	3.95 ^{a}	2.75 ^{b}	2.79 ^{b}	4.15 ^{a}	2.99 ^{b}	2.69 ^{b}	0.16	0.000
Feed conversion, kg/kg	1.80	1.74	1.65	1.74	1.81	1.66	1.73	0.08	0.752
Feed cost / kg gain, BGN	1.62 ^{b}	2.40 ^{a}	1.56 ^{b}	1.64 ^{b}	2.43 ^{a}	1.80 ^{b}	1.63 ^{b}	0.10	0.000
Net revenue/ kg gain, BGN	3.18 ^{a}	2.40 ^{b}	3.24 ^{a}	3.16 ^{a}	2.37 ^{b}	3.00 ^{a}	3.17 ^{a}	0.10	0.000
Economic efficiency (EE)	1.96 ^{ab}	1.00 ^{c}	2.08 ^{a}	1.93 ^{ab}	0.98 ^{c}	1.67 ^{b}	1.94 ^{ab}	0.12	0.000
Relative EE	100.0 ^{ab}	51.02 ^{c}	106.12 ^{a}	98.47 ^{ab}	50.00 ^{c}	85.20 ^{b}	98.98 ^{ab}	5.93	0.000

$^{a-d}$ -Means in the same row with different superscripts are significantly different P<0.05, C = Control Group , E7 = 0.02% *Matricaria chamomilla* oil, E8 = 0.02% *Rosmarinus officinalis* oil, E9 = 0.02% *Lavandula angustifolia* oil, E10 = 0.02*%, Origanum vulgare* oil, E11 = 0.02% *Thymus vulgaris* oil, E12 = 0.02% *Hypericum perforatum* oil

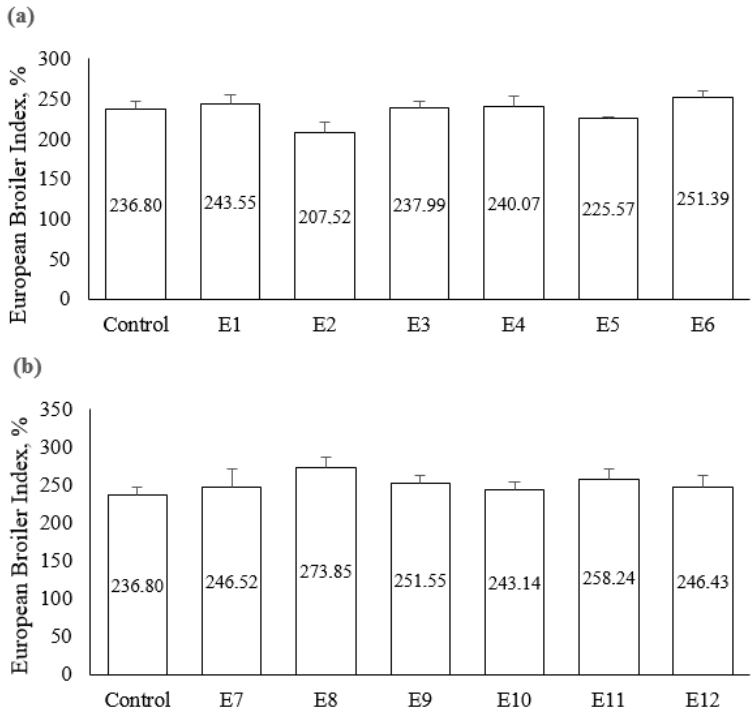

Figure 1. European Broiler Index in relation to dietary supplementation with dried herbs (a) and essential oils (b). C = Control Group, E1 = 2% *Matricaria chamomilla*, E2 = 2% *Rosmarinus officinalis*, E3 = 2% *Lavandula angustifolia*, E4 = 2% *Origanum vulgare*, E5 = 2% *Thymus vulgaris,* E6 = 2% *Hypericum perforatum, E7 = 0.02% Matricaria chamomilla oil, E8 = 0.02% Rosmarinus officinalis oil, E9 = 0.02% Lavandula angustifolia oil, E10 = 0.02%, Origanum vulgare oil, E11 = 0.02% Thymus vulgaris oil, E12 = 0.02% Hypericum perforatum* oil.

Wang et al. (2024) found that supplementing broiler diets with 2% rosemary powder resulted in a lower average daily gain over a 1–42 day period, despite an increase in daily feed intake. Rosemary's strong aroma, combined with its high crude fiber, tannins, and other potential interfering compounds, may limit its efficacy when used in large amounts. Yesilbag et al. (2013) also reported that rosemary affected average daily gain and feed conversion, though better outcomes were observed with rosemary essential oil rather than the whole herb. Dried *Hypericum perforatum* (St John's Wort) positively impacted broiler body weight at slaughter and feed conversion efficiency, while its powdered form did not significantly affect live weight or feed utilization. Similarly, İlhan et al. (2024) found that a *Hypericum perforatum* extract, with low production costs and environmental benefits, showed variable impacts on growth performance. The inclusion of thyme as a feed additive in broiler diets demonstrated the lowest cost per kilogram of weight gain, with the highest economic efficiency observed compared to unsupplemented diet (Osman et al., 2010). In duck diets, chamomile (*Matricaria chamomilla* L.) supplementation at low levels (0.25%, 0.50%, and 0.75%) significantly increased economic efficiency by 37%, 40%, and 64%, respectively, compared to the control group (Ibrahim et al., 2014). Diets containing 1.0 g/kg of chamomile flower powder for both ducklings and duck breeders demonstrated improved economic efficiency, likely due to enhanced feed conversion ratios and body weight gain (Gad et al., 2018; EL-Shhat et al., 2021). However, the tannin concentration in chamomile can impact feed intake and conversion rates, as highlighted by Dada et al. (2015), which suggests that careful attention to herbal composition is necessary when using this herb. Oregano, another widely studied herb, has also demonstrated specific advantages and limitations. Ismail et al. (2017) observed that providing dried oregano leaves to broiler chickens reduced feed intake, resulting in lower average body weight and daily weight gain. However, the feed conversion ratio improved, particularly in birds fed higher levels (9 g/kg diet) of dried oregano leaves, suggesting that the herb enhances gut efficiency, enabling broilers to convert feed into body mass more effectively despite lower overall intake. Ri et al. (2017) suggested that the positive effects of oregano powder supplementation were particularly beneficial during the grower phase of broiler development, indicating that specific growth phases may influence the efficacy of herbal additives. However, these findings were not observed in the present study. Some researchers found a significant reduction in net revenue and economic efficiency when thyme powder was added at the level of 8 g/kg, while statistically similar values were observed in birds fed diets supplemented with 2 and 5 g/kg thyme compared to the control group (Hassan and Awad, 2017). This was due to the fact that the improvement in growth occurred along with a significant concurrent increase in total feed cost. Similar to our results, Singh et al. (2018) reported that supplementing broiler diets with

phytogenic feed additives did not improve economic efficiency.

Although the addition of essential oils to broiler diets did not provide economic benefits in our study, they remain important from the perspective of consumers seeking high-quality meat, as they contain numerous bioactive compounds with antioxidant potential (Vlaicu et al., 2022). Adaszynska-Skwirzynska and Szczerbinska (2018) highlighted the advantage of supplementing lavender essential oil (EO) in broiler drinking water, particularly during the second rearing period (days 22–42), when the impact on production performance was more favorable. Additionally, Popović et al. (2016) found that a combination of thyme, rosemary, and oregano essential oils at concentrations of 0.05% and 0.1% improved broiler production performance, suggesting that specific EO blends may offer advantages over individual essential oils.

These inconsistencies in phytogenic feed additives responses across studies are often attributed to the variability of active compounds in herbs, which may be affected by factors such as plant species, harvest time, drying techniques, storage duration, and extraction methods (Hippenstiel et al., 2011). Furthermore, dosage, dietary interactions, and management conditions play significant roles in influencing PFA effectiveness (Behboodi et al., 2021). Research has also indicated that combining specific herbs or essential oils can produce synergistic effects, potentially enhancing the benefits beyond those observed with individual components (Hippenstiel et al., 2011).

5. Conclusion

The economic evaluation indicated better economic efficiency with the dietary supplementation of 2% dried St. John's wort herb, as well as 0.02% St. John's wort, rosemary, thyme, or lavender essential oil supplemented group compared to the corresponding other treated groups ($P<0.05$), but not when compared to the control group ($P>0.05$). Unsatisfactory results were observed when using dry herb lavender or essential oil of chamomile or oregano, expressing in a significant decrease in net revenue and economic efficiency due to the highest feed cost to produce 1 kg of live weight compared to other treatments ($P<0.05$). The European Broiler Index (EBI) values in the groups treated with dry herbs or corresponding essential oils did not show a significant increase compared to the control group ($P>0.05$). These results underline the need for a comprehensive evaluation of the economic implications associated with the inclusion of phytogenic feed additives in broiler diets.

Author Contributions

The percentages of the authors' contributions are presented below. The author reviewed and approved the final version of the manuscript.

	N.M.	M.O.	I.I.	P.H.
C	25	25	25	25
D	25	25	25	25
S	25	25	25	25
DCP	25	25	25	25
DAI	25	25	25	25
L	25	25	25	25
W	25	25	25	25
CR	25	25	25	25
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

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. All procedures, including the use of birds, management and care, were in compliance with the European Council Directive regulations on the protection of animals used for experimental and other scientific purposes (2010/63/EU), and national protocol (approval date: November 01, 2012, protocol code: 20).

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COMPARISON OF DIFFERENT LINSEED GENOTYPES MEAL IN TERMS OF FEED VALUE PROPERTIES

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Abstract: This study was conducted to determine the nutritional content and nutritive values of some varieties of linseed meal. For this purpose, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), crude ash (CA), and crude protein (CP) analyses were performed on meals obtained from 13 different linseed varieties (Clli 1423, Larkana, Milas, NewTurk, Dillman, Sari-85, Clli 1351, Clli 1370, Clli 1400, Clli 1412, Karakız, Beyaz Gelin, Noreum) in order to determine some nutritional content. Based on some data obtained from these analyses, nutritive values such as dry matter digestibility (DMD), dry matter intake (DMI), relative feed value (RFV), metabolizable energy (ME), and net energy for lactation (NEL) were calculated. In terms of nutritional content, the NDF, ADF, ADL, and CP contents of linseed meals were found to be significant, while the CA content was not found to be significant. The NDF, ADF, ADL, and CP contents of the meal varied within the ranges of %58.40-96.37, %18.62-35.64, %8.55-20.45, and %34.84-41.21, respectively. In terms of nutritive value characteristics, the DMD, ME, and NEL properties of linseed meal were found to be significant, while the DMI and RFV features were deemed insignificant. The DMD, ME, and NEL values of the meal varied within the ranges of %61.14-74.39, 9.35-11.91 MJ/kg, and 1.37-1.81 Mcal/kg, respectively. In conclusion, it was determined that among the linseed varieties, the highest results in terms of both nutritional content and nutritive value were provided by the NewTurk variety, while the worst results were generally observed in the Beyaz Gelin variety. However, it was concluded that the meals from all evaluated linseed varieties could be utilized in the intensive feed industry.

Keywords: Linseed, Linseed meal, Nutritional content, Nutritive value, Feed

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1. Introduction

Linseed (*Linum usitatissimum* L.) is a species cultivated for its fibers and seeds within the Linaceae family. While linseed genotypes are typically grown in continental climate regions, fiber linseed genotypes thrive in cool, moist climate conditions (Zuk et al., 2015). Presently, linseed is a versatile crop cultivated for its seeds, fibers, and for dual-purpose production (both seed and fiber), with a height range of 60-120 cm depending on the variety, planting density, and weather conditions (Muir and Westcott, 2003; Mert, 2020; Aygün and Mert, 2022). Its fibers are primarily used in the textile industry, while its seeds, rich in healthy oils and high in protein content, find application in various food products as well (Aygün et al., 2024).

While linseed seeds are consumed directly, they are also used to obtain oil through cold pressing methods (Nykter et al., 2006). Linseed oil is rich in omega-3 fatty acids, making it a recognized source of healthy fats widely used by people (Tripathi et al., 2013). After extracting oil from linseed seeds, the remaining residue is referred to as linseed meal or cake.

Meal is typically the term given to the residue left after extracting oil from oilseeds (Katok-Öztürk, 2020). This material is usually rich in nutritious components such as fibers, proteins, vitamins, minerals, and antioxidants (Onat et al., 2017). However, the exact composition of meal, including the proportions of different nutrients, can vary depending on the content and type of the oilseed from which it is obtained. Meal is commonly used in animal feed production, serving as an additional source of fiber and nutrients for animals (Şahin et al., 2018). It is particularly prevalent in the diets of cattle, goats, sheep, and poultry.

Research on the evaluation of linseed meal for animal nutrition, including its nutrient content and nutritive value, is quite limited. This study aims to investigate the nutrient content and feeding value of linseed meals obtained from various linseed genotypes.

2. Materials and Methods

In this study, 13 linseed (*Linum usitatissimum* L.) genotypes (Clli 1423, Larkana, Milas, NewTurk, Dillman, Sarı-85, Clli 1351, Clli 1370, Clli 1400, Clli 1412, Karakız, Beyaz Gelin, and Noreum) were used as plant materials.. This investigation was carried out according to randomized block design with three replications. The plants were sown in December 2020 at the Research and

Application Field of Mustafa Kemal University in Hatay, and harvested in June 2021. Experimental field has a Mediterranean climate located south of Turkiye and east of the Mediterranean region (annual mean rainfall is approximately 600 mm). The detailed chemical and physical properties of the soil at the experimental site are given in Table 1. The soil texture of the experimental field had a clay structure, and the lime content was moderate. Additionally, the organic matter, phosphorus and potassium contents of the soil at the experimental site were very low. Climatic data (Anonymous, 2022) from the experimental field for the growing season (2020-2021) and long-term averages are given in Figure 1. The April, May and June temperatures during the growing season were greater than those during the long-term years. The temperatures in the late autumn and winter months were similar to the long-term temperature data. The rainfall in December, January and March of the growing season was greater than that the long-term years. In the growing season, there was a higher rainfall value than that in the long-term years for all months except for January.

Each linseed genotype was cultivated in plots measuring 5 meters in length. The plots consisted of 5 rows, with row spacing set at 20 cm. During cultivation, 100 kg ha^{-1} of nitrogen was applied, with half applied at the time of sowing and the other half during the flowering stage. Additionally, phosphorus and potassium were applied at a rate of 50 kg ha^{-1} at the time of sowing. No irrigation was provided during the growing season, and the plant's water needs were met solely through natural rainfall. After harvesting, the seeds were removed from the capsules and ground. Afterward, the oil extraction of the seeds was conducted using the Soxhlet extraction method, following the AOAC guidelines from 2005.

The nutrient content and feeding value of the meal from the linseed genotypes were determined using the scientific methods outlined below:

The NDF (Neutral Detergent Fiber), ADF (Acid Detergent Fiber), and ADL (Acid Detergent Lignin) contents (% DM) of the obtained meal were analyzed using the protocols outlined by Van Soest et al. (1991) with the Ankom Fiber Analyzer device.

The crude protein (CP) and crude ash (CA) contents (DM%) of the meal were determined according to the AOAC (Association of Official Agricultural Chemists) guidelines from 1990. The CP content of the meal was determined using the Kjeldahl method, while the CA content was determined by incinerating the samples in a muffle furnace.

Dry Matter Digestibility (DMD), Dry Matter Intake (DMI), and Relative Feed Value (RFV): These characteristics were calculated using the formulas expressed in Equations 1-3, as outlined by Van Dyke and Anderson (2002).

$$DMD\ (\%) = 88.9 - (0.779 \times ADF\%) \quad (1)$$

$$DMI\ (\%) = \frac{120}{NDF\%} \quad (2)$$

$$RFV = DMD \times DMI \times 0.775 \quad (3)$$

Metabolizable Energy (ME) and Net Energy for Lactation (NEL): These characteristics were calculated using the formulas provided in Equationd 4 and 5:

$$ME\ (MJ\ kg^{-1}\ DM) = 14.70 - (0.150 \times ADF\%) \quad (4)$$

$$NEL\ (Mcal\ kg^{-1}\ DM) = \left(1.044 - (0.0119 \times ADF\%)\right) \times 2.205 \quad (5)$$

The data obtained from this study were subjected to analysis of variance (ANOVA) to determine the differences among the linseed genotypes via statistical program of Statistica. For characteristics found to be significant ($P<0.05$) in the analysis of variance, Tukey's honestly significant difference (HSD) test was applied for multiple comparisons (Genç and Soysal, 2018).

Table 1. Detailed chemical and physical properties of the soil at the experimental site

Properties	Method	Unit	Results	Results
pH	Potentiometric	-	7.43	Slightly alkaline
Conductivity	Potentiometric	µS cm^{-1}	328	Negligible
Useful phosphorus (P)	Spectrophotometric	kg da^{-1}	2.4	Low
Useful potassium (K)	Spectrophotometric	kg da^{-1}	85.4	High
Calcium (Ca)	Spectrophotometric	ppm	8900.0	High
Iron (Fe)	Spectrophotometric	ppm	14.0	Low
Copper (Cu)	Spectrophotometric	ppm	1.6	Low
Manganese (Mn)	Spectrophotometric	ppm	20.7	Low
Zinc (Zn)	Spectrophotometric	ppm	2.3	Low
Magnesium (Mg)	Spectrophotometric	ppm	1679.2	High
Organic matter	Walkley-Black	%	1.7	Low
Lime	Calcimetric measurement	%	2.4	Low
Saturation	Saturation with water	%	72.6	Clay

Figure 1. Monthly total rainfall and monthly mean temperature at the experimental site during the growing season (2020–2021 and 2021–2022) and in the long term (1940–2021).

3. Results and Discussion

The NDF contents of linseed meals have been significantly affected by genotype (P<0.05) (Table 2). The NDF content varied between 58.40% and 96.37%. It has been determined that the variability range of NDF content among genotypes (a difference of 37.97%) is quite high. The highest NDF content was observed in the Karakız genotype, while the lowest was found in the Dillman genotype. An NDF content exceeding 50% in directly fed feeds (concentrate or roughage) may lead to digestive problems (Yavuz, 2005). It has been reported that the most appropriate NDF content for feeds should be between 25-32% (Tekce and Gül, 2014). On the other hand, linseed meal s are used to complement insufficient NDF content in concentrating feeds. In this regard, it can be concluded that the genotypes assessed here may not be suitable for direct feed use but could be utilized as feed raw materials.

Table 2. Results of variance analysis and multiple comparison tests for nutrient content of linseed meal from various genotypes

Genotypes	NDF (% DM)	ADF (% DM)	ADL (% DM)	ASH (% DM)	CP (% DM)
Clli 1423	67.43±7.32 ^{ab}	20.42±2.24 ^{d}	9.50±1.41 ^{d}	6.65±0.38	39.69±0.49 ^{abc}
Larkana	69.10±7.66 ^{ab}	21.80±1.75 ^{cd}	10.29±0.83 ^{d}	7.08±0.22	37.08±1.97 ^{abc}
Milas	64.27±6.71 ^{ab}	19.31±0.36 ^{d}	8.66±0.22 ^{d}	7.65±0.50	38.69±0.38 ^{abc}
NewTurk	62.33±4.70 ^{ab}	18.62±2.17 ^{d}	8.55±1.56 ^{d}	7.40±0.94	41.21±0.83 ^{a}
Dillman	58.40±5.82 ^{b}	19.55±2.56 ^{d}	10.09±1.55 ^{d}	7.39±0.61	38.71±0.85 ^{abc}
Sarı-85	81.38±7.76 ^{ab}	22.14±1.84 ^{cd}	9.72±1.81 ^{d}	7.85±0.40	40.68±0.64 ^{ab}
Clli 1351	64.82±11.36 ^{ab}	22.02±2.77 ^{cd}	10.08±2.46 ^{d}	6.68±0.08	40.40±1.65 ^{abc}
Clli 1370	67.58±7.54 ^{ab}	22.21±2.60 ^{cd}	10.69±2.12 ^{cd}	6.78±0.12	40.78±0.70 ^{ab}
Clli 1400	71.79±8.39 ^{ab}	22.58±2.57 ^{bcd}	11.23±2.45 ^{bcd}	6.63±0.23	40.63±1.16 ^{ab}
Clli 1412	68.39±4.43 ^{ab}	25.17±2.29 ^{abcd}	13.25±1.21 ^{abcd}	6.50±0.39	39.68±1.46 ^{abc}
Karakız	96.37±3.48 ^{a}	33.01±0.99 ^{abc}	18.55±1.21 ^{abc}	6.32±0.14	35.69±0.51 ^{abc}
Beyaz Gelin	86.35±7.01 ^{ab}	35.64±3.52 ^{a}	20.45±2.32 ^{a}	6.34±0.16	34.84±0.19 ^{c}
Noreum	66.51±1.34 ^{ab}	33.72±0.94 ^{ab}	18.80±0.08 ^{ab}	6.53±0.06	35.46±1.16 ^{bc}
Coef. Var.	20.08	26.82	38.20	11.05	6.84
SEM	2.29	1.04	0.75	0.12	0.42
F	2.52*	7.16**	7.19**	1.66 ^{ns}	4.23**

*= P<0.05, **= P<0.01, ns: non-significant, Coef. Var.= coefficient of variation, SEM= standard error of the mean.

Table 3. Variance analysis and multiple comparison test results of feeding values for meals from various linseed genotypes

Genotypes	DMD (%)	DMI (%)	RFV	ME (MJ/kg KM)	NEL (Mcal/kg KM)
Clli 1423	72.99±1.74 ^{a}	1.82±0.19	103.48±12.96	11.64±0.34 ^{a}	1.77±0.06 ^{a}
Larkana	71.92±1.37 ^{ab}	1.78±0.19	99.54±12.52	11.43±0.26 ^{ab}	1.73±0.05 ^{ab}
Milas	73.86±0.28 ^{a}	1.91±0.22	109.54±12.82	11.80±0.05 ^{a}	1.80±0.01 ^{a}
NewTurk	74.39±1.69 ^{a}	1.95±0.16	112.78±11.83	11.91±0.33 ^{a}	1.81±0.06 ^{a}
Dillman	73.67±1.99 ^{a}	2.10±0.21	119.74±12.93	11.77±0.38 ^{a}	1.79±0.07 ^{a}
Sarı-85	71.66±1.43 ^{ab}	1.50±0.13	83.49±8.57	11.38±0.28 ^{ab}	1.72±0.05 ^{ab}
Clli 1351	71.75±2.16 ^{ab}	2.00±0.42	112.56±27.51	11.4±0.42 ^{ab}	1.72±0.07 ^{ab}
Clli 1370	71.60±2.02 ^{ab}	1.83±0.22	101.94±15.28	11.37±0.39 ^{ab}	1.72±0.07 ^{ab}
Clli 1400	71.31±2.00 ^{abc}	1.72±0.22	95.86±15	11.31±0.39 ^{abc}	1.71±0.07 ^{abc}
Clli 1412	69.29±1.78 ^{abcd}	1.77±0.11	95.29±8.29	10.92±0.34 ^{abcd}	1.64±0.06 ^{abcd}
Karakız	63.18±0.77 ^{bcd}	1.25±0.04	61.18±2.88	9.75±0.15 ^{bcd}	1.44±0.03 ^{bcd}
Beyaz Gelin	61.14±2.74 ^{d}	1.41±0.12	67.24±8.60	9.35±0.53 ^{d}	1.37±0.09 ^{d}
Noreum	62.63±0.74 ^{cd}	1.81±0.04	87.60±0.76	9.64±0.14 ^{cd}	1.42±0.02 ^{cd}
Coef. Var.	7.26	20.98	26.37	8.85	10.29
SEM	0.81	0.06	4.06	0.16	0.03
F	7.16**	1.53 ^{ns}	1.86 ^{ns}	7.16**	7.16**

*= P<0.05, **= P<0.01, ns: non-significant, Coef. Var.= coefficient of variation, SEM= standard error of the mean.

The ADF contents of the linseed meals have been significantly affected by genotype (P<0.01) (Table 2). The ADF content varied between 18.62% and 35.64%. The highest ADF content was observed in the Beyaz Gelin, while the lowest was found in the NewTurk (Table 2). Inadequate ADF content in ruminant diets can lead to various disorders (Tekce and Gül, 2014). The ADF contents obtained from this study are sufficient for animal feeding purposes. Direct feeding of ruminants with feeds containing higher ADF content, such as those with 28% ADF or more, may lead to various health problems (Yavuz, 2005). Therefore, it would be more appropriate to use the linseed meals from some of the genotypes assessed in this study as raw materials for concentrate feeds.

The ADL contents of the linseed meals have been significantly affected by genotype (P<0.01) (Table 2). The ADL content varied between 8.55% and 20.45%. The highest ADL content was found in Beyaz Gelin, while the lowest was in the NewTurk (Table 2). High ADL content is one of the significant factors adversely affecting digestion in ruminants (He et al., 2018). Based on the ADL contents obtained from this study, it can be inferred that genotypes with ADL content exceeding 10% are generally unsuitable for direct feeding. However, considering the cell wall components (structural carbohydrates) of all linseed genotypes, it can be suggested that meals from the NewTurk and Milas could be used directly for ruminant feeding. Nonetheless, it would be more appropriate to use the meals from these linseed genotypes as raw materials in the concentrate feed industry due to their cell wall component profiles.

The CA contents of the linseed meals were not significantly affected by genotype (P>0.05) (Table 2). In contrast, the CP contents were significantly affected by genotype differences (Table 2). The CP content ranged from 34.84% to 41.21%. The highest CP content was observed in the NewTurk, while the lowest was found in the Beyaz Gelin (Table 2). The CP contents in soybean, canola, cotton, sunflower, and peanut meals have been reported as 48%, 35%, 39%, 30%, and 46%, respectively (Willis, 2003). According to the results from this study, the CP contents of the genotypes are higher than those in sunflower meal. Furthermore, some genotypes exhibited higher CP content compared to both canola and cotton meals.

The variation and mean comparison results for DMD, DMI, RFV, ME, and NEL of the meals derived from different linseed genotypes are presented in Table 3. As shown in Table 3, significant differences in DMD, ME, and NEL properties were observed among the genotypes. The highest DMD value (74.39%) was obtained from the NewTurk genotype, although statistically similar values were found in other genotypes. The lowest DMD value (61.14%) was determined in the Beyaz Gelin genotype. Some DMD values for certain genotypes in this study (70%-75%) were found to be similar to the DMD values of alfalfa hay (Jančík et al., 2017). Thus, many of the genotypes used in the study were deemed significant. The ME values for the linseed meals ranged from 9.35 MJ kg^{-1} DM to 11.91 MJ kg^{-1} DM, with the highest ME value obtained from the NewTurk genotype and the lowest from the Beyaz Gelin genotype. Cordeiro et al. (2022) found the ME value of sunflower meal to be 12.13 MJ kg^{-1} DM, while Brand et al. (2000) reported it as 13.76 MJ kg^{-1} DM for canola meal. Although the ME values obtained in this study are close to literature reports, they are lower, which can be attributed to differences in the types of meals. The highest NEL value (1.81 Mcal kg^{-1} DM) was found in the NewTurk genotype, while the lowest (1.37 Mcal kg^{-1} DM) was in the Beyaz Gelin genotype. The NEL values of the genotypes were found to be similar to those of many roughage crops (Atasever et al., 2020; Yılmaz et al., 2018).

4. Conclusion

This study aimed to determine the nutritional content and feeding values of meals derived from different linseed genotypes. Among these genotypes, the NewTurk genotype yielded the most superior results in terms of both nutritional content and feeding values, while the Beyaz Gelin genotype showed the least favorable results. Overall, it was concluded that the meals obtained from all genotypes could potentially serve as good concentrate feed additives in terms of both nutritional content and feeding values.

Author Contributions

The percentages of the authors' contributions are presented below. The author reviewed and approved the final version of the manuscript.

	İ.E.	Y.Z.A.	M.M.
C	50	50	
D	50	50	
S			100
DCP	50	50	
DAI	100		
L	100		
W	50	50	
CR	25	25	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 there was no study on animals or humans.

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DETERMINATION OF ORGANIC CARBON CONTENT OF THE SOILS WITHIN THE GREENHOUSES BUILT ON PYROCLASTIC DEPOSITS IN ISPARTA SETTLEMENT AREA

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Abstract: Soil organic carbon (SOC) is an important indication of soil health and helps to sustain soil fertility. As a result, determining its composition and the factors that influence it is critical for long-term soil nutrient management, especially in controlled conditions such as greenhouses. This study utilizes machine learning to classify SOC content in greenhouses built on pyroclastic deposits in the Isparta region. A dataset of 276 samples and eight variables—clay (%), silt (%), sand (%), soil electrical conductivity (EC), pH, elevation, slope, and aspect—were used to model SOC values. SOC content was classified into five classifications: very low (<0.6%), low (0.6-1.2%), medium (1.2-1.8%), good (1.8-2.3%), and high (>2.3%). In this study, five machine learning models—Logistic Regression (LR), K-Nearest Neighbors (KNN), Support Vector Machine (SVM), Decision Tree (DT), and Random Forest (RF)—were evaluated using cross-validation to determine their classification accuracy, precision, recall, F-score, and ROC area. Random Forest (RF) and Decision Tree (DT) outperformed the other models, with RF achieving the highest overall accuracy (76.4%), precision (77.3%), and AUC (0.904), followed by DT at 75.4% and AUC of 0.874. This study shows the practicality of machine learning models in categorizing SOC content, highlighting their importance for long-term soil health and fertility control in greenhouse conditions. To improve model efficacy, future studies should include more auxiliary variables, such as soil physical and chemical qualities and lithological data, as well as a wider range of soil types.

Keywords: Soil organic carbon, Machine learning, Greenhouses, Topography, Soil properties, Volcanic materials

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1. Introduction

Soil organic carbon (SOC) is an important component of the global carbon cycle, serving as a crucial carbon store that helps to the management of atmospheric carbon dioxide levels. Within agricultural environments, SOC functions as an active pool that is heavily impacted by human activities. Climate change and soil moisture levels have a significant impact on SOC content, while global warming and population growth highlight the importance of natural (climate, soil parent material, land cover, and topography) and anthropogenic (land use, management, and degradation) factors in SOC dynamics (Hiederer and Köchy, 2011).

The Paris Agreement aims to reduce greenhouse gas emissions by 21% by 2030 when compared to the reference scenario (Genç, 2021). As a result, different projects have been launched globally and in Türkiye to examine SOC levels. Türkiye's SOC status is crucial to promoting sustainable land use and combatting climate change. The importance of estimating and monitoring SOC stocks was stressed at the 12th Conference of the Parties to the United Nations Convention to Combat Desertification in 2015, underlining SOC's role in combating land degradation. The "Türkiye Soil Organic Carbon (CARBON) Project," conducted in collaboration with the General Directorate of Combating Desertification and Erosion (ÇEM) and TÜBİTAK-BİLGEM- Software Technologies Research Institute (YTE), established a high-resolution SOC map using data from 21,061 sampling points. The Random Forest modeling estimated a total carbon stock of 3.51 billion tons in the soil at a depth of 30 cm (ÇEM, 2018). The high-resolution SOC maps and data generated by the CARBON Project aim to enhance agricultural sustainability and develop effective strategies to combat climate change. Several studies have investigated the application of machine learning techniques for predicting SOC, highlighting the effectiveness of various algorithms. For instance, Long Short-Term Memory (LSTM) models demonstrated a high predictive accuracy, with an R^2 value of 0.89 in Southern Xinjiang, China (Wang et al., 2023). Other research has emphasized the importance of spatial SOC distribution, utilizing advanced techniques such as meta-learning stacking to improve predictive performance (Taghizadeh-Mehrjardi et al., 2020). Additionally, recent advancements in digital soil mapping

have demonstrated the superiority of models like LSM-ResNet over traditional methods (Zeng et al., 2022). The spatial distribution of SOC has been projected using remote sensing, geographic information systems, and machine learning algorithms (Minasny et al., 2006; Grimm et al., 2008; Minasny et al., 2016; Minasny et al., 2018; Alaboz et al., 2021; Demir and Başayiğit, 2022; Bekana and Mohammed, 2022; Odebiri et al., 2022; Xie et al., 2022; Padarian et al., 2022; Demir, 2024a). In Türkiye, studies focused on SOC determination in greenhouse environments remain limited, necessitating further investigation into the impacts of both anthropogenic and natural factors on SOC levels. Given Türkiye's unique climatic characteristics and agricultural potential, exploring SOC dynamics within greenhouse settings is essential for optimizing soil management practices (TurkStat, 2023).

This study aims to determine the variability of SOC content in greenhouse environments situated on pyroclastic deposits around Isparta. The hypothesis posits that pyroclastic flows and deposits resulting from volcanic activity during the Pliocene and Quaternary periods significantly influence the region's soil composition, leading to notable changes in SOC content. Materials released into the atmosphere during volcanic activity and quickly deposited onto the Earth's surface make up pyroclastic deposits. These deposits can significantly influence soil structure and soil organic carbon (SOC) accumulation when incorporated into surface soils. Pyroclastic materials have a high surface area and fine grain size, which increases their ability to retain water. This helps to build up organic matter and foster plant growth (Elitok et al., 2009; Saputra et al., 2022). The study's primary goal is to develop classification models for SOC levels based on soil characteristics, thereby improving understanding of SOC dynamics and promoting sustainable agricultural practices. To achieve this, soil samples were collected from greenhouses on pyroclastic deposits and analyzed using the Modified Walkley-Black method to determine organic carbon content. Soil pH, electrical conductivity (EC), temperature, texture, and topographic characteristics were also evaluated. Based on the collected soil properties, classification models for SOC levels were constructed using modern technologies and machine learning techniques. This study intends to improve understanding of SOC dynamics in greenhouses built on pyroclastic deposits and to educate sustainable agricultural operations, laying the framework for more efficient and environmentally friendly farming methods.

2. Materials and Methods

2.1. Study Area

The study area is located in the northeastern section of the Central District of Isparta Province, Türkiye, and includes pyroclastic deposits created by volcanic activity near Gölcük Crater Lake (Figure 1). The area is depicted on the M24b3 and M25a4 sheets of the 1:2500 Türkiye Topographic Map. A land survey was undertaken in Deregümü Village and its surroundings, which are 3 kilometers from Isparta's settlement and 1125 meters above sea level.

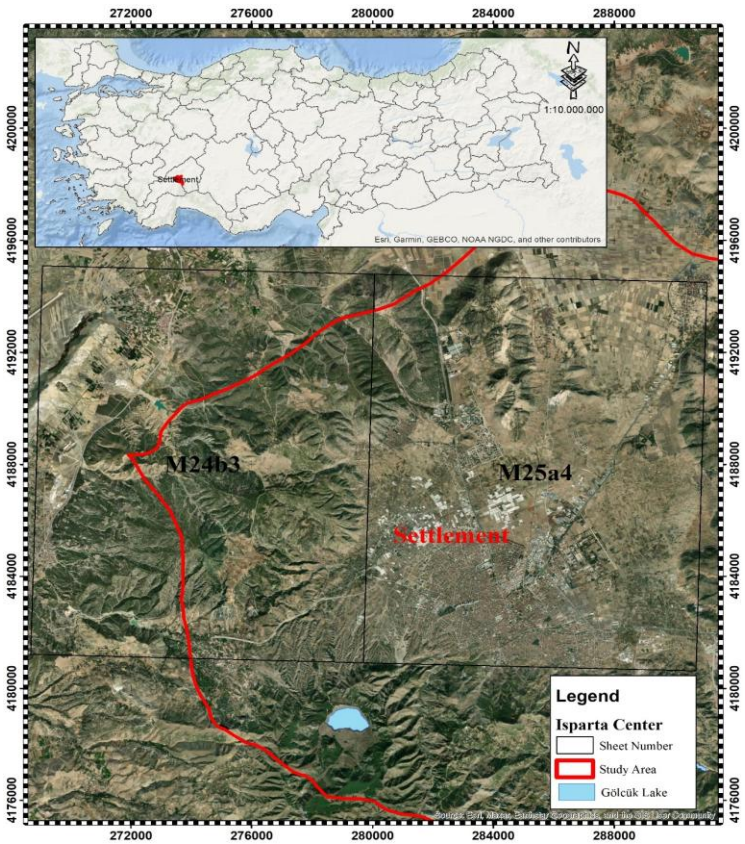

Figure 1. Study area (Isparta Settlement Area) map.

Climate data were obtained from the Turkish State Meteorological Service's Isparta Central Station (MGM). Based on long-term records from 1929 to 2023, the annual average temperature in Isparta was recorded as 12.3 °C, with the highest mean temperature reaching 18.5 °C and the lowest at 6.3 °C. The region receives an average of 7.1 hours of sunshine per day and experiences approximately 99.1 rainy days per year. The highest monthly precipitation was observed in December, totaling 86 mm, while the lowest was recorded in July at 15.5 mm. The maximum recorded temperature in the region was 40.3 °C in August, and the minimum was -21.0 °C in February (MGM, 2024).

According to Corine's 2018 land use data, forest and semi-natural areas account for 64.72% of the total area, covering 50,085.86 hectares. Agricultural land is the second largest land use type, accounting for 29.62% of the total area, covering 22,922.30 hectares. Artificial surfaces account for 5.55% of the total area, covering 4,298.01 hectares. Water bodies account for 0.10% of the total area, covering 80.26 hectares (Corine, 2024). The distribution indicates that the study area predominantly comprises natural and agricultural land uses, ensuring a smooth and clear representation of the region's characteristics.

The 2023 data from the Turkish Statistical Institute (TurkStat, 2023) presents a noteworthy overview of the distribution of agricultural lands and greenhouse activities in the study area. Cereals and other crops make up the largest use of agricultural land, covering 96,911 decares. Next, fallow lands are the second-largest, occupying 26,116 decares. Fruit, beverage, and spice crops follow, covering 21,516 decares. Vegetable cultivation takes up 6,123 decares, while farmers grow ornamental plants on 1,426 decares. In the study area, farmers grow crops in greenhouses that cover 2,506 decares, primarily using plastic for the structures. This indicates that plastic is the most common greenhouse covering material in the region. Farmers do not use other methods like low tunnels, glass greenhouses, or high tunnels (TurkStat, 2023). As a result, greenhouse activities rely solely on plastic structures, limiting the adoption of alternative techniques. These results highlight effective agricultural land use and suggest significant opportunities for expanding greenhouse cultivation in the future.

The Gölcük volcanic region, situated at the apex of the Isparta Angle, represents a geologically complex area influenced by Pliocene and Quaternary volcanic activity. The area is characterized by a combination of autochthonous and allochthonous units that have been intruded by volcanic material and covered with pyroclastic fall and flow deposits. These deposits, consisting of ash, lapilli, and pumice fragments, are associated with the volcanic eruptions of the Gölcük system and have contributed to the formation of surface soils. The pyroclastic material, through its fine-grained structure and high porosity, influences soil physical properties, such as water retention capacity and soil aeration, which are crucial for organic carbon accumulation. Additionally, the pyroclastic deposits are spatially distributed along faults and extensional structures within the Isparta region, further shaping soil development processes (Elitok et al., 2009; Canpolat and Turoğlu, 2019). The interaction of volcanic deposits with tectonic and climatic factors plays an important role in the geochemical genesis of soils in this area, enhancing the study's focus on soil organic carbon dynamics.

2.2. Greenhouses Soil Samples

This study used various cartographic materials in the Remote Sensing and Geographic Information Systems Laboratory of the Department of Soil Science and Plant Nutrition at the Faculty of Agriculture, Isparta University of Applied Sciences to determine the greenhouse areas. These include a 1:2500 scale topographic map, satellite images, major soil group maps, land use capability classes, geological maps, and numerical data (Demir, 2024b). Using Google Earth Pro, 288 greenhouse areas were identified for 2022.

Sample points for this population were calculated using the G-power test, especially the "Means: Equal sample sizes, two groups" test type. This test determines whether the difference in the means of the two groups is statistically significant. In the G-power calculation, the alpha value was set at 5%, and the power (1-beta) was set at 95%, resulting in a 5% margin of error and 95% test power. The effect size was presumably set at 0.5, indicating a medium effect size (Demir et al., 2024).

The calculated sample size was 92, meaning that at least 92 samples were required for analysis. This sample size was expected to detect a medium effect size with 95% power and a 5% margin of error. However, it is important to note that the effect size was estimated hypothetically, and the actual effect size may differ from this assumption. The spatial distribution of the selected sampling points is shown in Figure 2.

Topography characteristics such as elevation, slope, and aspect for each sample point were derived from Türkiye topographic maps. The M24b3 and M25a4 map sheets were digitized, and the corresponding elevation, slope, and aspect values were recorded as attribute data for each point using ArcGIS Pro software (Demir, 2024b). The Corrected Akaike Information Criterion (AICc) was considered, ensuring the ratio of observations to parameters exceeded 40 (Eyduran et al., 2015; Altay, 2022).

Greenhouse farming activities around Isparta are conducted between May and November. During the winter, the coverings are removed, and sampling was performed twice from 92 greenhouses identified for this study. Coordinates of the areas were recorded using GPS, and soil samples were taken from 0-30 cm depth at the end of winter and mid-July (Kacar, 2014). Stratified random sampling was conducted using ArcGIS Pro, identifying 30 points.

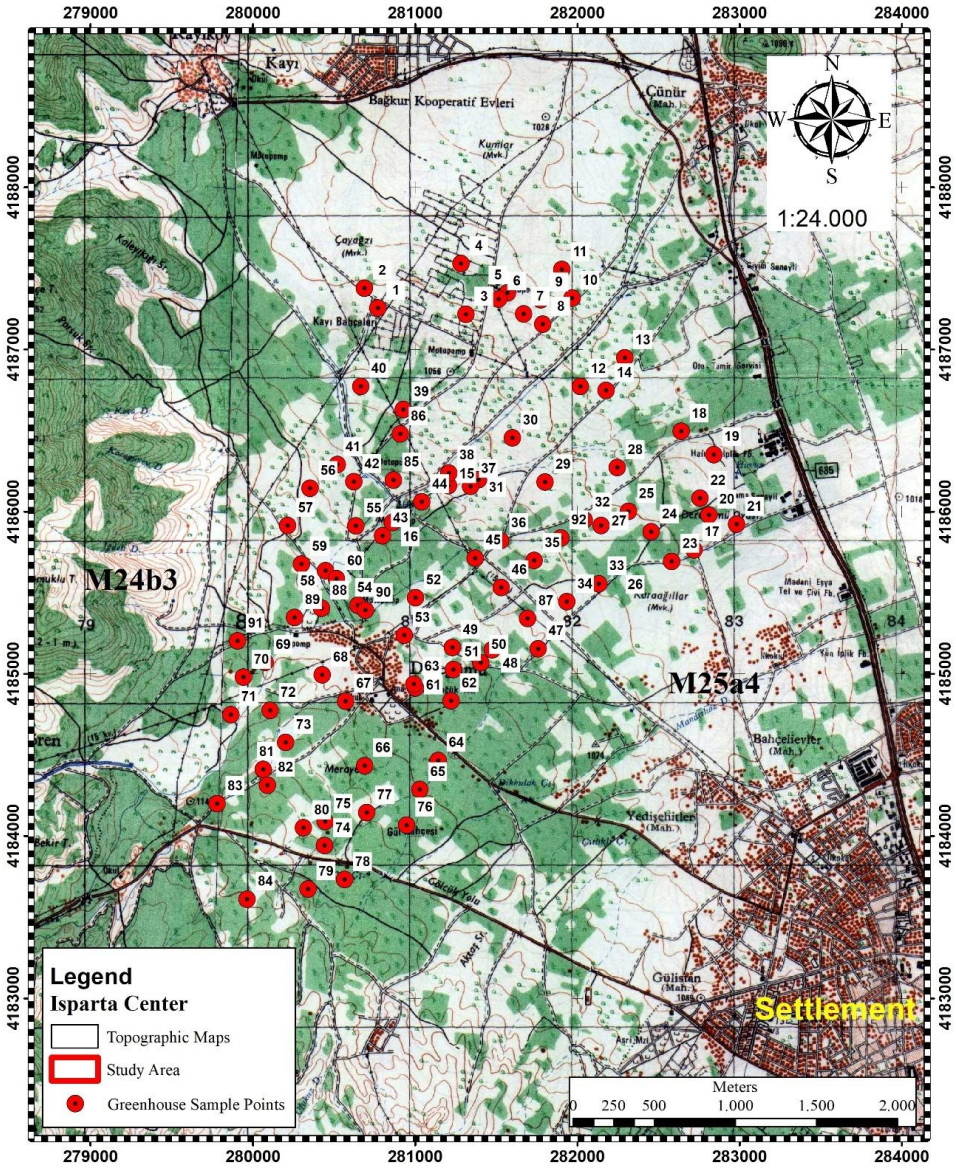

Figure 2. Greenhouse sample points map.

The sampling in two distinct stages was conducted to accurately assess the organic carbon dynamics in the region, particularly considering the practice of greenhouse cultivation in highland areas. During the winter months, production in the greenhouses ceases, and the cover materials are removed, resulting in a significant increase in organic matter inputs. Therefore, the study focuses on the July period, when remote sensing data can effectively capture the status of organic carbon levels, as it reflects the conditions post-cultivation (ÇEM, 2018). By analyzing samples from both periods, this research aims to provide a comprehensive understanding of SOC variations throughout the year, allowing for a better assessment of the seasonal impacts on soil health in the context of highland greenhouse agriculture. Soil sampling from the designated points during the first sampling period was completed between March 19-21, 2023. In the second sampling, additional soil samples were taken from nearby areas with different land uses in July 2023. Soil samples were collected from non-compacted, non-border areas of the plots, placed in polyethylene bags, labeled, and their GPS coordinates recorded. During the land survey, some greenhouses were found to have organic fertilizer added, particularly those growing tomatoes. Carnation greenhouses did not remove their covers in winter. Farmers reported irrigation issues and crop losses due to soil pathogens. Measurements for moisture, temperature, pH, and electrical conductivity (EC) were taken using a KC300 device. Using shading materials in greenhouses to control high temperatures caused changes in light conditions.

2.3. Laboratory Analysis

Soil samples collected from the study area were air dried after being sieved through a 2 mm mesh. The preparation process for the soil samples collected during the first and second sampling periods was completed before analysis. Soil texture analysis was performed using the hydrometer method (Demiralay, 1993). Electrical conductivity (EC) and pH measurements were carried out using a 1:2.5 soil-to-water suspension (Kacar, 2014).

For the determination of SOC air-dried soil samples were sieved through a 500 μm mesh and analyzed using the Modified Walkley-Black method (Kacar, 2014).

These analyses were conducted in the collected soil samples. Organic carbon content was determined on 92 soil samples collected during the first and second sampling periods, with three replicates for each sample. In addition, soil samples collected from adjacent parcels with different land-use types were analyzed with three replicates. Subsequently, calculations were carried out to determine the changes in organic carbon between the two periods, as well as to assess the difference in organic carbon content between the main parcels and adjacent land parcels.

2.4. Statistical Analysis and Modeling

A database was created for the soil samples collected during the first and second sampling periods. Additionally, a dataset was established for the organic carbon content of samples taken from neighboring parcels. The descriptive statistics of the soil samples were evaluated using Minitab 17 software. The normal distribution of the data was checked using the Kolmogorov-Smirnov test (Koşkan et al., 2011; Demir and Başayiğit, 2022). Levene's variance homogeneity test revealed significant differences among the regional types ($P<0.05$); therefore, the Tukey test, significant at $\alpha=0.05$, was employed for post hoc comparisons. This analysis was conducted on 276 different SOC contents and soil organic matter amounts from 92 samples (Demir, 2024a). The organic carbon class range identified through the Turkish Soil Organic Carbon Mapping Project was categorized as very low (<0.6%), low (0.6-1.2%), moderate (1.2-1.8%), good (1.8-2.3%), and high (>2.3%) (Sönmez et al., 2018). Based on these classifications, modeling was performed to classify the observations with the highest accuracy, considering parameters such as sand (%), silt (%), clay (%), pH, electrical conductivity (EC in mmhos/cm), elevation (meters), slope (%), and aspect (°). Classification analyses was conducted using the Weka software (Koçak, 2022). Machine learning models were developed and evaluated, including Logistic Regression (LR), K-Nearest Neighbors (KNN), Support Vector Machine (SVM), Decision Tree (DT), and Random Forest (RF). In the modeling process, a 10-fold cross-validation method was applied to evaluate the performance of the models more reliably, using the entire dataset. This technique ensures that the reported results are not biased by a particular data split, as each fold is used for training and validation. Model accuracies were determined based on the developed models' General Accuracy, Precision, Sensitivity, F-Score, and ROC area measurements (Demir and Başayiğit, 2022). Cross-validation also helps prevent overfitting by testing the models on multiple subsets of the dataset, providing a more comprehensive performance assessment (Demir et al., 2024).

3. Results

3.1. Soil Organic Carbon

SOC is an important measure of ecosystem health and soil fertility. It includes carbon compounds from plants and other organic materials. These compounds greatly affect the structure of the soil, its ability to hold water, and the nutrients it contains. Proper SOC identification and management are crucial for increasing agricultural production, decreasing erosion, and controlling greenhouse gas emissions. Understanding the link between soil organic carbon and topographic parameters is critical in establishing sustainable soil management and environmental protection methods.

Soil organic matter (SOM) refers to the total organic compounds in the soil and is an important factor in evaluating soil health. It consists of plant leftovers, microbial byproducts, and other organic components. SOC is a key component of SOM, indicating the amount of carbon in organic matter (Demir and Başayiğit, 2021). In general, SOC accounts for 58% of SOM, and this ratio is critical for estimating SOM content. As a result of their relationship with SOM, SOC estimations are critical when assessing soil health and fertility.

Table 1 shows descriptive statistics for soil samples taken in greenhouses. The results from the two sampling periods clearly show variations in SOC and SOM content over time. In the first period, the average SOC value was calculated to be 1.047%, with a standard deviation of 0.516 and a coefficient of variation (CV) of 49.313%. This shows that SOC is very variable, with values that deviate greatly from the mean. In the second period, the average SOC decreased to 0.757%, with a standard deviation of 0.543 and a coefficient of variation of 71.789% (Table 1). The larger CV in this phase indicates a wider range of variability in SOC levels, resulting in a more dispersed distribution than in the previous period. The average SOM content in the first period was calculated to be 1.805%, with a standard deviation of 0.890 and a coefficient of variation (CV) of 49.313%. These numbers suggest considerable variability in SOM, demonstrating that the values deviate significantly from the mean. The average SOM content during the second period was 1.304%, with a standard deviation of 0.936 and a CV of 71.789% (Table 1). The increase in the coefficient of variation during the second phase indicates increased variability and dispersion in SOM data. The variability in SOC and SOM rose significantly across the two periods. This shows that the organic content of the soil samples changed significantly over time, which should be considered when evaluating soil management strategies and greenhouse conditions. The notably high coefficients of variation in the second period highlight the importance of conducting a more in-depth investigation of the effects of soil management practices and environmental conditions on greenhouse soil organic content levels. The seasonal results of the soil samples collected from the greenhouse areas provide important insights into the distribution of SOC and SOM.

Table 1. Descriptive statistics of the dataset of greenhouses

Periods	Variable	*n*	Min.	Mean	Max.	StDev	CoefVar	Skew.	Kurt.
1. Period	SOC, %	276	0.104	1.047	2.650	0.516	49.313	0.400	0.259
	SOM, %		0.179	1.805	4.389	0.890	49.313	0.400	0.259
2. Period	SOC, %	276	0.072	0.757	3.601	0.543	71.789	1.677	1.001
	SOM, %		0.124	1.304	6.208	0.936	71.789	1.677	1.001

It can be observed that the skewness and kurtosis values for both periods align with normal distribution. In the first period, the calculated skewness values for SOC and SOM were both 0.400. These values indicate that the data distribution is symmetric, showing no significant positive or negative skew. The kurtosis values for SOC and SOM were also calculated as 0.259 (Table 1). These results suggest that the data distribution is quite close to normal, with neither a highly peaked nor a flat distribution. This means that the data sets tend to follow a normal distribution, without significant centralization or spreading. In the second period, the skewness and kurtosis values for both SOC and SOM were measured as 1.677. The skewness value of 1.677 indicates a positive skew, meaning the data distribution tends to shift to the right of the mean. Similarly, the kurtosis value of 1.677 suggests that the data distribution is slightly peaked compared to normal distribution, indicating that the data points are somewhat more concentrated around the mean (Table 1). However, these values remain within acceptable limits for normal distribution, indicating that the data set still generally conforms to a normal distribution with no major deviations in terms of skewness or kurtosis.

In conclusion, the skewness and kurtosis values for both SOC and SOM in both periods suggest that the data distribution largely follows a normal pattern. This regularity and consistency in the organic content of the soil samples support the assumption of normal distribution, enhancing the reliability of the statistical analyses. These results indicate that normal distribution assumptions are valid for analytically assessing soil organic carbon content.

During the second period of the study, soil sampling was conducted at points near 30 randomly selected greenhouse areas using a stratified random sampling method. These points were located in different land-use types adjacent to the greenhouses. These areas' SOC and SOM values were compared with those observed under greenhouse conditions. The distribution of land-use types for the sampling points is presented in Table 2. Identifying the various land-use types within the project area is crucial for understanding their impact on SOC and SOM. Each land-use type can have distinct effects on soil health and ecosystem dynamics. For example, vineyards and orchards may enhance soil organic matter content, while fallow land and greenhouse fallow practices play a significant role in soil improvement and sustainable agricultural practices. Other land-use types, such as vegetable and rose gardens, may impact soil fertility and organic matter content in addition to serving aesthetic and commercial purposes.

A detailed analysis of these different land-use types contributes to the development of effective soil management strategies and ecosystem management. Furthermore, the results obtained from the different-sized parcels in agricultural areas provide a broad perspective on the soil organic matter and carbon content, supporting the development of more effective management strategies for these parcels. This evaluation provides valuable insights into the effects of various land-use types on soil properties, which are critical for developing optimized agricultural and ecosystem management practices.

Table 2. The land use type in the side plots of some greenhouse areas

Greenhouse ID Number	Land Use Types
48-49-64-65-70-76-79-80	Vineyard
33-40-52-55-66-83-87-89-90-92	Bare fallow
21-36-88	Greenhouse fallow
5-9	Rose garden
16-26-45	Mixed fruit orchard
7-12	Cherry orchard
1	Walnut orchard
14	Vegetable garden

The descriptive statistics results for the 30 greenhouse sampling points and their adjacent plots have been calculated and are presented in Table 3. In the first period, the average values for SOC and SOM were found to be 1.03% and 1.78%, respectively. The coefficient of variation for SOC and SOM during this period was determined to be 51.92% for both, indicating a wide distribution of the data. The skewness and kurtosis values for both components were measured at 0.47 and 0.39, respectively (Table 3). These values suggest that the data distribution is symmetric and exhibits a tendency close to normal distribution, with data points evenly distributed around the mean. In the second period, the average values for SOC and SOM were calculated as 0.73% and 1.26%, respectively. The coefficients of variation for SOC and SOM were found to be 81.25% for both, indicating a broader variation in the data during this period. The skewness and kurtosis values for SOC were 1.48 and 1.59, and for SOM, they were also 1.48 and 1.59 (Table 3). The positive skewness values indicate a positive trend in the data distribution, with values tending to shift right relative to the mean.

Table 3. Descriptive statistics results in the adjacent plot dataset

Periods	Variable	*n*	Min.	Mean	Max.	StDev	CoefVar	Skew.	Kurt.
1. Period	SOC, %	90	0.104	1.034	2.520	0.537	51.921	0.467	0.392
	SOM, %		0.179	1.783	4.344	0.926	51.921	0.467	0.392
2. Period	SOC, %	90	0.075	0.730	3.367	0.593	81.253	1.480	1.586
	SOM, %		0.129	1.259	5.805	1.023	81.253	1.480	1.586
Adjacent Plot	SOC, %	90	0.047	0.631	2.161	0.467	74.018	1.407	1.725
	SOM, %		0.081	1.088	3.726	0.805	74.020	1.408	1.725

The slightly higher kurtosis values suggest that the data distribution is more peaked than a normal distribution, with data points clustering closer to the mean. For the adjacent plot, the average values for SOC and SOM were calculated as 0.63% and 1.09%, respectively. The coefficients of variation for SOC and SOM in this plot were both 74.02%, indicating a significantly wide distribution of these data. The skewness and kurtosis values for SOC were measured at 1.41 and 1.72, respectively, and for SOM, they were also 1.41 and 1.72 (Table 3). The high values of skewness and kurtosis indicate that the data distribution deviates more from normality, with data points exhibiting a rightward shift and a more peaked distribution compared to a normal distribution.

In conclusion, the skewness and kurtosis values obtained from both periods and the adjacent plot indicate that the data distributions exhibit a certain degree of normal distribution tendency. However, some periods and plots show tendencies of deviation from normality. This suggests that soil organic content varies over time and may exhibit significant differences across different areas. These results provide important insights for soil management and assessment.

The sampling conducted in greenhouse areas resulted in an analysis of SOC content over two different periods. In the first period, the average SOC content was determined to be 1.047% ± 0.516, while in the second period, this value was measured at 0.757% ± 0.543. According to the results of the variance analysis, the difference between the two periods was found to be statistically significant at the 95% confidence level. Based on the Tukey post-hoc test, the SOC content in the first period was classified as "A," and that in the second period as "B" (Table 4). These results indicate that the SOC content in greenhouse areas varies seasonally, and this variation is statistically significant. A higher SOC content was observed in the first period, while this value showed a marked decrease in the second period. This reduction may suggest the influence of seasonal changes, agricultural management strategies employed, or other environmental factors on SOC content. The results indicate that during the winter months in highland conditions, lower temperatures lead to reduced soil biological activity, resulting in decreased soil organic carbon decomposition. However, in open greenhouses, organic matter applications and precipitation contribute to an increase in soil organic carbon levels during this period. In contrast, during the summer months, rising temperatures and heightened soil biological activity are observed, which, coupled with the elevated temperatures within greenhouse conditions, can lead to a reduction in organic carbon content. The determined change in SOC content emphasizes assessing and optimizing greenhouse management and soil improvement measures. Furthermore, it is recommended that more comprehensive studies be conducted to determine whether the seasonal variations are associated with microenvironmental conditions within the greenhouse or changes in agricultural practices. These results could provide a significant foundation for making strategic decisions in greenhouse management and optimizing SOC content.

Table 4. Seasonal SOC content variance analysis results of greenhouse areas

Periods	*n*	Means ± StDev
1. Period	276	$1.047 \pm 0.516^{A*}$
2. Period	276	0.757 ± 0.543^{B}

*= a statistically significant difference exists between the groups ($P<0.05$).

SOC content in greenhouse areas has been assessed using investigations performed over multiple periods and on adjacent plots. The average SOC content obtained from the adjacent plot is significantly lower (0.631%) than the periodical data gathered from the greenhouse, especially when compared to the SOC content obtained during the first period (1.034%). Furthermore, the SOC content in the second period (0.730%) is consistent with the samples collected from the adjacent plot. However, the initial period's values are significantly higher, indicating a considerable divergence from the SOC contents reported in subsequent periods (Table 5).

Table 5. Variance analysis results of SOC in some greenhouse plots and adjacent plots

Periods	n	Means ± StDev
Adjacent Plot	90	$0.631 \pm 0.467^{B*}$
1. Period	90	1.034 ± 0.537^{A}
2. Period	90	0.730 ± 0.593^{B}

*= a statistically significant difference exists between the groups ($P<0.05$).

These differences between periods may stem from changes in greenhouse management practices, the effects of agricultural activities, or other environmental

conditions. Notably, the high SOC content during the first period suggests an accumulation of organic matter under specific conditions of that period, whereas a significant reduction in SOC content is observed in subsequent periods and the adjacent plot. It is important to consider that the SOC content in the adjacent plot exists at a different level compared to the conditions within the greenhouse and displays similar seasonal variations.

These results underscore the need for assessing the effectiveness of in-greenhouse practices and soil management strategies, as well as evaluating soil characteristics across different plots. Additionally, this data provides a critical foundation for strategic planning aimed at monitoring and improving soil organic carbon levels.

SOC content under greenhouse conditions exhibits significant variations based on periodic analyses. During the first period, the SOC content was determined to be 1.034%, indicating a high accumulation of organic matter in the greenhouse environment. However, in the second period, the SOC content decreased to 0.730%, reflecting a reduction in organic carbon levels. The SOC content obtained from the adjacent plot was found to be 0.631%, which is comparable to the values recorded during the second period. These results demonstrate that the organic carbon content within the greenhouse varies over time. This variability highlights the need to review greenhouse management practices and strategies for organic matter addition.

3.2. Machine Learning Models for Greenhouse SOC

The dataset for classifying SOC content under greenhouse conditions consisted of 276 observations and 8 variables. The variables used in the prediction included clay content (%), silt content (%), sand content (%), electrical conductivity (EC) (mmhos/cm), soil pH value, elevation (meters), slope percentage (%), and aspect direction (degrees). These variables were utilized in the modeling process to predict the soil organic carbon (%OC) content. The descriptive statistics results for these variables are shown in Table 6. The mean clay content was 12.551%, with a Coefficient of Variation of 14.81%, ranging from 9.986% to 15.104%. The clay distribution was relatively symmetrical, indicated by a skewness of -0.03 and a kurtosis of -1.41. The mean silt content was 21.633% (CoefVar = 8.79%), with values ranging from 18.989% to 24.633%. A slight positive skewness of 0.19 and a kurtosis of -0.96 suggested a mild tendency towards higher silt values. Sand content averaged 65.815% (CoefVar = 4.28%), ranging from 62.686% to 71.025%, with a rightward skewness of 0.97 and a kurtosis of -0.30, indicating a distribution close to normal. Electrical conductivity averaged 0.59391 mS/m (CoefVar=25.11%), with values between 0.32 and 0.72 mS/m, demonstrating a negatively skewed distribution (skewness=-1.13) and a kurtosis of -0.66, indicating extreme values. The mean pH level was 7.0773 (CoefVar=8.85%), ranging from 6.0200 to 7.7990, reflecting a negatively skewed distribution (skewness=-0.73) and a kurtosis of -0.92. The average elevation was 1077.0 meters (CoefVar=2.67%), with a range of 1035.0 to 1151.0 meters, showing a mild positive skewness (skewness=0.45) and a kurtosis of -0.73. The mean slope was 2.4096% (CoefVar=53.93%), with values between 0.0000% and 5.6290%, indicating a slight positive skewness (skewness=0.45) and a kurtosis of -0.49. Finally, the average aspect was 115.13° (CoefVar=95.72%), with values ranging from -1.00° to 354.81°, displaying a positive skewness of 1.11 and a kurtosis of -0.09, suggesting a relatively uniform distribution.

In the Türkiye Soil Organic Carbon Mapping Project, the organic carbon content classes were defined as follows: very low (<0.6%) as Class I, low (0.6-1.2%) as Class II, moderate (1.2-1.8%) as Class III, good (1.8-2.3%) as Class IV, and high (>2.3%) as Class V [29] (ÇEM, 2018). The accuracy of the developed machine learning models was evaluated using the Cross-Validation method. This method allows for more reliable testing of each model's performance by repeatedly training and testing on various subsets of the dataset. LR, KNN, SVM, DT, and RF algorithms were systematically tested on the data partitions defined in this process. Cross-Validation helped provide clearer measurements of each model's overall accuracy, precision, sensitivity, F-score, and ROC area, revealing how the models performed across different data subsets.

The advantages and disadvantages of the models can be summarized as follows: LR, while being a simple and fast model, may be limited in capturing complex relationships. KNN can offer high accuracy but may slow down with large datasets.

Table 6. Descriptive statistics results of independent variables dataset (n=276)

Variable	Min.	Mean	Max.	StDev	CoefVar	Skew.	Kurt.
Clay, %	9.99	12.55	15.10	1.86	14.81	-0.03	-1.41
Silt, %	18.99	21.63	24.63	1.90	8.79	0.19	-0.96
Sand, %	62.69	65.82	71.03	2.81	4.28	0.97	-0.30
EC, mmhos/cm	0.32	0.59	0.72	0.15	25.11	-1.13	-0.66
pH,	6.02	7.08	7.80	0.63	8.85	-0.73	-0.92
Elevation, meters	1035.00	1077.00	1151.00	28.80	2.67	0.45	-0.73
Slope, %	0.00	2.41	5.63	1.30	53.93	0.45	-0.49
Aspect, °	-1.00	115.13	354.81	110.20	95.72	1.11	-0.09

SVM achieves high success in classification but may require parameter tuning. DT provides high interpretability but poses a risk of overfitting. RF generally offers high accuracy and minimizes overfitting risk, but the model's explainability can be more challenging. This evaluation process provides a solid foundation for selecting the most suitable approach for predicting organic carbon content by analyzing the basic logic and performance of each model.

When evaluating the performance of the developed classification models, different results were obtained in terms of each model's ability to predict soil organic carbon (%OC) content. The evaluation results are reported here using weighted averages from several categorization algorithms.

The LR model has limitations due to missing metrics such as precision and F-score, making it difficult to fully assess its overall performance. However, it achieved a sensitivity value of 50.7% and an ROC area of 0.595 (Figure 3). These results indicate that the LR model has limited success in predicting soil organic carbon content.

The KNN algorithm reached a sensitivity of 52.5% and an ROC area of 0.611. The performance of KNN could not be fully evaluated due to missing precision and F-score metrics (Figure 3). Nonetheless, KNN showed a slightly higher overall accuracy compared to LR.

The SVM model exhibited lower performance with a sensitivity of 49.6% and an ROC area of 0.552 (Figure 3). The lack of precision and F-score values limited SVM's ability to predict soil organic carbon content.

The DT model demonstrated the highest performance. It achieved a precision of 75.3%, a sensitivity of 75.4%, and an F-score of 75.1. Additionally, the ROC area was determined to be 0.874, with an overall accuracy of 75.4% (Figure 3). These results indicate that the DT model is effective in classifying soil organic carbon content successfully.

The RF model exhibited the highest performance. It attained a precision of 77.3%, a sensitivity of 76.4%, and an F-score of 75.1. The ROC area was determined to be 0.904, with an overall accuracy of 76.4% (Figure 3). RF was observed to be the most successful model in predicting soil organic carbon content.

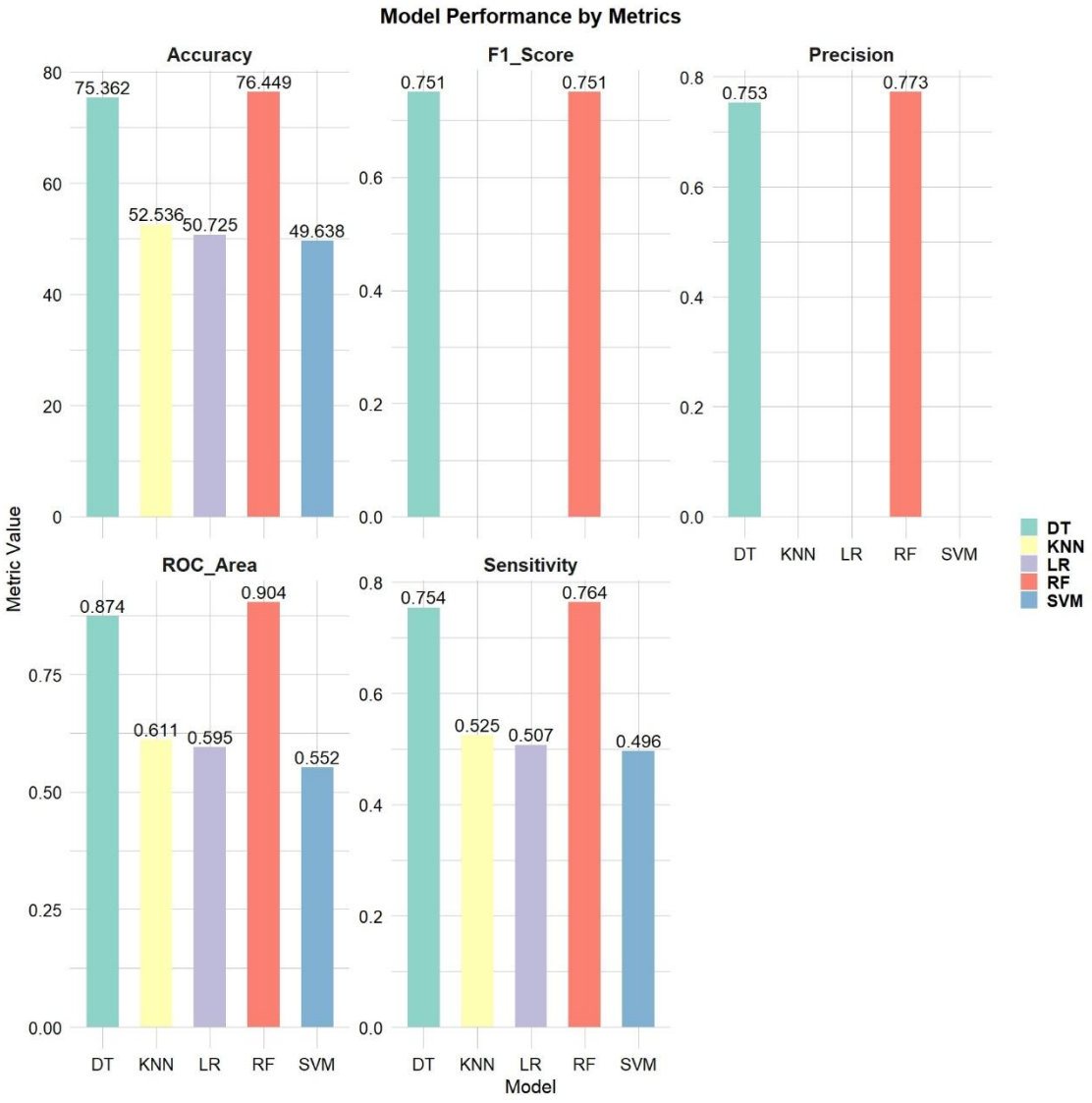

Figure 3. Performance results of SOC machine learning models.

In conclusion, the RF and DT models outperformed other models in classifying soil organic carbon content (Figure 3). These models are regarded as the most suitable methods for effectively predicting soil organic carbon content due to their high accuracy rates and robust performance metrics (Demir and Başayiğit, 2022). Future studies could conduct more detailed analyses on the applicability and improvement of these models.

The model with the highest accuracy in classifying organic carbon in greenhouses is the RF model. The confusion matrix representing the classification performance of the RF model is presented in Figure 4.

The RF model has demonstrated a higher accuracy in classifying organic carbon content compared to other models. The accuracy rate of the RF model is determined to be 76.449%, which reflects the model's overall performance quite successfully.

The confusion matrix illustrates the relationship between the RF model and the class labels:

I. Class (Very Low): In this class, 96 samples were correctly classified, while 20 samples were misclassified. This result indicates that the RF model identifies this class with high accuracy (Figure 4).
II. Class (Low): In this class, 103 samples were correctly classified, whereas 23 samples were misclassified. This indicates that the RF model effectively identifies this class with a high degree of accuracy (Figure 4).
III. Class (Medium): In this class, 7 samples were correctly classified, and 16 samples were misclassified. This suggests that while the model recognizes this class relatively well, it encounters some challenges (Figure 4).
IV. Class (Good): In this class, 4 samples were correctly classified, and 5 samples were misclassified. This result shows that the RF model classifies this class with reasonable accuracy but with some instances of misclassification (Figure 4).
V. Class (High): In this class, 1 sample was correctly classified, and 5 samples were misclassified. This indicates that the model faces challenges in classifying this class (Figure 4).

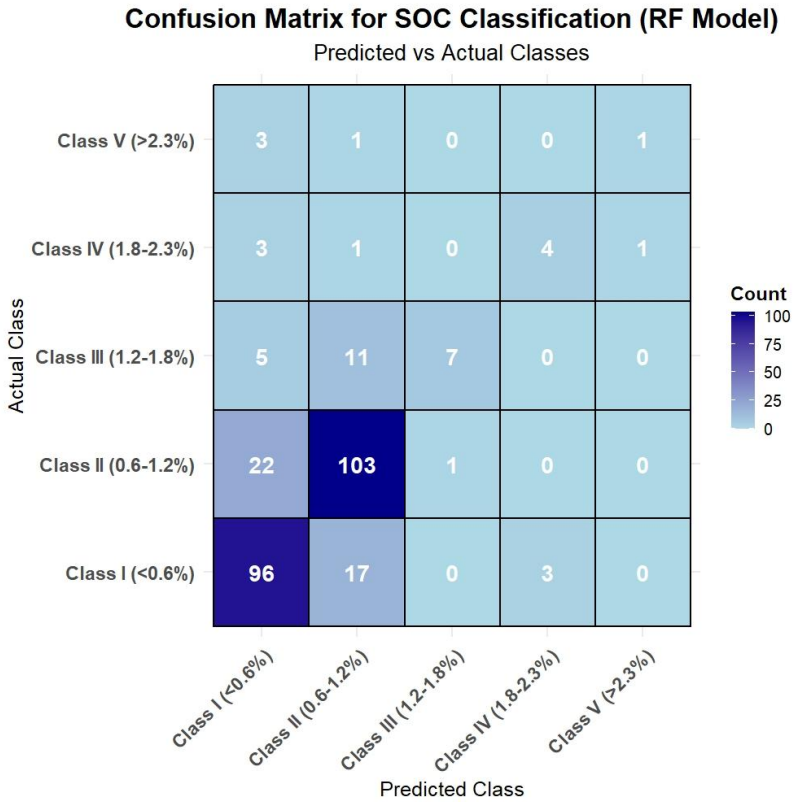

Figure 4. RF model confusion matrix results.

Figure 4 shows the classification results of the RF model, which was the most accurate in recognizing soil organic carbon and indicated overall efficacy. Although the model performs well in certain classes, such as Classes I and II, it is more likely to misclassify others. These results confirm the RF model's effectiveness as a powerful tool for detecting organic carbon soil, but they also highlight the need for enhancements in specific classes. Thus, reviewing model parameters and training data may help improve classification accuracy.

4. Discussion

This study presents substantial results on the performance of SOC content classification using several machine learning models, such as RF, LR, KNN, DT, and SVM. The results show that the RF model has the highest

accuracy (76.449%), showing its applicability for SOC classification (John et al., 2020; Zhang et al., 2024). The investigation used a complete dataset that included land and laboratory data as well as topographic features, allowing for high-precision categorical classification of SOCs (Fathizad et al., 2022). A significant asset of this study is the comparison of multiple machine learning methods, which enhances our understanding of their effectiveness in identifying SOC material (John et al., 2020). Regarding the study's advantages, some restrictions were noted. The dataset was geographically restricted, and the sample numbers for several classifications were limited. For instance, Classes III, IV, and V had poorer prediction accuracy, suggesting that it would be challenging to discern between these groups. These results highlight the necessity of more investigation to improve categorization methods and deal with these issues in future studies (Fathizad et al., 2022).

While previous study suggests that the RF model performs well with similar environmental data (Yang et al., 2016; Wang et al., 2018; Fathizad et al., 2022; Loria et al., 2024), it is crucial to note that other algorithms, such as SVM and DT, may perform better under certain scenarios (Bernardini et al., 2024; Agaba et al., 2024). This shows that choosing a suitable model should be context-dependent, taking into consideration the dataset's and study area's unique characteristics. Future research should focus on using larger and more diverse datasets to back up the conclusions of this study. Furthermore, hybrid models and deep learning techniques are proposed to improve model performance (Odebiri et al., 2021; Saporetti et al., 2022; Pouladi et al., 2023; Moharana et al., 2024). It is noteworthy that pyroclastic deposits play a crucial role in soil formation, particularly in the context of SOC development. These deposits, characterized by their rich mineral content and unique physical properties, provide an essential substrate for soil organic matter accumulation (Elitok et al., 2009; Saputra et al., 2022). The influence of pyroclastic materials on nutrient availability and moisture retention can significantly enhance SOC levels, particularly under greenhouse conditions. The study of SOC content under greenhouse conditions on pyroclastic deposits provided valuable insights, emphasizing the necessity for better model parameters and larger datasets for the reliable monitoring of organic carbon levels. While this study did not specifically address economic statistics, it does acknowledge the enormous economic benefits of SOC classification in terms of agricultural output and sustainable farming techniques. Future research should consider these economic implications to develop a more comprehensive understanding of SOC management (Stockmann et al., 2013; Mayer et al., 2020; Derrien et al., 2023).

The conclusions of this study contribute to the development of effective soil management strategies for greenhouse agriculture in the Isparta region. Additionally, the results offer valuable insights into the impact of global warming and climate change on soil organic carbon dynamics, enhancing our understanding of these critical environmental processes.

5. Conclusion

The study successfully proved greenhouse cultivation's effects on organic carbon soil under plateau circumstances in the Isparta region. The information, which included field and laboratory data and topographic features, was evaluated using machine learning techniques, and soil organic carbon concentrations were accurately classified categorically. The results enabled a more precise assessment of soil organic matter levels in greenhouse-growing regions, overcoming the inadequacies of prior approaches and representing a substantial advancement.

The analysis of soil organic carbon content under greenhouse conditions revealed that the RF model was the most accurate, confirming its efficiency in organic carbon classification. However, several misclassifications were observed, particularly in the high and well-represented classes. Based on these results, it is recommended to optimize the model parameters and use larger datasets for more accurate and reliable monitoring of organic carbon in greenhouse conditions. Additionally, a review of greenhouse management practices aimed at increasing organic carbon levels is necessary.

This study contributes to soil management practices for greenhouse horticulture in the region while enhancing our understanding of the impacts of global warming and climate change on soil organic carbon in Türkiye. Further research into the interactions between pyroclastic deposits and organic matter is essential for optimizing soil health and quality. The results offer valuable insights into future land management and sustainable agricultural strategies and serve as a foundation for similar research in other ecosystems.

Author Contributions

The percentages of the authors' contributions are presented below. The author reviewed and approved the final version of the manuscript.

	S.D.	M.E.Ç.
C	90	10
D	100	
S	100	
DCP	50	50
DAI	80	20
L	50	50
W	80	20
CR	60	40
SR	80	20
PM	50	50
FA	50	50

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

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

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DETERMINATION OF SOME MORPHOLOGICAL PARAMETERS OF *Anoplophora chinensis* (FORSTER) (COLEOPTERA: CERAMBYCIDAE)

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Abstract: The citrus longhorned beetle, *Anoplophora chinensis* (Forster), native to East Asia, poses a significant threat to various crops and tree species in our country, including hazelnuts, due to its invasive nature and wide host range. In this study, the morphological characteristics of *A. chinensis* collected from infested hazelnut fields in Arifiye district, Sakarya province in Türkiye, during June, July, and August of 2024, were examined. Various parameters, such as body size, antenna length, elytra structure, and pronotum length, were analyzed on 200 adult beetles (100 ♀, 100 ♂), and distinct differences in morphological parameters between the sexes were observed. Measurements revealed that the body length, width, and elytra length of female individuals averaged 30.94 mm, 11.50 mm, and 22.89 mm, respectively, while male individuals measured 27.68 mm, 10.02 mm, and 20.27 mm. The number of antenna segments was determined to be 11 in both sexes, with the antenna length averaging 38.18 mm in females and 47.85 mm in males. It was observed that females had larger body sizes than males, but males possessed longer antennae. These findings are provided descriptions of some morphological parameters related to the sexes of *A. chinensis*.

Keywords: Invasive species, Citrus longhorned beetle, Morphological analysis, Description

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1. Introduction

The Citrus longhorned beetle (CLB) [*Anoplophora chinensis* (Forster) (Coleoptera: Cerambycidae)], native to East Asia, has been reported in China, Japan, and Korea, as well as Vietnam, Taiwan, the Philippines, Myanmar, Malaysia, and Indonesia (Gressitt, 1951; Lingafelter and Hoebeke, 2002). CLB is a polyphagous, invasive species, and known as a pest of citrus, but over 100 tree and shrub species from 36 families have been reported as host plants (Haack et al., 2010). In the areas it invades, in addition to citrus (*Citrus* spp.) and hazelnut (*Corylus* spp.), CLB bores galleries into economically important trees such as *Acer* spp., *Betula* spp., *Malus pumila*, *Pyrus* sp., *Salix* sp., *Melia azedarach*, and *Casuarina equisetifolia*. It spends its pre-adult stages within these trees, consuming xylem tissue, and causing dieback in the affected areas (Hérard et al., 2006; Liu, 2013; Ge et al., 2014). Although CLB typically completes its life cycle within one year, this cycle can extend to two years depending on factors such as host species and environmental conditions (Adachi, 1994).

International plant trade eliminates biogeographical barriers, allowing invasive pest species like CLB to be rapidly transported to different regions of the world (Hulme, 2009; Venette and Hutchison, 2021). CLB was first detected outside its natural range in the Netherlands in 1980, and since then, it has spread to many countries via ornamental plants imported from areas where it naturally occurs and from regions with established populations (Haack et al., 2010; Loomans et al., 2013). The beetle was first added to the quarantine lists in the EPPO region in 1994 and is currently listed in the quarantine lists of many countries in Africa, America, Asia, and Europe due to the destructive damage it caused (EPPO, 2024). The pest has been detected in Croatia, Denmark, France, Germany, Guernsey, Italy, Lithuania, the Netherlands, Switzerland, Türkiye and the United Kingdom. Although eradication efforts have successfully controlled it in many countries, it still persists in some areas of Croatia, Italy, and Türkiye (Hérard and Maspero, 2019). CLB was first detected in 2014 at the Kumbaba Nursery in Şile, Istanbul, Türkiye (Hızal et al., 2015). Initial detections have also been reported in various provinces, including Bartın (Yildiz, 2017), Trabzon, Antalya (Eroğlu et al., 2017; Topakcı et al., 2017), Sakarya (TOB, 2021), and Diyarbakır (Özdikmen and Şeker, 2021). The pest was first detected in Turkish hazelnut orchards in 2018 and has been highlighted as a serious threat to sustainable hazelnut production (Bozkurt, 2018; Tuncer et al., 2020). Following the detection of CLB in hazelnut orchards in Türkiye, quarantine measures

were rapidly implemented, and as a result of eradication efforts carried out in hazelnut orchards infested with CLB, approximately 2 million dollars in compensation was paid to farmers (Turan and Erdoğan, 2022; Dogan et al., 2024).

In this study, the morphological characteristics of CLB populations in the Arifiye district of Sakarya province were examined, and various parameters such as body size, antenna length, elytra structure, and pronotum length were evaluated to reveal the differences between sexes of the insect.

2. Materials and Methods

2.1. Collection of Insects

As part of the research, healthy and alive adult individuals of CLB (100 ♀, 100 ♂) were collected from hazelnut fields in the Arifiye district of Sakarya province in Türkiye, which were found to be infested with the pest, during June, July, and August of 2024.

2.2. Morphological Parameters and Measurements

The identification of the collected adult CLBs was carried out with the help of literature (Gyeltshen and Hodges, 2005; EPPO, 2021). The collected insects were killed by placing them in a deep freezer at -20 °C for 5 minutes, then thawed for 5 minutes in the laboratory before being subjected to morphological examination. Any individuals with physical defects were excluded from the analyses. In the morphological analyses, parameters such as the sex of the adult individuals, body length, body width, antenna length, elytra length, pronotum length, number of antenna segments, number of spots on the elytra, and presence of tubercles were examined (Lingafelter and Hoebeke, 2002; EPPO, 2021). Measurements were taken using a digital caliper with ±0.01 mm accuracy.

3. Results

In this study, various morphological characteristics of adult CLB individuals collected from different hazelnut fields in the Arifiye district of Sakarya province were examined. According to the results, significant differences were identified between the sexes (Figure 1). The average body length of female individuals was recorded as 30.94±0.38 mm, while that of males was 27.68±0.32 mm. Additionally, the average body width of females was 11.50±0.18 mm, whereas in males, this value was recorded as 10.02±0.13 mm. The average antenna length was 38.18±0.46 mm in females, while it was found to be 47.85±0.69 mm in males. It was determined that the antenna length in females was 1.2 times the body length, while this ratio was 1.7 times in males. These results show that the antenna length of males is significantly greater than that of females, while males are shorter in terms of body length and width (Table 1).

Table 1. Sex-based morphological measurements of *Anoplophora chinensis*

Sex	Body Length (mm)	Body Width (mm)	Antennal Segment Count	Antenna Length (mm)	Elytra Length (mm)	Pronotum Length (mm)	Number of White Spots on Elytra	Number of Individuals (n)
Female	30.94±0.38	11.50±0.18	11±0	38.18±0.46	22.89±0.30	4.91±0.12	35±1	100
	Min: 17	Min: 7.1		Min: 25.7	Min: 13.5	Min: 3.6		
	Max: 36	Max: 16.7		Max: 45.8	Max: 29.1	Max: 9.1		
Male	27.68±0.32	10.02±0.13		47.85±0.69	20.27±0.26	4.31±0.09	34±1	100
	Min: 20.1	Min: 8		Min: 36	Min: 11.5	Min: 2.7		
	Max: 32.8	Max: 12.8		Max: 58.4	Max: 23.8	Max: 7.3		

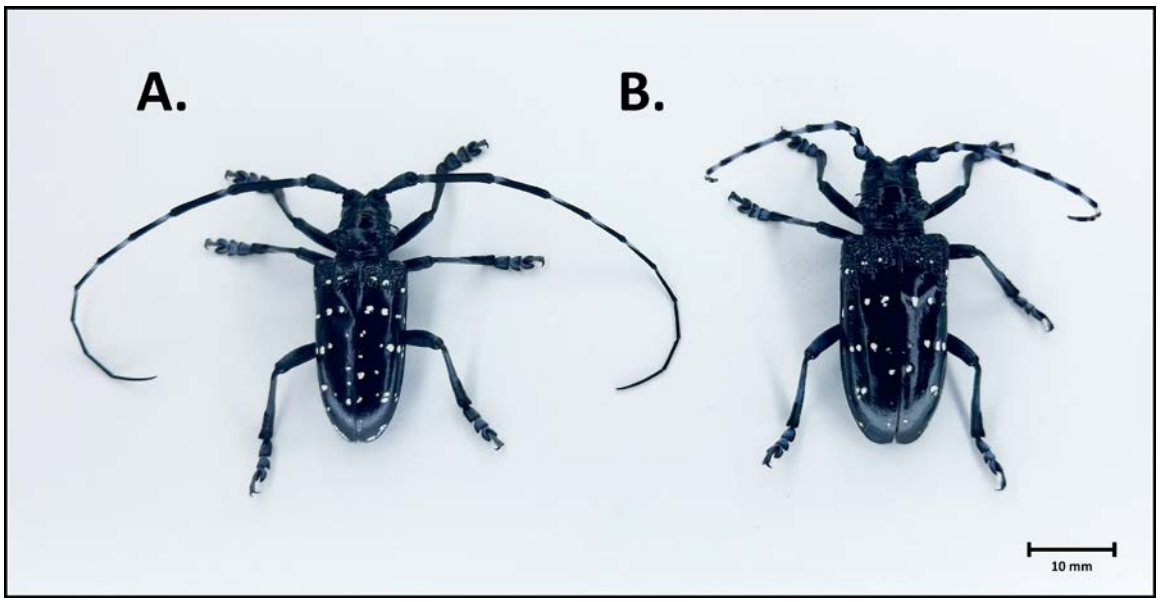

Figure 1. Adult male (A) and female (B) of *Anoplophora chinensis*.

The pronotum length in females was found to be an average of 4.91±0.12 mm, while in males it was 4.31±0.09 mm. Additionally, the elytra length was measured as 22.89±0.30 mm in females and 20.27±0.26 mm in males. An increase in elytra length was observed in correlation with the increase in body length. The number of white spots on the elytra was recorded as an average of 35 in females and 34 in males, with no significant difference between the sexes. Both sexes were found to have tubercles on the elytra (Figure 1).

4. Discussion

CLB adults were glossy black in color and have irregularly shaped spots on their elytra. It has been noted that the body length of adult beetle's ranged from 17 to 40 mm, that tubercles were present in the basal quarter of the elytra, and that there were 10 to 20 irregularly shaped spots on the elytra, though in rare cases, this number can exceed 60 (Lingafelter and Hoebeke, 2002; Haack et al., 2010). In the study conducted in Trabzon province in Türkiye, Eroğlu et al. (2017) measured 14 female and 9 male individuals and reported that females had an average of 11-12 spots and males had 14-15 spots on their elytra. They also found that the average body length of females was 28.6 mm (24-33.5 mm) and their body width was 10.3 mm, while the average body length of males was 25.4 mm (23-30.1 mm) and their body width was 8.9 mm. In another study, Hızal et al. (2015) reported that the sizes of adult CLBs collected from Istanbul ranged from 27 to 34 mm. Our data revealed a significant difference in the number of spots on the elytra compared to other studies. Although the number of tubercles did not recorded in this present study, the presence of these structures confirms that the beetle population is CLB. While our data on body length aligns with the findings of Hızal et al. (2015), the average body sizes we recorded 30.94±0.38 mm for females and 27.68±0.32 mm for males were larger than those reported by Eroğlu et al. (2017). These differences may be due to the smaller number of specimens examined in their study compared to ours. Additionally, a significant morphological differences were observed in terms of body size and antenna length. Female individuals were found to have a significantly larger body size compared to males, while males had longer antennae. These findings are consistent with previous studies on the morphology of CLB (Hızal et al., 2015; Eroğlu et al., 2017). The antennae of adult CLB are long and consist of 11 segments, with each segment having a white or light blue band at the base and black tips. Similarly, in our study, we found a maximum of 11 segments in the adult beetles examined, and the observed colors matched the white or light blue colors specified in EPPO (2021). According to previous studies, antenna lengths in females and males were reported to be 33.1 mm and 44.1 mm, respectively (Eroğlu et al., 2017). In our study, antenna lengths were measured as 38.18±0.46 mm in females and 47.85±0.69 mm in males, indicating that the antennae were longer in both sexes. Another difference between males and females is that in males, the tip of the abdomen is completely covered by the elytra, while in females, a portion of the abdomen is exposed (Lieu, 1945). This difference was also observed in the specimens examined in our study. Additionally, in adult CLB individuals, the pronotum narrows towards both the top and bottom and is characterized by sharp spines extending laterally.

5. Conclusion

This study revealed some morphological parameters of *Anoplophora chinensis*. The differences, particularly in antenna length and body size are elucidated important information for sex determination and thus aid in the identification of the species.

Author Contributions

The percentages of the authors' contributions are presented below. The author reviewed and approved the final version of the manuscript.

	F.D.	İ.O.Ö.	S.K.
C	20	40	40
D	60	40	
S		100	
DCP	100		
DAI	70	30	
L	40	40	20
W	40	40	20
CR	20	60	20
SR	40	40	20
PM	40	40	20
FA	40	40	20

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 due to the use of research materials that did not fall under the definition of experimental animals (The Scientific and Technological Research Council of Türkiye, Animal Experiments Local Ethics Committee Directive, 2018, Article 3-c).

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INVESTIGATION OF FEED VALUE OF NATURAL PASTURES IN ŞANLIURFA REGION AND FATTENING PERFORMANCE OF AWASSI LAMBS GRAZING IN PASTURES

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Abstract: In this study, samples were collected from natural pastures in the districts of Haliliye and Eyyübiye, where pasture-based sheep breeding is practised in the Tek Tek Mountains region, at different times (1 March, 1 April, 1 May, 1 June, 2020). For the purpose of sampling, quadrats measuring 50 x 50 cm (equivalent to 0.25 m^2) were positioned in four randomly selected areas at four different points in time. The grasses were manually harvested at ground level. The objective of this study was to ascertain the dry matter (DM), crude ash (CA), crude protein (CP), ether extract (EE), neutral detergent insoluble fibre (NDF) and acid detergent insoluble fibre (ADF) values in the pasture samples collected. In addition, during the study, the live weights of lambs from 30-31 lambs (Pasture1 F/M:18/12; Pasture2 F/M:15/15 Pasture3 F/M:15/15 Pasture4 F/M:15/16) reared in these areas and born during the same period were recorded at the start and end of the grazing period. The data obtained from the research were subjected to analysis according to the Least Squares method. The Duncan multiple comparison test was employed to ascertain the existence of any significant differences between the groups. The results of the analysis of the raw nutrient contents of four different pasture regions in March, April, May and June were presented. Accordingly, the levels of DM, CA, CP, CF, NDF and ADF were determined to be between 24.3 and 43.3, 7.64 and 10.48, 7.72 and 14.85, 2.28 and 2.64, 33.34 and 60.88, and 28.66 and 41.97, respectively. While the average DM, NDF and ADF levels increased over the periods, the CP level decreased. The greatest increase in live weight was observed in male lambs in pasture 3 herd. The highest live weight gain in female lambs was detected in the herd in pasture4. It can be posited that the aforementioned values pertaining to live weight gain in pasture-based feeding will enable breeders to augment their production through supplementary feeding.

Keywords: Natural pastures, Nutrient content, Forage, Awassi, Live weight

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1. Introduction

Fodder plants are produced to meet the nutritional needs of animals to continue their life activities also have qualities such as protecting soil and water, improving the yield of subsequent agricultural products in crop rotation, and are plants that are dried or made into silage after being harvested. Many species belonging to the legume and wheat family are among the forage plants. On the other hand, many plant species outside these families are used as a source of animal feed in the world. Some of these are produced as agricultural products, while others grow naturally in nature (Temel and Tan, 2012). Especially in feeding ruminant animals, production cost cover 60-70% of feed cost. This situation is of great importance in terms of meeting the demand for quality roughage of the animal, despite the feed cost having 70% input for the enterprises dealing with animal husbandry. The primary sources of quality roughage are meadows and pastures, forage crops and silage (Şeker, 2006). This situation clearly shows how important roughage is for economic animal production in ruminant nutrition. In our country, the source from which the need for roughage is mostly met is our pastures (Avcıoğlu, 2000). Our natural meadows and pastures, which are the most important sources of nutrition for our country's livestock, have decreased significantly compared to the past. While they covered almost half of our country's land with 44 million hectares in the 1940s, these rates have now decreased to approximately 14-15 million hectares. Due to excessive, unplanned and early grazing in these natural forage areas for years, imbalances have been observed in the natural vegetation and have become erosive. In 1940, this pasture area per animal was 3.38 ha, and in recent years, it has decreased to 1.18 ha, and with the increase in animal husbandry in the country, there has been a three-fold increase in the number of animals grazing per unit area (Kuşvuran et al., 2011). Small ruminant farming has an important place in the livestock activities of the GAP region. The presence of sheeps and goats in the Southeastern Anatolia Region

covers approximately one-fourth of the small ruminant animals in Türkiye (TUIK, 2019).

The total area of our pastures in Şanlıurfa is 234,357 hectares. Our pastures have poor pasture quality due to insufficient rainfall. While the green grass yield in the pasture area in the region is 680 kg/hectare, this rate is approximately 200 kg/hectare for dry grass yield (General Directorate of Agriculture and Forestry, Turkish abbreviation of OGM, 2016). The quality of pasture grasses varies depending on the vegetation period, fauna, climate factors, growth conditions, suitability for irrigation and fertilization. The surface area of Şanlıurfa province is 1,858,400 ha and 234,357 ha of this area is meadow / pasture area. (Turkish abbreviation of OGM, 2016). Considering the vegetation cover of pastures and the geographical conditions of Türkiye, ovine husbandry stands out as the most suitable livestock breeding activity. As fewer people live in rural areas, there has been a corresponding decline in demand for products made from sheep and goats (Cedden et al., 2020). Instead, a large percentage of Türkiye's livestock consists of sheep and goats. Not only does Türkiye have the highest density of sheep in Europe, but the percentage of cattle considered small ruminants ranges from 50 to 85 percent, with some variation between regions (Oral and Yıldız 2023). Türkiye's red meat production (estimated at 2 million tons in 2021), 24.6% is from ovine meat, with 19.8% coming from sheep and 4.8% from goats (Gül Varış and Pınar 2024).

In Şanlıurfa province, sheep breeding is in the form of family businesses, and the sheeps raised in the region spend the night in the pasture depending on the climate conditions and are brought to the farm in the morning for milking and feeding the lambs. The milked and rested sheeps are taken back to the pasture. Cultivation is carried out in pasture conditions without additional feed between March and early April, when the climate and pasture vegetation are suitable, and until September, when the vegetation is insufficient. Therefore, research on the nutrient content of pastures in sheep breeding regions in Şanlıurfa province is limited. This study aims to determine the nutrient content of pastures located in individual mountains in the region and to determine their effects on the fattening performance of newborn small ruminants.

2. Materials and Methods

2.1. Material

2.1.1. Trial location

In the study, samples were collected from natural pastures in the villages of Kargalı (pasture1), Karaca (pasture2), Sarpdere (pasture3) and Uğurlu (pasture4) in the Tek Tek Mountains of Şanlıurfa province, where pasture-based sheep breeding is carried out, at different periods (March 1, 2020, April 1, 2020, May 1, 2020, June 1, 2020). In sampling, quadrats of 50x50 cm (0.25 m^2) were placed on the ground in 4 randomly selected regions and the remaining grass was mowed at the soil level. The dried samples were ground through a 1 mm sieve and bagged for analysis of dry matter, crude ash, crude protein, ether extract, crude cellulose, ADF, NDF. The total area of Tek Tek Mountains National Park is 19,335 hectares. Tek Tek Mountains National Park is 40 km away from Şanlıurfa city center and is located in the east of the city (Figure 1). It lies between Viranşehir Plain and Harran Plain in the north-south direction, south of the Şanlıurfa-Mardin Highway. According to the habitat classification in the National Park area, there are habitat types as Perennial Calcareous Meadows and Simple Steppes, Small Groves and Habitats with Sparse or No Vegetation, Young Cultivation Areas, Temporary Streams, Agricultural Areas Where Single Species Crops Are Grown (Şanlıurfa ÇED, 2015).

Figure 1. Field location where the study was conducted.

Table 1. Temperature and precipitation averages for 2020 (DMI, 2020)

Months	Temperature (ºc)			Mean Humidity (%)	Amount Of Rainfall (Mm)
	Min.	Max.	Mean		
January	-2.3	14.2	6.9	-	54.3
February	-7.4	19.6	7.1	-	13.4
March	2.4	24.2	14.1	26.5	65.5
April	3	28	16.5	19.2	39.1
May	8.5	37.8	22.7	43.2	19.7
June	12.6	40.7	28.1	30	0.2
July	20.9	43.6	33.4	24.6	-
August	18.4	42.3	31	30	-
September	16.1	41.2	29.7	32.5	-
October	12.9	33.5	24	27.5	-
November	5.3	26.8	14.1	53.3	46.8
December	7.6	17.8	12	64.9	2.3

Table 2. Nutrient contents of pastures in different periods (%DM)

Pasture	Periods	DM	CA	CP	EE	NDF	ADF
	March	24.7±1.0^{a}	7.88±0.25^{a}	14.85±0.28^{d}	2.46±0.15	35.23±0.40^{a}	29.61±0.39^{a}
	April	31.8±0.4b^{b}	8.12±0.52^{a}	12.97±0.23^{c}	2.44±0.17	40.98±0.68^{b}	34.14±0.60^{b}
1	May	35.8±0.9^{c}	9.30±0.34^{b}	9.84±0.26^{b}	2.36±0.09	52.25±0.78^{c}	37.28±0.74^{c}
	June	41.2±0.7^{d}	9.96±0.29^{b}	7.89±0.25^{a}	2.61±0.17	59.22±0.27^{d}	41.11±1.06^{d}
	P	**	**	**	ns	**	**
	March	25.7±1.1^{a}	7.64±0.32^{a}	13.88±0.36^{c}	2.39±0.10	34.11±0.80^{a}	28.66±0.72^{a}
	April	30.6±0.5^{b}	8.52±0.46^{ab}	13.14±0.28^{c}	2.28±0.08	40.39±0.37^{b}	34.21±0.59^{b}
2	May	36.1±0.8^{c}	8.80±0.25^{b}	9.82±0.21^{b}	2.40±0.10	53.08±0.59^{c}	37.15±0.52^{c}
	June	43.3±0.6^{d}	10.34±0.27^{c}	7.82±0.40^{a}	2.28±0.09	60.88±1.24^{d}	41.97±1.04^{d}
	P	**	**	**	ns	**	**
	March	25.4±0.8^{a}	8.25±0.24^{a}	14.25±0.49^{d}	2.45±0.12	33.34±0.95^{a}	29.55±0.59^{a}
	April	31.1±1.0^{b}	8.12±0.26^{a}	12.95±0.33^{c}	2.44±0.12	40.80±0.71^{b}	34.43±0.34^{b}
3	May	33.4±0.4^{c}	8.80±0.17^{a}	10.28±0.22^{b}	2.50±0.14	53.22±0.67^{c}	37.96±0.72^{c}
	June	42.9±0.9^{d}	10.48±0.33^{b}	7.72±0.15^{a}	2.64±0.14	60.61±1.11^{d}	41.05±1.35^{d}
	P	**	**	**	ns	**	**
	March	24.3±1.1^{a}	8.42±0.31^{a}	14.32±0.40^{d}	2.45±0.08	34.17±0.98^{a}	29.51±0.63^{a}
	April	31.7±0.7^{b}	8.28±0.57^{b}	13.06±0.36^{c}	2.33±0.15	40.94±0.55^{b}	34.23±0.76^{b}
4	May	33.6±0.8^{b}	9.10±0.08^{bc}	9.84±0.26^{b}	2.44±0.15	52.87±0.67^{c}	38.24±0.51^{c}
	June	42.1±0.6^{c}	10.02±0.26^{c}	8.08±0.24^{a}	2.58±0.16	60.62±0.83^{d}	39.54±0.40^{c}
	P	**	**	**	ns	**	**

^{a,b}= the difference between groups in the same column is statistically significant.**P<0.01 ns= no significant, DM= dry matter (%), CA= crude ash (% of DM), CP= crude protein (% of DM, EE= ether extract (% of DM), NDF= ceutral detergent fiber (% of DM), ADF= ccid detergent fiber (% of DM).

2.1.2. Climate characteristics

According to the temperature and precipitation averages of the Turkish State Meteorological Service (Turkish abbreviation of DMİ) for 2020, no precipitation was observed in July, August, September and October. The months with the most precipitation were January, March and November. The lowest temperatures are in December, January and February, while the highest temperatures are in June, July, August and September. The climate features a climate where the influence of the Mediterranean and Eastern Anatolia Regions and the arid-tropical region in the southern parts were seen. Winters are rainy, cold and humid. With the arrival of June, desert conditions in the south begin to be felt in the region and the drought level reaches its maximum. The drought period is long and is effective between June and September. The Southeastern Anatolia region generally has a steppe climate and evaporation is very common in these months. This steppe climate is different from the steppe climate seen in the Central Anatolia Region in that summers are hot and dry Turkish State Meteorological Service (Turkish abbreviation of DMİ, 2020) (Table 1).

2.2. Methods

For the samples examined within the scope of the study, dry matter determination was calculated by drying the samples at 105 °C for 3 hours based on (%DM). The crude ash values of the forage plant samples were calculated after burning them in the oven at 525 °C for 8 hours. Organic matter contents were determined by the calculation method (AOAC, 1990).

Crude protein is based on the calculation of nitrogen amount by converting nitrogen into ammonia by first adding ammonium sulphate and then adding alkali (sodium hydroxide) with the Kjeldahl method, which is found as a result of burning the ground leaves with concentrated H_2SO_4, and titrating with 0.1 N HCl. The values found were calculated as CP, N×6.25. (AOAC, 1990). ADF and NDF content of the collected samples were determined by the method of (Van Soest et al., 1991). Record information was obtained from a total of 120 animals, 30-31 lambs (Female (F)/Male (M) ratio is given in the Table 2) from each region, grazing in the pastures in the Tek Tek Mountains of Şanlıurfa region in March, April, May and June and giving birth in the same period. In line with the data obtained in the study, the live weights of the lambs at the beginning of the pasture and at the end of the pasture were recorded. A weighing machine with a sensitivity of 50 g was used for live weight weighing. The animals were not subjected to additional feeding and were fed entirely based on pasture.

Effects on the growth characteristics of the environment are determined using the Least Squares method and GLM procedure. Growth traits were adjusted for the fixed effects of herd, sex, and maternal age. The model for adjusting for growth changes is as given in Equation 1:

$$Y_{ijkl} = \mu + F_i + MA_j + S_k + e_{ijkl} \quad (1)$$

Here Y=observed values of traits, μ=general mean, F=effect of herd (1, 2, 3, 4), MA=effect of dam age (2, 3, and 4) and S = effect of gender of lambs (male or female) was included in the model as a covariate for the other two characteristics and e = random error N (0,σ) run with each observation. Analyzes were carried out using the SPSS 9.0 package program. The means of the groups were compared using the Duncan multiple comparison test.

3. Results and Discussion

In the pasture1 region given in Table 2, DM, NDF and ADF levels increased significantly (P<0.01) while CP levels decreased significantly (P<0.01) according to the periods. Two separate groups emerged between March-April and May-June, and CA levels between the groups were found to be significant (P<0.01). On the other hand, statistical differences between periods in EE levels were found to be insignificant. The results of the study conducted by Aydogan et al. (2014) when we analyzed the results of our study, it was seen that they were parallel to the results of our study and accordingly, as the plant matured towards the last forms decreased protein value, dry matter, the amount of cellulose, NDF and ADF amounts were found to increase were found. It is thought that the changes in nutritional value are due to the change in harvest time.

Nutrient content varied depending on vegetation. When the samples taken from Pasture2 were evaluated, DM, NDF and ADF levels increased significantly (P<0.01) according to the periods, while CP levels were similar in March and April, these two months were found to be significantly higher than the other months (P<0.01). In CA levels, April was similar to March and May, while the difference in June was found to be significant (P<0.05), while in EE levels, the difference between the periods was found to be insignificant. Erkovan et al. (2009) showed similarity in their study, and while CP decreased according to vegetation, ADF and NDF levels increased, showing similarity. In Pasture3 region, DM, NDF and ADF levels increased significantly (P<0.01) while CP levels decreased significantly (P<0.01) according to the periods. CA levels were found to be significantly higher in June than other months (P<0.01), while other months were similar. On the other hand, the difference in CF levels between periods was due to chance. Tuna et al. (2013) found that ADF and NDF rates were similar while HP rates were high in their study. Aydoğan et al. (2014) observed that the CA ratio was low in their study and our study, and it is in line with other nutrient contents. The nutrient content changed depending on the vegetation. In the pasture4 region, NDF levels increased significantly (P<0.01) according to the periods, while CP levels decreased significantly (P<0.01). DM levels were similar in April and May, the highest DM level was found in June and the lowest in March, and the difference was found to be significant. CA levels were similar in May to June and April, and the lowest CA level was found in March. ADF levels were found to be similar in May and June and these two months were significantly higher than other months (P<0.01). On the other hand, the difference in EE levels between the periods was not found to be significant.

In the study, in Table 3, it was determined that the difference in DM levels of the averages of four different pasture regions for the periods of March, April, May and June was insignificant in all months. The DM rate obtained from our study is lower than Aydoğan et al. (2014), and is in agreement with Işık and Kaya (2011). In our study, the general averages of the CA level contents of four different pasture regions in the periods of March, April, May and June were calculated. It was determined that the differences in the CA levels of the averages of four different pasture regions in the periods of March, April, May and June was insignificant in all months. In our study in terms of CA content, it was observed that the CA rate was similar to Arslan and Tufan (2011) and higher than Canbolat and Karaman (2009). The general averages of the CP level contents of four different pasture regions for the periods of March, April, May and June, the difference in CP levels was found to be significant (P<0.05) in March for pasture1 and pasture2, while pasture3 and pasture4 were similar among themselves, while pasture1 and pasture2 were close to CP levels.

Table 3. Changes in nutrient content of pastures in different periods (%DM)

	Periods	Pastures				P	Mean
		1	2	3	4		
DM	March	24.7±1.0	25.7±1.1	25.4±0.8	24.3±1.1	ns	25.0
	April	31.8±0.4	30.6±0.5	31.1±1.0	31.7±0.7	ns	31.3
	May	35.8±0.9	36.1±0.8	33.4±0.4	33.6±0.8	ns	34.7
	June	41.2±0.7	43.3±0.6	42.9±0.9	42.1±0.6	ns	42.3
CA	March	7.88±0.25	7.64±0.32	8.25±0.24	8.42±0.31	ns	8.04
	April	8.12±0.52	8.52±0.46	8.12±0.26	8.28±0.57	ns	8.26
	May	9.30±0.34	8.80±0.25	8.80±0.17	9.10±0.08	ns	9.00
	June	9.96±0.29	10.34±0.27	10.48±0.33	10.02±0.26	ns	10.02
CP	March	14.85±0.28^{a}	13.88±0.36^{b}	14.25±0.49^{ab}	14.32±0.40^{ab}	*	14.33
	April	12.97±0.23	13.14±0.28	12.95±0.33	13.06±0.36	ns	13.03
	May	9.84±0.26	9.82±0.21	10.28±0.22	9.84±0.26	ns	9.95
	June	7.89±0.25	7.82±0.40	7.72±0.15	8.08±0.24	ns	7.88
EE	March	2.46±0.15	2.39±0.10	2.45±0.12	2.45±0.08	ns	2.43
	April	2.44±0.17	2.28±0.08	2.44±0.12	2.33±0.15	ns	2.38
	May	2.36±0.09	2.40±0.10	2.50±0.14	2.44±0.15	ns	2.43
	June	2.61±0.17^{a}	2.28±0.09^{b}	2.64±0.14^{a}	2.58±0.16^{a}	*	2.53
NDF	March	35.23±0.40	34.11±0.80	33.34±0.95	34.17±0.98	ns	33.46
	April	40.98±0.68	40.39±0.37	40.80±0.71	40.94±0.55	ns	40.78
	May	52.25±0.78	53.08±0.59	53.22±0.67	52.87±0.67	ns	52.86
	June	59.22±0.27	60.88±1.24	60.61±1.11	60.62±0.83	ns	60.33
ADF	March	29.61±0.39	28.66±0.72	29.55±0.59	29.51±0.63	ns	29.33
	April	34.14±0.60	34.21±0.59	34.43±0.34	34.23±0.76	ns	34.25
	May	37.28±0.74	37.15±0.52	37.96±0.72	38.24±0.51	ns	37.66
	June	41.11±1.06^{ab}	41.97±1.04^{a}	41.05±1.35^{ab}	39.54±0.40^{b}	*	40.92

^{a,b}= the difference between groups in the same column is statistically significant, **P<0.01 ns= no significant, DM= dry matter (%), CA= drude ash (% of DM), CP= drude protein (% of DM, EE= ether extract (% of DM), NDF= neutral detergent fiber (% of DM), ADF= acid detergent fiber (% of DM).

In terms of CP content, the CP level in our study is lower than the values found by Arslan (2008) and Canbolat and Karaman (2009), and the values found by Marinas and Gonzalez (2006) and Karabulut et al. (2006) was found to be lower than their study, while Aschalew et al. (2006) was observed to be higher.

By calculating the general averages of the contents of the EE level, it was determined that the difference in the EE level of the averages of four different pasture regions belonging to the periods of March, April, May and June was insignificant in March, April and May. In June, while pasture1, pasture3 and pasture4 were similar, they were higher than pasture2 in EE level and the statistical differences were found to be significant (P<0.05). By calculating the general averages of the contents of the EE levels, it was determined that the difference in the EE level of the averages of four different pasture regions belonging to the periods of March, April, May and June was insignificant in March, April and May. In June, while pasture1, pasture3 and pasture4 were similar, they were higher than pasture2 in EE level and the statistical differences were found to be significant (P<0.05). In our study in terms of NDF content, it was observed that NDF rates were in accordance with the studies of Tuna et al. (2013), Arslan and Tufan (2011) and Martinson et al. (2011). By calculating the general averages of the ADF level contents, it was determined that the differences in ADF levels of the averages of four different pasture regions belonging to the periods of March, April, May and June was insignificant in March, April and May. In June, pasture2 and pasture4 ADF levels were found to be significant (P<0.05) between two pastures, while it was determined that both pastures were similar to other pastures. In our study in terms of NDF content, it was observed that NDF rates were in accordance with the studies of Tuna et al. (2013), Arslan and Tufan (2011) and Martinson et al. (2011). By calculating the general averages of the ADF level contents, it was determined that the differences in ADF levels of the averages of four different pasture regions belonging to the periods of March, April, May and June was insignificant in March, April and May. In June, pasture2 and pasture4 ADF levels were found to be significant (P<0.05) between two pastures, while it was determined that both pastures were similar to other pastures. It was determined that the DM levels increased linearly in the periods of March, April, May and June (R^2>0.98). Karslı et al., (2003) found that the DM levels were lower in our study than their study. While the DM rate decreased, it increased in our study. Karslı et al., (2003). In their study, it is seen that the CA ratios did not change, but it increased in our study. It is seen that the CP, ADF and NDF levels are similar to our study. Kaya et al., (2004) in their study, it is seen that the CP ratio is high and the CF ratio is low

compared to our study, while it is in harmony with the DM and NDF levels. Avcı et al. (2006), the results of their study are in harmony with our study in terms of DM, NDF, ADF and CP, while the EE ratio decreased, it did not change in our study. Arslan and Tufan (2011) in their study, it is seen that the CA, ADF, NDF and CP levels are in harmony with our study, while the EE level is higher than our study. In their study, Worrell et al. (1990) found that DM and NDF levels were higher than our study, while ADF levels were similar. In their study, Aschalew et al. (2006) found that CA levels were consistent with our study, CP levels were low, and ADF and NDF levels were high.

In this study, Kaya and Işık (2011) investigated the effect of the quality of change in the nutrient content of the pasture during the grazing period (22 May-09 October) of Tuj sheeps and lambs raised in Kars province on the fattening performance. In the weightings, an average live weight gain of 164.9 g/day and 181.6 g/day was observed in female and male lambs of Tuj sheeps and lambs during the grazing season, reaching 23.10 and 25.47 kg, respectively (P>0.05). In Tuj broodstock sheep, an average live weight gain of 35 g/day was achieved, with a live weight gain of approximately 4.9 kg. In our study, it was observed that Awassi lambs gained less than Tuj lambs. Kaya et al. (2004) investigated the effects of grazing and concentrate feed addition during the pasture green period and the nutrient content of pasture plants on the body weight gain (BWG) increase, rumen pH, total volatile fatty acids (VFA) and ammonia nitrogen (NH3-N) values in Morkaraman and Tuj breed lambs. No significant effect of grazing and concentrate feed addition on BWG was found between breeds. During the total trial period (70 days), the group grazed on pasture gained 14.52 kg, the group supported with the addition of concentrated feed gained 17.40 kg BW, and the difference between the feeding groups in terms of BWG was found to be significant (P<0.05).

When Table 4 was examined, it was determined that the dry matter (DM) content of the pasture grazed depending on the vegetation was 23.08-45.34%, the CP ratios were 6.72-17.18%, and the EE ratios were 23.78-36.45. As a result of our study, BWG is similar to the group grazed in pasture in terms of BWG averages. In the study conducted by Özkan (2009), the current structure of sheep breeding in Viranşehir district and its villages was investigated. In the study, a total of 184 Akkaraman sheeps, including 174 sheeps and 10 rams, were observed from 4 villages. In this study, where birth weights were observed, the average lamb weight was 3.32 kg, 90-day weaning average weight was 26.5 kg and the average daily BWG in the 0-90 day period was 294.4 grams. By determining these characteristics, it was determined that the effect of gender and birth type was important. As a result, it was determined that the maintenance and feeding conditions in the in-house breeding were effective in the yields obtained from Akkaraman sheep. In this study, it was observed that gender did not have much effect on live weight gain. In the study conducted by Çalışkan (2019), the values of birth weight (BW), weaning weight (WW) and daily live weight gain (DLWG) in the examined Awassi sheeps were found to be 3.879±0.0139, 20.648±0.0824, kg 186.297±0.9298 g, respectively. The coefficients of variation were determined as 21.88%, 23.81 and 29.80, respectively. In Awassi herds, the survival rate was determined as 95%. In our study, since the daily feeding was based only on pasture, the body weight gain was less than in the study conducted by Çalışkan (2019). In their study, Gül and Ekici (2020) investigated the effects of weaning at different ages in Awassi sheeps on the developmental characteristics of lambs and milk production in dams. The animal material of the study consisted of Awassi sheeps and lambs in a private farm in Kilis province. The lambs born were divided into 3 groups of 35 lambs each, taking into account their gender. The lambs in the first group were weaned on the 60th day (Group I), the lambs in the second group were weaned on the 75th day (Group II), and the lambs in the third group were weaned in the traditional time (90th day) (Group III). At the end of the study, the mean birth weights were determined as 3.4 ± 0.09 kg, 3.6 ± 0.08 kg, 3.4 ± 0.09 kg in Group I, and it was determined that weaning at an early age (60 days) had a significant effect on the development of the lambs when compared to the traditional method (90 days) (P<0.05).

Table 4. Descriptive statistics of total live weight at the beginning of the pasture and at the end of the pasture

			Live weight at the beginning of the pasture			Live weight at the end of the pasture			Total live weight gain			
Herd	Gender	N	Mean	Std Dev	CV%	Mean	Std Dev	CV%	Mean	Std Dev	CV%	DLWG
Pasture 1	female	18	14.430	0.4657	13.69	30.158	0.588	8.267	15.728	0.4352	11.740	131.07
	male	12	13.571	0.6962	17.77	30.080	0.948	10.914	16.508	0.4697	9.858	137.57
Pasture2	female	15	17.787	0.6737	14.66	32.534	0.778	9.247	14.747	0.4364	11.462	122.89
	male	15	16.383	0.9005	21.28	31.190	1.002	12.446	14.807	0.5827	15.242	123.39
Pasture 3	female	15	10.210	0.6179	23.44	25.871	0.807	12.081	15.660	0.4955	12.257	130.5
	male	15	15.197	0.6599	16.81	30.224	10.302	13.201	15.027	0.5947	15.330	125.23
Pasture 4	female	15	12.143	10.447	33.31	28.377	1.317	17.969	16.233	0.6340	15.128	135.28
	male	16	12.163	0.9676	31.82	26.539	1.036	15.615	14.375	0.3708	10.320	119.79

Number of samples (N), mean, standard deviation, and coefficient of variation (CV), daily live weight gain (DLWG).

However, it should also be taken into account that weaning at an early age and raising lambs on quality pastures will increase the profitability of the enterprise. In our study, it was detected that the effect of pasture is greater. Descriptive statistics of live weight at the beginning of pasture, live weight at the end of pasture and total live weight gain are given in Table 4. While the highest live weight gain was observed in male lambs in pasture 3, the highest live weight gain in pasture 4 was detected in female lambs.

4. Conclusion

In ruminant nutrition; a more efficient feeding in terms of animal physiology and economy is possible with the quality of the roughage used. According to the studies, it is known that there are significant differences between the distribution of pasture and meadow areas in different regions and the quality and quantity of grass yield in these regions. The quality of pasture grass varies depending on the vegetation period, growth conditions, climatic factors, botanical composition, irrigation and fertilization, vegetation period and geographical structure. Therefore, research on the nutrient content of pastures in sheep breeding regions in Şanlıurfa region is limited. For this purpose, scientific studies on determining the nutrient content of pastures in the region should be increased. It has been observed that pastures in Şanlıurfa Tek Tek Mountains meet the nutrient needs of lambs to a significant extent during the periods when the grass is green between March 1 and June 30, and it is seen that there is a positive increase due to the fact that pasture-based breeding is more economical than livestock farming and that there is less labor. This live weight increase is provided, and it will allow breeders to increase live weight gain with additional feeding. The importance of supporting the Awassi sheep, which are highly adaptable to regional conditions, within the scope of the breeding project in the hands of the public is great. The Awassi breed, known as yellow gold in this region, makes very good use of the pasture capacity due to its characteristics. The protection and increase of this animal stock is of great importance in terms of further developing and advancing the regional animal husbandry.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	F.T.	A.B.K.
C	50	50
D	100	
S	100	
DCP		100
DAI	100	
L	50	50
W	50	50
CR	50	50
SR	100	
PM	80	20

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

Since this study does not involve animals or humans, ethics committee approval is not required. Within the scope of the "Small Ruminant Breeding Project in Public Hands" carried out by the General Directorate of the Ministry of Agriculture and Forestry (TAGEM), the live weight data of 120 lambs were officially recorded in 2020. A permit document for the data used in the study has been submitted.

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NUTRACEUTICAL EFFECTS OF SNOT APPLE POWDER ON TRIIODOTHYRONINE, OXIDATIVE STRESS MARKERS, HAEMATOLOGY AND GROWTH OF BROILER CHICKENS

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Abstract: This study investigated the effects of incorporating snot apple powder with or without probiotics on performance, hematological indices, serum protein profile, oxidative markers, and triiodothyronine levels in chickens. The treatments included a control (0% snot apple powder) and varying levels of snot apple powder (1%, 2%, and 3%), all supplemented with probiotics at a rate of 0.5%. Over a 42-day trial period, parameters such as feed intake, weight gain, feed conversion ratio (FCR), mortality, and blood samples for hematological and serum analyses were collected. Growth indices revealed significant variations ($P<0.05$) among treatments, with birds in T3 exhibiting the highest final body weight, followed by T1, while T4 recorded the lowest. Weight gain and feed intake were also significantly influenced by treatment, with T3 demonstrating superior performance in weight gain and T1 in feed intake. The feed conversion ratio was notably efficient in birds on T3 and T4 compared to T1. The serum protein profile indicated higher total protein and globulin levels in probiotic-treated groups (T2, T3, and T4) compared to T1, whereas albumin and uric acid varied significantly among treatments. Enhanced total antioxidant capacity in T2 and higher superoxide dismutase activity was observed in T2, T3, and T4. Triiodothyronine levels differed significantly among treatments, with T1 and T3 showing higher values compared to T2 and T4. Incorporating snot apple powder and probiotics in broiler diets positively impacted growth performance, health markers, and antioxidant capacity, suggesting potential benefits for poultry nutrition and health management strategies.

Keywords: Antioxidant enzymes, Feed efficiency, Immune response, *Azanza garckeana*, Thyroid function

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1. Introduction

The poultry industry is continually exploring new strategies to enhance production efficiency and poultry health, while meeting consumer demands for nutritious products. Recently, nutraceuticals, including plant-derived compounds and probiotics, have gained attention for their potential benefits in poultry diets. Snot apple, known for its antioxidant, antimicrobial, anti-inflammatory, and anticancer properties due to bioactive compounds like acetogenins, flavonoids, and phenolics, has been studied for its health-promoting effects (Jung et al., 2010; Li et al., 2015; Ferdous et al., 2019; Feng et al., 2023). These compounds can modulate physiological processes such as immune function, oxidative stress, and metabolism (Ryszard, 2007; Tungmunnithum et al., 2018), making snot apple a promising addition to poultry feed. Probiotics, beneficial live microorganisms, enhance poultry health by improving gut microbiota and boosting immune responses (Beski and Al-Sardary, 2015; Kikusato, 2021). They prevent pathogen colonization, enhance nutrient absorption, and reduce intestinal inflammation, improving broilers' growth efficiency (Rocha et al., 2022). The combined use of snot apple powder and probiotics may offer synergistic benefits, promoting health and performance through complementary mechanisms. Snot apple's antioxidant and immunomodulatory properties (Alozieuwa et al., 2022) may reduce oxidative stress, improve immune function, and enhance nutrient metabolism in broilers. Despite increasing interest, research on the combined effects of snot apple powder and probiotics on triiodothyronine (T3) levels, oxidative stress markers, hematological parameters, and growth performance in broilers is limited. Triiodothyronine, a crucial thyroid hormone, regulates poultry's metabolism, growth, and development (Jiang et al., 2020). However, Snot apple fruit includes nutrients, minerals, and phytochemical substances such as flavonoids, steroids, triterpenes,

saponins, phenols, and tannins that are helpful to human and animal health (Maroyi and Cheikh-Youssef, 2017), Probiotics also shown to improve immune functions by interacting with various immune cells and having a favorable impact on the composition of intestinal microbiota (Azcárate-Peril et al., 2011; Adel et al., 2017; Umair et al., 2022). Thus, probiotics are known to have immunomodulatory and health-promoting characteristics (Ashraf and Shah, 2014; Peng et al., 2022) revealing how they influence broilers' physiological and metabolic processes. Evaluating growth performance metrics like body weight gain, feed intake, and feed conversion ratio will provide practical insights into the economic viability of snot apples and probiotic supplementation in broiler diets (Ravindran and Abdollahi, 2011). Integrating snot apple powder and probiotics into broiler diets appears to be a promising strategy for boosting poultry health, productivity, and sustainability. Their combined effects on metabolism, immune function, and gut health could significantly enhance growth performance, oxidative balance, and overall well-being. Thus, comprehensive research is essential to optimize their use in commercial broiler production and advance sustainable poultry farming practices. The present study is distinguished from others by its focus on the combined effects of snot apple powder and probiotics on various critical parameters in broilers, including growth performance, oxidative stress markers, hematological indices, serum protein profiles, and triiodothyronine (T3) levels. While previous studies have investigated the individual benefits of plant-derived compounds and probiotics, this research uniquely explores their potential synergistic impact in poultry nutrition. Additionally, the study delves into the under-researched area of how these dietary components influence thyroid function (via T3 levels) and antioxidant capacity, providing insights into their mechanisms of action and practical implications for improving poultry health and production efficiency. This comprehensive approach addresses gaps in existing research, offering new perspectives for sustainable poultry farming practices.

2. Materials and Methods

This study was carried out in Teaching and Research Farm, The Federal Polytechnic Ado-Ekiti, Ekiti State in the Southwest of Nigeria. The sample size was calculated at the level of significance of 5% and power of study at 80% as described by Charan and Biswas (2013) and the G Power tool (Faul et al., 2009).

2.1 Preparation of Test Ingredient

Ripe Snot apple (*Azanza garckeana*) fruits were procured at a local market in Tula, Northern Nigeria. The identification of the plants was confirmed by Mr Ayodele S.O. (a Principal Technologist) in the Department of Agricultural Technology, Federal Polytechnic Ado Ekiti. They were air-dried and ground to powder and designated as Snot apple powder. Phytochemical analysis and proximate composition of the powder were carried out using Association of Analytical Chemistry Standard Procedures 1990 and presented in Table 1 and 2.

A proprietary probiotic (Natupro®) was procured from a reputable veterinary store in Ibadan, Oyo State. It contains 150 CFUx10^6/g each of *Bacillus subtilis*, *Bacillus licheniformis*, *Bacilus amyloliquefaciens* 390 and *Bacillus amyloliquefaciens* 700.

2.2 Experimental Animals and Management

200-a-day-old arbor-acre broiler chickens were randomly allotted to four treatments in a completely randomized design. The birds were allotted 10 replicates, 5 birds per replicate. The probiotics was administered to all four treatments at a uniform rate of 0.5% Naturpro (as recommended) and Snot apple meal were incorporated 0%, 1%, 2% and 3%, respectively into T_1, T_2, T_3 and T_4, and were thoroughly mixed in the feed shown in Table 3 and 4. Standard experimental starter and finisher rations were formulated as shown in Table 1 and 2, respectively. Birds were fed *ad libitum* and fresh water was offered daily throughout a 42-day trial. The vaccination program as recommended by the hatchery was followed and no medication was offered throughout the study.

2.3 Data Collection

The feed intake and weight changes were monitored throughout the study to evaluate their growth in a 42-day trial. Mortality was recorded, and feed conversion was computed per replicate.

2.3.1 Performance evaluation

Records of feed intake, weight gain, and mortality were taken weekly. The feed conversion ratio (FCR) was obtained by calculation. Total Feed Intake (g) = Total Feed supplied (g) - Total feed left over (g), Average feed intake (g/bird) = Total Feed Intake/Number of birds, Total weight gain = Final weight – Initial weight, Feed conversion ratio = Total Feed intake (g)/Total weight gain (g), %Mortality = (Number of dead birds/ Total number of stocked birds) ×100.

2.3.2 Blood sample collection

At the end of the feeding trial, blood samples were collected from 3 birds per replicate into the plain samples and heparinized bottles for serum and hematology, respectively. Hematology samples were assayed using standard procedures. For serum, blood samples in plain tubes were centrifuged and serum was obtained using standard procedures and stored at -20^{o}C until analysis. The serum was assayed for total protein, albumin, globulin and Uric acid carried out using Fortress diagnostics commercial assay kits and its procedures.

Determination of serum total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase activities and lipid peroxidation were assays as outlined in Jimoh (2019).

Serum triiodothyronine was assayed using enzyme linked immunosorbent assay, with commercial ELISA kits and its protocol. Triiodothyronine (T3) ELISA Catalog No. T3225T (Calbiotech Inc., 1935 Cordell Ct., El Cajon, CA 92020)

2.4 Statistical Analysis

All data were subjected to one way analysis of variance and significant means were separated using Duncan multiple range test SAS (version 2003).

3. Results

The growth indices of chicken fed snot apple powder with/or without probiotics is presented in Table 5. The final body weight of chicken was significantly ($p<0.05$) highest in T3 (2604.58 g) followed by T1 (2545.42 g), and the significantly ($P<0.05$) least values was obtained in birds on T4 (2383.21 g). Weight gain (g/bird/day) was significantly ($p<0.05$) higher in T3 (61.09) and T2 (59.69) compared to T4 (55.81). Feed intake (g/bird/day) was significantly ($P<0.05$) higher in T1 (78.74) compared to those on T4 (64.32). Feed conversion ratio (FCR) was ($P<0.05$) better in birds on T3 (1.19) and T4 (1.17) compared to those T1 (1.32). The hematological indices of chickens fed snot apple powder with/without probiotics are presented in Table 6. The Packed cell volume, Haemoglobin, erythrocytes, leukocytes, thrombocytes and lymphocytes of birds on the different treatments were statistically ($P>0.05$) similar. The Heterophils of birds on T1 differed significantly ($P<0.05$) from T2 and T3. Monocytes of birds on T2 were significantly ($P<0.05$) higher than those on other treatment. Eosinophils of birds on T3 were significantly ($P<0.05$) higher than other treatment. There were no significant ($P<0.05$) differences between mean obtained for basophils. The Heterophil: Lymphocyte ratio of birds on T2 and T3 were higher ($P<0.05$) than those from T1. The serum protein profile of chickens fed snot apple powder with/without probiotics is presented in Table 7. The serum protein of birds fed snot apple powder with probiotics (T2, T3, and T4) were higher than those on T1. Chickens on T2, T3, and T4 had significantly ($P<0.05$) higher globulin levels compared to those T1. Albumin values of birds on T1 were significantly ($P<0.05$) lower than those T2, but birds on T3, and T4 share statistically similar values with other treatment. The values obtained for Uric Acid in birds on T1 were least and was significantly ($P<0.05$) lower than those on T2, T3, and T4. The oxidative markers in chickens fed snot apple powder with/without probiotics are presented in Table 8. The total antioxidant capacity showed that birds administered 1% snot apple meal (T2) exhibited the highest total antioxidant capacity (14.34 mM) and significantly ($P<0.05$) least was found in those on T1. The lipid peroxidase activity was highest in T2 and T3 compared to those in T1 and T4. Birds on T2, T3 and T4 had higher ($P<0.05$) SOD activity when compared to those on T1. Birds on control had the highest catalase activity (148.63 u/ml mins), while birds on T2, T3, and T4 had significantly ($P>0.05$) similar catalase activity. The estimated values of glutathione peroxidase (GPx) showed that T2 had the highest GPx activity (36.01 U/L), but significantly ($P>0.05$) similar values were obtained amongst birds on T1, T3, and T4. Triiodothyronine of chickens fed snot apple powder with/without probiotics is presented in Figure 1. Chickens on T1 and T3 had significantly ($P<0.05$) higher triiodothyronine than birds on T2 and T4.

Table 1. Proximate and phytochemical analysis of snot apple powder

	Snot Apple
Dry Matter (%)	2.64
Crude Protein (%)	25.59
Ash (%)	7.57
Ether Extract (%)	13.60
Crude Fiber (%)	3.00
Nitrogen Free Extract (%)	50.32
Alkaloid (%)	1.13
Terpenoid (%)	0.90
Tannin (%)	1.19
Flavonoid (%)	5.56
Phytate (%)	0.22
Phenol (%)	10.17
Saponin (%)	1.17

Table 2. Phytochemical screening of snot apple powder

Secondary metabolites	Test	Inference
Alkaloid	Wagner's test	+ve
Tannin	Braymer's test	++ve
Glycoside	Keller's test	-ve
Saponin	Frothing test	++ve
Flavonoid	Alkaine reagent test	++ve
Terpenoid	Salkowski test	+ve
Phenol	Ferric chloride test	+++ve
Anthraquinone	Carbon tetrachloride test	+ve

-= absent, += trace, ++= moderate, +++= abundant.

Table 3. Gross composition of broiler starter ration

Feedstuff	T1	T2	T3	T4
Maize	51.25	50.2	49.2	48.2
Soyabean Meal (42% CP)	41.5	41.5	41.5	41.5
Fishmeal (65% CP)	3	3	3	3
Bonemeal	2	2	2	2
Limestone	0.5	0.5	0.5	0.5
Premix	0.25	0.25	0.25	0.25
Methionine	0.5	0.5	0.5	0.5
Lysine	0.2	0.2	0.2	0.2
Salt	0.3	0.3	0.3	0.3
Vegetable oil	0.5	0.5	0.5	0.5
Probiotics	0	0.05	0.05	0.05
Snot Apple Powder	0	1	2	3
TOTAL	100	100	100	100
Calculated Nutrient analysis				
Dry Matter %	85.21	84.29	83.41	82.53
Crude Protein (%)	24.72	24.61	24.51	24.41
Metabolizable Energy (kcal/kg)	2966.23	2930.17	2895.83	2861.49
Ether Extract %	3.64	3.6	3.56	3.52
Crude Fibre (%)	3.75	3.73	3.71	3.69
Lysine (%)	1.61	1.61	1.61	1.61
Methionine (%)	0.89	0.89	0.89	0.89
Calcium (%)	1.19	1.19	1.19	1.19
Available Phosphorus (%)	0.69	0.68	0.68	0.68

* Premix composition per kg diet: Vit A:400000 IU, Vit D:80000 IU, Vit E:40000 ng, Vit k3:800 mg, Vit B1:1000MG, Vit B2:6000 mg, Vit B6:500 mg, VitB12:25 mg, Niacin:6000 mg, Panthothenic acid:2000 mg, Folic acid: 200 mg, Biotin:8 mg, Manganese: 300000 g, Iron:8000 mg, Zinc:20000 g, Cobalt:80 mg, Iodine:400 mg, Selenium:40 mg, Choline:800000 g.

Table 4. Gross composition of broiler finisher ration

Feedstuff	T1	T2	T3	T4
Maize	58.25	57.2	56.2	55.2
Soyabean Meal (42% CP)	31.5	31.5	31.5	31.5
Rice Offal	3	3	3	3
Fishmeal (65% CP)	3	3	3	3
Bonemeal	2	2	2	2
Limestone	0.5	0.5	0.5	0.5
Premix	0.25	0.25	0.25	0.25
Methionine	0.5	0.5	0.5	0.5
Lysine	0.2	0.2	0.2	0.2
Salt	0.3	0.3	0.3	0.3
Vegetable oil	0.5	0.5	0.5	0.5
Probiotics	0	0.05	0.05	0.05
Snot Apple Powder	0	1	2	3
Total	100	100	100	100
Calculated Nutrient analysis				
Dry Matter %	85.07	84.15	83.27	82.39
Crude Protein (%)	21.58	21.47	21.37	21.27
Metabolizable Energy (kcal/kg)	3022.41	2986.35	2952.01	2917.67
Ether Extract %	3.94	3.9	3.86	3.82
Crude Fibre (%)	3.62	3.6	3.58	3.56
Lysine (%)	1.37	1.36	1.36	1.36
Methionine (%)	0.85	0.85	0.85	0.85
Calcium (%)	1.17	1.17	1.17	1.17
Available Phosphorus (%)	0.65	0.64	0.64	0.64

* Premix composition per kg diet: Vit A:400000 IU, Vit D:80000 IU, Vit E:40000 ng, Vit k3:800 mg, Vit B1:1000MG, Vit B2:6000 mg, Vit B6:500 mg, VitB12:25 mg, Niacin:6000 mg, Panthothenic acid:2000 mg, Folic acid: 200 mg, Biotin:8 mg, Manganese: 300000 g, Iron:8000 mg, Zinc:20000 g, Cobalt:80 mg, Iodine:400 mg, Selenium:40 mg, Choline:800000 g.

Table 5. Growth indices of chickens fed probiotics and snot apple inclusive diets

	T1	T2	T3	T4	SEM
Initial Weight (g)	38.54	37.80	38.66	39.03	1.30
Final weight (g)	2545.42^{b}	2461.39^{bc}	2604.58^{a}	2383.21^{c}	41.76
Weight gain (g/b/d)	59.69^{a}	57.70^{ab}	61.09^{a}	55.81^{b}	0.99
Feed Intake (g/b/d)	78.74^{a}	71.29^{ab}	72.75^{ab}	64.32^{b}	2.28
Feed conversion ratio	1.32^{a}	1.23^{ab}	1.19^{b}	1.17^{b}	0.03

a,b= means along the same row with different superscripts, differ significantly (P<0.05), SEM= standard error of mean.

Table 6. Haematological indices of chickens fed probiotics and snot apple powder inclusive diets

	T1	T2	T3	T4	SEM
Packed cell volume (%)	30.00	27.33	29.33	29.67	0.92
Haemoglobin (g/dL)	9.43	8.63	9.43	9.60	0.31
Erythrocytes (x 10^{6}/L)	3.02	2.70	2.75	3.28	0.15
Leukocytes (x10^{8}/L)	15.38	14.37	17.12	15.18	0.48
Thrombocytes (x10^{7}/L)	1.27	1.23	1.29	1.28	0.02
Lymphocyte (%)	63.33	59.67	60.33	63.33	1.29
Heterophils (%)	29.67^{b}	33.33^{a}	33.00^{a}	31.67^{ab}	1.26
Monocyte (%)	3.00^{b}	4.00^{a}	3.00^{b}	2.33^{b}	0.34
Eosinophils (%)	3.33^{b}	3.00^{b}	5.00^{a}	2.67^{b}	0.44
Basophils (%)	0.67	0.00	0.67	0.00	0.14
Heterophil: Lymphocyte Ratio	0.47^{b}	0.56^{a}	0.55^{a}	0.50^{ab}	0.01

a,b= means along the same row with different superscripts, differ significantly (P<0.05), SEM= standard error of mean.

Table 7. Protein of chickens fed probiotics and snot apple powder inclusive diets

	T1	T2	T3	T4	SEM
Protein (g/dL)	27.82^{b}	36.30^{a}	33.82^{a}	33.95^{a}	1.14
Globulin (g/dl)	1.48^{b}	1.92^{a}	1.91^{a}	1.93^{a}	0.08
Albumin (g/dL)	1.30^{b}	1.71^{a}	1.47^{ab}	1.46^{ab}	0.05
Uric Acid (mg/dl)	11.68^{b}	18.53^{a}	17.59^{a}	18.82^{a}	1.34

a,b= means along the same row with different superscripts, differ significantly (P<0.05), SEM= standard error of mean.

Table 8. Oxidative markers of chickens fed probiotics and snot apple powder inclusive diets

	T1	T2	T3	T4	SEM
Total antioxidant capacity (mM)	7.37^{b}	14.34^{a}	9.79^{ab}	11.72^{ab}	1.04
Lipid Peroxidation (MDA uM)	0.87^{b}	1.00^{a}	0.92^{a}	0.86^{b}	0.03
Superoxide dismutase (U/mL)	1.16^{b}	2.83^{a}	2.59^{a}	2.78^{a}	0.29
Catalase (u/ml mins)	148.63^{a}	106.98^{b}	102.75^{b}	103.08^{b}	10.86
Glutathione peroxidase (U/L)	25.13^{b}	36.01^{a}	28.88^{b}	24.38^{b}	2.39

a,b= means along the same row with different superscripts, differ significantly (P<0.05), SEM= standard error of mean.

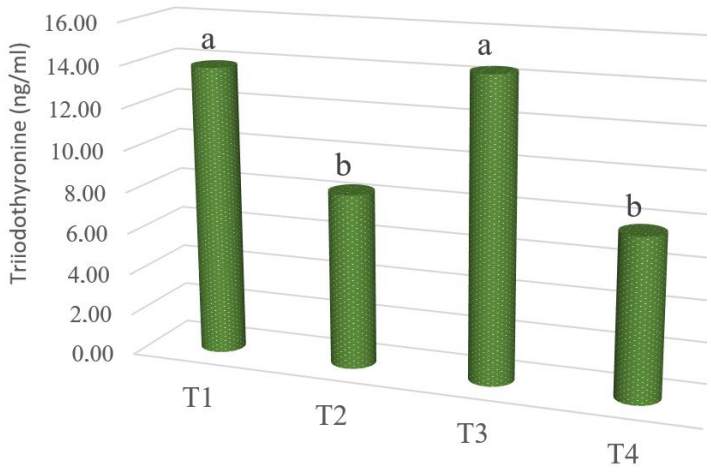

Figure 1. Triiodothyronine of chickens fed probiotics and snot apple powder inclusive diets. a,b= means values with different superscripts, differ significantly (P<0.05).

4. Discussion

In this study, notable distinctions emerged in weight gain, feed intake, and FCR, where T_3 displayed the highest final body weight, indicating its potential for maximizing overall growth, followed closely by T_1 and T_2, with T_4 exhibiting the lowest final body weight. The results obtained in this study concurred with those obtained by Jimoh et al. (2022a) recording birds fed on moringa and mistletoe supplements had higher performance indices than birds without supplementation during heat stress condition. However, other factors may influence final body weight beyond diet alone. The birds on 1% (T_2) and 2% (T_3) inclusion levels outperformed those on 3% (T_4) in weight gain, showcasing the positive impact of their respective diets on growth rates. Although weight gain indices were lower than that reported for fennel seed powder supplementation in broiler chicken (Al-Sagan et al., 2020). It is crucial to note that feed intake provides insights into dietary consumption patterns, suggesting potential differences in palatability or nutrient utilization between these diets (Rocha et al., 2022). The FCR is a key indicator of feed efficiency, where lower values denote better utilization of feed for growth (Feng et al., 2023). Here T_3, T_4, and T_2 with probiotics demonstrated significantly better FCRs than T_1 without probiotics administration, indicating that their diets were more efficiently converted into body mass. This suggests that the inclusion of probiotics and phytobiotics in T_3, T_4, and T_2 may have positively influenced nutrient absorption and metabolism, leading to improved feed efficiency. The observed variations in weight gain, feed intake, and FCR seem to be an integral part of dietary interventions on growth performance and these are key areas that commercial production needs to leverage on so as to meet demands in the broiler meat production enterprise (Ilaboya et al., 2024). Birds on 2% snot apple powder had higher final body weight compared to those on 0% and 3%. This suggests that moderate inclusion of snot apple powder enhances body weight in broiler chickens. In summary, weight gain of birds on 1% snot apple powder showed significantly higher weight gain compared to 3%, indicating that higher concentrations of snot apple powder (3%) may not be as effective for weight gain. Birds on 0% snot apple powder had significantly higher feed intake compared to 3%, possibly due to differences in palatability or digestibility influenced by the snot apple powder. Birds on 2% and 3% snot apple powder exhibited better FCR values compared to 0%, indicating improved feed efficiency with snot apple powder supplementation. The improved feed conversion ratio (FCR) and growth performance in birds supplemented with 1-2% snot apple powder (T2 and T3) may be attributed to the bioactive compounds in snot apple, such as flavonoids and phenolics. These compounds are known to enhance nutrient absorption and gut health by modulating intestinal morphology and enzyme activity. Probiotics contribute by stabilizing gut microbiota, reducing harmful pathogens, and enhancing nutrient utilization, which synergistically improve feed efficiency when combined with snot apple powder.

The variations across treatment groups (T_1 to T_4) suggest potential differences in blood volume or red blood cell mass in chickens subjected to different dietary interventions. Similarly, variations in Hb concentration and erythrocyte count further reveal the oxygen-carrying capacity of the blood. Leukocyte counts were in line with values obtained for broiler chickens (Beski and Al-Sardary, 2015) and quail (Ufele and Ebenebe 2017) and are crucial for assessing immune function, with variations indicating potential differences in immune responses among treatment groups. Higher leukocyte counts may suggest enhanced immune activity or a response to stressors, while lower counts could indicate a less robust immune response (Berghof et al., 2018). Higher lymphocyte percentages in this study may indicate a stronger immune response or better overall health status. Variations in percentages of heterophils, monocytes, eosinophils, and basophils suggest potential immune system modulation by dietary interventions, with birds on probiotic treatment potentially promoting specific types of immune responses over others (Lee et al., 2012). The heterophil:lymphocyte ratio serves as an indicator of stress and immune function balance. The observed variations in this ratio across treatment groups suggest differential impacts on stress levels and immune function among the dietary interventions. Overall, the hematological parameters (packed cell volume, hemoglobin, erythrocytes, leukocytes, thrombocytes, lymphocytes) did not significantly differ among treatments, suggesting that neither snot apple powder nor probiotics had adverse effects on blood parameters.

The result of this study indicates that birds in treatments with snot apple powder and probiotics (T2, T3, T4) had higher total protein levels compared to T1, indicating better protein utilization and potentially enhanced growth. This finding corroborates the results obtained by Jimoh et al. (2018) who reported mistletoe inclusion significantly ($P<0.05$) influenced serum total protein of pullets across the treatment, Pullets fed diets with 6% inclusion of MLM had significantly ($p<0.05$) highest serum total protein. Globulin levels were significantly higher in T2, T3, and T4 compared to T1, which suggests improved immune response and overall health status. Albumin levels varied but generally favored treatments with snot apple powder. This finding disagrees with Jimoh et al. (2018) whose result revealed that serum globulin and albumin of laying pullets were not influenced by mistletoe leaf meal. Birds on T1 had significantly lower uric acid levels compared to T2, T3, and T4, indicating potential differences in metabolic processes influenced by the additives. The observed variations in protein profile parameters highlight the diverse effects of probiotics and phytobiotics supplementation on chicken physiology and metabolism (de Vries et al., 2022). T_2 emerges as a promising treatment with enhanced total protein and globulin

levels, suggesting improved protein metabolism and immune function. T_3 and T_4 also show positive effects on protein profile parameters, indicating potential benefits for overall health and performance. Chickens on T_2 exhibited the highest globulin level, suggesting its diet may enhance immune function or stimulate the production of immune-related proteins, while values obtained were lower than that reported by Tóthová et al. (2019) in broiler chicken and in grower turkey (Szabó et al., 2005). The elevated serum protein and globulin levels in T2, T3, and T4 could indicate enhanced protein metabolism and immune function due to the immunomodulatory effects of both snot apple powder and probiotics. These effects may be mediated by increased production of immune-related proteins and improved nitrogen metabolism. Probiotics could stimulate immune cells in the gut-associated lymphoid tissue (GALT), while the phytochemicals in snot apple may act as immunostimulants, enhancing lymphocyte proliferation and activity. The intermediate uric acid levels in T_3 and T_4 suggest their diets may impacts on nitrogen metabolism differently compared to T_1 (without probiotics) and T_2.

Birds on 1% snot apple powder exhibited the highest TAC, indicating superior antioxidant defense in these birds, which could contribute to better health and stress resilience. Higher lipid peroxidase activity in 1% and 2% suggests potential oxidative stress, although within manageable levels. Oxidative stress refers to any situation where there is a serious imbalance between the production of free radicals (FR) or reactive oxygen species (ROS), called the oxidative load, and the antioxidant defense system (Jimoh, 2022b). Birds on T2, T3, and T4 showed higher SOD activity compared to T1, indicating enhanced antioxidant enzymatic activity. Catalase activity was similar among treated groups, possibly indicating balanced oxidative status. Birds on T2 had the highest GPx activity, further supporting snot apple powder's antioxidative benefits. The significantly higher TAC observed in birds fed with 1% snot apple meal (T_2) suggests that this dietary intervention enhances the birds' antioxidant capacity. Similarly, T_3 and T_4 also exhibit elevated TAC levels compared to the control group without probiotics treatment and values obtained were higher than that obtained for Astathanxin supplementation in broilers in Asia (Hosseindoust et al., 2020), and the result obtained by Jimoh et al. (2020; 2022a) who recorded that Birds on phytogenic supplements tend to have lower lipid peroxidation and better antioxidant profile than birds on control treatment during heat stress conditions, Serum lipid peroxidation of birds on treatments phytogenic supplements were statistically ($P>0.05$) similar and significantly ($P<0.05$) lower than birds on treatment control indicating the potential benefits of probiotics and phytobiotics supplementation in bolstering antioxidant defenses. However, T_1 and T_4 exhibited lower lipid peroxidation levels, indicating potential protective effects against lipid oxidation conferred by their respective diets. SOD is a critical antioxidant enzyme that catalyzes the conversion of superoxide radicals into less harmful molecules, thus playing a crucial role in reactive oxygen species (ROS) detoxification (Xue et al., 2018; Jafari et al., 2021). The significantly higher SOD activity observed in T_2 suggests that this diet enhances the birds' ability to neutralize superoxide radicals. Similarly, T_3 and T_4 also exhibit elevated SOD activities compared to the control group (without probiotics), indicating the potential antioxidant effects of probiotics and phytobiotics supplementation (Jung et al., 2010; Kim et al., 2016). Catalase is another important antioxidant enzyme responsible for breaking down hydrogen peroxide into water and oxygen, thereby protecting cells from oxidative damage (Wang et al., 2022). The lower catalase activity observed in birds fed with probiotics and phytobiotics-enriched diets (T_2, T_3, and T_4) compared to the control group (T_1) suggests that these dietary interventions may modulate catalase activity to maintain cellular redox balance. The significantly higher GPx activity observed in T_2 and T_3 indicates that this diet enhances the birds' ability to detoxify peroxides (Daun and Akesson, 2004), suggesting the potential benefits of probiotics and phytobiotics supplementation in enhancing GPx-mediated antioxidant defenses. The higher total antioxidant capacity (TAC), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activity observed in birds fed snot apple powder and probiotics likely result from the antioxidant properties of the bioactive compounds in snot apple, such as tannins and saponins, which neutralize reactive oxygen species (ROS). Probiotics may enhance the production of endogenous antioxidants by supporting gut health and reducing systemic inflammation, further bolstering oxidative defenses.

In this study, chickens fed 2% treatment levels seem to have the most active thyroid hormone and knowing fully well the role this hormone plays in regulating metabolism, growth, and development. The values obtained were within the expected range for healthy broiler chickens (Jiang et al., 2020). Birds on 0% and 2% snot apples had significantly higher T3 levels compared to T2 and T4, suggesting differential effects on thyroid function possibly influenced by the dietary additives. In broiler chickens, thyroid hormones play a crucial role in regulating metabolism, growth, and development (Hyeong-Kyu and Rexford, 2023). The result in this study partially agrees with Jimoh et al. (2023) that triiodothyronine of birds fed phyllanthus and mistletoe were significantly ($P<0.05$) higher than birds on basal diet. The increased triiodothyronine (T3) levels observed in birds on 0% and 2% snot apple powder diets suggest that the additives may influence thyroid function. This could be linked to improved energy metabolism and growth, as thyroid hormones are critical regulators of these processes. The bioactive compounds in snot apple may modulate thyroid hormone synthesis or activity through antioxidant or anti-inflammatory pathways.

However, 1-3% inclusions of Snot apple meal seem essential for proper growth, feed efficiency, and overall health in broilers. Any significant deviations from these levels could indicate underlying health issues, stress, or nutritional imbalances that may impact the birds' performance and well-being. Anwar et al (2018) reported the need to monitor thyroid hormone levels in broiler chickens as it is important for optimizing production outcomes and ensuring the welfare of the birds.

5. Conclusion

This study demonstrates that incorporating snot apple powder, particularly at 1% and 2% levels, along with probiotics in broiler chicken diets, positively influences growth performance, serum protein profiles, antioxidant status, and potentially thyroid function. Specifically, 2% snot apple powder showed optimal performance outcomes, while maintaining favorable hematological parameters and oxidative balance. These findings suggest that snot apple powder can be a beneficial dietary supplement in poultry nutrition, enhancing both performance and health parameters. To refine dietary recommendations for commercial application and fully understand the benefits of snot apple powder in poultry nutrition, further research should explore the mechanisms of action, optimal dosages, species-specific effects, interactions with feed components, long-term impacts on health and productivity, environmental and economic implications, and consumer acceptance to refine the use of snot apple powder in poultry diets.

Author Contributions

The percentages of the authors' contributions are presented below. The author reviewed and approved the final version of the manuscript.

	A.O.J.	U.D.O.	S.O.A.	U.G.D.I.
C	50	50		
D	100			
S	100			
DCP			50	50
DAI			50	50
L			80	20
W	40		60	
CR	60		20	20
SR	60		20	20
PM	20	80		
FA	20	50	10	20

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

The authors confirm that the ethical policies of the journal as noted on the journal's author guidelines page have been adhered to and the institutional ethics committee for care and use of animal for research approved the study (approval date: November 01, 2023, protocol code: AP/REC/2023/0864). The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

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EFFECT OF POTASSIUM OPTIMIZATION ON WHEAT DROUGHT TOLERANCE IN CONTROLLED CONDITIONS

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Abstract: Wheat (*Triticum* spp.) is an important cereal crop consumed worldwide, but it is highly susceptible to drought. Potassium plays an essential role in osmotic regulation, photosynthesis, and nitrogen assimilation, all of which are critical for maintaining plant growth and productivity under stress conditions. The aim of this study is to investigate how different potassium levels, including sufficient potassium (SK, 1 mM) and low potassium (LK, 0.05 mM), affect the drought tolerance of wheat during the early stages of seedling development under PEG-induced drought stress. Plant physiological development, canopy temperature, photosynthetic efficiency, antioxidant defense enzymes, and nitrogen assimilation enzymes were assessed in the experiment. In non-drought conditions, LK increased canopy temperature and reduced dry matter yield and photosynthetic performance, with these effects becoming more pronounced under drought stress. SK-treated plants exhibited higher biomass, chlorophyll content, maximum quantum efficiency of photosystem II, and lower canopy temperatures, even under drought conditions. Furthermore, LK restricted the accumulation of key osmotic regulators, including proline, amino acids, and soluble sugars. Under drought stress, LK plants also showed increased hydrogen peroxide and superoxide anion levels, while SK plants had lower reactive oxygen species accumulation and higher antioxidant enzyme activities (catalase and superoxide dismutase). Additionally, LK resulted in reduced activity of nitrogen assimilation enzymes (nitrate reductase, NR, and nitrite reductase, NiR) under both normal and drought conditions. In contrast, SK-treated wheat seedlings maintained higher NR and NiR activities and higher soluble protein content during drought stress. These findings underscore the critical role of potassium management in enhancing wheat yield, particularly in water-scarce regions, as optimal potassium supply strengthens essential physiological and biochemical mechanisms that improve plant tolerance to drought stress.

Keywords: Drought stress, Potassium, Nitrate assimilation, Oxidative stress

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1. Introduction

Wheat (*Triticum* spp.) is a significant cereal crop extensively produced globally and is essential for human nutrition (Igrejas and Branlard, 2020). Wheat, which is prone to higher yields under irrigated conditions (Mansour et al., 2020), is a highly water-sensitive plant (Zhao et al., 2020). Experiencing drought during various growth stages leads to considerable reductions in wheat yield (Hu et al., 2021). Drought stress is a critical environmental challenge that endangers global food security, particularly in areas with limited water resources and irregular precipitation (Begna, 2020; Pequeno et al., 2021). Drought stress severely impacts crop productivity by disrupting basic physiological processes of plants, including water uptake, nutrient absorption, and photosynthesis (Pamungkas and Farid, 2022; Wahab et al., 2022; Qiao et al., 2024; Ramazanoglu et al., 2024).

Plants subjected to drought stress experience significant physiological disturbances, primarily due to water loss, which leads to osmotic stress and a reduction in turgor pressure (Yang et al., 2021; Hemati et al., 2022). In the later stages of water scarcity, plants close their stomata to minimize water loss through transpiration, but this also restricts CO_2 uptake, reducing photosynthesis and inhibiting plant growth (Song et al., 2020; Shohat et al., 2021). Additionally, drought stress induces oxidative stress, which harms cellular structures such as lipids, proteins, and DNA by promoting the accumulation of reactive oxygen species like hydrogen peroxide and superoxide anion (Zheng et al., 2023). This oxidative stress amplifies the detrimental impact of drought, resulting in disrupted cellular function and decreased biomass (Seleiman et al., 2021). In response, plants activate antioxidant defense systems to mitigate oxidative stress (Akbari et al., 2022) and accumulate osmotic regulators like proline, amino acids, and soluble sugars to preserve cellular homeostasis (Ahmad et al., 2020; Choudhary et al., 2023; Mehta and Vyas, 2023).

Nitrate, an important nitrogen source for plants in agricultural soils (Shafreen et al., 2021), is converted into ammonium within plant cells through the actions of the enzymes nitrate reductase and nitrite reductase (Kumari et al., 2022). The ammonium produced from nitrate

reduction is utilized in the synthesis of amino acids and proteins with the assistance of various enzymes (Taria et al., 2022). Drought stress impairs nitrate uptake and assimilation by diminishing the activity of these enzymes, thereby reducing the nitrogen use efficiency in plants (Khatoon et al., 2024; Wei et al., 2024). This reduction in nitrate assimilation leads to a decline in photosynthetic capacity and carbon uptake, adversely impacting plant health and yield (Zayed et al., 2023). Consequently, regulating nitrogen metabolism under stress conditions is crucial for improving plant tolerance (Baslam et al., 2020; Ye et al., 2022).

Potassium is a vital macronutrient that helps plants adapt to stress conditions by regulating key physiological and biochemical processes (Ul-Allah et al., 2020). Research shows that potassium enhances drought tolerance by promoting root growth, controlling stomatal movements, supporting photosynthesis, facilitating protein synthesis, and maintaining cellular osmotic balance (Xu et al., 2021; Johnson et al., 2022; Mostofa et al., 2022). Additionally, a positive relationship has been found between potassium and nitrate uptake and transport in plants. Ye et al. (2022) found that potassium boosts nitrate uptake and assimilation, while nitrate, in turn, facilitates potassium uptake and transport from roots to shoots. Feng et al. (2020) also observed that potassium improves plant resistance by regulating nitrogen uptake and assimilation. Furthermore, potassium reduces oxidative stress in plants by activating antioxidant enzymes that neutralize reactive oxygen species (Taha et al., 2020; Tittal et al., 2021). However, potassium availability can be limited by factors such as low potassium reserves (Reimer et al., 2020), leaching (Dianjun et al., 2022), and potassium fixation (Celik et al., 2023), all of which can contribute to potassium deficiency in plants.

Due to potassium's ability to regulate key physiological and biochemical processes under drought stress, plant responses to drought conditions largely depend on the availability of this nutrient. However, information on the effects of K deficiency on nitrogen metabolism, oxidative stress, and osmotic adjustment in plants under drought stress is limited. Therefore, this study aims to investigate the impact of potassium nutritional status on the stress tolerance of wheat plants exposed to drought stress during the early stages of seedling development. The research focuses on assessing the role of potassium under both optimal growth conditions and drought stress simulated using polyethylene glycol (PEG).

2. Materials and Methods

This study was conducted in the plant growth chambers at the Faculty of Agriculture, Harran University in 2023, using the wheat variety 'Edessa' as the research plant. Seedlings germinated in perlite were transferred to 5 L PVC containers filled with aerated Hoagland solution, which was refreshed every two days throughout the experiment. Half of the containers were supplied with a nutrient solution containing low potassium (0.05 mM), while the other half received a sufficient potassium concentration (1 mM K_2SO_4).

Polyethylene glycol 6000 (PEG 6000) was chosen in this study to simulate drought stress. PEG 6000, as an inert and non-ionic molecule, reduces water potential uniformly without entering plant cells, making it a reliable option for creating drought stress under controlled conditions (Mohi-Ud-Din et al., 2021; Batool et al., 2022). PEG 6000 has been extensively used in drought tolerance studies across various plant species (Bukhari et al., 2021; Guo et al., 2022; Mahpara et al., 2022; Qi et al., 2023). However, the water deficit created by PEG does not fully capture all aspects of natural drought conditions, such as fluctuations in temperature and humidity. Therefore, the findings reflect a drought simulation under controlled conditions and should be further validated under natural settings.

After a 10-day growth period, PEG 6000 was added to the pots at a concentration of 200 g L^{-1} (20%) to induce drought stress, and the plants were harvested six days later. The experiment consisted of 12 pots, with 4 treatments and 3 replicates for each treatment.

2.1. Biomass Yield and Potassium Content Measurement

Shoots and roots were washed with distilled water and kept in a 70 °C oven until they reached constant weight, and then their dry weights were recorded. The samples were then ashed at 500 °C, digested with HCl, and analyzed using a flame photometer to determine their potassium content (Kacar and Inal, 2008).

2.2. Chlorophyll Content and Chlorophyll Fluorescence

Chlorophyll content was measured by homogenizing fresh plant leaves in 80% acetone and measuring absorbance at 663 and 645 nm (Arnon, 1949). Chlorophyll fluorescence was assessed using a photosynthetic efficiency analyzer (Walz, Germany) after dark-adapting leaves for 30 minutes.

2.3. Proline, Total Free Amino Acids, and Soluble Sugar Content

Proline levels were measured following the method outlined by Bates et al. (1973). Leaf samples were homogenized in 3% sulfosalicylic acid and filtered through filter paper. An aliquot of the supernatant was then mixed with acid ninhydrin solution and acetic acid. The mixture was heated at 100ºC for 1 hour and subsequently cooled in an ice bath. To isolate free proline, 4 ml of toluene was added and vortexed. The absorbance of the mixture was measured at 520 nm using a microplate spectrophotometer (Epoch, SN: 1611187, ABD), with pure toluene as the blank. Proline concentration was determined using a standard curve prepared with L-proline.

The method described by Lee and Takahashi (1966) was used to determine total free amino acids. Fresh plant leaves were incubated in distilled water at 45 °C for 1 hour. After incubation, 100 µl of the centrifuged sample

was transferred to a new tube, and 1.9 mL of ninhydrin-glycerol-citrate buffer was added. The mixture was heated in boiling water for 12 minutes, then cooled to room temperature in a water bath. The absorbance was measured at 570 nm using a microplate spectrophotometer. Total free amino acids were quantified using a standard curve prepared with glycine.

The soluble sugar content of leaf samples was determined following the method of Dubois et al. (1956). Leaf samples were treated with 5 ml of 2.5 N HCl and incubated in a water bath at 80 °C for 2 hours. After cooling, concentrated sulfuric acid and 5% phenol were added, and the mixture was shaken. The absorbance of the solution was measured at 490 nm using a microplate spectrophotometer.

2.4. Oxidative Stress Markers

The hydrogen peroxide (H_2O_2) concentration was determined following the method described by Loreto and Velikova (2001). Fresh plant leaves were homogenized in 0.1% TCA and centrifuged at 15,000 rpm at 4 °C for 15 minutes. The H_2O_2 content was measured by adding 10 mmol L^{-1} K-phosphate buffer and 1 M KI to the homogenate, and the absorbance was recorded at 390 nm.

Superoxide anion levels were measured according to the method by Elstner and Heupel (1976). Fresh leaf samples were homogenized in 65 mM phosphate buffer (pH 7.8) and centrifuged at 5000 x g for 10 minutes. The supernatant was treated with 10 mM hydroxylamine and homogenization buffer, then incubated at 25 °C for 20 minutes. Following incubation, 1 mL of aminobenzene sulfonic acid and α-naphthylamine were added and incubated again at 25 °C for 20 minutes. The absorbance was measured at 530 nm using a spectrophotometer, and the results were calculated using a standard curve prepared with $NaNO_2$.

2.5. Activities of Antioxidant Enzymes and Total Soluble Protein Content

Fresh plant leaves were homogenized by adding 0.1 mol L^{-1} K-phosphate buffer (containing 1% PVP and 0.1 mmol L^{-1} EDTA). The extract was centrifuged at 15,000 rpm and +4 °C for 15 min.

Superoxide dismutase (SOD) activity was measured following the method of Beauchamp and Fridovich (1971). The activity was determined by measuring the absorbance at 560 nm using a microplate spectrophotometer after incubating the reaction mixture (0.05 M K-phosphate buffer (pH 7.8), 0.05 M sodium carbonate, 13 mM L-methionine, 100 µM EDTA, 75 µM NBT, 5 µM riboflavin, and 25 µL supernatant) under a fluorescent lamp for 15 minutes. A tube without the enzyme extract served as the control, while a complete mixture that was not irradiated and did not develop color was used as the blank.

Catalase (CAT) enzyme activity was determined following the procedure described by Aebi (1984). The absorbance change of the reaction mixture (50 mmol L^{-1} K-phosphate, 10 mmol L^{-1} H_2O_2, 4 mmol L^{-1} Na_2EDTA and 10 µL supernatant) at 240 nm was measured for 5 min.

Total soluble protein content was measured using the Bradford method (1976). Briefly, 200 µL of Bradford reagent (prepared according to Bradford (1976) recipe) was added to 10 µL of protein extract. Samples were kept at room temperature for 15 min and their absorbance at 595 nm was determined by microplate spectrophotometer. The results were calculated with a standard curve prepared with Bovine Serum Albumin (BSA).

2.6. Nitrate Reductase (NR) and Nitrite Reductase (NiR) Activity

The NR activity was determined using the method described by Jaworski (1971). Fresh leaf samples were placed in test tubes with 0.1 M phosphate buffer (comprising 20 mM KNO_3 and 5% propanol) and incubated for 2 hours at 25 °C in darkness. At the conclusion of incubation, 0.3 mL of a mixture containing 1% sulfanilamide and 0.02% naphthylenediamine dihydrochloride was introduced to a 0.4 mL aliquot. After 20 minutes, the absorbance of samples diluted with 0.4 mL of deionized water was measured at 540 nm using a microplate spectrophotometer. Nitrite production was quantified with a $NaNO_2$ calibration curve.

To measure NiR activity, the method of Ramirez et al. (1966) was used. Leaf samples were homogenized in potassium phosphate buffer prepared as described in the method. 1.4 ml potassium phosphate buffer, 5 mmol L^{-1} KNO_2 and 100 µl 2 mg/ml methyl viologen and 100 µl enzyme extract were mixed and the volume was completed to 1.8 ml with distilled water. The reaction was initiated by adding 200 µl sodium dithionite and incubated at 30 °C for 30 min. After incubation, 100 µl of the mixture was added with 1.9 ml water and vortexed. Then, 2 ml of 1% sulfanilamide and 0.05% (w:v) N-(1-naphthyl)-ethylenediamine hydrochloride solution was added. The solution was incubated again, and its absorbance was measured at 540 nm.

2.7. Statistical Analysis

All data obtained were analyzed using SPSS (Version 22.0) program with variance analysis (ANOVA) and Duncan Multiple Comparison Test at a significance level of $P<0.05$ and presented in graphs (Genç and Soysal, 2018).

3. Results

3.1. Effect of Potassium Availability and Drought Stress on Plant Temperatures, Potassium Uptake and Biomass Accumulation

Thermal imaging revealed that potassium (K^+) deficiency elevated plant canopy temperature (Figure 1A). When K^+ deficiency was combined with drought stress (DS), plant temperatures rose even further. However, supplementing K^+ to the growth medium seemed to mitigate temperature increases under DS. Overall, low potassium (LK) levels tended to result in higher plant temperatures.

Shoot K^+ content (Figure 1B) significantly increased in

the sufficient potassium (SK) treatment compared to the LK treatment, under both control and DS conditions. In control conditions, the shoot K^+ content of SK-treated plants was approximately twice that of LK-treated plants, whereas under drought stress, this ratio decreased to 1.4-fold.

The treatments also significantly changed the shoot dry weight (DW) and root DW (Figure 1C, D). Without drought stress, SK-treated plants had 34% higher shoot DW and 15% higher root DW than LK-treated plants. DS reduced shoot DW by 46% in LK plants and by 42% in SK plants, with root DW decreasing by 44% in both treatments. The reduction in biomass was more severe in LK plants under DS.

3.2. Effects of Potassium Deficiency and Drought Stress on Chlorophyll Content and Chlorophyll Fluorescence

Total chlorophyll (Chl) content significantly decreased under DS in both LK and SK treatments (Figure 1E). Under control conditions, there was no significant difference in Chl content between LK and SK treatments. However, under DS, Chl content in SK-treated plants was approximately 35% higher than in LK-treated plants. Notably, LK-treated plants experienced a pronounced reduction in Chl content when exposed to drought. Compared to control conditions, Chl content decreased by 54% in LK-treated plants and by 44% in SK-treated plants under DS.

Photosystem II efficiency (*Fv/Fm*) followed a similar trend, with DS reducing *Fv/Fm* values in both treatments (Figure 1F). LK plants showed a 39% reduction under DS, while SK plants had a smaller decrease of 25%, indicating that potassium helps maintain photosynthetic efficiency under drought conditions.

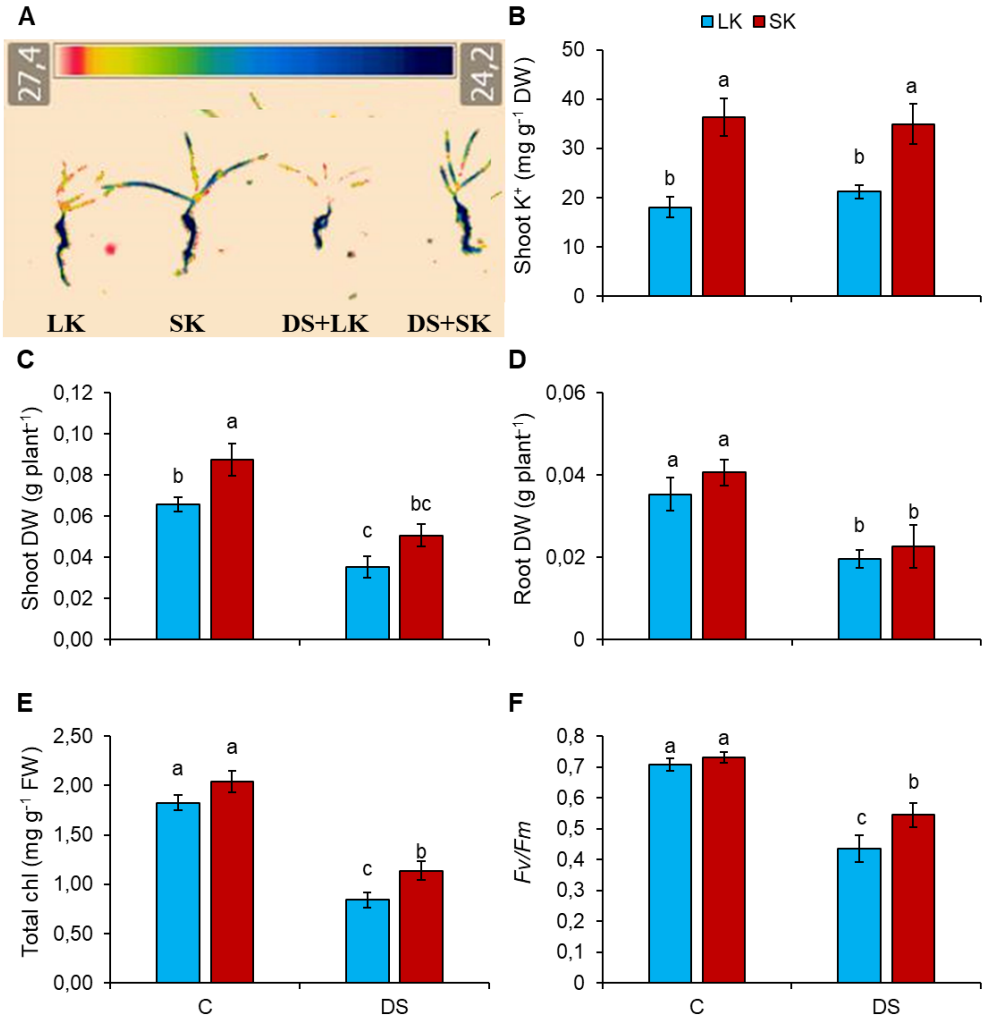

Figure 1. A) Thermal image, B) Shoot potassium (K^+) content, C) Shoot DW, D) Root DW, E) Total chlorophyll (chl) and F) Photosystem II quantum efficiency (*Fv/Fm*) in wheat seedlings grown under drought stress (DS) and low K (LK) or sufficient K (SK). Error bars represent the standard error of each treatment (n=3). Letters above the bars indicate statistical differences ($P<0.05$) between treatments.

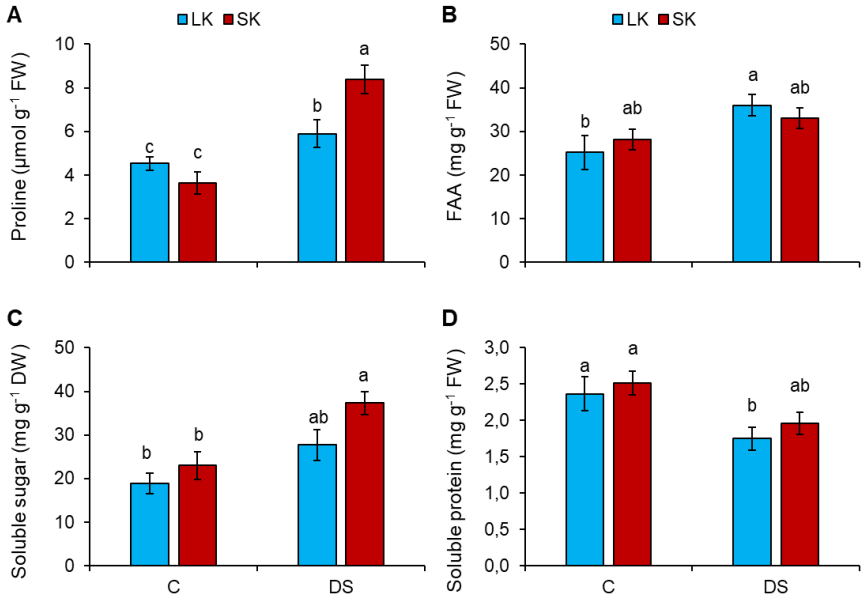

Figure 2. A) Proline, B) total free amino acids (FAA), C) soluble sugar and D) Soluble protein content in wheat seedlings grown under drought stress (DS) and low K (LK) or sufficient K (SK). Error bars represent the standard error of each treatment (n=3). Letters above the bars indicate statistical differences (P<0.05) between treatments.

3.3. Effects of Potassium Deficiency and Drought Stress on Osmolyte Accumulation and Soluble Protein Levels

Under DS, proline content increased approximately 2.3-fold in SK plants, while it rose by about 1.3-fold in LK plants, indicating that potassium enhances proline accumulation for drought tolerance (Figure 2A). Total free amino acids (FAA) increased modestly under drought (Figure 2B), with SK and LK plants showing increases of 4% and 5.6%, respectively, compared to control conditions. Under DS, LK plants exhibited 8% higher FAA content than SK plants. Soluble sugars also increased significantly under DS, with SK showing a 62% rise compared to control, while LK increased by 46% (Figure 2C). Consequently, SK plants accumulated 35% more soluble sugars than LK plants under DS. No significant difference in soluble protein content was observed under control conditions (Figure 2D). However, under DS, protein content decreased by 22% in SK plants and 26% in LK plants, with SK plants having 12% higher protein content than LK plants.

3.4. Oxidative Stress and Antioxidant Enzyme Activity in Wheat Seedlings under Potassium Deficiency and Drought Stress

The results in Figure 3 demonstrate that DS and potassium availability significantly affect oxidative stress markers and antioxidant enzyme activities in wheat seedlings.

Hydrogen peroxide (H_2O_2) levels increased under DS in both LK and SK treatments (Figure 3A), with H_2O_2 content in LK-treated plants being approximately 21% higher than in SK-treated plants under DS. Superoxide anion (O_2^-) production rates also rose significantly under drought, with SK plants showing a 47% decrease in O_2^- levels compared to LK plants under stress (Figure 3B).

Catalase (CAT) activity increased under DS in both LK and SK treatments (Figure 3C), with LK-treated plants exhibiting approximately 37% higher CAT activity than SK plants. Superoxide dismutase (SOD) activity also increased slightly under drought conditions, with LK-treated plants showing about 14% higher SOD activity compared to SK under DS (Figure 3D).

3.5. Nitrogen Metabolism Enzymes in Wheat Seedlings under Potassium Deficiency and Drought Stress

The results for nitrate reductase (NR) and nitrite reductase (NiR) activities under potassium deficiency and DS are shown in Figures 3E and 3F. Under control conditions, NR activity in SK-treated plants was approximately 28% higher than in LK-treated plants. DS significantly reduced NR activity in both treatments, with activity decreasing by 47% in SK and 58% in LK compared to control. However, even under DS, SK plants had 59% higher NR activity than LK plants.

NiR activity followed a similar pattern to NR. Under control conditions, SK plants exhibited 41% higher NiR activity than LK plants. DS led to a 31% reduction in LK plants and a 38% reduction in SK plants. Despite the decline, SK plants maintained 25% higher NiR activity under DS compared to LK plants.

4. Discussion

Potassium (K^+) is essential for plants because it regulates stomatal openings (Ahammed et al., 2022), enhances gas exchange efficiency (Simões et al., 2020), maintains water balance (Lu et al., 2022), activates enzymes critical for photosynthesis and protein synthesis (Mostafa et al., 2022; Rawat et al., 2022), and boosts tolerance to environmental stresses such as drought (Hasanuzzaman et al., 2018).

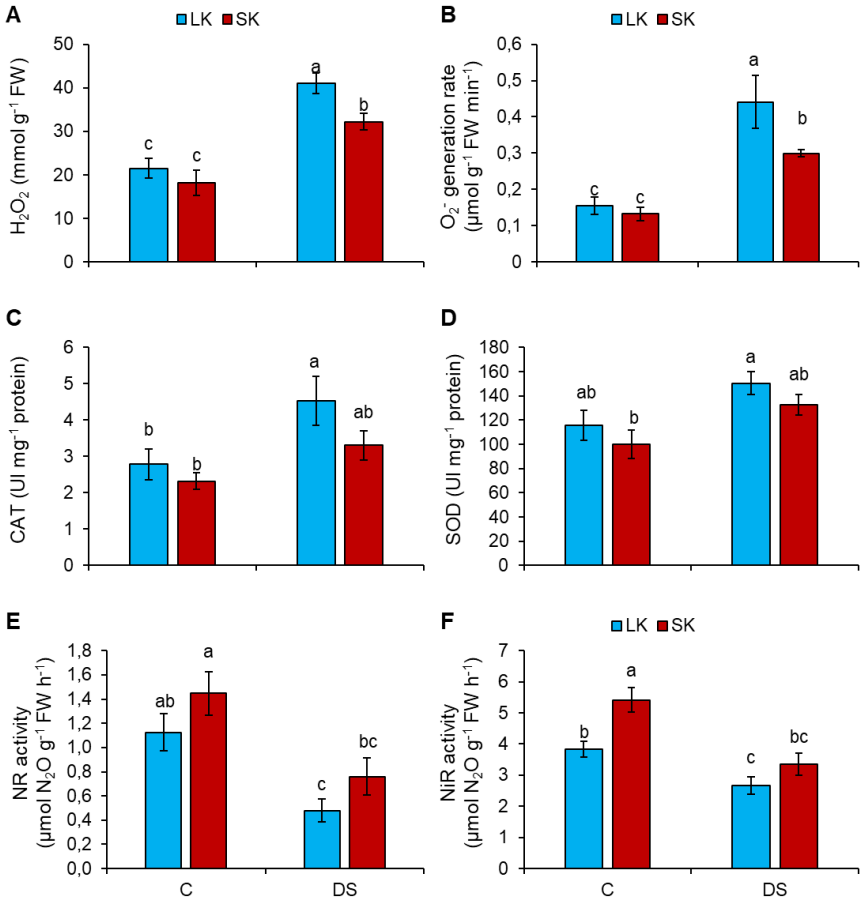

Figure 3. A) Hydrogen peroxide (H_2O_2) content, B) superoxide (O_2^-) generation rate, C) Catalase (CAT) activity, D) Superoxide dismutase (SOD) activity, E) Nitrate reductase (NR) activity and E) Nitrite reductase (NiR) activity in wheat seedlings grown under drought stress (DS) and low K (LK) or sufficient K (SK). Error bars represent the standard error of each treatment (n=3). Letters above the bars indicate statistical differences ($P<0.05$) between treatments.

Numerous studies have observed that potassium-enriched plants exhibit greater resistance to environmental stressors (Wang et al., 2013; Sardans and Peñuelas, 2021; Johnson et al., 2022; Pantha et al., 2023). Potassium deficiency significantly reduced biomass accumulation, especially under drought stress. The reduction in biomass observed in potassium-deficient plants can be attributed to impaired water relations (Tavakol et al., 2018) and decreased photosynthetic capacity (Pan et al., 2017), both of which are exacerbated under drought stress. Potassium is necessary to regulate stomatal activity and supports cooling through transpiration (Hasanuzzaman et al., 2018). In LK-treated plants, reduced K^+ availability likely led to impaired stomatal function, causing higher canopy temperatures and reduced water use efficiency. This resulted in lower shoot and root biomass, likely limiting carbon assimilation and reducing the energy available for growth (Tighe-Neira et al., 2018). Additionally, the decrease in biomass can be linked to potassium's role in maintaining chlorophyll content and photosynthetic efficiency (Tighe-Neira et al., 2018; Ju et al., 2021). Bo et al. (2022) demonstrated that potassium deficiency reduces chlorophyll content and the quantum efficiency of Photosystem II (*Fv/Fm*), which aligns with our findings under drought stress. The decrease in photosynthetic parameters is probably due to increased oxidative stress or impaired chlorophyll synthesis (Khodabakhshi et al., 2023). In contrast, plants treated with adequate potassium with higher chlorophyll levels and photosynthetic efficiency were more resistant to drought stress, thus resulting in higher biomass compared to potassium deficient plants. Therefore, adequate nutrition of plants with potassium plays a critical role in maintaining plant growth in both normal and drought conditions by supporting photosynthesis and water relations (Wang et al., 2013; Rawat et al., 2022; Fang et al., 2023).

Plants accumulate organic substances such as proline, soluble sugars, and soluble proteins to reduce damage caused by drought stress, protect cellular structures, and maintain metabolic activities (Fang et al., 2022; Alagoz et al., 2023). These compounds play a key role in reducing the effects of reactive oxygen species (ROS), aiding osmotic adjustment, maintaining cell water balance, and enhancing stress tolerance (Afzal et al., 2021; Alagoz et al., 2023). In our study, drought stress induced the accumulation of proline, soluble sugars, and free amino acids. Many researchers have reported similar increases in these compounds under drought stress in various

plants (Holmstrup et al., 2015; Gurrieri et al., 2020; Nguyen et al., 2020; Semida et al., 2020; Živanović et al., 2020). Potassium enhances osmoregulation in plants by increasing cell turgor and maintaining water balance (Kumar et al., 2020; Pandey and Mahiwal, 2020). In our study, plants with adequate potassium under drought stress accumulated higher levels of proline and soluble sugars, emphasizing potassium's key role in regulating osmotic potential. Similar patterns have been reported in cotton (Zahoor et al., 2017), wheat (Ahanger et al., 2017), and sunflower (Shehzad et al., 2020), highlighting potassium's contribution to osmotic adjustment under stress. These results emphasize the importance of maintaining optimal potassium levels to enhance plant resilience to drought.

Drought stress induced oxidative stress in wheat seedlings, as indicated by increased levels of hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-). These reactive molecules damage cellular structures such as lipids, proteins, and nucleic acids, leading to the disruption of cellular functions (Zheng et al., 2023). In our study, plants exposed to potassium deficiency exhibited higher O_2^- and H_2O_2 production rates, indicating that adequate potassium reduces the extent of oxidative damage under drought (Johnson et al., 2022). Similar patterns of increased ROS under potassium deficiency have been observed in barley (Tavakol et al., 2021), rapeseed (Zhu et al., 2020), tomato (Siddiqui et al., 2021), and sorghum (Tittal et al., 2021), reinforcing potassium's critical role in managing oxidative stress. Plants activate antioxidant defense mechanisms to mitigate ROS-induced damage (Nowroz et al., 2024). Superoxide dismutase (SOD), a widespread metalloenzyme, converts O_2^- into molecular oxygen and H_2O_2, providing the primary defense against oxidative stress (Mishra and Sharma, 2019). Increased catalase (CAT) activity under stress conditions breaks down H_2O_2 into harmless compounds, playing a crucial role in safeguarding plants against oxidative stress (Dikilitas et al., 2016). Although both treatments showed increased CAT and SOD activity under drought, LK-treated plants had higher antioxidant enzyme activity than SK-treated plants, possibly as a compensatory response to elevated oxidative stress. Nevertheless, the results indicate that potassium sufficiency reduces oxidative stress, likely by maintaining better cellular homeostasis and membrane stability (Sardans and Peñuelas, 2021).

Nitrate (NO_3^-) is an important nitrogen source for plants in agricultural soils and must be reduced to ammonium (NH_4^+) form for the synthesis of proteins and other organic compounds in plants (Shafreen et al. 2021; Islam et al., 2022). Nitrate reductase (NR) reduces NO_3^- to nitrite (NO_2^-) in plant cells, and this NO_2^- is reduced to NH_4^+ by the nitrite reductase (NiR) enzyme (Kumari et al., 2022; Chen et al., 2023). NH_4^+ is assimilated into amino acids and proteins by glutamine synthetase-glutamate synthase enzymes or glutamate dehydrogenase in the plant (Taria et al., 2022). Stress exposure leads to a reduction in the transcript levels of nitrogen-assimilating enzymes, including NR, NiR, and glutamate synthase (Sahay et al., 2021; Sathee et al., 2021). The suppressive effect of drought stress on NR and NiR enzyme activities has been previously reported in many plants such as soybean (Du et al., 2020; Qu et al., 2023), maize (Majeed et al., 2020), Brassica juncea (Sahay et al., 2021) and pepper (Kaya and Shabala, 2023). Our results showed that drought stress decreased NR and NiR enzyme activities in wheat plants, like other reports. However, potassium deficiency worsened the negative effects on nitrogen metabolism by further decreasing NR and NiR activities under both normal and drought stress conditions. This aligns with the findings of Hu et al. (2016), who reported that potassium deficiency significantly reduced NR activity and nitrogen assimilation in cotton, leading to impaired nitrogen metabolism and reduced growth. This reduction in nitrate assimilation not only disrupts nitrogen metabolism but also directly affects photosynthetic efficiency, as it limits CO_2 availability (Ivanov et al., 2023). Potassium supplementation, on the other hand, improved NR and NiR activities, leading to better nitrogen assimilation and photosynthetic efficiency under drought stress. This demonstrates potassium's vital role in regulating nitrogen metabolism, especially under stress conditions (Zhong et al., 2017; Liu et al., 2022). Consequently, maintaining sufficient potassium levels is crucial for enhancing stress tolerance by supporting nitrogen use efficiency and sustaining overall plant growth and productivity.

5. Conclusion

This study highlights that potassium plays an important role in regulating wheat plant responses to drought stress. Potassium supply alleviates the adverse effects of drought by regulating water relations in plants, increasing photosynthetic efficiency, promoting osmolyte accumulation, reducing oxidative damage, and maintaining nitrogen metabolism. The findings highlight the need to maintain potassium levels in agricultural soils to enhance wheat resilience in water-limited environments. However, the findings are based on controlled conditions using PEG to simulate drought, which does not fully capture natural environmental variables like temperature and humidity fluctuations. Thus, future studies should focus on evaluating the effects of potassium supplementation under field conditions where multiple environmental factors interact. Moreover, while the study focused on the early seedling stage of wheat, potassium's effects on drought tolerance at later developmental stages should be further investigated.

Author Contributions

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

	F.U.
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, as it did not involve any research on humans or animals.

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EVALUATING THE PERFORMANCE OF THE CARROT SLICER MACHINE

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Abstract: The study was carried out to evaluate the performance of the carrot slicer machine. An experiment was conducted with a multi-factor factorial design under a randomized complete block design. Using the Statistix 8 software, the experiments' collected data were statistically examined. The analysis of variance indicated that the effects of machine speed and feed rate on throughput capacity, efficiency, and percentage loss of the machine were significant at the 5% probability level. The results of the least significant difference pairwise comparison tests revealed that the treatment combination means did not differ significantly from one another at the 5% level. The key physical properties of the carrot including moisture content, angle of repose, bulk density, porosity, coefficient of friction, geometric mean diameter, arithmetic mean diameter, equivalent mean diameter, sphericity, surface area and aspect ratio were obtained as 84.3%, 39.4°, 469.5 kg m^{-3}, 59.8%, 0.78, 47.8 mm, 62.18 mm, 83.7 mm, 0.55, 57.67 cm^2, and 0.27, correspondingly. The results showed that the maximum throughput capacity of 621.4 kg h^{-1} was recorded at 550 rpm machine speed while the minimum throughput capacity of 511.6 kg h^{-1} was recorded at 350 rpm machine speed. It has been found that the maximum machine efficiency was 96.03% at 550 rpm machine speed whereas the minimum machine efficiency was 92.5% at 350 rpm machine speed. The investigation results revealed that the minimum percentage loss was 4.2% at 550 rpm machine speed whereas the maximum percentage loss was 7.8% at 350 rpm machine speed. The test results suggested that the carrot slicer machine was found to be very effective for processing the vegetable root crop of carrots for end users.

Keywords: Carrot, Carrot slicer, Evaluation, Performance indicators, Physical properties

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1. Introduction

Carrot (*Daucus carota L.*) is a type of vegetable plant that belongs to the Apiaceae family and grows throughout the globe. It is one of the top ten vegetable crops in the entire world in terms of economic significance (Simon, 2021). Carrots are one of the most widely consumed agricultural products by millions of people worldwide (Nagraj et al., 2020). It is also a vital agricultural export that is good for a large number of global nations (Tabor and Yesuf, 2012)

Carrots have long been a mainstay of potlucks, get-togethers, and even snack time. They are dependable vegetables that are very adaptable and essential to vegetable trays. It is a useful food crop that is frequently eaten by the general public in the form of fresh veggies or drinks containing juice, as well as processed into various culinary delights (Ikram et al., 2024). Because it can be made into a variety of food products, the adaptable crop known as carrot is essential in tropical climates (Paparella et al., 2024b). It is regarded as a vegetable that is essential to global food security and the advancement of human health and well-being (Sharma et al., 2023).

Carrots are a highly valued root vegetable used extensively in cooking, raw, and juice forms (Ahmad et al., 2019).

The many health benefits of carrots include potential improvements to blood sugar regulation, weight management, cancer risk reduction, blood pressure regulation, heart disease prevention, immunity building, and brain health (Varshney and Mishra, 2022). Carrot cakes, cookies, and mashed carrots are just a few of the snacks that use carrot as a main ingredient. Carrots are mostly used as vegetables but can also be found in some limited supply as snacks like carrot chips. Carrot slices and chips are made by frying, drying, or baking (Laily et al., 2023). In addition to being a fantastic source of minerals and antioxidants, carrots are an intriguing source of carotenoids, vitamins, and dietary fiber. Carrots are a common food because they are an inexpensive source of vitamins, especially vitamin A, minerals, and fiber for the human diet (Ikram et al., 2024). Carrots are root vegetables that are typically orange in color, though they can also be red, white, yellow, or purple in appearance.

In tandem with the growing world population, there has been and is expected to be a continued increase in the

demand for carrots. On an area of 1,137,738 ha, an average of 42,158,403 tonnes of carrots and turnips are produced globally each year, with an average yield of 37 tons per hectare (Papoutsis and Edelenbos, 2021). Asia accounted for about 61.8% of global carrot production, with Europe coming in second with 22.6%, the Americas third with 9.1%, and Africa contributing 5.4% (Ahmad et al., 2019). China presently contributes worldwide in the generation and consumption of carrots; the country harvests nearly half of the world's 42 million tons of carrots, through considerably China is surpassed by the United States of America, Uzbekistan, and Russia. Global consumption of turnips and carrots in 2020 was 46.3 million metric tonnes (Hamed and Mohamed, 2023).

In Ethiopia, carrots are grown in a variety of agro-ecologies, from the lowlands to the highlands (Fufa et al., 2020). The Shewa province and the central-northern regions surrounding Addis Ababa are the main production areas. Most developing countries, including Ethiopia, whose average fresh carrot yield per hectare is 5.6 t, have carrot yields per unit area that are lower than the average worldwide (Muhie and Yimer, 2023). Ethiopia's high altitude of 1800 to 2,500 meters above sea level makes it a promising place to produce premium carrots. During the growing season, carrots need 500 mm of evenly-spaced rainfall. 16 to 21 degrees Celsius are suitable for growth. It needs sandy to loamy, deep, loose, well-drained soils with a pH of 6 to 6.5. Grown from seed, carrots can reach maturity in as little as four months; however, in ideal circumstances, most cultivars reach maturity in 70–80 days (Noor, 2020).

Postharvest problems related to carrots include the most common storage decays, which include watery soft rot, sour rot, gray mold rot, bacteria soft rot, and Rhizopus soft rot (Papoutsis and Edelenbos, 2021). The shelf life of freshly harvested carrots is usually 3 to 4 weeks at 0 °C and 2 to 3 weeks at 3 to 5 °C (Asgar, 2020). While each variety of carrot crop has a different potential for storage, in general, fresh carrots don't keep well. Traditional methods may produce uneven drying or infected dried slices because the slices are not uniformly produced. Because manual slicing requires a lot of labor, takes a long time, and is prone to human error, the thickness and dimensions of the slices produced are erratic (Alabi et al., 2023).

The purpose of processing or slicing carrot crops is to extend shelf-life and shorten the drying period by increasing the surface area's exposure to air. The majority of processed root products must have their moisture content reduced to a point where microorganisms cannot grow there to be preserved (Leneveu-Jenvrin et al., 2022). A substitute for storing produce in its fresh state is to process it into staple, non-perishable, and transportable forms. The best way to preserve carrots for human consumption is to process them into flour, chips, and pellets while they are dried (Haq and Prasad, 2015). Before being cooked and steam-tested, carrots are frequently sliced, either for immediate consumption or as a first step in a processing system. Carrot crops are processed through a series of size-reduction steps, such as washing, peeling, chipping, slicing, and drying.

Nowadays, machine processing is increasingly supplanting manual processing because life for people is becoming more competitive and fast-paced Technology-induced automation has significantly reduced the amount of time and effort that humans must spend. The use of carrot slicer machines by consumers to cut vegetable carrots has become more and more important as technology has advanced. The necessity of using a slice machine to slice carrot crops arose from the increased demand for these crops for a variety of household purpose. Therefore, the main objective of this study was to assess the vegetable root crop carrot slicer machine's performance to reduce the technology disparity in carrot processing in Oromia regions that produce carrots, make the carrot slicer available for upcoming involvements, and provide the performance indicating data about machine for end users.

2. Materials and Methods

2.1. Study Area

The study of the evaluating the carrot slicer machine's performance was conducted at the Melkassa Agricultural Research Center, which is situated 17 km southeast of Adama town and 117 km east of Addis Ababa in the Adama Woreda, East Shewa Zone, Oromia Regional State, close to the town of Awash Melkassa. At 1560 meters above sea level, it is located between 8° 24' 0" and 8° 30' 12'' N and 39° 21' 0" and 39° 35' 14'' E. The average yearly rainfall is 763 mm, and the average high and low temperatures are 28.4 °C and 14 °C.

2.2. Machine Description

The carrot vegetable crop slicer machine (Figure 1) was driven by a 3.73 kW motor which revolves at a continuous machine speed. The main components of the carrot slicer machine are the feed inlet, cover, disc, cutting blade, blade holder, chute, main frame, engine set, shaft, pulley, V-belt, and motor. The mainframe is the unit of the machine on which all other components of the machine were supported. All of the machine's other parts are supported by the main frame. Mild steel was used to fabricate the other components, while stainless steel was used for the processing portion that came into direct contact. The carrot vegetable crop slicer machine is simple to use, requires only two operators to manage the entire process, and is less complicated to operate. For farmers who grow carrots as a vegetable crop, this carrot vegetable crop slicer machine was a great option because of its easy-to-use mechanism. For small-scale farmers working alone or in groups, the carrot slicer machine was cost-effective, endured for an extended period, sliced carrots uniformly, and was sufficiently quick.

Figure 1. Carrot slicer machine.

2.2.1. Working principles

The carrot slicer machine is operated by an electric motor and power is transmitted to the blades through the shaft via the pulley's V-belt. Before it is operated, all the parts must be properly set and fixed together. The slicing machine works by using a combination of a rotating blade and manual feeding to cut material into slices. As the shaft rotates, it turns the slicing disc in the anti-clockwise direction. The rotation of the slicing disc would perform an impact action on the root and the sharp blade would cut the tubers by impact shear force to the designed thickness. The centrifugal force of the rotating disc forced the fallen root on a fixed cutter to accomplish the slicing process. The clearance between the casing plate and the fixed cutting stainless steel blade was adjusted to get slices of the desired thickness.

The clearance on the blade predetermines the thickness of the slices and this is greatly enhanced by the rotating speed of the blade. The roots are manually fed through the inlet by the operator against the rotating blades and to be sliced to meet the rotating blade. This directs it to the slicing disc then the sliced chips then proceed to the discharge chute by gravity where they are collected into the receptacle below the outlet. During working the slicer, to prevent the carrot slice from being flown away by the effect of centrifugal force on the cutting blade, a circular disc cover was provided which helps to direct the chips through a discharge chute.

2.2.2. Cutting unit and its shape features

The cutting blades (knife type) are sharpened at one side and were positioned at a tension through adjustable screws and bolts to prevent. It was made up of stainless steel material with a dimension of 300x60x6 mm. Stainless steel blades are suited for everyday use and can slice both hard and soft crops. The 18° champers were given to make edges sharpened. Each of the blades has holes of Ø 6 mm at one end to facilitate bolting it to the circular disc of diameter 500 mm. The blade holder rotated continuously at the face of the disc when the carrot came in contact with blade holder it sliced the carrot to a precise thickness. The thickness of the slices is predetermined by the clearance on the blade to the fixed disc and this is greatly enhanced by the rotating speed of the blade. The centrifugal force of the rotating disc forced the fallen root on a fixed cutter to accomplish the slicing process. The operation could be based on a slicing blade as the processing unit. The clearance between the casing circular plate and the fixed cutting stainless steel blade was adjusted to get slices of the desired thickness.

Cutting units or blades of slicer can have a variety of shapes and features, including straight, circular, curved, and custom shapes. A cutting blade's shape features depend on the blade type, desired cutting performance, and the intended task. In this case, a straight blade was selected, considering the sharpening's ease and availability. The slicer cutting unit or blade considers the following shape features: the edge of the blade, can be straight; the blade angle, can vary over the curvature of the blade to achieve different accelerations of the slice and blade material, which should be appropriate for the cut material, hardness, wear resistance, and environment.

2.3. Materials

The carrot crop sample was taken from the Kulumssa Agricultural Research Center experimental field in the east-western regions of Oromia, east Arsi, to compute the physical attributes and conduct comprehensive tests on the carrot slicer machine. A Nantes-variety vegetable carrot crop served as the experiment's resources. Since it is readily available and has a high production capacity in the chosen area as well as other growing regions of Ethiopia, this variety ought to be chosen. The most

popular variety in Ethiopia is Nantes as well.

2.4. Device for Measurement

In the experiment, a digital tachometer, tape measure, digital Vernier caliper, and digital weighing scale were utilized. Several measurements were taken of the carrot crops using these different instruments. The vegetable carrot crop's length and dimensions were measured with a tape measure. The minimum length that this measuring tool can measure is 5 mm. We used a Vernier caliper with a resolution of 0.01 mm to measure the carrots' diameters and thickness. The mass of the carrot could be measured using the digital scale both before and after processing. This 0.01 g accurate digital scale (GF600, USA model) was utilized. A non-contact kind of tachometer was used to measure the speeds of the slicer machine. With a measurement range of 2 r min^{-1} to 99,999 r min^{-1} and a measuring distance of 50 mm to 500 mm, this tachometer has a sensitivity of 0.044 v rad sec^{-1}.

2.5. Methods

2.5.1. Determining physical properties of carrot (*Daucus carota L.*)

Studies on the different important physical characteristics of carrots have provided important information essential for the design and development of processing machinery and equipment for sorting, washing, grading, processing, and cleaning (Nithyalakshmi, 2024). Determining the physical characteristics of agricultural materials is crucial for designing conveying machinery and processes, feed hoppers, metering systems, and storage facilities (Fred, 2014). It also necessitates knowledge of how to turn these materials into products. The characteristics of carrots are helpful when designing equipment for processing, handling, and storing them. These physical characteristics are essential for designing the carrot slicer's hoppers and outlets.

The carrots used in this study were harvested when they reached their maximum ripeness. From 25 to 100 carrot samples were chosen at random to determine each of the physical characteristics of the carrot, including its moisture content, angle of repose, bulk density, porosity, coefficient of friction, geometric mean diameter, arithmetic mean diameter, equivalent mean diameter, sphericity, surface area and aspect ratio. The following characteristics of carrots were studied:

Weight was reached. Then, the moisture content of carrot fruit was calculated by Equation 1.

$$Mc = \frac{Mw - Md}{Mw} \times 100 \quad (1)$$

where Mc is the moisture content of carrot (%), M_w is the initial mass of carrot (g), and M_d is the mass of dried carrot (g).

Angle of repose

One of the key parameters for identifying the machine's convening component is the angle of repose. The carrot sample's radius (r) and one opposing side (L) formed a conical shape, which was used to calculate the angle of repose. The angle formed by the carrot and the horizontal surface when piled from a known height is also known as the angle of repose. The angle of the repose for carrot fruit was calculated by the formula given in Equation 2 (Nithyalakshmi, 2024).

$$\theta = cos^{-1}\frac{r}{l} \quad (2)$$

where Θ is the angle of repose in (°), l is the height in (cm), and r is the radius of conical shape in (cm).

Bulk density

The bulk density was calculated by dividing the volume of a container by the mass of carrot fruit per unit. A measuring cylinder was filled with carrots, and the volume of the carrots was then calculated. The bulk density of the carrot was calculated using the following formula (Nithyalakshmi, 2024) given in Equation 3.

$$\rho_b = \frac{M}{V} \quad (3)$$

where ρ_b is the bulk density of the carrot (kg m^{-3}), M is the mass of the carrot (kg), and V is the volume of the carrot (m^{-3}).

Porosity

Porosity was determined by dividing the fruit density value by the difference between the fruit and bulk densities, expressed as a percentage, as per methodologies described by (Vursavuş et al., 2006) as given in Equation 4:

$$\varepsilon = \frac{\rho_f - \rho_b}{\rho_f} \quad (4)$$

where ε is the porosity, ρ_b is the bulk density, and ρ_f is the fruit density.

Coefficient of friction

The coefficient of static friction is crucial for the carrots to slide or move. Carrot surfaces are kept from moving when they come into contact with a machine convening part by the frictional force ratio. The coefficient of friction for carrot related different surfaces namely stainless, galvanized and mild steel can be calculated using Equation 5 (Nithyalakshmi, 2024).

$$\mu S = \frac{Sin\theta}{Cos\theta} \quad (5)$$

where μs is the coefficient of static friction.

Geometric mean diameter

Utilizing the major length (L), width (W), and thickness (T), the cub root of the carrot fruit was used to express the geometric mean diameter, which was taken into consideration as the size criterion. An average amount for the geometric mean diameter of the carrot can be determined using Equation 6 (Jahanbakhshi et al., 2018).

$$D_g = \sqrt[3]{L \times V \times T} \quad (6)$$

where D_g is the geometric mean diameter (mm), L is the major length (mm), W is the width (mm), and T is the thickness (mm).

Arithmetic mean diameter

The Arithmetic mean diameter of carrot fruit samples was determined using the formula (Jahanbakhshi et al., 2018) in Equation 7.

$$D_a = \frac{(L + T + V)}{3} \quad (7)$$

where D_a is the arithmetic mean diameter (mm), L is the major length (mm), W is the width (mm), and T is the thickness (mm).

Equivalent mean diameter

The equivalent mean diameter of carrot fruit samples was determined using the formula (Jahanbakhshi et al., 2018) given in Equation 8.

$$D_e = \frac{(D_g + D_a + D_s)}{3} \quad (8)$$

where D_e is the equivalent mean diameter, D_g the is geometric mean diameter (mm), D_a is the arithmetic mean diameter (mm), and D_s is the square mean diameter (mm).

Sphericity

The ratio of the carrot's surface area to the surface area of a sphere with the same volume as the carrot fruit is termed its sphericity. The sphericity of carrot fruit samples was determined using the formula by (Jahanbakhshi et al., 2018) given in Equation 9.

$$\Phi = \frac{D_g}{L} \quad (9)$$

where Φ is the sphericity of the carrot (mm), D_g is the geometric mean diameter (mm), and L is the major length (mm).

Surface area

To calculate the surface area, the carrot's four sides were traced on a graph sheet, and the number of squares inside the traced outline was counted. The surface area for the carrot were determined as per the following Equation 10 (Nithyalakshmi, 2024).

$$S = \pi D_g^2 \quad (10)$$

where S is the surface area (mm^2), and D_g is the geometric mean diameter (mm).

Aspect ratio

It was calculated as the ratio of the width of carrot fruit to the major length of carrot fruit then it can be determined by the expression as reported by (Jahanbakhshi et al., 2018) as given in Equations 11.

$$R_a = \frac{W}{L} \times 100 \quad (11)$$

Where R_a is the aspect ratio, L is the major length (mm) and W is the width (mm).

2.6. Experimental Procedures

The experimental sample of carrots was cleaned and washed by hand to eliminate adhering soil, hairs and extraneous matter before slicing it. An electronic balance was used to measure the mass of each type of carrot crop, and each sample was fed into the slicer machine through the feeding inlet in a specific way while the machine operated at different preset machine speeds. The test materials were manually pushed through the inlet into the slicing unit, where they were then sliced to the appropriate thickness by pushing them up against the cutting blade. The carrot slicer's effectiveness could have been evaluated throughout the experiment using the full weight of 320 kg of carrot crop. The machine's ideal speed was ascertained through preliminary testing. A tachometer was used to set the machine speeds to 350, 450, and 550 rpm after the carrot slicer was powered on. These speeds were selected by (El-Haq et al., 2016) to test the machine's performance on carrot fruit.

2.7. Performance indicators

The evaluation was performed on a carrot slicer machine in terms of performance indicators to predetermine the machine's effectiveness at different levels of machine speed and material feed rate. After being collected from the machine outlet, the output materials were divided into two categories: sliced and unsliced. An electronic balance was used to determine each category's mass. A stopwatch was used to record the amount of time needed for each test run. The throughput capacity, machine efficiency, percentage loss, and time required to forecast the machine's performance were all taken into account when evaluating the machine. The test parameters such as throughput capacity, machine efficiency, percentage loss, and time taken for carrot slicer were assessed utilizing the following formulas given in Equations 12-14 by Ezeanya (2020).

$$\text{Throughput capacity (kg h}^{-1}) = \frac{W_f}{t} \quad (12)$$

$$\text{Machine efficiency} = \frac{W_c - W_n}{W_c} \times 100 \quad (13)$$

$$\text{Percentage loss} = \frac{W_i - W_c}{W_i} \times 100 \quad (14)$$

where W_f is the weight of the total sliced carrot in (kg), t is the time required to slice carrot in (h), W_c is the weight of carrot collected in (kg), W_i is the weight of carrot feed in (kg), and W_n is the weight of a non-uniform slice in (kg).

2.8. Statistical analysis

An experiment was carried out with a multi-factor factorial design under a randomized complete block design and the levels of machine speed with feed rate levels were taken as treatment combinations for an experiment. As independent variables or factors, the material feed rate and machine speed were taken into account. Each treatment is replicated three times in this experiment (3x2x3 = 18). Using the Statistix 8 software, the experiments' collected data were statistically examined. The 95% confidence interval was utilized to demonstrate the significant impact of independent variables on dependent variables. The least significant difference was used at the 5% level to compare the treatment means. An analysis of variance (ANOVA) was used to test the effects of the experiment.

3. Results and Discussion

3.1. Physical Properties of Carrot (*Daucus carota L.*)

As Table 1 shows, the Nantes variety of carrot crop samples' mean, deviation, maximum, and minimum value for moisture content, angle of repose, bulk density, porosity, coefficient of friction, geometric mean diameter, arithmetic mean diameter, equivalent mean diameter, sphericity, surface area and aspect ratio were calculated. The findings showed that, on a wet basis, the mean moisture content of the carrot was 84.3% with standard deviations of 2.06 and 81.7% to 86.7% was the range of this moisture content. The vegetable root crop of carrot had a mean angle of repose of 39.4° with a standard deviation of 1.08, and a range of 38° to 40.8°.

The findings showed that the bulk density of the carrot crop ranged from 456.8 to 480.6 kg m^{-3}, with a mean and standard deviation of 469.5 kg m^{-3} and 10.6, respectively. The carrot porosity varied from 65% to 53%. The calculated mean porosity was 59.8% with a standard deviation of 5.12. The average static coefficient of friction for carrot crop on stainless, galvanized, and mild steel surfaces was 0.78, 0.82, and 0.87 with standard deviations of 0.06, 0.03, and 0.05, respectively.

The carrot crop varied in length from 133 to 191 mm, width from 27.1 to 39.4 mm, and thickness from 30.8 to 38.4 mm, according to the results. The mean values for thickness, width, and length were found to be 34.5 mm, 33.06 mm, and 161.8 mm, respectively. The calculated values of the standard deviation were 2.83, 4.53, and 24.3. The findings indicated that the geometric mean diameter of carrot ranged from 42 mm to 53.7 mm. The corresponding mean values and standard deviations were found to be 47.82 mm and 4.64. Additionally, the arithmetic mean diameter ranged from 55 mm to 76 mm as well as the mean values of 62.18 mm and standard deviations of 7.39 were found. Between 80.9 and 87.3 mm was the range of the equivalent mean diameter moreover, the standard deviations and mean values were 2.54 and 83.7 mm, respectively.

The sphericity of the carrot ranged from 0.49 to 0.59, with corresponding means and standard deviations of 0.55 and 0.04. The carrot crop surface area was determined to be between 46 and 69 cm^2 with means and standard deviations of 57.67 cm^2 and 9.66. The range of aspect ratios for carrot crops was 0.21 to 0.34, additionally, the computed mean value and standard deviations were found to be 0.27 and 0.05, respectively. Nithyalakshmi (2024) noted a similar pattern for the property of carrot fruit.

The results showed that slicing worked better when vegetable carrots had a higher moisture content. According to the test, the most important element preserving the carrot crop's capacity to be sliced was its moisture content. The obtained angle of repose indicated that to feed the carrot on the machine simultaneously, a small inclination was needed at the discharge chute. It was very helpful for conveying systems, processing equipment, handling and development of machine to examine the physical properties of the vegetable crop of carrot. A similar pattern was observed by Nithyalakshmi (2024) regarding the carrot fruit's properties.

3.2. Evaluation of the machine

The performance of the machine was evaluated based on the speed of the machine and the material feed rate at which it cleans on a variety of carrots. The tests were repeated thrice, and the outcome was measured in terms of the machine's performance indicators. The carrot slicer was evaluated in terms of throughput capacity, machine efficiency, percentage loss, and time required at a mean moisture content of 84.3% at the wet basis for vegetable root crop carrot Nantes variety at three different machine speed levels and different feed rate levels. Weighing of the entire sliced carrot, the weight uniformly sliced carrot, the weight non-uniformly sliced carrot, and the time requirement were recorded after the machine finished processing the carrot crop.

Table 1. Various physical characteristics of carrot

Properties of carrot	N	Mean value	SD	Mean±SD	Max	Min	CV
Mass (g)	100	122.2	85.9	122.2 ±85.9	221	26	32
Moisture content (%)	60	84.3	2.06	84.3±2.06	86.7	81.7	2.4
Angle of repose (°)	40	39.4	1.08	39.4±1.08	40.8	38	2.8
Bulk density (kg m^{-3})	50	469.5	10.6	469.5±10.6	480.6	456.8	2.3
Porosity (%)	36	59.8	5.12	59.8±5.12	65	53	8.5
Coefficient friction							
Stainless steel	45	0.78	0.06	0.78±0.06	0.84	0.7	7.6
Galvanized steel	45	0.82	0.03	0.82 ±0.03	0.85	0.79	3.5
Mild steel	45	0.87	0.05	0.87±0.05	0.91	0.83	5.3
Geometric mean diameter (mm)	30	47.82	4.64	47.82±4.64	53.7	42	9.7
Arithmetic mean diameter (mm)	30	62.18	7.39	62.18±7.39	76	55	11.8
Equivalent mean diameter (mm)	30	83.7	2.54	83.7±2.54	87.3	80.9	3.0
Sphericity	40	0.55	0.04	0.55±0.04	0.59	0.49	7.6
Surface area (cm^2)	32	57.67	9.66	57.67±9.66	69	46	16.8
Aspect ratio	25	0.27	0.05	0.27±0.05	0.34	0.21	17

Table 2. Analysis of variance for throughput capacity

Source	DF	Sum of squares	Mean squares	F-value	P-value
Replication	2	4.96	2.48		
Machine speed	2	27263.4	13631.7	326.58	0.000
Feed rate	1	448.7	448.7	10.75	0.006
Speed ×Feed	2	576.5	288.25	6.9	0.012
Error	10	417.4	41.74		
Total	17	28710.96			

P<0.05, significant at 5% probability level, P>0.05, non-significant at 5% probability level.

It was observed during the test that the machine cut the carrot crop into slices that were between 3 and 5 mm thick. The test results showed that the carrot slicer machine was found to be very effective for processing the vegetable root crop of carrots.

3.2.1. Effects of machine speed and feed rate on throughput capacity

Table 2 shows the results of the analysis of variance for the effects of machine speed, material feed rate, and their interaction on the throughput capacity of the carrot slicer machine. According to the results, analysis of variance showed that material feed rate, machine speed, and their interactions had significant effects at a 5% probability level because the p-values were less than 0.05 (Table 2). The findings indicated that the output of a machine was influenced by feed rate, machine speed, and their interaction effect.

As shown in Figure 2, the machine's mean throughput capacity ranged from 511.6 kg h^{-1} to 621.4 kg h^{-1} when the machine speed increased from 350 rpm to 550 rpm. As increasing the throughput capacity from 511.6 kg h^{-1} to 621.4 kg h^{-1} while the material feed rate caused the machine output capacity to decrease then machine speed caused it to increase. This finding revealed that the throughput capacity had a direct relationship with the machine speed and an inverse relationship with the feed rate of carrot crop.

The results showed that the maximum throughput capacity of 621.4 kg h^{-1} was recorded at 550 rpm machine speed and 10 kg min^{-1} material feed rate, while the minimum throughput capacity was recorded at 350 rpm machine speed and 15 kg min^{-1} feed rate of material. There were previous studies in the literature relating to slicing machines capacity for carrot crop.

Tanwar et al. (2021) reported similar findings for slicer machine capacity. Ezeanya (2020) reports the throughput capacity of the machine ranged from 20.52 to 44.28 kg h^{-1}, during evalutating the slicer. Agbetoye and Balogun (2009) reported a capacity of machine as 48.9 kg h^{-1} when assessing slicer performance for slicing the carrot.

3.2.2. Effects of machine speed and feed rate on efficiency

Table 3 displays the results of the analysis of variance for the effects of machine speed, feed rate, and their interaction on the efficiency of the machine. The analysis of variance indicated that the effects of machine speed and feed rates were significant at the 5% probability level as given (Table 3) that the p values were less than 0.05 and the interaction effects were non-significant (P>0.05). Regardless of the outcome, feed rate and machine speed, excluding their relative interactions, had an impact on the machine's efficiency.

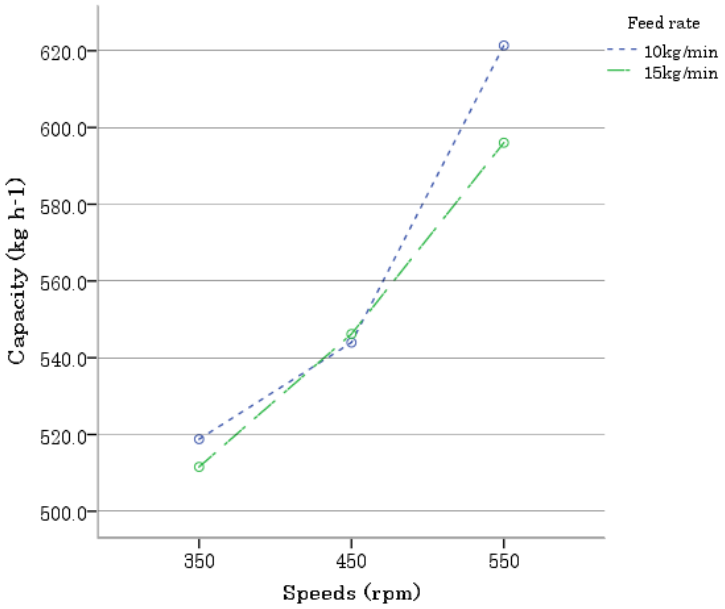

Figure 2. Effects of machine speed and feed rate on throughput capacity.

Table 3. Analysis of variance for machine efficiency

Source	DF	Sum of squares	Mean squares	F-value	P-value
Replication	2	0.0156	0.0078		
Machine speed	2	30.5613	15.2807	21.46	0.0002
Feed rate	1	4.2522	4.2522	5.97	0.0330
Speed ×Feed	2	3.7876	1.894	2.66	0.1224
Error	10	7.1157	0.712		
Total	17	45.7324			

P<0.05, significant at 5% probability level, P>0.05, non-significant at 5% probability level.

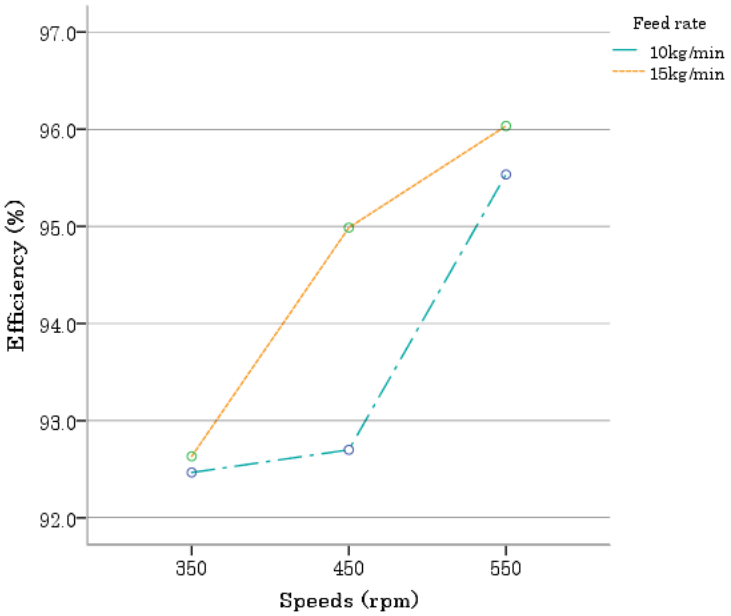

Figure 3. Effect of machine speed and feed rate on machine efficiency.

Table 4. Analysis of variance for percentage loss

Source	DF	Sum of squares	Mean squares	F-value	P-value
Replication	2	0.0212	0.0106		
Machine speed	2	30.5325	15.2663	21.25	0.0003
Feed rate	1	4.3311	4.3311	6.03	0.0311
Speed ×Feed	2	3.8172	1.909	2.66	0.1215
Error	10	7.1836	0.71836		
Total	17	45.8856			

P<0.05, significant at 5% probability level, P>0.05, non-significant at 5% probability level.

As shown in Figure 3, the test's findings showed that a machine's mean efficiency ranged from 92.5% to 96.03%. When machine speed increased from 350 rpm to 550 rpm, the machine efficiency increased from 92.5% to 96.03%. As machine speed and the material feed rate increased, the machine's efficiency was expected to increase as well. This suggests a clear relationship between the efficiency and the independent variable or machine speed and feed rate of carrot.

It has been found that the maximum machine efficiency was 96.03% at 550 rpm machine speed and 15 kg min^{-1} material feed rate, whereas the minimum machine efficiency was 92.5% at 350 rpm and 10 kg min^{-1} material feed rate. There were also previous investigation relating to slicing machines efficiency for carrot crop. Tanwar et al. (2021) reported the same trend for slicer machine efficiency. Ezeanya (2020) states the efficiency of machine ranged from 60.7 to 87.8% when testing the machine. Agbetoye and Balogun (2009) reported a efficiency of machine as 95.4% when assessing slicer performance for slicing the carrot.

3.2.3. Effects of machine speed and feed rate on percentage loss

Table 4 displays the results of analysis of variance for the effects of feed rate, machine speed, and their interaction on the percentage loss of the machine. According to the results, analysis of variance showed that the influence of feed rate and machine speed was significant at the five percent (5%) significance level because the P-values were less than 0.05, but the effect of their interaction was non-significant, meaning the result was greater than 0.05 as given (Table 4). The results demonstrated that feed

rate and machine speed affected a machine's percentage loss when their interactions were uninvolved.

As shown in Figure 4, a machine's mean percentage loss ranged from 4.2% to 7.8% based on the test results. As the machine speed increased from 350 rpm to 550 rpm, the percentage loss decreased from 7.8% to 4.2%. The machine's percentage loss was expected to decrease as machine speed and the material feed rate rose. This suggests that the carrot crop feed rate and machine speed were negatively related with percentage loss.

The investigation results showed that the minimum percentage loss was 4.2% at 550 rpm machine speed and material feed rate of 15 kg min^{-1} whereas the maximum percentage loss was 7.8% at 350 rpm machine speed and feed rate of 10 kg min^{-1}. The test results revealed that the 550 rpm machine speed had a negligible percentage loss when compared to the other machine speeds. There were previous findings relating to percentage loss of machine.

Ezeanya (2020) reports the percentage loss of machine was 10.9% during evalutating the slicer. Agbetoye and Balogun (2009) reported a percentage loss of machine as 8.7% when assessing slicer performance for slicing the carrot crop.

3.2.4. Effects of machine speed and feed rate on time

Table 5 displays the results of the analysis of variance for the effects of feed rate, machine speed, and their interaction on the time taken by the machine. An analysis of variance revealed that the interaction between machine speed and feed rate was non-significant (P > 0.05) whereas the effects of each were significant at the 5% probability level due to values for p smaller than 0.05 (Table 5). According to the outcome, feed rate and machine speed had an impact on the machine's time taken, but their impact interactions had no effect observed.

Figure 5 shows that when material feed at 10 kg min^{-1} with 550 rpm machine speed and at 15 kg min^{-1} with 350 rpm machine speed, the mean time taken of a machine varied between 106 and 180 seconds. The results specifically demonstrated that when machine speed increased and material feed rate decreased, the machine's time taken to process tended to decrease. This is because faster machine speed outperformed slower ones, indicating that the time taken was negatively connected with machine speed but strongly correlated with the rate at which carrot crop was fed.

Based on test results, a minimum time taken of 106 seconds was achieved with machine speed of 550 rpm and a material feed rate of 10 kg min^{-1}, while a maximum time taken of 180 seconds was achieved with machine speed of 350 rpm and a material feed rate of 15 kg min^{-1}. With higher machine speed of 350 rpm to 550 rpm, the time taken was shortened from 180 seconds to 106 seconds.

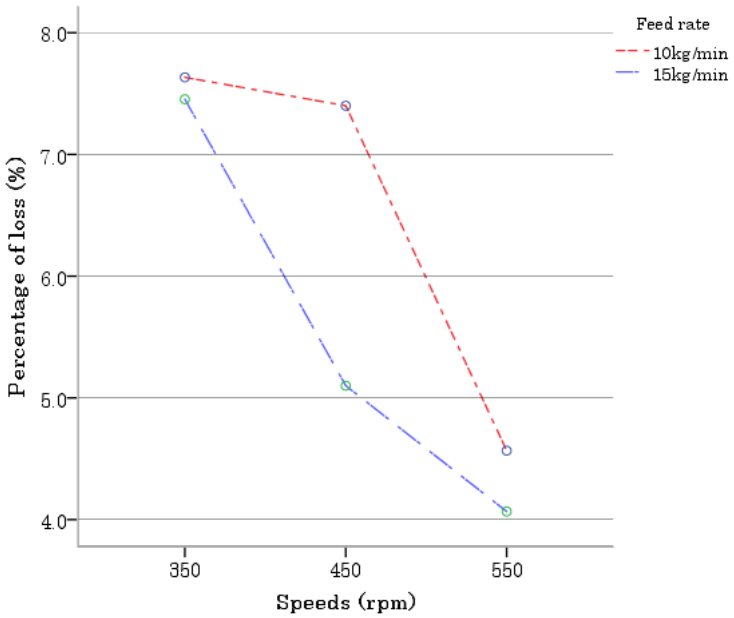

Figure 4. Effect of machine speed and feed rate on percentage loss.

Table 5. Analysis of variance for time taken

Source	DF	Sum of squares	Mean squares	F-value	P-value
Replication	2	0.3	0.15		
Machine speed	2	1571.6	785.8	116.07	0.000
Feed rate	1	11341	11341	1675.2	0.000
Speed ×Feed	2	25.1	12.55	1.854	0.146
Error	10	67.7	6.77		
Total	17	13005.7			

P<0.05, significant at 5% probability level, P>0.05, non-significant at 5% probability level.

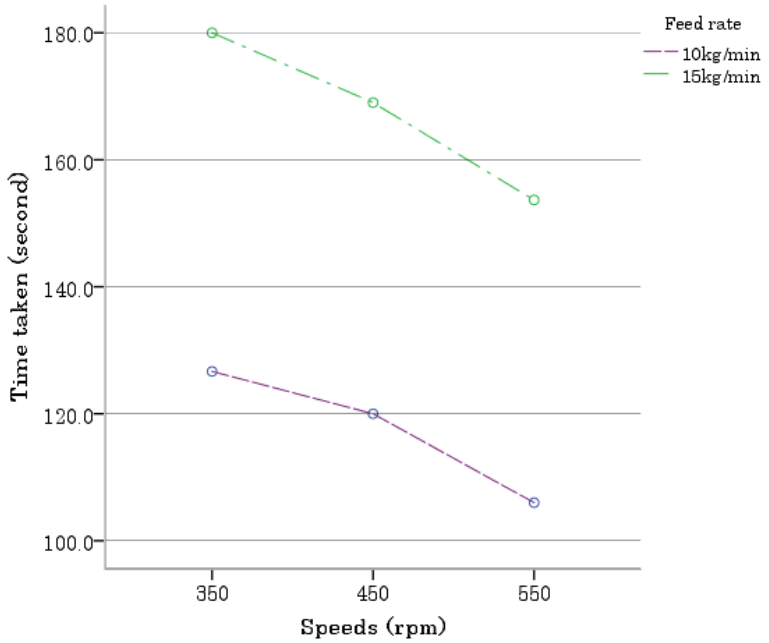

Figure 5. Effect of machine speed and feed rate on time.

Table 6. Comparisons of treatment means

No.	Treatment combination	Speeds (rpm)	Feed (kg min^{-1})	Throughput capacity (kg h^{-1})	Machine efficiency (%)	Percentage loss (%)	Time (sec)
1	V1F1	350	10	518.8^{D}	92.5^{B}	7.8^{A}	126.7^{D}
2	V1F2	350	15	511.6^{D}	92.6^{B}	7.6^{A}	180^{A}
3	V2F1	450	10	543.9^{C}	92.7^{B}	7.5^{A}	120^{E}
4	V2F2	450	15	546.2^{C}	94.98^{A}	5.3^{B}	169^{B}
5	V3F1	550	10	621.4^{A}	95.5^{A}	4.7^{B}	106^{F}
6	V3F2	550	15	596^{B}	96.03^{A}	4.2^{B}	153.7^{C}
7	Grand mean			556.3	94.06	6.5	142.6
8	CV			1.18	0.98	16	1.84

V = speed, F = feed rate, V1 = 350 rpm, V2 = 450 rpm, V3 = 550 rpm, F1 = 10 kg min^{-1}, F2 = 15 kg min^{-1} and CV = coefficient of variation.

3.2.5. Mean separation

Each treatment combination underwent the least significance difference all pairwise comparison tests for throughput capacity, machine efficiency, and percentage loss, as indicated below in Table 6. To determine whether there were significant differences between treatment means, this analysis was then subjected to least significance difference all pairwise comparison tests for dependent variables for levels of machine speed and for feed rates. The results of least significance difference's pairwise comparison tests indicated that, at the 5% level, the treatment combination means of four groups (A, B, etc.) did not differ significantly from one another at 5% probability level.

4. Conclusion

The carrot slicer performance was evaluated at three different machine speeds (350, 450, and 550 rpm) and different feed rate levels at a mean moisture content of 84.3% for the carrot Nantes variety. The performance was carried out in terms of throughput capacity, machine efficiency, percentage loss, and time requirement. It was very helpful for conveying systems, processing equipment, handling and development of machine studying the physical properties of the crop carrot. The key physical properties of the carrot including moisture content, angle of repose, bulk density, porosity, coefficient of friction, geometric mean diameter, arithmetic mean diameter, equivalent mean diameter, sphericity, surface area and aspect ratio were studied. The assessment's findings indicated that when machine speed increased from 350 to 550 rpm, the throughput capacity increased from 511.6 kg h^{-1} to 621.4 kg h^{-1}, the machine efficiency increased from 92.5% to 96.03%, and the percentage loss decreased from 7.8% to 4.2%. Analysis of variance showed that the carrot slicer machine's performance indicators were impacted by the main effects of feed rate and machine speed, but that throughput capacity was the only thing that was affected by their interactions.

Author Contributions

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

	A.E.
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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FARM ASSISTANT COUNTS SHEEP

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Abstract: Small livestock farming in our country is mostly based on pasture. The most important advantage of this situation is that it reduces feed expenses and increases our profitability within the farm. However, the most important problem is in the counting of animals when they come from the pasture to the pen and when they go from the pen to the pasture. This situation depends on the shepherd's attention and follow-up. However, finding experienced shepherds in our country is becoming more and more difficult every day. It may be difficult or even impossible for a sheep giving birth in the pasture to follow the herd when the geographical conditions become difficult. Quick counting of sheep and lambs as the animals enter and exit the pen depends on the shepherd's practice and experience. In order for this situation to be more realistic and to prevent personal mistakes, different alternatives should be considered. For this reason, a system has been developed using deep learning techniques to automatically count the animals in the herd when entering the pen. This system will automatically count the animals at the entrance and exit of the farm, and in case of missing animals, the system users will automatically notify the system users via web and mobile applications. With the implementation of this system, it will be possible to determine the losses that will occur on the farm with an early warning system. In our study, animals will be detected with the deep learning-based YoloV8 pre-trained model on images taken from fixed cameras that will be placed at the entrance and exit of the pen. Counting results obtained from the developed system can be used on different devices by providing multi-platform support. By disseminating this practice, losses of sheep and lambs in the pasture can be prevented.

Keywords: Sheep counting, Object detection, Object counting, YoloV8, Object tracking

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1. Introduction

The practice of small livestock farming represents a significant source of income for farmers residing in rural areas. In countries with extensive pastures, such as Türkiye, small ruminants, including sheep and goats, are predominantly fed using natural pastures. This has the dual benefit of reducing feed costs and allowing animals to grow in a more natural environment. However, pasture-based small ruminant farming also presents certain difficulties in terms of herd management. Accurate counting and monitoring of the herd has become even more complicated, especially in herds with many animals (Joshi et al., 2008). At this point, new opportunities provided by technology offer a promising solution to these problems.

The unrestricted movement of animals in pastures can give rise to significant challenges in regions where the geographical conditions are particularly challenging. A sheep may become separated from the herd as a result of predator attacks or other unforeseen circumstances. The occurrence of animals being separated from the herd is only identified following the completion of the count. It is therefore important to conduct a rapid count. Technological techniques have been employed to render the process of animal counting autonomous. While traditional technologies, such as RFID, are employed in the tracking of animals, the advancement of deep learning-based algorithms has the potential to significantly enhance the efficiency of this process (Esen and Onan, 2022; Canga et al., 2022). The implementation of automated animal counting techniques based on image analysis from fixed cameras has the potential to enhance herd management practices, reducing the likelihood of human error (Kavurur, 2023; Özden et al., 2023).

The present study is concerned with the enumeration of sheep. The counting process is performed in two distinct ways. The first counting process is that of a regional count. This process entails the enumeration of sheep within a specified region of the image. The second counting process is the determination of the number of sheep that traverse a specific line. In order to perform these processes, both object recognition and real-time object recognition techniques were employed. In the course of the tests, the Yolo v8 pre-trained model was employed, demonstrating optimal efficiency in the execution of these processes. This study hypothesizes that the YOLOv8-based system will outperform traditional counting methods in terms of accuracy and efficiency.

2. Materials and Methods

The objective of this study is to enumerate sheep within a specified area and at designated transition points. The system is based on deep learning and object detection algorithms, with training conducted using various versions of the YOLOv8 model. Furthermore, object tracking algorithms have been employed to monitor the movements of sheep and enhance the precision of counting operations. This section will provide a comprehensive overview of the utilized dataset, deep learning models, object tracking methodologies, and evaluation metrics. The experimental studies and the specifics of the techniques utilized to assess the accuracy and performance of the developed system will be presented subsequently.

2.1. Dataset

In this study, a single-class sheep dataset was employed. The YoloV8 pre-trained model is capable of detecting sheep autonomously. It is crucial to capture images of sheep from diverse angles to enhance the efficacy of object detection algorithms (Lin et al., 2014). To this end, the model was augmented with the RoboFlow sheep detection dataset, encompassing images captured from varying angles. The RoboFlow Sheep Detection dataset comprises 200 training, 200 validation, and 175 test images. Figure 1 shows sample images for the dataset.

2.2. Models Used and Object Detection

In order to facilitate the counting process, it is necessary to employ a model that is capable of performing real-time object detection. Accordingly, the YOLO (You Only Look Once) architectural approach is employed in the present study. YOLO is an algorithm that provides high-speed and real-time object detection (Redmon et al., 2016). YOLOv8 has advanced feature extraction layers and an optimised architecture (Bochkovskiy et al., 2020). YOLOv8 models have nano (n), small (s), medium (m), and large (x) architectures and are optimised for different difficulty levels. The decision to select a model was made by comparing these four versions of YOLOv8 (Dandıl et al., 2024). The sheep sample detected with yolov8 is shown in Figure 2.

Figure 1. Sample images of the dataset.

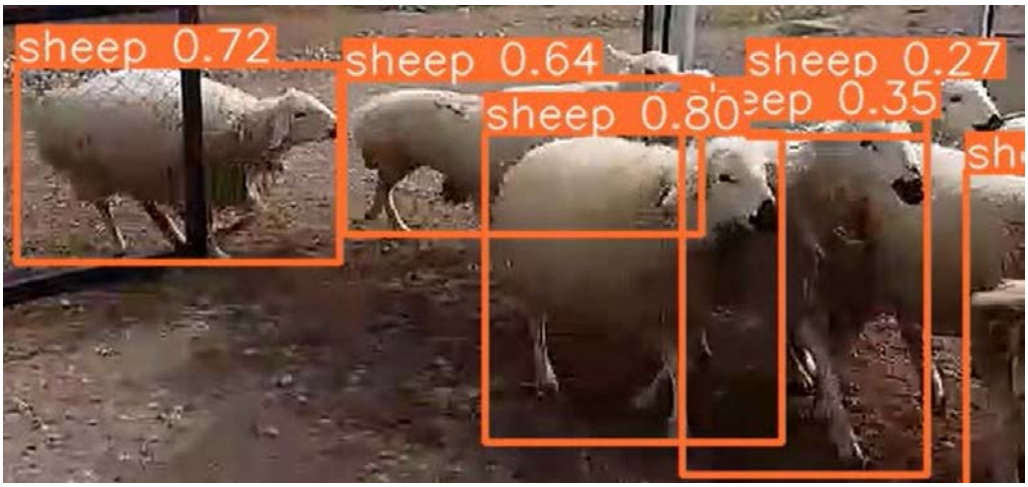

Figure 2. Sheep sample detected with yolov8.

In the training of the models, the AdamW optimiser, a robust optimisation method, was employed. AdamW is a variant of the Adam optimisation algorithm. It prevents overfitting of the model by adding L2 regularisation (Kingma and Ba, 2014). During the training phase, the batch size was set to 8, the epoch number to 50, and the image size to 640. The application of data augmentation techniques, including horizontal flipping and colour alteration, served to enhance the diversity of the dataset and reinforce the model's capacity for generalization (Lin et al., 2014).

2.3. Object Tracking

Object tracking is a crucial aspect in the monitoring of sheep movements, thereby enhancing the precision of counting operations. In the present study, two distinct object tracking algorithms were employed. The ByteTrack and BoTSort algorithms.

The ByteTrack algorithm is a modern approach to object detection and tracking that has demonstrated particular efficacy in multi-object tracking tasks (Zhang et al., 2022). The ByteTrack algorithm enables the tracking of detected objects based on their positions in previous frames.

BoTSort is an algorithm that offers a balance of high accuracy and speed and can be integrated with modern object detection architectures such as YOLOv8 (Wang et al., 2023). A comparison of the performance of the two algorithms revealed no significant difference. The decision was taken to use BoTSort. Tracking the movements of a sheep between frames is shown in Figure 3.

2.4. Sheep Counting Types

The system is capable of performing two distinct counting operations. Regional counting is employed to ascertain the number of sheep within a specified area. This operation is primarily based on the counting of sheep from a single image subsequent to their ingress into the pens.

The process of counting passing sheep is the determination of the number of sheep entering and exiting through a specified boundary line (e.g., a gate) in real time. Counting passing sheep is of paramount importance for the monitoring of animal movements in a farm environment. Therefore, real-time sheep tracking can be employed in conjunction with a multitude of operations other than this counting.

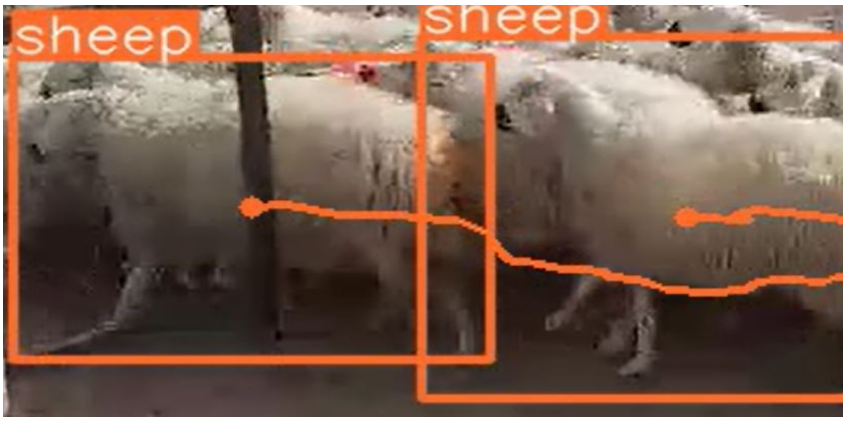

Figure 3. Tracking the movements of a sheep between frames.

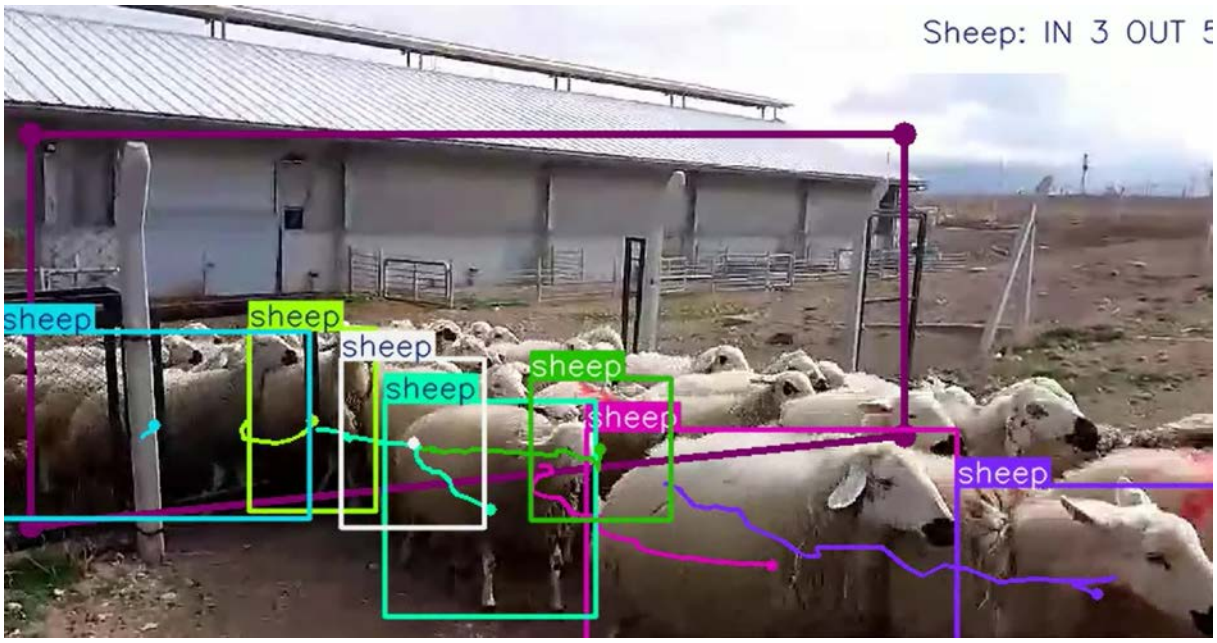

Figure 4. Regional counting process example image.

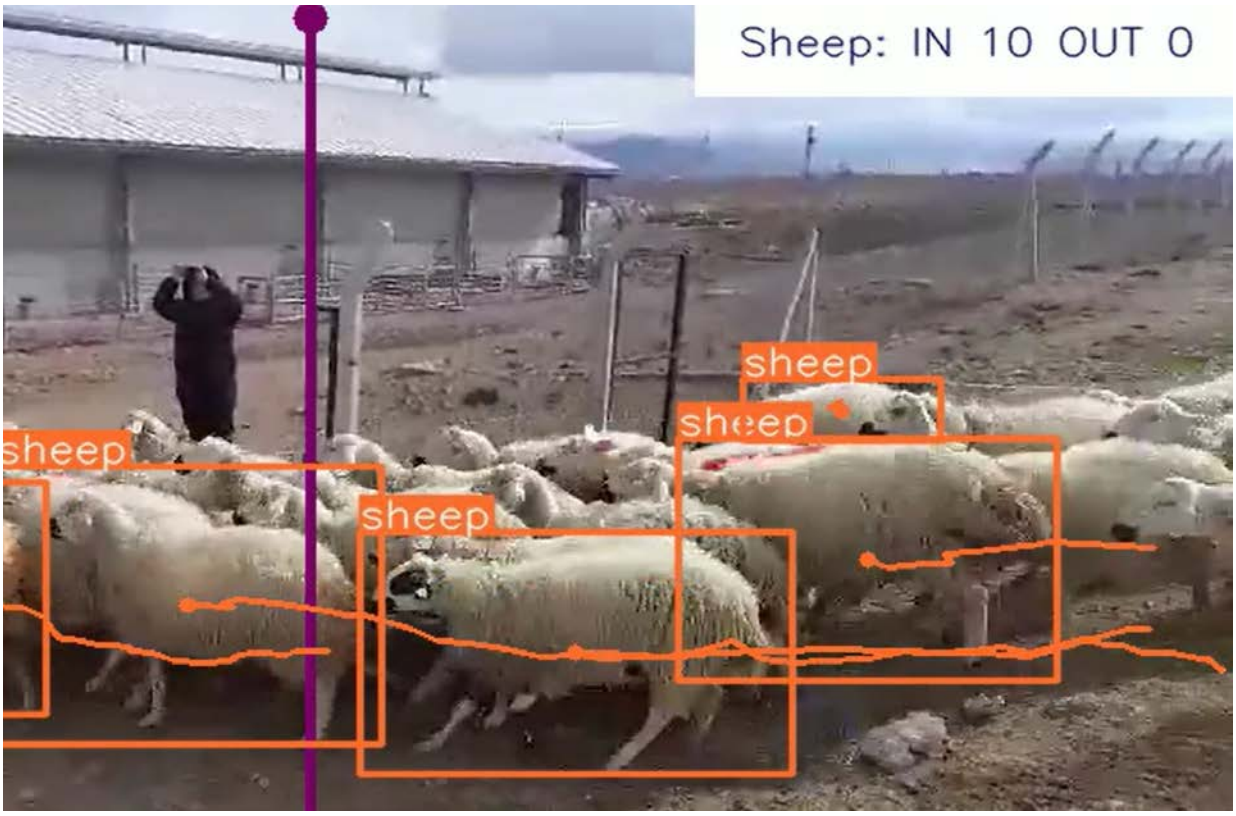

Figure 5. The process of counting passing sheep image.

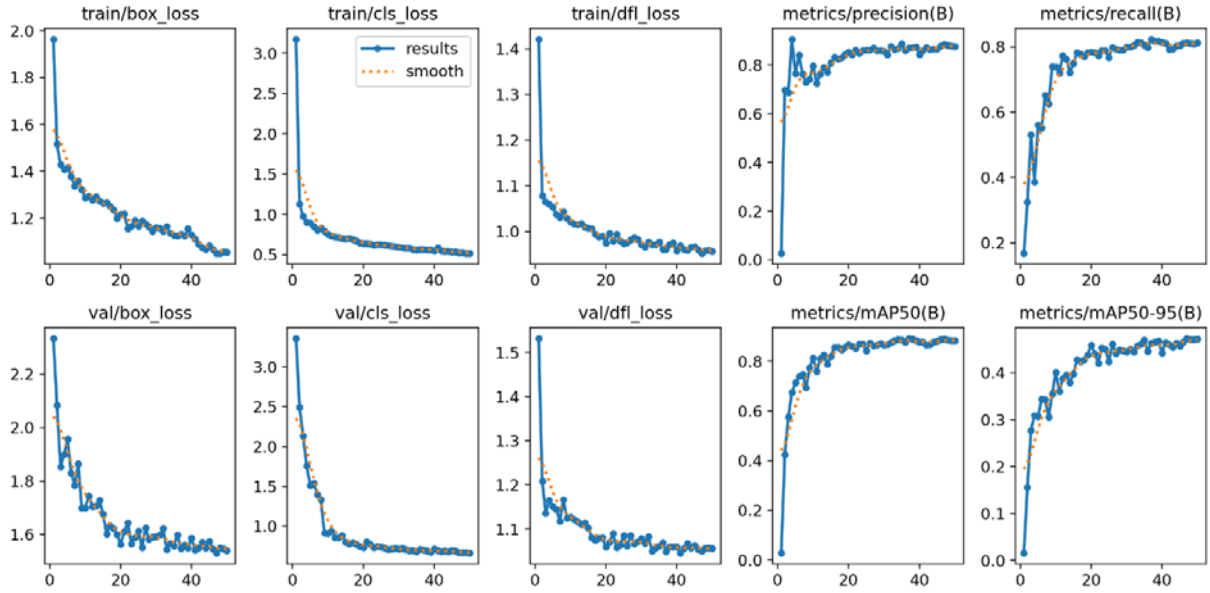

Figure 6. The training results for Yolov8n.

2.5. Evaluation Metrics

In order to evaluate the performance of the models, mean Average Precision (mAP) and training/validation losses were taken into account. In particular, mAP50 and mAP50-95 are the two main metrics used to measure the object detection performance of the models (Padilla et al., 2020). While mAP50 measures whether the detected objects are correctly counted with a 50 percent overlap rate, mAP50-95 takes into account a wider overlap range (from 0.5 to 0.95)(Everingham et al., 2010). In addition, the balance between the accuracy and losses of the model was monitored with the loss values used during training (Yuksel and Tan, 2023).

3. Results

Yolov8 has introduced different versions in response to varying demands, including those related to hardware costs, accuracy, and speed. In our study, four distinct versions of YOLOv8 were evaluated. These were YOLOv8n, YOLOv8s, YOLOv8m, and YOLOv8x. All were

trained using the same dataset, and a comparative analysis was conducted.

In the training results for Yolov8n, the highest values were reached as 0.89174 mAP50 and 0.47129 mAP50-95. The training results for Yolov8n are shown in Figure 6.

In the training results for Yolov8s, the highest values were reached as 0.93040 mAP50 and 0.53200 mAP50-95. The training results for Yolov8s are shown in Figure 7.

In the training results for Yolov8m, the highest values were reached as 0.92553 mAP50 and 0.52822 mAP50-95. The training results for Yolov8m are shown in Figure 8.

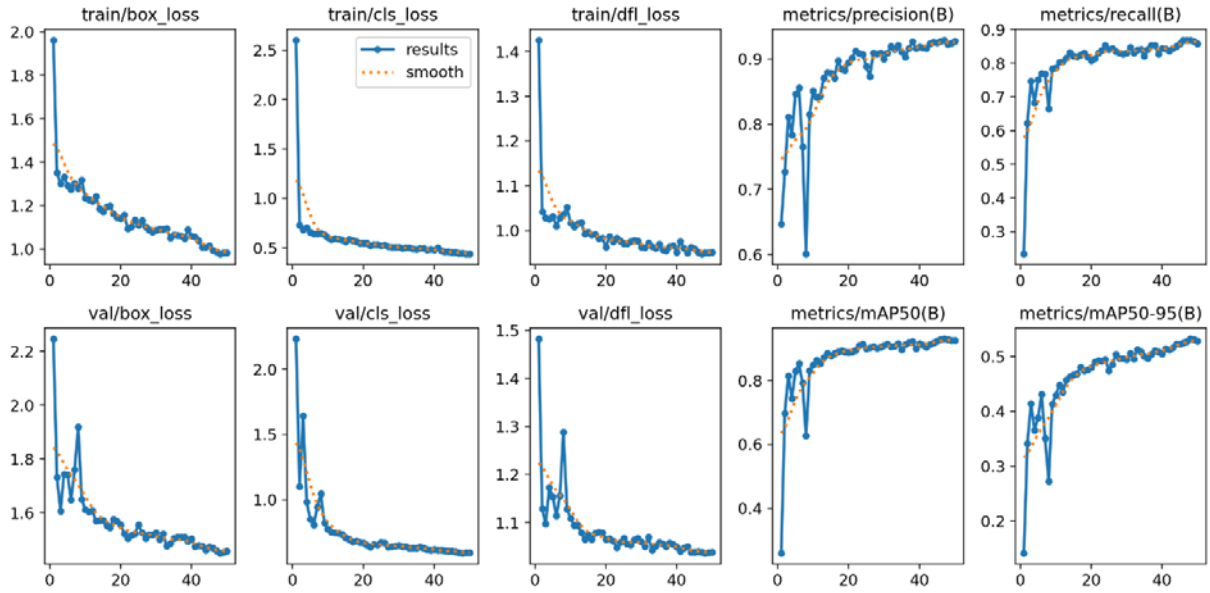

Figure 7. The training results for Yolov8s.

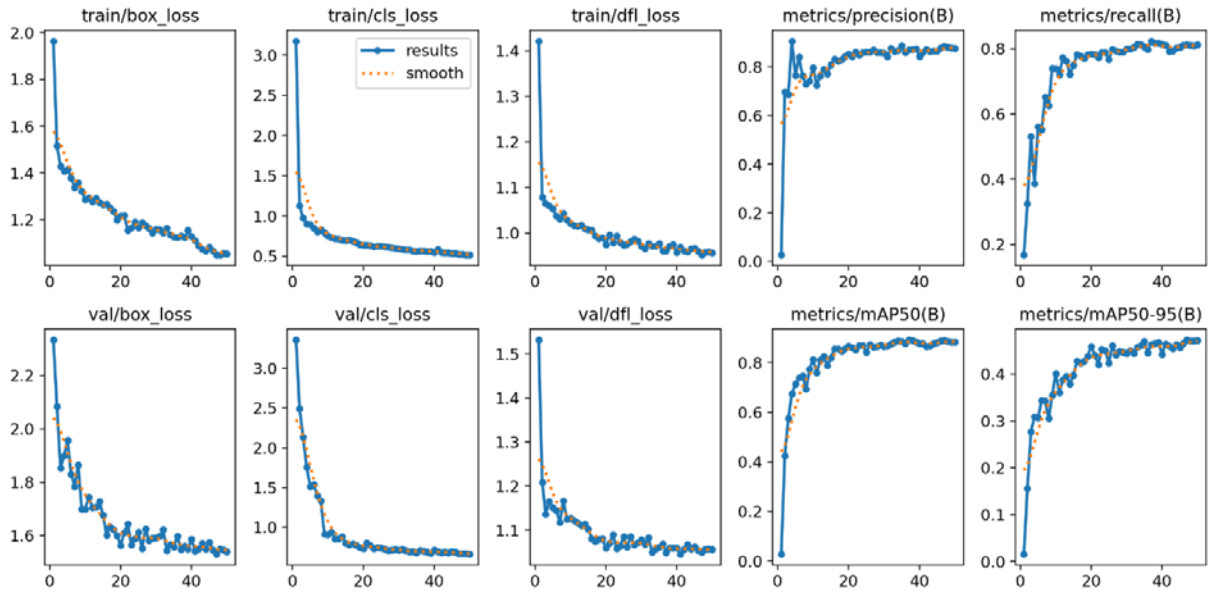

Figure 8. The training results for Yolov8m.

In the training results for Yolov8x, the highest values were reached as 0.92286 mAP50 and 0.53836 mAP50-95. The training results for Yolov8x are shown in Figure 9.

The findings indicate that the YOLOv8 Small model demonstrated the highest accuracy rates in both the mAP50 and mAP50-95 metrics. In particular, the mAP50 value yielded the most successful result, with a value of 0.93040. While the YOLOv8 Extra model attained the highest result of 0.53836 according to the mAP50-95 metric value, the YOLOv8 Small model demonstrated the most balanced and high-performance characteristics in general.

The YOLOv8 Nano, Medium and extra models demonstrated inferior performance in comparison to the mAP50-95 results. In object recognition problems, a

mAP50 value of 0.9 indicates a high level of success (Lin et al., 2017). Additionally, mAP50-95 values of 0.6 indicate a high level of success of a model (Huang et al., 2017). The YOLOv8s model is considered successful in generally accepted scales. For this reason, YOLOv8s was used as the object detection model in this study.

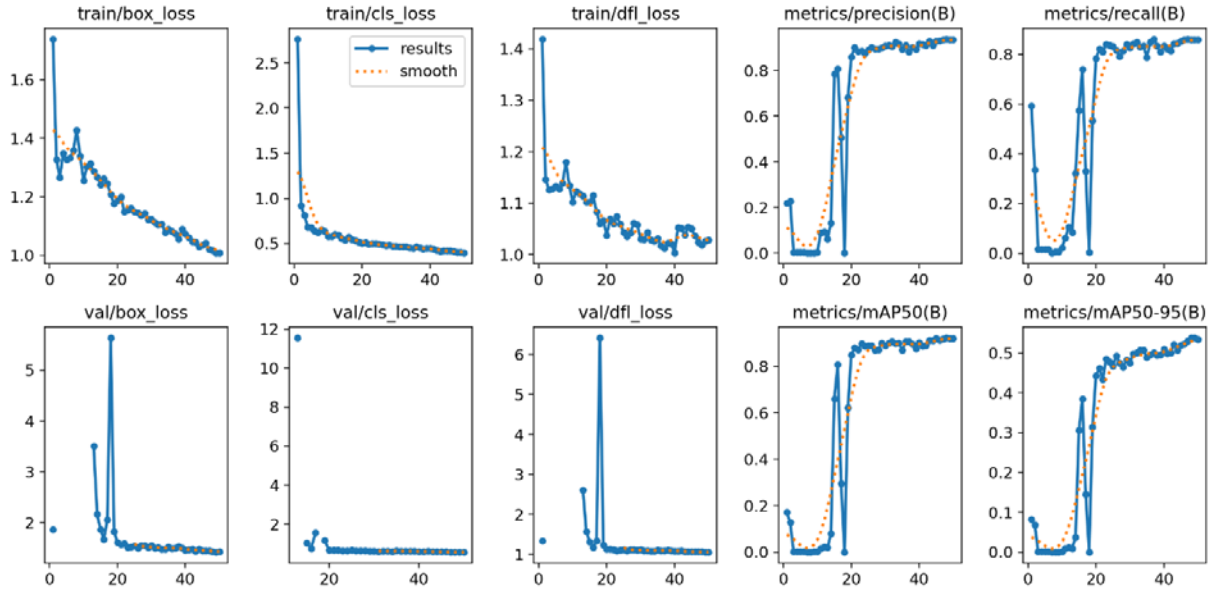

Figure 9. The training results for Yolov8x.

4. Discussion

The objective of this study was to assess the viability of a system based on the YOLOv8 model for the secure monitoring and enumeration of animals in pastures and surrounding enclosures in small livestock farming. In light of the inherent challenges associated with conventional shepherding techniques and the scarcity of available personnel in Türkiye, the integration of automated and digital tracking systems holds immense promise for the advancement of this sector. The four distinct models (n, s, m, x) of the YOLOv8 algorithm utilized in the study were evaluated in terms of their performance, and the most optimal model was selected based on the training process and accuracy rates. Comparative results of the models are shown in Table 1.

Table 1. Comparative results of the models

Model	mAP50	mAP50-95
YOLOv8n	0.89174	0.47129
YOLOv8s	0.93040	0.53200
YOLOv8m	0.92553	0.52822
YOLOv8x	0.92286	0.53836

In order to enhance the efficacy of the model, the AdamW optimisation algorithm and data augmentation techniques were employed. The ByteTrack and BoTSort algorithms were also evaluated for their suitability for object tracking. Following a comparison of their respective performances, the latter was selected as the most appropriate for the task.

The counting and monitoring methods developed in this research make a significant contribution to small livestock enterprises in terms of animal safety and counting accuracy. The capacity to monitor the daily movements of animals diminishes the workload of shepherds and facilitates the secure supervision of animals. Furthermore, the automation of counting processes enhances efficiency in activities such as shepherding and herd management and provides alternative solutions in instances where there is a shortage of qualified personnel in shepherding. In this regard, the functionality of the system offers an operational convenience and security advantage in Türkiye and other regions where livestock sectors are prevalent.

Future work should focus on improving the performance of the system under different weather and lighting conditions. In particular, the effects of adverse weather conditions such as heavy fog, rain, snow and daylight differences on the accuracy of the system can be studied in detail. Methods such as thermal camera data or night vision technologies, which perform better in low light conditions, can be investigated. In addition, extending the data sets to cover such challenging environmental scenarios can help to make the model more robust. Such improvements will allow the system to be used more effectively in real-world applications.

5. Conclusion

The objective of this study was to develop a digital monitoring and counting system that would ensure the safe and accurate counting of sheep in small livestock farming. The system, which was developed based on the YOLOv8 model, demonstrated high accuracy in monitoring small livestock and contributed to automatic counting and security monitoring, which is a crucial requirement in local livestock farming. The integration of

object tracking algorithms employed in the study enabled the effective tracking of sheep movements, thereby facilitating the implementation of a monitoring mechanism that enhanced numerical accuracy.

The development of a more accurate and reliable system, based on an improved dataset and model, represents a significant opportunity for countries such as Türkiye, where there is a shortage of personnel in small livestock farming. Such technological solutions have the potential to both facilitate shepherding and provide an effective alternative for ensuring animal safety.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	M.B	M.A.K.
C	50	50
D	30	70
S	50	50
DCP	100	0
DAI	0	100
L	50	50
W	20	80
CR	80	20
SR	80	20
PM	50	50
FA	50	50

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

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

Acknowledgments

This work was previously presented in summary form at VIII. International Congress on Domestic Animal Breeding Genetics and Husbandry, 2024 as a conference abstract. The current paper significantly expands upon the preliminary findings presented in that abstract, incorporating additional data, advanced analysis methods, and comprehensive discussion to provide a more in-depth exploration of the research topic.

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Research Article
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DETERMINING THE BIOLOGICAL EFFECTS OF SOME PLANT PROTECTION PRODUCTS TO CONTROL BROWN PSEUDOBUTTERFLY, *Pochazia shantungensis* (CHU & LU, 1977) (HEMIPTERA: RICANIIDAE)

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Abstract: *Pochazia shantungensis,* an invasive pest originating from Far East Asia, poses a threat to numerous commercially significant agricultural crops and ornamental plants. This insect species was initially discovered in Istanbul province in 2019 and has since spread extensively across a significant part of the ecosystem in the Marmara Region of Türkiye. The primary strategy for managing this pest relies heavily on chemical control methods. Given the lack of a registered plant protection product (PPP) specifically for *P. shantungensis* in Türkiye, various chemical and biological formulations were evaluated in this study. The chosen preparations were those that have received official approval for use against other harmful species in Türkiye, which exhibit damage patterns similar to those caused by *P. shantungensis*. The study assessed the recommended dosage along with two sub-doses of these registered formulations for their effectiveness against this pest. Efficacy testing was conducted in pots fitted with muslin cloth cages, employing a randomized block design with three replications for each formulation at three different dosages. The findings indicated that the 0.025% concentration of the acetamiprid 20% formulation (Mospilan 20 SP) achieved the highest efficacy, resulting in a 100% effect within five days post-application. Additionally, a 0.05% concentration of deltamethrin 25 g/L (Deltharin 2.5) demonstrated an efficacy of 97.65% after seven days, while a 0.5% concentration of azadirachtin 0.3 g/L (Nimbecidine) reached an efficacy of 69.51% after eleven days. Minimal effects were noted one day after application, with recorded efficacies of 1.14% and 2.27% for the 0.05%, 0.15%, and 0.25% doses of 1.5% *Verticillium lecanii* strain bb-1 1.5% - 1x10 over 8 cfu/ml min (Nibortem SL) and the 0.2% concentration of sulfur 80-WG, respectively.

Keywords: *Pochazia shantungensis*, Ricaniidae, Chemical control, Plant protection product

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1. Introduction

The climate in the Marmara Region is described as a transitional climate, located between the Mediterranean and continental climate zones. A wide variety of fruit and vegetable species, including olives, peaches, cherries, figs, strawberries, chestnuts, quinces, kiwi fruits, grapes, tomatoes, potatoes, garlic, onions, eggplants, zucchinis, and peppers, are cultivated extensively in this region. The region's lands have significant agricultural potential, with income from agricultural products making a substantial contribution to the country's economy. Numerous biotic and abiotic elements adversely influence the quality and yield of agricultural produce. A significant factor to consider is also the presence of harmful organisms and the issues they lead. Certain detrimental organisms possess the capability to alter their environments and migrate to new locations in response to food shortages influenced by global climate change. The recent rise in international trade, along with the enhanced human-induced movement between countries, has made it feasible to transport harmful organisms across boundaries. One of the invasive species being carried is the Brown pseudobutterfly, *Pochazia shantungensis* (Chu & Lu, 1977) (Hemiptera: Ricaniidae), a new pest to the fauna of Türkiye, with its initial discovery in Istanbul Province (Hizal et al., 2019). *P. shantungensis* belongs to the Ricaniidae family. This family's species are mostly common in tropical regions. In the Palearctic region, only species from the genus Pochazia are found (Demir, 2009). The Ricaniidae family comprises a total of 45 genera and approximately 450 species across the world (Chou et al., 1985; Xu et al., 2006; Ginezdilov and Sugonyaev, 2009). Only four species are known to exist in Europe. Among these species, *Ricania hedenborgi* Stal, 1865, has been recorded in Greece and Türkiye (Demir, 2009), while *Ricania japonica* Melichar, 1898, occurs in

Türkiye, Bulgaria, and Ukraine (Demir, 2009; Gjonov, 2011). Additionally, *Ricania limbata* Lallemand, 1935, is found in France (Bourgoin, 2013), and *Ricania speculum* Walker, 185, is present in Italy (Mazza et al., 2014). Currently, three species of Ricania have been identified in Türkiye: *Ricania hedenborgi* Stal, 1865 (Lodos and Kalkandelen, 1981; Tezcan and Zeybekoglu, 2001); *Ricania simulans* Walker, 1851 (Ak et al., 2015; Göktürk and Mıhlı, 2015); and *Ricania aylae* Dlabola, 1983 (Demir and Demirsoy, 2009). Nonetheless, Demir (2009) and Öztemiz (2018) assert that *R. japonica* was incorrectly identified as *R. simulans*, while *R. aylae* is deemed a synonym of *R. hedenborgi*. This destructive insect species, first identified in China and subsequently observed in South Korea, causes significant damage to jujube, blueberry, apple, chestnut, plum, and quince (Choi et al., 2011). Brown pseudobutterflies, similar to their Far Eastern relative *Orosanga japonica*, lead wounds on the shoots during the egg-laying phase. In the nymphal stage, their feeding on plant sap results in the accumulation of honeydew on the plants (Ak et al., 2015; Altaş and Ak, 2019). Additionally, the presence of sugary materials stimulates the proliferation of mold fungus, which can further impair plant respiration and photosynthesis (Choi et al., 2011). This species, which spreads rapidly through the formation of substantial populations, has a diversified host range and is detrimental to 138 plant species in 62 families (Kim et al., 2015). This pest, similar to the Pseudobutterfly (*Orosanga japonica*) species found in the Eastern Black Sea Region, causes damage to agricultural crops and has an undesirable effect on public places such as homes, cafes, restaurants, parks, and gardens due to its high population. Accordingly, this study was conducted to find solutions to the previously mentioned issues. As no licensed plant protection product is available in Türkiye for the chemical management of this newly identified pest, a trial was carried out to determine the effective doses of some registered chemical and organic preparations that have been effective against numerous insects with similar damage characteristics. The study was supported by the General Directorate of Agricultural Research and Policies, affiliated with the Ministry of Agriculture and Forestry.

2. Materials and Methods

2.1. Material

The main materials of the study were the biological stages of *Pochazia shuntungensis*, *Ligustrum vulgare* L. (Lamiales: Oleaceae) and several plant protection products including 1.5% *Verticillium lecanii* strain bb-1 (Nibortem SL), azadirachtin 0.3 g/L (Nimbecidine), sulfur 80% WG (Diaflex 80 WG), acetamiprid 20% SP (Mospilan) and 25% G/L deltamethrin 25% G/L (Deltharin 2.5). Other supplies comprised personal protective clothing, a 2-liter hand sprayer from HP Garden Tools, drift prevention material (a cardboard box), pots and cages with muslin cloth, soil, insect repellent, black mulch (jute), sterile purified water, petri dishes, Eppendorf tubes, soft-tipped brushes, and clear polyethylene bags.

2.2. Method

The biological efficacies of plant protection products against the second and third instar nymphs of *P. shantungensis* were determined by using pot tests in net cages at the institute's field in Yalova Province.

Table 1. Plant protection products licensed against other pests in Türkiye tested in this study and their recommended dosages, in 2021

Active substance name	Trade name	Plant for which it is licensed	Pest for which it is licensed	Recommended Dosage/ Concentration
1.5% *Verticillium lecanii* strain bb-1 1.5% - 1×10^8 kob/ml min	Nibortem SL	Cucumber	*Bemisia tabaci* (Gennadius 1889) *Frankliniella occidentalis* (Pergande 1895)	2500 ml/ha larvae, pupae, adult
Azadirachtin 0.3 g/l	Nimbecidine	Tobacco, grape, peach, cultivated mushroom, olive, pepper	*Bemisia tabaci* (Gennadius 1889) *Frankliniella occidentalis (*Pergande 1895)	500 ml/100 L water
Sulphur %80	Diaflex 80 WG	A variety of fruits and vegetables	Powdery mildew and mites	400 g/l 100 L water
Acetamiprid %20	Mospilan 20 SP	Pepper, Watermelon, Cherry, Tomato, Pistachio, Peach, Tobacco, Cotton, Potato	*Myzus persicae,* (Sulzer 1776) *Bemisia tabaci* (Gennadius 1889) *Aphis pomi*, (DeGeer. 1773) *Empoasca* spp. *Leptinotarsa decemlineata*, Say,1824 *Agonoscena pistaciae* Burckhardt and Lauterer, 1989	25 g/100 L water (adult-nymph)
Deltamethrin 25 G/L	Deltharin 2,5	A variety of fruits and vegetables	*Cacopsylla pyricola*, (Förster, 1848) *Agonoscena pistaciae*, Bur. and Lau.1989 *Bemisia tabaci* (Gennadius 1889)	50 ml/100 L water

Table 2. Plant protection products and their doses tested against *Pochazia shantungensis* on common privets in Yalova province in 2021

The name of plant protection production	D/C	D/C	D/C
1.5% *Verticillium lecanii* strain bb-1 1.5% - 1×10^8 kob/ml min	0.25%	0.015%	0.05%
Azadirachtin 0.3 g/l	0.5%	0.4%	0.3%
Sulphur %80 WG	0.4%	0.3%	0.2%
Acetamiprid %20 SP	0.025%	0.02%	0.015%
Deltamethrin 25 G/L	0.05%	0.035%	0.02%

D= dosage, C= concentration.

The trial was established in a randomized block trial design with three replications using two sub-doses and the registered dose of each plant protection product against the pest for which it has a license in Türkiye. Data relating to the plant protection products utilized in the trial are provided in Table 1, along with details about the doses tested in Table 2 and the trial design in Table 3.

Table 3. Trial plan established against *Pochazia shantungensis* on common privets in Yalova province in 2021

Order	I. Block	II. Block	III. Block
1	A1	A3	A2
2	C1	B3	C2
3	E1	E3	E2
4	D1	C3	K3
5	B1	D3	B2
6	A2	A1	D2
7	C2	C1	A3
8	E2	E1	B3
9	K1	D1	E3
10	B2	B1	C3
11	D2	A2	D3
12	A3	C2	A1
13	B3	E,2	C1
14	E3	K2	E1
15	C3	B2	D1
16	D3	D2	B1

A=1.5% *Verticillium lecanii* strain bb-1 1.5% - 1×10^8 kob/ml min (Nibortem SL), B=Azadirachtin 0.3 g/l (Nimbecidine), C=Sulphur 80% WG (Diaflex 80 WG), D=Acetamiprid 20% SP (Mospilan), E=Deltamethrin 25 G/L (Deltharin).

2.2.1. Cultivation of common privets

The biological effects of PPPs were determined on second- and third-instar nymphs feeding on leaves and shoots of common privets. Therefore, the plants required for the experiment were cultivated in 300 x 300 mm pots. Firstly, the pots were filled with a mixture of sterile soil, peat, and barnyard manure (1:1:1, v/v/v), and then rooted plant cuttings were planted in these pots in pairs. The plants were left to grow naturally, and they were placed in 40 x 40 × 80 cm net cages when they reached a height of 70 cm and had grown enough leaves and shoots for the nymphs to feed on. The floor of the cage was covered with black mulch (jute).

2.2.2. The collection of *Pochazia shantungensis* nymphs

The nymphs for the experiment were gathered using an insect aspirator from heavily identified locations from May to June. The collected nymphs were placed using an insect aspirator on the 50-60 cm tall common privets in the cages. Following the introduction of 30 nymphs into each cage, the plants in the cage were sprayed with the recommended dosages and two sub-doses of the plant protection agents listed in Table 2, which are registered against pests comparable to *P. shantungensis* in Türkiye, using a hand sprayer (2 L). Prior to the experiment's setup, an ant repellent insecticide was applied on the ground and surrounding area. Nymphs in control plots (cages) were only applied sterilized water. The pots used in the study were spaced 70 cm apart in rows, 30 cm apart in rows, and a 1 m wide safety strip was between plots. In addition, a 50 x 50 x 100 cm spray screen (cardboard box) was placed inside the cages in order to prevent the sprayed water from drifting during application to the plot. The application involved 300 milliliters of the plant protection product mixed with water for every individual plant. The counts and evaluations of nymphs, both alive and dead, were executed on the 1st, 3rd, 5th, 7th, 9th, and 11th days after the spraying occurred. Throughout the trial period, temperature and humidity data were provided from the Provincial Directorate of Meteorology.

2.2.3. Statistical analysis

The percentage efficacy of plant protection products was calculated by using the Abbot (1925) formula given in Equation 1 on the data obtained from counting and evaluation.

$$\% \text{ Mortality} = \frac{\text{LC} - \text{LT}}{\text{LC}} \times 100 \quad (1)$$

Where, LC is % of living individuals in the control plot and LT is % of living individuals in the treated plot.

In addition, variance analysis (ANOVA) and multiple comparisons were performed using the JMP 5 statistics program, and differences between means were compared using the Tukey ($P<0.01$) test (Genç and Soysal, 2018).

3. Results

Under natural conditions, nymph emerging occurred on 1 May 2021, from 10 egg clusters, which were detected on the annual shoots of common privets a year ago (15-20 November 2020) and marked with colored raffia, and monitored daily from April the following year. Subsequently, the nymphs were gathered out of various outdoor ornamental plants in May and June, when they were in their second and third stages, and placed in trial cages. The trial was established on 18 June 2021, when nymphs transferred to potted common privets in muslin cages began consuming leaves and shoots, and the results obtained in the trial are shown in Table 4, and the percentage effects of the PPPs included in the trial are given in Table 5. Meteorological data on the dates when the experiment was established and when counts were conducted to assess the experiment are presented in Table 6.

Table 4. Counting results from the plant protection products trial conducted against *Pochazia shantungensis* on the common privets in Yalova provinces in 2021

				1. Day		3. Day		5. Day		7. Day		9. Day		11. Day	
Active ingredient name		D. / C.	Rep.	19.06.2021		21.06.2021		23.06.2021		25.06.2021		27.07.2021		29.06.201	
				D.	A.	D.	A.	D.	A.	D.	A.	D.	A.	D.	A.
1.5% *Verticillium lecanii* strain bb-1 1.5% - 1×10^8 kob/ml min	Nibortem SL	500 ml/ha	I.	1	29	1	29	2	28	4	26	5	25	5	25
			II.	1	29	1	29	3	27	4	26	4	26	4	26
			III.	1	29	1	29	2	28	3	27	5	25	5	25
			Ave.	1	29	1	29	2.3	27.7	3.7	26.3	4.7	25.3	4.7	25.3
		1500 ml/ha	I.	1	29	1	29	3	27	6	24	6	24	8	22
			II.	1	29	2	28	4	26	5	25	6	24	7	23
			III.	0	30	1	29	3	27	4	26	4	26	5	25
			Ave.	0.67	29.3	1.33	28.7	3.33	26.7	5	25	5.3	24.7	6.7	23.3
		2500 ml/ha	I.	1	29	1	29	3	27	8	22	10	20	10	20
			II.	1	29	2	28	5	25	7	23	9	21	9	21
			III.	0	30	1	29	5	25	6	24	8	22	8	22
			Ave.	0.67	29.3	1.3	28.7	4.3	25.7	7	23	9	21	9	21
Azadirachtin 0.3 g/l	Nim.	300 ml/100 L W.	I.	7	23	8	22	8	22	10	20	11	19	12	18
			II.	6	24	8	22	9	21	10	20	10	20	12	18
			III.	6	24	10	20	11	19	13	17	13	17	13	17
			Ave.	6.3	23.7	8.7	21.3	9.3	20.7	11	15.7	11.3	18.7	12.3	17.6
		400 ml/100 L W.	I.	9	21	11	19	12	18	14	16	16	14	17	13
			II.	7	23	9	21	11	19	14	16	16	14	17	13
			III.	7	23	10	20	12	18	13	17	15	15	18	12
			Ave.	7.7	22.3	10	20	11.7	18.3	13.7	16.3	15.7	14.3	17.3	12.7
		500 ml/100 L W.	I.	10	20	14	16	19	11	19	11	19	11	20	10
			II.	7	23	10	20	17	13	20	10	22	8	23	7
			III.	8	22	10	20	15	15	19	11	21	9	22	8
			Ave.	8.3	21.7	11.3	18.7	17	13	19.3	10.7	20.7	9.3	21.7	8.3
Sulphur 80 % WG	Dia. WG 80%	200 g/100 L W.	I.	2	28	2	28	4	26	4	26	4	26	4	26
			II.	1	29	2	28	3	27	3	27	3	27	4	26
			III.	1	29	3	27	5	25	5	25	5	25	5	25
			Ave.	1.3	28.7	2.3	27.7	4	26	4	26	4	26	4.3	25.7
		300 g/100 L W.	I.	2	28	3	27	4	26	4	26	5	25	5	25
			II.	2	28	2	28	3	27	3	27	4	26	5	25
			III.	1	29	3	27	3	27	4	26	5	25	6	24
			Ave.	1.7	28.3	2.7	27.3	3.3	26.7	3.7	26.3	4.7	25.3	5.3	24.7
		400 g/100 L W.	I.	3	27	5	25	5	25	5	25	5	25	6	24
			II.	2	28	4	26	5	25	6	24	6	24	6	24
			III.	3	27	6	24	6	24	7	23	7	23	7	23
			Ave.	2.7	27.3	5	25	15.3	24.7	6	24	6	24	6.3	23.8
Acetamiprid 20%	Mospi. 20 SP	15 ml/100 L W.	I.	9	21	19	11	22	8	24	6	24	6	24	6
			II.	10	20	21	9	23	7	25	5	25	5	25	5
			III.	11	19	22	8	23	7	24	4	24	6	24	6
			Ave.	10	20	20.7	9.3	22.7	7.3	24.3	5	24.3	5.7	24.3	5.7
		20 ml/100 L W.	I.	12	18	21	9	25	5	27	3	27	3	27	3
			II.	14	16	20	10	26	4	28	2	28	2	28	2
			III.	16	14	21	9	26	4	28	2	28	2	28	2
			Ave	14	16	20.7	9.3	25.7	4.3	27.7	2.3	27.7	2.3	28.3	2.3
		25 ml/100 L W.	I.	20	10	26	4	30	0	30	0	30	0	30	0
			II.	24	6	28	2	30	0	30	0	30	0	30	0
			III.	22	8	29	1	30	0	30	0	30	0	30	0
			Ave.	22	8	27.7	1.7	30	0	30	0	30	0	30	0

Table 4. Counting results from the plant protection products trial conducted against *Pochazia shantungensis* on the common privets in Yalova provinces in 2021 (continue)

Active ingredient name		D. / C.	Rep.	1. Day		3. Day		5. Day		7. Day		9. Day		11. Day	
				19.06.2021		21.06.2021		23.06.2021		25.06.2021		27.07.2021		29.06.201	
				D.	A.	D.	A.	D.	A.	D.	A.	D.	A.	D.	A.
Deltamethrin 25 G/L	Delth. 2.5	20 ml/100 L W.	I.	4	26	9	21	18	12	19	11	21	9	22	8
			II.	5	25	11	19	20	10	20	10	20	10	20	10
			III.	5	25	10	20	19	11	20	10	19	11	19	11
			Ave.	4.7	25.3	10	20	19	11	19.7	10.3	20	10	20.3	9.76
		35 ml/100 L W.	I.	9	21	18	12	25	5	25	5	25	5	26	4
			II.	11	19	20	10	23	7	24	6	24	6	24	6
			III.	10	20	20	10	21	8	23	7	24	6	24	6
			Ave.	10	20	19.3	10.7	23	6.7	24	6	24.3	5.7	24.6	5.3
		50 ml/100 L W.	I.	14	16	22	8	27	3	28	2	28	2	28	2
			II.	16	14	24	6	29	1	30	0	30	0	30	0
			III.	17	13	24	6	29	1	30	0	30	0	30	0
			Ave.	15.7	14.3	23.3	6.7	28.3	1.7	29.3	0.7	29.3	0.7	29.3	0.7
Control			I.	0	30	0	30	1	29	1	29	2	28	3	27
			II.	1	29	1	29	1	29	2	28	3	27	3	27
			III.	1	29	1	29	1	29	2	28	2	28	2	28
			Ave.	0.66	29.3	0.66	29.3	1	29	1.66	28.3	2.33	27.6	2.66	27.3

D. / C.= dosage /concentration, D= dead, A= alive, Ave.=average, Delth.= deltamethrin, Dia.= Diaflex, Mospi.= Mospilan, Nim.= Nimbecidine, W.= wate.

Table 5. Effects of plant protection products tested against *Pochazia shantungensis* on common privets in Yalova province in 2021

Tested plant protection product		Efficiacy (%)					
		1. Day	3. Day	5. Day	7. Day	9. Day	11. Day
1.5% *Verticillium lecanii* strain bb-1 1.5% - 1×10^8 kob/ml min	Nibortem SL 500 ml	1.14 G	1.14 F	4.60 HI	7.06 GH	8.43 FG	7.32 HI
	Nibortem SL 1500 ml	1.14 G	2.27 F	8.05 GHI	11.76 FG	10.84 F	14.63 GH
	Nibortem SL 2500 ml	1.14 G	2.27 F	11.49 GH	18.82 F	24.10 E	23.17 G
Azadirachtin 0.3 g/l	Nimbecidine 300 ml	19.32 DE	27.27 D	28.74 F	32.94 E	32.53 E	35.37 F
	Nimbecidine 400 ml	23.86 CDE	31.82 D	36.78 F	42.35 D	48.19 D	53.66 E
	Nimbecidine 500 ml	26.14 CD	36.36 D	55.17 E	62.35 C	66.27 C	69.51 CD
Sulphur 80% WG	Diaflex WG 80 200 g	2.27 G	5.68 EF	10.34 GH	8.24 GH	6.02 FG	5.80 HI
	Diaflex WG 80 300 g	3.41 FG	6.82 EF	8.05 GHI	7.06 GH	8.43 FG	9.76 HI
	Diaflex WG 80 400 g	6.82 FG	14.77 E	14.94 G	15.29 FG	13.25 F	13.41 H
Acetamiprid 20%	Mospilan 20 SP 15 ml	31.82 C	68.18 BC	74.71 D	79.52 B	79.52 B	79.27 BC
	Mospilan 20 SP 20 ml	45.45 B	68.18 BC	85.06 BC	91.76 A	91.57 A	91.46 A
	Mospilan 20 SP 25 ml	72.73 A	92.05 A	100.00 A	100.00 A	100.00 A	100 A
Deltamethrin 25 G/L	Deltharin 2.5 20 ml	13.64 EF	31.82 D	62.07 E	63.53 C	63.86 C	64.63 D
	Deltharin 2.5 35 ml	31.82 C	63.64 C	77.01 CD	78.82 B	79.52 B	80.49 B
	Deltharin 2.5 50 ml	51.14 B	77.27 B	94.25 AB	97.65 A	97.65 A	97.65 A
Control	Control 1	0.00 G	0.00 F	0.00 I	0.00 H	0.00 G	0.00 I
	Control 2	0.00 G	0.00 F	0.00 I	0.00 H	0.00 G	0.00 I
	Control 3	0.00 G	0.00 F	0.00 I	0.00 H	0.00 G	0.00 I
P<0.01; CV =%32							

* Means containing the same letter are not statistically different according to the Tukey test (P<0.01).

Table 6. Meteorological data relating to the experiment conducted against *Pochazia shantungensis* on the common privets in Yalova province in 2021

Counts	Date	Average temperature (˚C)	Maximum temperature (˚C)	Minimum temperature (˚C)	Average humidity (%)
Date of trial setup	18.06.2021	21.0	23.9	16.5	82.4
1. Day	19.06.2021	20.4	22.8	19.1	85.5
3. Day	21.06.2021	21.7	25.1	19.9	82.1
5. Day	23.06.2021	22.9	26.7	18.4	82.1
7. Day	25.06.2021	24.6	28.3	19.8	82.0
9. Day	27.06.2021	25.0	29.2	20.3	79.4
11.Day	29.06.2021	24.8	29.4	19.7	77.1

The results presented in Table 4 indicate that various statistical groups formed regarding the effects observed among the trial characters in the days following application. In the trial, the most effective experimental character was acetamiprid 20% (Mospilan 20 SP), which was applied at a concentration of 25 ml per 100 L and achieved a complete effect within five days of application. The second strongest effect was recorded at 97.65% within 7 days following the application of deltamethrin 25 g/l (Deltharin 2.5) at a concentration of 50 ml per 100 L. Subsequently, 11 days after the application of azadirachtin 0.3 g/L (Nimbecidine) at a concentration of 500 ml per 100 L, the third highest effect was achieved, resulting in an efficacy of 69.51%. In contrast, the least effects were recorded in the evaluations performed one day after the treatment with 1.5% *Verticillium lecanii* strain bb-1,110 CFU/ml (Nibortem SL), and sulfur 80% WG (Diaflex WG 80). Namely, the 1.5% *Verticillium lecanii* strain bb-1,110 CFU/ml (Nibortem SL) demonstrated an efficacy of 1.14% at application rates of 500, 1500, and 2500 ml/ha, whereas sulfur 80% WG (Diaflex WG 80) exhibited an effect of 2.7% at a concentration of 200g per 100 L (Table 5). Throughout the experiment, the average temperature fluctuated between 20.4 °C and 24.8 °C. The minimum temperatures were recorded between 16.5 °C and 20.3 °C, while the maximum temperatures ranged from 22.8 °C to 29.4 °C. Additionally, the average relative humidity varied from 77.1% to 85.5% (Table 6).

4. Discussion

The trial results indicate that the use of acetamiprid 20% (Mospilan 20 SP) at a concentration of 25 ml per 100 liters, deltamethrin 25 G/L (Deltharin 2.5) at 50 ml per 100 liters, and azadirachtin 0.3 g/L (Nimbecidine) at 500 ml per 100 liters of water can be recommended for the management of this pest. Among the three preparations that exhibited effectiveness, two were chemical formulations: Acetamiprid at 20% and deltamethrin at 25 g/L. Furthermore, azadirachtin, an organic plant protection product at a concentration of 0.3 g/L, was also effective. As this research represents the first investigation in Türkiye focused on the chemical management of this pest, a comparison was performed with studies on *Ricania simulans*/*Orosanga japonica* (Walker, 1851), a species from the same family that inflicts comparable harm on its hosts. In Türkiye's Eastern Black Sea Region, azadirachtin (Neem-azal) demonstrated a 30% effectiveness against *R. simulans/O. japonica* when utilized at a concentration of 400 ml per 100 L, according to the findings of (Ak et al. 2013). Furthermore, another study indicated that Neem-azal achieved more than 40% efficacy against nymphs of the same species in two consecutive years (Öztemir, 2014). On the other hand, in this study, Nimbecidine, a commercially available formulation of azadirachtin at a concentration of 0.3 g/l, demonstrated a higher efficacy of 69.5%. In another study, utilizing Nimbecidine at the same concentration on *R. simulans/O. japonica* in the Eastern Black Sea Region demonstrated an efficacy rate of 75% (Göktürk, 2020). Likewise, another investigation revealed that neem extract -1 and neem extract -2, both at the same concentration, exhibited effectiveness rates of 70% and 76.9% against *P. shantungensis*, respectively (Choi. et al., 2012). Deltamethrin EC was found to be 100% effective against nymphs on the 3rd day at a concentration of 0.1% of the recommended dosage (Choi et al., 2012), an another research conducted using permethrin (Restron), a synthetic pyrethroid belonging to the same category as deltamethrin, revealed an effectiveness rate of 100% (Öztemir, 2014). Due to the preference of sunflowers as plants to lay *P. shuntengensis*'s eggs, the application of a 0.1% concentration of acetamiprid SL 20% resulted in a 90.3% effectiveness (Choi et al., 2017). In the present study, using this formulation as a spray against 3rd and 4th stage nymphs resulted in 100% efficacy. On the other hand**,** the findings of the study reveal that the biological preparations (Nibortem SL) had the minimal biological effect as detailed in Table 5. Conversely, *Beauveria bassiana* isolates extracted from *Orosanga japonica* adults in the Black Sea Region, where it was prevalent, were assessed as effective and have been noted to significantly support the biological control of this species (Erper et al., 2022).

5. Conclusion

The study's findings led to the conclusion that the following concentrations can be suggested for the control of the pest: Acetamiprid 20% (Mospilan 20 SP) preparation at 25 ml/100 L, deltamethrin 25 G/L (Deltharin 2.5) at 50 ml/100 L, and azadirachtin 0.3 g/l (Nimbecidine) at 500 ml/100 L. Since *Pochazia shantungensis* (Brown pseudobutterfly), an invasive species originating from Far East Asia, was first discovered in Türkiye in 2019, the application of chemical and organic preparations plays an important role in its short-term solution. Therefore, the determination of chemical and organic insecticides and their effective doses that can be recommended in the control of the pest formed the basis of this study. In addition, the removal and pruning of annual shoots that contain egg clusters on the primary host plants of the pest in the spring and autumn, prior to the hatching of the eggs, could be an effective pest control method and should not be overlooked as a topic for further investigation. This is due to the fact that the pest overwinters within the annual shoots of the host plants during the egg stage.

Author Contributions

The percentages of the authors' contributions are presented below. All author reviewed and approved the final version of the manuscript.

	G.Ç.	Ki.A.	Ka.A.
C	50	30	20
D	60	30	10
S		80	20
DCP	70	10	20
DAI	60	20	20
L	60	20	20
W	60	20	20
CR	60	20	20
SR	60	20	20
PM	60	20	20
FA	60	20	20

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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PHYSICOCHEMICAL, FUNCTIONAL, SENSORY, AND RHEOLOGICAL PROPERTIES OF TRADITIONAL TARHANAS FROM THE CENTRAL ANATOLIAN REGION

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Abstract: Tarhana, produced in Anatolia using a variety of production techniques, is a traditional fermented product made from a mixture of yogurt, wheat flour, vegetables, and spices. Tarhana, with its high nutritional value, makes a positive contribution to human nutrition in terms of health. In this study, the chemical (moisture, ash, protein, fat, titratable acidity, salt, water activity, total sugar), functional (total phenolic content, total antioxidant capacity, water and fat holding capacity), viscosity and sensory properties of tarhana produced in Aksaray, Ankara, Eskişehir, Kayseri, Konya, Nevşehir and Sivas provinces of Central Anatolia region were determined. The moisture contents of the tarhana samples were found to be 17.36-7.52%, protein contents 12.62-8.88%, ash contents 7.4-3.66%, fat contents 4.23-0.81%, titratable acidity values 21.5-7.5%, pH values 5.4-4.04. The highest viscosity value was found in Sivas tarhana with 1.721 Pa.s Kayseri and Konya tarhanas had the highest total phenolic content and antioxidant capacity. In terms of sensory properties, the most admired tarhana was the tarhana from Aksaray province, and the least admired tarhana was the tarhana from Ankara province. In conclusion, when we compared the tarhana commonly consumed as soup in the Central Anatolian region, it was found that the physical and chemical properties, as well as the sensory preferences, varied regionally.

Keywords: Central Anatolia, Functional, Traditional, Tarhana

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1. Introduction

Definition of tarhana according to TS 2282: It is a food with a high nutritional value which is obtained by mixing wheat flour or cracked wheat or semolina or their mixture with yogurt, pepper, salt, dried onion, onion, tomato, herbal substances which are neutral in taste and smell, kneading and fermenting, then drying, grinding and sieving. Tarhana, which has been produced and consumed since ancient times, came to Anatolia thanks to the Turks who migrated from Central Asia. From Anatolia, tarhana spread to eastern countries such as Iran and Iraq, which were neighbors of the Ottoman Empire, and to western countries such as Hungary and Greece via Rumelia (Temiz, 2011). The names of tarhanas by country are shown in Table 1.

Tarhana is a widely consumed product group in our country due to its easy production, low cost, long shelf life, and high nutritional value. Tarhana is a food with both vegetable and animal content due to the presence of flour and yogurt in its composition (Erbaş et al., 2004). Tarhana, which is extremely rich in nutritional value, contains many minerals such as protein, calcium, iron, sodium, potassium, magnesium, zinc, and copper. In addition, it is extremely rich in group A and B vitamins and is easily digestible (Yıldırım and Güzeler, 2016). Although there is no standard method for producing tarhana, it is generally made by mixing, drying, and grinding its primary components. In tarhana made in our country, various types have been developed by incorporating a range of additional ingredients into the dough. Adding some spices (mint, thyme, dill, etc.) creates aromatic flavors in tarhana dough (Özçam, 2012). Although the amounts and types of ingredients added to tarhana dough vary, they are produced in the same stages in home and industrial production (Özdemir et al., 2007; Keşkekoğlu, 2009).

According to TS 2282, tarhana is classified into four main groups based on the raw materials used: Göce tarhana, flour tarhana, semolina tarhana, and mixed tarhana. Additionally, approximately 50 different types of tarhana are known to exist in our country (Aksu et al., 2012). Tarhana types differ according to the basic production technique or the variety of ingredients added. The different products added affect the fermentation time, and tarhana with different flavors, tastes, and smells are obtained in each region. Table 2 shows the fermentation times of tarhana produced in the different areas.

Beyşehir Tarhana: This type of tarhana is made by

preparing buttermilk from strained yogurt, to which butter, milk, water, and wheat are gradually added (Coşkun, 2014). In tarhana production, buttermilk is made from strained yogurt, transferred to copper kettles, and heated. Wheat (göce) is added to the heated buttermilk with continuous stirring and cooking. The wheat used should be thinner than bulgur (Anonymous, 2020a). Butter is then added to the mixture and left to cool. Depending on the consistency of the dough obtained, buttermilk, water, or milk is added. When the dough reaches the desired consistency, it is shaped and dried on reeds (Kahraman, 2009).

Table 1. Tarhana names according to countries (İbanoğlu et al., 1999)

Countries	Nomenclature
Syria, Egypt, Lebanon	Kishk
Iraq	Kushuk
Hungary	Tahonya
Finland	Talkuna
Greece	Trahana
Scotland	Atole
Albania	Trahana and trahan
Macedonia	Tarana
Bulgaria	Trahan and tarhana

Table 2. Fermentation times of tarhanas produced in the central Anatolian region

Region	Fermentation Time (days)	References
Sivas	2-5	Gürdaş, 2002
Ankara/Beypazarı	6-7	Anonymous, 2023
Kırşehir	3-5	Anonymous, 2020a
Aksaray	2	Anonymous, 2019
Nevşehir	1-7	Yıldırım and Güzeler, 2016
Eskişehir	1	Anonymous, 2020b
Kayseri	7	Anonymous, 2022

Sivas Tarhana: It is known that various fruits from the province of Sivas, such as apples, pears, and quince, are added to the local tarhana (Gürdaş, 2002). Sivas tarhana is mostly made with yogurt. Yogurt or buttermilk is added to the pre-cooked yogurt and mixed until it thickens. It is dried on fences in the form of flat round balls or balls with a hole in the middle (gilik). This tarhana, called tarhana with additives, is soaked and left to soften before it is prepared as soup (Üçer, 2006).

Kayseri Tarhana: After washing the durum wheat to be used in the production of tarhana, water and salt are added and boiled. Chickpeas are added to the boiling paste, and the mixture is kneaded. Once the salt ratio is balanced, the yogurt flour mixture prepared beforehand is added to the kettle. The mixture is cooked until it reaches a thick consistency. The dough is left to rest and left to dry (Anonymous, 2022).

Ankara Tarhana: This type of tarhana, which originating from the province of Ankara, is made in many districts using different techniques. In Çamlıdere tarhana, the wheat is first sorted and washed and then ground into semolina. The yeast used in Çamlıdere tarhana is sourdough. Yeast, salted yogurt, and wheat are mixed and kept for 3 days, then wrapped in cloths and dried (Anonymous, 2023).

Eskişehir Tarhana: The yogurt used to make tarhana is salted and filtered in bags. Onions are fried in vegetable oil in a cauldron, and milk is added after adding a little water. Göce (ground wheat) is added to the boiling cauldron with constant stirring, and the mixture is left to simmer. The strained yogurt is mixed with the egg. The cooked wheat and yogurt are mixed and kneaded like dough. The dough is left to rest for a day, then cut into small pieces and dried (Anonymous, 2020b).

Nevsehir Tarhana: Onion, tomato, capia pepper, red pepper, coriander, parsley, and garlic are added as vegetables in this tarhana from Nevsehir province. After all the vegetables are cooked, pre-cooked chickpeas are added. After the mixture has cooled, it is mashed, yogurt and flour are added and left to ferment. The fermented tarhana dough is placed on a clean cloth. As the tarhana dries, it is rubbed by hand, and powdered tarhana is obtained by drying it (Anonymous, 2020c).

Aksaray Tarhana: Tarhana, which is specific to the province of Aksaray, is made by kneading yogurt, flour, mint, red and green pepper, and finely chopped onion into a smooth dough. The dough is covered with a cloth and left to ferment. The longer the fermentation time, the sourer the tarhana will taste, while the shorter the fermentation time, the sweeter the tarhana will taste. When the dough has reached the desired taste, it is placed on cloths and dried (Anonymous, 2019).

There are studies in the literature that have investigated the properties of tarhana from different regions. In the study conducted by Güler (1993), the nutritional composition of 10 different tarhana samples from Adana, Hatay, and Maraş provinces was analysed. Moisture 13.1-18.8%, total acidity 9.0-17.5%, ash 2.0-8.0%, starch 41.6-56.4%, protein 17.2-21.9%, sugar 4.7%, salt 0.7-0.9% and fat 1.3-9.6% were determined. In the study in which the chemical composition of 21 different tarhana samples collected from the provinces of Afyonkarahisar, Burdur, Bolu, Eskişehir, Kütahya, and Tekirdağ was analysed, it was reported that the moisture, ash, salt, protein, fat, and acidity contents of the samples were between 9. 35-66.4%, 1.36-9.40, 0.62-9.01, 6.77-28.55, 0.43-15.78 and 1.7-4.7%, respectively (Tamer et al., 2007). In the study conducted by Soyyiğit (2004), 27 different home-made flour tarhana in Isparta region were analysed and pH 3.61-4.86, acidity 4.91-36.62%, moisture 8.46-15.38%, fat 1.35-7.90%, protein 12.79-21.58%, salt 1.29-12.43% and ash content 1.63-13.19% were determined. Several studies have investigated the properties of tarhana produced in different regions. These include 51 traditional homemade tarhanas from Edirne, Kırklareli, and Tekirdağ provinces (Coşkun, 2002), 13 different

tarhanas from Kahramanmaraş province (Yörükoğlu and Dayısoylu, 2016), homemade tarhanas from Bilecik, Zonguldak, Eskişehir, Kütahya, Van, Afyon, İstanbul and Ankara provinces (Funda, 2009), commercially produced tarhanas from Konya province (Bilgicli et al., 2006) and tarhanas from different regions of Antalya province (Erbaş et al., 2003).

It is important to determine the quality and technological characteristics of traditional/commercial tarhanas produced with different methods and raw materials in different regions of Türkiye to ensure standard production conditions, sustainability, and marketing. In the literature review, there are studies on tarhanas produced in different provinces. However, studies that analyse and compare tarhanas regionally are limited. Additionally, no studies have focused on determining the viscosity properties of traditional tarhanas. This study aimed to evaluate the chemical, functional, sensory, and rheological properties of traditional tarhanas produced in several provinces within the Central Anatolia region.

2. Materials and Methods

2.1. Material

Ten different tarhana samples were obtained from local producers in Ankara, Konya, Kayseri, Sivas, Eskişehir, Nevşehir, and Aksaray provinces in Central Anatolia. Tarhana samples were stored in sealed glass containers in a refrigerator at +4 °C in an odourless environment until analysis. The chemicals used were of analytical grade and were purchased from Sigma (Sigma Chemical Company, MO, USA) and Merck (Merck KGaA, Darmstadt, Germany).

2.2. Chemical Analysis

The moisture and ash content of tarhana samples were determined using AACC standard methods 44-01.01 and 08-01.01, respectively (AACC, 2004). Total nitrogen was determined by micro-Kjeldahl (AOAC, 2000) and crude fat by the Ankom method (AOCS, 2005). Total sugar content was determined spectrophotometrically by the phenol-sulphuric acid method using the xylose standard (Dubois et al., 1956). The colour of the samples was measured using a Minolta Chroma Meter (CR-300 Minolta Japan).

2.3. Titration of Acidity

To 10 g of tarhana sample, 50 mL (20 °C) of 67% neutralised ethyl alcohol was added and stirred at 150 rpm for 5 minutes. The mixture is then filtered through filter paper, and 10 mL of the filtrate is titrated with 0.1 N NaOH solution (Anonymous, 2004).

2.4. pH

5 g of tarhana sample was homogenised in 50 mL of distilled water. The pH was then measured using a pH meter (WTW inolab, Germany).

2.5. Salt Analysis

The tarhana samples were filtered through ashless filter paper (Whatman No:42). The pink colour was removed with 0.1 N H_2SO_4 solution after a few drops of 1% phenolphthalein were dropped on the filtrate. The neutralised filtrate was titrated with 0.1 N $AgNO_3$ solution until a brick red colour was obtained, and the % salt content was determined using the following Equation 1 (Anonymous, 2010).

$$\% \text{ Salt} = V \times mEq \times F \times 100 / m \quad (1)$$

V: Amount of 0.1 N $AgNO_3$ solution used in the titration, mL

mEq: Millivalent weight of NaCl, 0.0585 g

F: Factor of $AgNO_3$ solution

m: Sample quantity (g)

2.6. Water Activity

The a_w values of the samples were measured using an Aqua Lab Model Series 3TE (USA) water activity meter set at 20 °C (Hughes et al., 2002).

2.7. Analysis of Total Phenolics and Total Antioxidant Capacity

Tarhana samples were weighed at 1 g and extracted with 20 mL acidified methanol (methanol/hydrochloric acid/distilled water, 8:1:1, v/v) at room temperature (24±1 °C) for two h in a shaking water bath. The extracts obtained were centrifuged at 3000 rpm for 10 min. The collected extracts were used to determine the total phenolic content and total antioxidant capacity (Beta et al., 2005).

The total phenolic content of tarhana was determined using 2 N Folin-Ciocalteu phenol reagent according to the method of Singleton and Rossi (1965). 2 N 100 µL Folin-Ciocalteu phenol reagent, 100 µL extract or 100 µL standard gallic acid solutions, 2.3 mL distilled water and 1 mL 7% aqueous sodium carbonate solution were mixed and kept at room temperature for 2 h. The absorbance was measured at a wavelength of 750 nm, and the results were reported as being "gallic acid equivalent" (Cingöz, 2018).

The total antioxidant capacity values of the samples were determined by two methods. The ferric reducing antioxidant power (FRAP) was determined according to the method developed by Benzie and Strain (1996). The samples were mixed with the obtained FRAP working solution and kept in the dark for 30 min. At the end of the time, the absorbance values were recorded at 593 nm in a spectrophotometer, and the results were expressed as "Trolox equivalent." The determination of antioxidant capacity by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method was performed according to the method described by Brand-Williams et al. (1995). 1.95 mL of 100 µM DPPH was added to 50 µL of extract or Trolox standard solutions (50 µL), mixed and allowed to stand for 10 min. The absorbance values were then read at 517 nm, and the results were expressed as 'Trolox equivalent.'

2.8. Viscosity

20 g of tarhana sample was mixed with 200 mL of water and boiled over medium heat for 12 minutes with continuous stirring. The whole mass was homogenised for 1 min with ultraturax before measurement. Then, the viscosity of the soup was measured with a viscometer

(IKA Rotavisc me-vi, Seoul, Korea) at 100 rpm at 60 °C using Mil no:5 (Bayrakçı and Bilgiçli, 2015). To maintain the measurement temperature at 60 °C, silicone tubing was wrapped around the outside of the measurement vessel, and 60 °C water was circulated through the tubing.

2.9. Water and Oil Holding Capacity

A 5.0 g tarhana sample was weighed into 50 mL centrifuge tubes; 25 mL of water and 25 mL of sunflower oil were added to assess water and oil holding capacities, respectively The mixture was mixed for 60 minutes and then centrifuged (20 minutes, 4.000×g). The water and oil holding capacities were expressed as the amount (grams) of water or oil absorbed per gram of tarhana (Bayrakçı and Bilgiçli, 2015).

2.10. Sensory Analysis

Sensory evaluations were carried out according to TS 5525, method 2.2.4, with a 1-5 point scale (1=least liked, 5=most liked) using the scoring method of descriptive analysis methods. Taste, odour, consistency and general flavour characteristics of the samples served as soup were evaluated by 15 panelists.

2.11. Statistical analysis

The results were obtained in 2 parallel three replicates. SPSS statistical computer software (SPSS, Inc., Chicago, IL, USA) was used to analyse the results, which are presented as mean±standard deviation. The values obtained in the experiments were evaluated by Duncan's multiple comparison test (Genç and Soysal, 2018).

3. Results and Discussion

The chemical analysis results of tarhana samples from Ankara, Konya, Kayseri, Eskişehir, Nevşehir, Aksaray, and Sivas provinces of the Central Anatolia region are presented in Table 3. The moisture content ranged from 17.36% in Ankara (the highest) to 7.52% in Nevşehir (the lowest). Four tarhana samples exceeded the upper limit of 10% moisture contents specifieed in TS 2282, and 3 different tarhana samples did not exceed this limit. In a study of tarhana enriched with oat bran and sugar beet fibre, the moisture content of the samples was reported to be between 9.5% and 13.9%. It was reported that the moisture content did not vary proportionally with the amount of fibre added (Karaman, 2020). Yücecan et al. (1988) found the moisture content of 15 tarhana samples from different regions of our country to be 9-12.1%. They reported that the moisture content of 134 tarhana samples varied between 6.4-13.9% (Siyamoğlu, 1961). The highest ash value of 7.40% belongs to Eskişehir province, and the lowest value of 3.66% belongs to Sivas province. The percentage of husk in cereals has a significant effect on the ash content. The aleurone layer and the peripheral layers of the endosperm in wheat grains added to the tarhana composition contain high amounts of ash. The amount of ash is high in samples using whole wheat flour (Aktaş, 2018). In the study that included the determination of chemical and microbiological conditions of tarhanas of Kahramanmaraş province, the ash content was determined to be between 3.46-5.35% (Dayısoylu et al., 2003). The highest titratable acidity of 21.50% was found in Nevşehir tarhana, and the lowest acidity of 7.50% was found in Aksaray province. The acidity of tarhana (Table 3) was reported to be in the range of 10-35 according to TS 2282 tarhana standard (Anonymous, 2004). The acidity values of 27 domestic tarhanas from the Isparta region were between 4.91-36.62% (Soyyiğit, 2004). In another study, the total acidity values of 16 commercial tarhana samples were measured between 9.65-28.00% (Göçmen et al., 2003). The highest pH value of 5.40 was found in Aksaray tarhana and the lowest pH value of 4.04 was found in Nevşehir. There is no value for pH in the TS 2282 standard, and values of 3.8-4.2 are accepted as the optimum range in the literature (Dağlıoğlu, 2000). Another factor affecting the shelf life of food is water activity. If the water activity exceeds certain limits, undesirable conditions such as mold may occur in the products (Özçam, 2012). The highest water activity was 0.66 in Aksaray tarhana, and the lowest was 0.40 in Konya tarhana. A significant correlation was found between the moisture content of the tarhana samples and the water activity value. In a study, water activity values of 22 domestic tarhanas were reported in the range of 0.28-0.63 (Çağındı et al., 2016).

Table 3. Physicochemical analysis results of tarhana samples

Samples	Moisture (%)	Ash (%)	Titration acidity (%)	pH	Water activity (a_w)	Protein (%)	Fat (%)	Salt (%)	Total Sugar (mg xylose/100 g tarhana)
Ankara	17.36±0.00^{a}	5.36±0.01^{c}	8.75±0.01^{f}	5.28±0.00^{b}	0.43±0.02^{d}	11.64±0.06^{c}	1.12±0.05^{d}	5.82±0.02^{b}	12.79±0.19^{c}
Konya	12.38±0.02^{c}	4.49±0.05^{e}	11.00±0.20^{d}	4.70±0.12^{cd}	0.40±0.11^{d}	11.22±0.17^{d}	0.81±0.11^{e}	4.25±0.05^{d}	15.58±0.95^{a}
Kayseri	7.55±0.01^{f}	4.63±0.11^{e}	20.00±0.03^{b}	4.12±0.05^{e}	0.57±0.04^{c}	12.19±0.13^{b}	1.65±0.04^{b}	4.48±0.04^{c}	15.60±0.60^{ab}
Eskişehir	16.64±0.00^{b}	7.40±0.04^{a}	10.50±0.21^{de}	4.85±0.08^{c}	0.75±0.00^{a}	8.88±0.08^{f}	1.27±0.03^{c}	8.21±0.11^{a}	14.93±0.29^{b}
Nevşehir	7.52±0.03^{f}	5.17±0.04^{d}	21.50±0.08^{a}	4.04±0.10^{e}	0.45±0.06^{d}	11.57±0.04^{c}	4.23±0.04^{a}	4.17±0.12^{d}	16.21±1.17^{a}
Aksaray	11.55±0.01^{d}	5.48±0.02^{b}	7.50±0.06^{g}	5.40±0.00^{a}	0.66±0.01^{b}	10.42±0.01^{e}	0.91±0.12^{e}	5.86±0.08^{b}	9.66±0.30^{d}
Sivas	7.98±0.00^{e}	3.66±0.04^{f}	12.30±0.11^{c}	4.76±0.10^{c}	0.54±0.03^{c}	12.62±0.01^{a}	1.63±0.02^{b}	2.30±0.01^{e}	15.36±0.92^{b}

a,b= Means marked with different letters in the same column are statistically different (P<0.05).

The protein content of the tarhana samples ranged from 8.88 to 12.62%, the fat content from 0.81 to 4.23%, the salt content from 2.30 to 8.21%, and the total sugar content from 9.66 to 16.21 mg xylose/100 g tarhana (Table 3). According to the tarhana standard, the protein content should be at least 12% (in dry matter), and tarhanas from Sivas and Kayseri met the standard (Anonymous, 2004). It was reported that the protein values of 27 domestic tarhanas in the Isparta region were in the range of 21.58-12.79% (Soyyiğit, 2004). The fat content of the tarhana samples ranged from 0.81 to 4.23% (Table 3). The fat ratio of all samples was statistically different (P<0.05). The use of oil in the production of tarhana, differences in the ingredients and the presence of fat or non-fat yogurt in the composition cause differences in the fat ratio. There are studies in the literature that have measured the fat ratios of different tarhanas. In a study conducted on 15 home tarhanas and five commercial tarhanas from different regions of Türkiye, the fat ratios of commercial tarhanas were found to be in the range of 3.05-3.56%, while those of home tarhanas were found to be in the range of 0.45-4.97% (Esimek, 2010). In a similar study, 22 pieces of home tarhanas and 14 pieces of commercial tarhanas were examined, and the fat content was reported to be in the range of 0.21-7.00% for commercial tarhanas and 0.25-4.12% for home tarhanas (Çağındı et al., 2016). In other studies in which the fat content of tarhana samples was determined, the % fat ratio was found to be in the range of 0.39-30.2 (Siyamoğlu, 1961; Çolakoğlu and Bilgir, 1977; Yücecan et al., 1988). The salinity of the tarhana samples ranged from 2.30 to 8.21% (Table 3). While Nevşehir and Konya tarhana samples were statistically similar in terms of salt content (P<0.05), other tarhana samples were different. The highest salt content was found in Eskişehir province and the lowest in Sivas province. It can be seen that the differences in salt content vary according to the diversity of tarhana composition. Salt contents of Maraş tarhana samples were reported in the range of 3.30 to 5.59% (Yörükoğlu, 2012), salt contents of tarhana samples collected from 21 different regions were reported in the range of 0.62 to 9.01% (Tamer et al., 2007), and salt contents of 96 tarhana samples collected from different regions were reported in the range of 0.32 to 6.64% (Ersoy Ömeroğlu et al., 2023). The total sugar content of tarhana samples was found to be in the range of 9.66-16.21 mg xylose/100 g tarhana. The total sugar contents of tarhana samples from Konya, Kayseri, and Nevşehir provinces were statistically similar (P<0.05). No study was found in the literature search that determined the total sugar content of tarhana samples. To estimate the GI values of tarhana samples, it is useful to know the total sugar content.

One of the primary factors influencing consumer food preferences is the presence of desirable colour attributes (Cingöz, 2018). Bulk images of tarhana samples collected from different provinces of Central Anatolia are shown in Figure 1. L* values of tarhana samples range between 72.34-81.41, a* values varied between 0.31-9.64, and b* values range between 17.69-42.27 (Table 4). It was found that the products used in the composition of the samples, such as tomato, pepper paste, hot red pepper, red pepper powder, and tomato paste were effective on the color values. Köse and Çağındı (2002) found L* values between 52.71-63.03, a* values between 14.41-18.72, b* values between 33.41-44.14, Esimek (2010) found L* values between 60.6-85.6, a* values between 0.00-19.2 and b* values between 7.30-30.40, Çağındı et al. (2016) reported L*, a* and b* values between 54.61-88.57,-0.14-28.10 and 1.43-52.88 respectively.

Table 4. Colour analysis results of tarhana samples

Samples	L*	a*	b*
Ankara	78.39±0.48^{b}	1.49±0.17^{d}	25.85±0.61^{e}
Konya	75.94±1.08^{c}	9.34±0.53^{a}	42.27±1.01^{a}
Kayseri	79.65±1.09^{b}	8.29±0.27^{b}	36.62±0.94^{b}
Eskişehir	72.34±1.03^{d}	9.64±0.54^{a}	34.11±0.91^{c}
Nevşehir	81.41±0.63^{a}	0.88±0.15^{e}	17.69±0.74^{f}
Aksaray	72.91±0.59^{d}	3.17±0.21^{c}	30.36±0.92^{d}
Sivas	76.61±1.08^{c}	0.31±0.17^{f}	26.29±0.93^{e}

a,b= Means marked with different letters in the same column are statistically different (P<0.05).

The total phenolic content and total antioxidant capacity of tarhana samples were analysed and the results are presented in Table 5. The total phenolic content of tarhana samples was 131.40-337.40 mg GAE/100 g, and the total antioxidant capacity was 28.05-40.97 µM TE/100 g (FRAP) and 60.31-62.45 µM TE/100 g (DPPH). The results of phenolic content and antioxidant activity of all samples were statistically different (P<0.05). Kayseri tarhana stands out with the highest total phenolic content. In the study where tarhana samples from different regions were analysed, it was reported that the total phenolic content varied between 572.47-1851.83 µg GAE/g (Esimek, 2010).

Table 5. Total antioxidant capacity and total phenolic content analysis results of tarhana samples

Samples	Total phenolic content (mg GAE/100 g tarhana)	Total antioxidant capacity	
		FRAP (µM TE/100g)	DPPH (µM TE/100g)
Ankara	174.73±3.41^{d}	37.00±0.25^{c}	61.44±0.42^{b}
Konya	231.73±3.12^{b}	40.97±0.08^{a}	61.44±0.11^{b}
Kayseri	337.40±3.12^{a}	37.01±0.31^{c}	60.61±0.19^{c}
Eskişehir	153.73±2.80^{e}	30.73±0.09^{e}	62.42±0.15^{a}
Nevşehir	131.40±3.44^{f}	28.05±0.10^{f}	62.45±0.06^{a}
Aksaray	140.40±6.18^{f}	31.17±0.10^{d}	62.42±0.21^{a}
Sivas	218.07±6.09^{c}	38.55±0.31^{b}	60.31±0.12^{c}

a,b= Means marked with different letters in the same column are statistically different (P<0.05).

Tarhana, one of the traditional products, is usually consumed by making soup. Therefore, the rheological properties of tarhana in the cooked state are important. The viscosity values and functional properties of tarhana samples are presented in Table 6. Viscosity is a recognised measure of the consistency of liquid foods such as soups. The viscosity values of the samples were found to vary between 1.447-1.721 Pa.s. While Sivas tarhana had the highest viscosity value, Konya tarhana had the lowest viscosity value. The viscosity values of all the samples were statistically different (P<0.05). The decrease in viscosity value in products such as soups is considered to be an indicator that the product has become thicker / darker, and the consistency of tarhana soups is a situation demanded by consumers. The water and oil holding capacity, which is one of the important criteria in the cooking stage, is also related to the composition. Water and oil holding values are similar to viscosity. It was found that Sivas tarhana had the highest water and oil holding capacity, and Konya tarhana had the lowest. The results of the sensory analysis of tarhana samples by 15 semi-trained panelists are presented in Table 7. Aksaray and Eskişehir tarhanas had the highest rating, while Ankara tarhanas had the lowest overall rating. Aksaray, Eskişehir and Sivas tarhanas received the highest scores in terms of taste and aroma, which is one of the most important sensory criteria of food.

Table 6. Rheological and functional properties of tarhana samples

Samples	Viscosity (Pa.s)	Water holding capacity (g/g)	Oil holding capacity (g/g)
Ankara	1.503±0.018^{d}	0.93±0.03^{e}	1.88±0.03^{e}
Konya	1.447±0.014^{e}	0.90±0.01^{f}	1.82±0.01^{f}
Kayseri	1.666±0.032^{b}	1.11±0.02^{b}	2.24±0.02^{b}
Eskişehir	1.512±0.011^{d}	0.95±0.02^{e}	1.92±0.04^{d}
Nevşehir	1.586±0.016^{c}	0.99±0.01^{d}	2.00±0.01^{c}
Aksaray	1.605±0.023^{c}	1.02±0.01^{c}	2.06±0.05^{c}
Sivas	1.721±0.021^{a}	1.18±0.03^{a}	2.38±0.04^{a}

a,b= Means marked with different letters in the same column are statistically different (P<0.05).

Table 7. Sensory analysis results of tarhana samples

Samples	Colour	Taste and Aroma	Odour	Sourness	Consistency	Overall Rating
Ankara	2.21	2.64	2.79	3.29	2.75	2.76
Konya	2.89	3.29	3.43	2.71	3.75	3.26
Kayseri	2.82	2.64	3.71	2.36	3.86	3.23
Eskişehir	3.82	3.39	3.75	2.96	4.04	3.71
Nevşehir	3.93	3.21	3.46	2.64	3.57	3.54
Aksaray	4.43	3.43	3.36	3.04	3.86	3.75
Sivas	3.25	3.32	3.39	3.86	3.86	3.43

Figure 1. Tarhana samples from central Anatolian region.

4. Conclusion

In this study, the chemical, functional, rheological, and sensory properties of tarhana from Aksaray, Eskişehir, Nevşehir, Sivas, Konya, Kayseri, and Ankara provinces of Central Anatolia region were determined. It was found that the tarhana of each region has different characteristics due to the differences in the raw materials used in tarhana production, production conditions, and methods. All these values are similar to those found in studies of tarhana. Except for the moisture values, the other parameters are within the accepted TSE criteria. This study will guide future research on traditional tarhana and address gaps in the literature. It also provides valuable insights into the standard commercial production of traditional tarhanas. Furthermore, this study serves as a reference for books, almanacs, and other publications related to traditional tarhana.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	A.C.	Z.E.
C	50	50
D	100	
S	100	
DCP	50	50
DAI	20	80
L	50	50
W	80	20
CR	80	20
SR	80	20
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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DETERMINATION OF QUALITY CHARACTERISTICS IN MATURE PARSLEY (*Petroselinum hortense*) PLANTS, PARSLEY MICROGREENS, AND PRIMED PARSLEY MICROGREENS

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Abstract: The term "microgreen" describes tiny seedlings of edible plants that have cotyledon leaves that form in the first 1-2 weeks after planting. Microgreen is a new topic in vegetable growing and has the potential to provide significant profits in a short time if marketing opportunities are found. For this reason, it is an important issue to compare microgreens with their mature forms and evaluate them in terms of their contribution to our health. In this study, mature parsley plants, parsley microgreens and primed parsley micro greens were compared in terms of yield and some biochemical properties. The study was carried out in greenhouse conditions at Çanakkale Onsekiz Mart University, Faculty of Agriculture, Dardanos Farm in spring and summer of 2023. Seeds of a standard parsley variety (*Petroselinum hortense* cv. Toros) were used as plant material in the experiment. In the study, ascorbic acid (mg/100g), pH, Titratable Acidity (TEA), water-soluble dry matter (WSDM), apigenin amount and yield (g/m^2) parameters were examined. At the first harvest, parsley microgreens had more yield in a shorter time compared to mature parsley plants. The yield has increased especially with the priming application. The amount of ascorbic acid was found to be statistically ($P<0.05$) less in parsley micro greens than in mature parsley plants. The highest amount of apigenin was obtained from primed parsley microgreens.

Keywords: Apigenin, Ascorbic acid, Microgreens, *Petroselinum hortense*, Yield

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1. Introduction

The microgreens market is expected to reach $3.4 billion by 2030, showing a bright future for the industry. While North America dominates the market, the Asia Pacific region is experiencing rapid growth (Anonymous. 2024). Microgreens, which include various vegetables, medicinal plants, and herbaceous plants characterized by thin structures, cotyledon leaves, and early harvesting, are highly diverse in terms of color, structure, and flavor (Bhatt and Sharma, 2018). These plants possess delicate tissues and can be incorporated into a variety of dishes such as salads, soups, and sandwiches (Choe et al., 2018). The cultivation of microgreens can be a profitable business opportunity when a market demand exists. Although microgreen cultivation is not widely recognized in the Çanakkale region and current production is limited, there is a growing potential for marketing these products, particularly within local restaurants, as awareness of microgreens increases.

Priming is a process that involves soaking seeds under controlled conditions (imbibition) to improve germination and promote the early onset of germination events, followed by drying the seeds to their initial moisture content (Varier et al., 2010). Microgreens are closely related to seed priming, as their production involves the sowing of various seeds, and priming is known to enhance seed germination potential. In a study conducted by Tok and Kurt (2019), parsley seeds were collected from the Arsuz and Samandağ districts in Hatay province, and it was reported that 80% of the healthy, non-infected parsley seeds successfully germinated. In another study (Khan et al., 2023) on curly-type parsley seeds, germination was tested at 25±2°C and the control group showed a germination rate of approximately 70-80%, while seeds subjected to 24-hour hydropriming exhibited a germination rate of 78.7%. In a study by Dimita et al. (2022), it was observed that the total phenolic contents in the microgreen stage of Chinese red basil (*P. frutescens* var. *crispa*) and Chinese green basil (*P. frutescens* var. *frutescens*) varieties were lower than in their mature stages. However, the amount of volatile organic compounds during the microgreen stage of Chinese red basil (*P. frutescens* var. *crispa*) was approximately twice as high (236.83 ng/g FW) compared to the mature stage (127.16 ng/g FW) (Dimita et al., 2022). In lettuce microgreens (*L. sativa* L. var. *capitata*), the contents (g kg^{-1}) of several minerals (Ca, Fe, Mn, Zn, Mo, Se) were found to be statistically higher than in their

mature stages (Pinto et al., 2015).

Farzaei et al. (2013) mentioned in their studies that parsley has been used in the treatment of diseases such as hypertension, heart disease, and diabetes, and that flavonoid compounds, especially apigenin, apiin, and 6"-Acetilapiin have been detected in parsley. Abid et al. (2022) described apigenin as a flavonoid that has long been recognized as a traditional immune-stimulating agent, with health-promoting properties against various cancers, cardiovascular diseases, and other ailments. Considering the study by Farzaei et al. (2013), it is important to investigate the changes in apigenin content in parsley, which is particularly rich in flavonoids such as apigenin, apiin, and 6"-acetylapiin as a result of various treatments. At the same time, it is important to evaluate the topic of microgreens through comparisons made with the mature form of the plants.

The aim of the study is to compare the yield and certain quality parameters of both microgreens and microgreens derived from seeds subjected to priming treatment, with those of mature parsley plants (*Petroselinum hortense* cv. Toros).

2. Materials and Methods

The study was carried out in greenhouse conditions at Çanakkale Onsekiz Mart University, Faculty of Agriculture, Dardanos Farm in Spring and Summer of 2023. In the study, parsley plants, parsley microgreens and primed parsley microgreens were determined as the subjects and these three were compared in terms of yield and some biochemical properties. In the experiment, the seeds of a standard parsley variety *(Petroselinum hortense* cv. Toros) were used as the plant material.

The priming treatment applied to parsley microgreens was conducted by soaking parsley seeds (*Petroselinum hortense*) in water at 10°C for 12 hours, which was found to be the most effective treatment for germination rate among the priming methods for parsley (Dursun and Ekinci, 2010).

The seeds were weighed before priming, and after priming, they were spread on filter papers in a 40×40 cm tray in a shaded and ventilated environment and dried to their initial weight (Varier et al., 2010).

The germination capacity of the vegetable seeds was determined according to Ellis and Roberts (1980). The germination rate of seeds without priming was determined to be 70%, while the germination rate of primed seeds was found to be 80%.

The growing medium in the experiment was prepared by mixing 2/3 peat with 1/3 perlite to retain the applied water.

Rectangular containers with dimensions of 36×27×7 cm were used in the experiment. Two holes were made at the bottom of each container using a soldering iron to allow for excess water drainage.

The containers have a surface area of 36×27 = 972 cm^2. The mature vegetables were planted in each container with 6 plants spaced evenly, providing each plant with a surface area of 13.5×12 = 162 cm^2. The seeds of the vegetables, considered to be mature, were first sown in the compartments of the seedling trays. Once germination occurred, the seedlings were transferred to containers containing the same growing medium, with the root zone remaining undisturbed, along with all the growing medium from the seedling tray. Prior to planting, an equal amount of growing medium, equivalent to the growing medium volume in the seedling trays, was added to the containers where the microgreens would be grown. This ensured that an equal amount of growing medium was used in each container. During the microgreens cultivation stage, parsley seeds were sown in containers with a surface area of 972 cm^2 at a seeding density of 5 seeds per cm^2 (Carillo et al., 2022). Initially, the required number of seeds per container was calculated as 5 × (972 cm^2) = 4860. The amount of seeds to be sown was determined based on their germination capacity. Additional seeds were added in proportion to the amount of seeds that do not germinate. The added seeds are assumed to be selected from those with a germination rate of less than 100%, and the calculations were made accordingly.

The germination capacity of the seeds used was determined to be 70%, and the thousand seeds weight was 2.3 g. As a result, for microgreens cultivation, 15.96 g of seeds were used per container (with a surface area of 972 cm^2). 4860 (972 x5) seeds were sown in a container with the calculation of 5 seeds per cm^2. The same amount of seed (15.96 g) was used for the subject to which priming was applied, and then priming was applied. It was observed that 164.2 g of seeds should be used for 1 m^2 area. The primed and non-primed seeds were evenly spread in the container filled with growing medium, and their surfaces were covered with a 0.5 cm layer of growing medium. To enhance uniform distribution, the surface area of the container and the seed quantity were divided into eight sections, with the seeds being spread separately in each section.

Daily, in the evening, water was applied to the containers until water emerged from the evenly distributed holes at the bottom of the containers. To promote the elongation of microgreens and prevent excessive thickening of the hypocotyls, a 40% shaded mesh material was used 20 days after sowing. Microgreens can be harvested when the cotyledon leaves are fully grown or when the plant has its first two true leaves (Gerovac et al., 2016; Waterland et al., 2017; Li et al., 2021). Di Gioia et al. (2023) defined the commercial harvesting stage of microgreens as the time when the cotyledons are fully developed and the first true leaves begin to grow. In our study, microgreens were harvested at the stage when the cotyledons were fully grown and the first true leaves began to grow (Di Gioia et al., 2023).

2.1. Measurements and analyses performed in the study

Ascorbic Acid Content (mg/100g): The ascorbic acid (vitamin C) content of the microgreens was analyzed

according to the Pearson and Churchill (1970) method. For each sample, 175 ml of 0.4% Oxalic Acid was added to 25 g of sample. L1 value was determined by reading of Oxalic acid/2.6 Diclorophenol indophenol: 1/10 solution in response to Oxalic acid/Pure water: 1/10 solution at 520 transmittance value. L2 value was determined by reading of filtered sample/2.6 Diclorophenol indophenol: 1/10: solution in response to Oxalic acid/Pure water: 1/10 solution at 520 transmittance value. In this way, ascorbic acid content was calculated by using the formulation.

Water-Soluble Dry Matter (%): It was found by direct reading as a percentage value with a hand refractometer, with 3 readings in each repetition. pH Value and Titratable Acidity (TETA) (g/100g): It was determined according to the titration method using 0.1 N NaOH in the samples. The titratable total acidity (g/100g) was calculated by formula in terms of citric acid (Jadczak et al., 2019) by finding the NaOH value detected when the pH value was 8.1 with the help of burette and digital desktop pH meter (WTW, Bavaria, Germany) (Anonymous, 1968).

Calculation of Apigenin Content (mg/kg): HPLC Method: System Used: Shimadzu Prominence Brand HPLC, CBM: 20ACBM, Detector: DAD (SPD-M20A), Column Oven: CTO-10ASVp, Pump: LC20 AT, Autosampler: SIL 20ACHT, Computer Program: LC Sotution; Mobile Phase: A: 3% Formic acid B: Methanol (The method of Gomes et al. (1999) was modified and used in HPLC analysis).

Sample preparation: 2 g sample was taken. 10 ml of 96% ethanol was added to it. It was mixed in the homogenizer for 2 minutes. It was kept in a water bath at 45°C for 1 night. At the end of this period, it was centrifuged at 4000 rpm for 5 minutes. The supernatant was taken and evaporated in a rotary evaporator at 45°C until it was completely dry. The extracts were then dissolved in 1 ml of methanol and used in phenolic compound analyzes (Kiselev et al., 2007).

Yield at First Harvest (g/m^2): The yield for the first harvest has been determined, along with the time required to achieve it.

All analyses and measurements in microgreens were conducted at harvest maturity (Di Gioia et al., 2023). In parsley, all analyses and measurements were performed on leafy and stemmed portions (up to the first point of leaf-stem attachment, excluding thick stems not suitable for consumption) for the first harvest.

The experiment was designed according to a randomized complete block design (Table 1) with three replications, each replication consisting of one container (each container containing 6 parsley plants or microgreens). Statistical analyses were performed using the SAS 9.0 software package, with analysis of variance (ANOVA) conducted, and differences between means were compared using the LSD test (P<0.05). Biplot analysis was used to interpret data for different quality parameters, and the data were also evaluated graphically.

Table 1. Trial design of the study.

Replication I	Replication II	Replication III
Mature Parsley	Parsley Microgreens	Primed Parsley Microgreens
Parsley Microgreens	Primed Parsley Microgreens	Mature Parsley
Primed Parsley Microgreens	Mature Parsley	Parsley Microgreens

3. Results and Discussion

Microgreens reached sufficient maturity in 25 days, while mature parsley reached maturity in 50 days. Yield values were given in Table 2. The yield at the first harvest was highest to lowest in the following order: primed parsley microgreens, parsley microgreens, and mature parsley (P<0.05).

Table 2. Yield values (g/m^2) at the first harvest of parsley at maturity stage, microgreens and primed microgreens*

Subjects	Yield Values (g/m^2) at the First Harvest
Mature Parsley	667.7 C
Parsley Microgreens	765.6 B
Primed Parsley Microgreens	886.4 A
LSD (P<0.05):	60.278

*Means followed by the same letter within a column are significantly different according to the least significant difference (LSD) test.

In a study (Carillo et al., 2022), parsley microgreens were grown in a peat-based growth medium with Hoagland solution under different light-emitting lighting systems in a climate chamber (5 seeds cm^{-2}), and the yield ranged from 1 to 2 kg m^{-2} (fresh weight). In another study (Thuong and Minh, 2020), the highest yield in red radish (*Raphanus sativus*) microgreens was not obtained from the highest seed planting density. This suggests that a gradual increase in seed planting density may not necessarily result in a corresponding gradual increase in yield.

It has been determined that the priming application in parsley microgreens increased the yield compared to non-primed microgreens (P<0.05) in the current study. Dursun and Ekinci (2010) conducted priming treatments on parsley (*Petroselinum crispum* L.) seeds using different materials at various temperatures and durations. In their study, the germination rate was determined to be 49.25% at 25°C, while the highest germination rate (90%) was achieved with a 12-hour hydropriming treatment at 10°C. In our study, it was observed that subjecting parsley seeds to a 12-hour priming treatment at 10°C had a positive effect on the yield of parsley microgreens. In a study conducted by Tamindzic et al. (2023), hydropriming (10 hours at room

temperature) was applied to pea varieties (*Pisum sativum* L. cv. E-244, Dukat, Partner), and it was found that hydropriming increased germination (%) and fresh shoot weight (g) in the Dukat pea variety. Additionally, hydropriming treatment on curly-type parsley seeds led to an increase in germination percentage (Khan et al., 2023).

In the current study, the lowest ascorbic acid content was obtained from the microgreens that did not undergo priming. It was observed that the microgreens subjected to priming and the mature parsley plants had statistically (P<0.05) the same ascorbic acid content. However, the highest ascorbic acid content was obtained from the mature plants (Table 3).

Table 3. Ascorbic acid contents (mg/100g) at the first harvest of parsley at maturity stage, microgreens and primed microgreens*

Subjects	Ascorbic Acid Contents (mg/100g)
Mature Parsley	234.94 A
Parsley Microgreens	176.15 B
Primed Parsley Microgreens	214.07 A
LSD (P<0.05):	28.231

*Means followed by the same letter within a column are significantly different according to the least significant difference (LSD) test.

In a study conducted by Karkleliene et al. (2014), the ascorbic acid content in five different parsley varieties (*Petroselinum crispum* cv. Moss Curled, Astra, Festival, Gigant D'Italia, and Average) ranged from 138.4 to 162.8 mg/100g. In another study conducted by Jadczak et al. (2019), the ascorbic acid content in the leaves of seven parsley varieties varied between 132.41 and 210.52 mg/100g (fresh weight).

Parsley (*Petroselinum crispum* cv. Comune) microgreens were grown in a peat-based growth medium with Hoagland solution, using different light-emitting lighting systems in a climate chamber. The ascorbic acid content ranged from 12.9±0.7 to 37.27±0.24 mg/100g fresh weight (Carillo et al., 2022).

Parsley is known to be a vegetable rich in ascorbic acid (vitamin C), as demonstrated in the studies mentioned above (Karkleliene et al., 2014; Jadczak et al., 2019; Carillo et al., 2022). The ascorbic acid values closest to those observed in our study (176.15 – 234.94 mg/100g) were reported in the study conducted by Jadczak et al. (2019), where values ranged from 132.41 to 210.52 mg/100g. When examining studies that used either mature plants or microgreens of parsley (Karkleliene et al., 2014; Jadczak et al., 2019; Carillo et al., 2022), it was observed that ascorbic acid levels in mature parsley plants were generally higher than in microgreens (Carillo et al., 2022). However, it is also known that the ascorbic acid content can vary depending on the variety, cultivation environment, and other external factors.

The titratable acidity (TEA), water-soluble dry matter (WSDM), and pH walues were given in Table 4). The highest TEA value was determined in mature parsley plants. The TEA values of parsley micro greens and primed parsley micro greens were in statistically similar groups (P < 0.05). The same result was observed for the water-soluble dry matter (WSDM) parameter too. The study showed that parsley microgreens had the highest pH value, while mature parsley plants had the lowest pH value (P<0.05).

Table 4. Titratable acidity (g/100g), water-soluble dry matter (%), and pH values at the first harvest of parsley at maturity stage, microgreens and primed microgreens*

Subjects	WSDM (%)	TA (g/100g)	pH
Mature Parsley	7.8 A	0.22 A	6.06 C
Parsley Microgreens	4 B	0.16 B	6.58 A
Primed Parsley Microgreens	5 B	0.16 B	6.29 B
LSD (P<0.05):	1.854	0.0305	0.1653

*Means followed by the same letter within a column are significantly different according to the least significant difference (LSD) test. WSDM= water-soluble dry matter, T= titratable acidity.

In the study performed by Can (2022), parsley seeds were planted in a mixture of peat and perlite, then transferred to hydroponic culture in trays, where different applications of fulvic acid, amino acids, and chitosan were tested. In the second experiment of the study, soluble solids content (%), pH and acidity (%) were determined. The control group had soluble solids content (%), pH and acidity (%) values of 8.9, 6.2, and 0.57 respectively. In another study on parsley (*Petroselinum crispum* (Mill.) Nyman ex A. W. Hill), where different plant growth regulators were applied (Gonzales, 2021), the control group showed soluble solids content values ranging from 0 to 1 °Brix, with pH values between 6 and 7. In another study on parsley conducted under greenhouse conditions, different vermicompost applications were tested (Peyvast et al., 2008), and the control group showed a soluble solids content of 2.2%. When evaluating the aforementioned studies on parsley (Peyvast et al., 2008; Gonzales, 2021; Can, 2022), the values closest to our study were obtained in Can (2022). In our study, as the plants matured, both The TEA and WSDM increased.

The apigenin contents at the first harvest of parsley at maturity stage, microgreens and primed microgreens were given in Table 5. The highest apigenin content in the study was obtained from the priming-treated parsley microgreens (P<0.05). Both mature parsley and parsley microgreens showed statistically similar values (P<0.05).

Tablo 5. Apigenin contents at the first harvest of parsley at maturity stage, microgreens and primed microgreens (mg kg^{-1}) *

Subjects	Apigenin (mg kg^{-1})
Mature Parsley	18.534 B
Parsley Microgreens	23.657 B
Primed Parsley Microgreens	97.776 A
LSD (P<0.05):	5.5948

*Means followed by the same letter within a column are significantly different according to the least significant difference (LSD) test.

In a study (Carillo et al., 2022) which different light systems were used for growing parsley microgreens, apigenin levels ranged from 2.12 to 4.6 mg kg^{-1} (dry weight). However, no priming treatment was applied in the study. In another study (Cao et al., 2010), flavonoid contents were determined in various vegetables and fruits in which the apigenin content in parsley recorded as 4.4±0.1 mg kg^{-1} (fresh weight). The apigenin levels observed in our study were found to be higher than those reported in the studies (Cao et al., 2010; Carillo et al., 2022).

It has been observed that the priming treatment significantly improved parsley germination in the current study. Similar to our findings, Dursun and Ekinci (2010) reporthed that among the priming treatments applied to parsley (*Petroselinum crispum* L.), the highest germination rate was observed when parsley seeds were soaked in water for 12 hours at 10°C. This could have positively influenced the synthesis of the apigenin flavonoid in the microgreens. However, it can also be noted that further studies are needed on this topic.

Bi-plot presenting the correlation between the tested parameters of parsley plants was given in Figure 1. The Component 1 and Component 2 components explained 100% of the variance for the three different topics and five parameters in our study. However, the biplot was used because it provided a visual opportunity to evaluate all the topics together.

The ascorbic acid, Water-Soluble Dry Matter and titratable acidity parameters, along with the mature parsley subject (OMA), are positioned in the positive direction of PC1 (opposite direction of the other subjects) in the figure, indicating that these parameters have higher values in mature parsley. The priming-treated (PMIP) and non-treated (MIP) microgreen subjects are located in the same direction as the yield in first harvest and pH parameters and in the opposite direction of the mature parsley subject (PC1<0), which suggests that the yield and pH parameters in the microgreens are higher compared to those of mature parsley plants. The priming-treated microgreen subject (PMIP) is the only subject aligned along the same axis as the yield in the first harvest parameter (PC1<0; PC2>0), indicating that the highest yield value was obtained from this subject. The Apigenin parameter is found in the opposite direction of the mature parsley subject (MAP) and in the same direction as the other subjects (MIP, PMIP) (PC1<0), showing that the lowest apigenin content was obtained from mature parsley. The priming-treated parsley microgreen subject (PMIP) being positioned along the same axis as the apigenin parameter (PC1<0; PC2>0) indicates that this subject has the highest apigenin content (Figure 1).

4. Conclusion

Under the conditions of the study, it was possible to obtain higher yields from parsley microgreens in half the time compared to mature parsley plants during the first harvest. This yield was further increased with the application of priming. The ascorbic acid content in the mature parsley microgreens was found to be higher than that in the parsley microgreens. In terms of the ascorbic acid parameter, the priming-treated parsley microgreens were statistically grouped with the mature parsley plants. The mature parsley plants showed the highest statistical values for Titratable Acidity and Water-Soluble Dry Matter parameters. No significant effect of priming on the Titratable Acidity and Water-Soluble Dry Matter values was observed in the microgreens. In the study, the apigenin content was similar in the mature parsley and parsley microgreens subjects, but it increased significantly in the priming-treated parsley microgreens subject, highlighting the importance of priming when growing parsley microgreens.

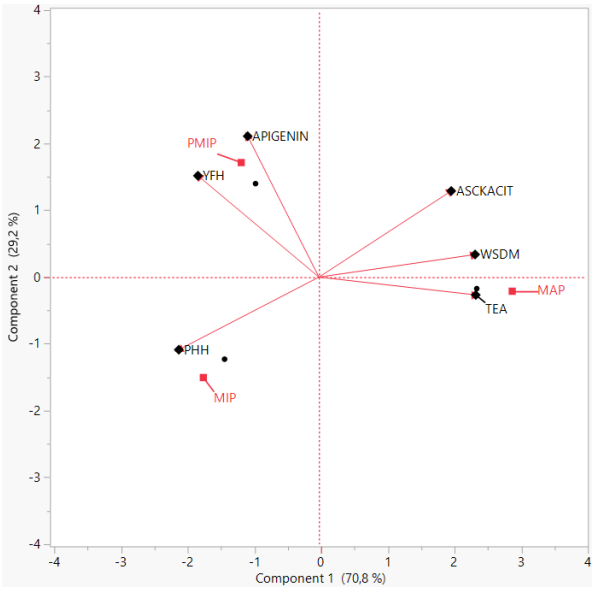

Figure 1. Bi-plot presenting the correlation between the tested parameters of parsley plants. MAP= mature parsley, MIP= Parsley Microgreen, PMIP= primed parsley microgreen, ASCKACIT= ascorbic acid, WSDM= water-soluble dry matter, TEA= titratable acidity, PHH= pH, YFH= yield in first harvest, APIGENIN= apigenin

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	A.Ç.	T.S.	N.Ö.
C		100	
D	30	50	20
S			100
DCP	90	10	
DAI	40	50	10
L		100	
W		70	30
CR			100
SR		70	30
PM	90	10	
FA	90	10	

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

Conflict of Interest

The authors declared that there is no conflict of interest.

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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Review
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LINKAGE DISEQUILIBRIUM IN ANIMAL GENETICS – DEFINITION, MEASURES AND APPLICATIONS

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Abstract: The non-random connection of alleles at various loci is known as linkage disequilibrium (LD). Combinations of alleles inside haplotypes occur at frequencies that differ from those expected on independence when two alleles at two distinct loci are in LD. When genetic variation at a locus is linked to a trait, it means that either the genetic variation at that locus directly impacts the phenotype of interest or the locus is in LD with the causal mutation. The level of LD, which dictates how many markers should be typed in a genome scan to discover a quantitative trait locus (QTL) using LD, is critical to the practicality of association studies. This review explores the origin of LD in genetics and how it applies to animal breeding and genetics.

Keywords: Linkage disequilibrium, Quantitative trait loci, Alleles, Haplotypes, Genetic variation

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1. Introduction

Consider 2 hypothetical markers, A and B that are on the same chromosomes. Alleles A1 and A2 are present in A, and alleles B1 and B2 are present in B. A1 B1, A1 B2, A2 B1, and A2 B2 are the four potential haplotypes of markers. If the population's frequencies of alleles A1, A2, B1, and B2 are all 0.5, we can anticipate the population's frequencies of the four haplotypes to be 0.25. Linkage disequilibrium (LD) occurs when the haplotype frequencies deviate from 0.25, indicating that the genes are not in random association. Two loci unlinked are possible to be in linkage disequilibrium in some populations (Kavuncu, 2021) - in fact, linkage disequilibrium between a marker and a QTL is essential if the QTL is to be found in either type of analysis (Mueller, 2004). The distinction is that linkage analysis only takes into account linkage disequilibrium within families, which can span tens of thousands of cM and is broken down by recombination after only a few generations. A marker must be in linkage disequilibrium (LD) with a QTL throughout the entire population for linkage disequilibrium mapping. The relationship must have persisted for a significant number of generations to be a property of the entire population; hence, the marker(s) and QTL must be closely related.

2. Measures of Linkage Disequilibrium

According to Hill (1981), one measure of LD is D, which can be calculated as (Equation 1):

$$D = Freq\ (A1B1) \times Freq\ (A2B2) - Freq\ (A1B2) \times Freq\ (A2B1) \quad (1)$$

where *Freq (A1alt indis$_{B1}$)* is the population frequency of the $A1_{B1}$ haplotype, and similarly for the other haplotypes.

The D statistic is highly reliant on the frequencies of individual alleles, making it ineffective for assessing the degree of LD between numerous loci (for example, at different points along the genome). Hill and Robertson (1968) suggested the r^2 statistic (Equation 2) is less dependent on the allele frequencies metric.

$$r^2 = \frac{D^2}{Freq\ (A1) \times Freq\ (A2) \times Freq\ (B1) \times Freq\ (B2)} \quad (2)$$

The frequency of the A1 allele in the population is *Freq (A1)*, and the same is true for the other alleles in the population. The value of r^2 ranges from 0 for a pair of loci with no linkage disequilibrium to 1 for a pair of loci in complete linkage disequilibrium.

For example, consider the following hypothetical allelic frequencies.

$Freq\ (A1) = Freq\ (A2) = Freq\ (B1) = Freq(B2) = 0.5$

The haplotype frequencies are:

$Freq\ (A1_{B1}) = 0.1$
$Freq\ (A1_{B2}) = 0.4$
$Freq\ (A2_{B1}) = 0.4$
$Freq\ (A2_{B2}) = 0.1$

$D = 0.1 \times 0.1 - 0.4 \times 0.4 = -0.15$

$D^2 = 0.0225$

The value of r^2 is then

$$\frac{0.0225}{(0.5 \times 0.5 \times 0.5 \times 0.5)} = 0.36$$

This is a moderate level of r^2.

D' is another often used pair-wise LD measure. The value of D is standardized by the highest value it can achieve to determine D' (Equation 3).

$$D' = \frac{|D|}{D_{max}} \quad (3)$$

where if D > 0, (Equation 4)

$$D_{max} = \min[Freq(A1)\{1 - Freq(B2)\}, \{1 - Freq(A2)\} Freq(B1)] \quad (4)$$

If D < 0 (Equation 5)

$$D_{max} = \min[Freq(A1) \times Freq(B2), \{1 - Freq(A2)\}\{1 - Freq(B2)] \quad (5)$$

For two reasons, the statistic r^2 is recommended over D' as a measure of the amount of LD. Firstly, the *r2* between a marker and a (unobserved) QTL is the fraction of variation generated by alleles at a QTL that can be explained by markers. The decrease in r^2 with distance represents how many markers or phenotypes are needed to discover QTL in an initial genome scan using LD. When compared to the sample size for testing the QTL itself, the sample size for detecting an ungenotyped QTL must be raised by a factor of $1/r^2$. D', on the other hand, performs a terrible job of forecasting needed marker density for a genome scan using LD. The second rationale for using r^2 instead of D' to determine the level of LD is that D' is prone to be overstated when sample sizes are small or allele frequencies are low (McRae *et al*. 2002).

The LD measurements mentioned above are for bi-allelic markers. While they can be applied to multi-allelic markers like microsatellites, Zhao *et al*. (2005) suggested using the $\chi^{2'}$ (Equation 6) measure of LD for multi-allelic markers.

$$\chi^{2'} = \frac{1}{l-1}\sum_{i=1}^{k}\sum_{j=1}^{m}\frac{D_{ij}^2}{Freq(A_i)Freq(B_j)} \quad (6)$$
$$D_{ij}^2 = Freq(A_iB_j) - Freq(A_i)Freq(B_j)$$

Freq (Ai) is the frequency of the ith allele at marker A, *Freq (Bj)* is the frequency of the jth allele at marker B, and *l* is the minimum of the number of alleles at marker A and marker B. Note that for bi-allelic markers, $\chi^{2'} = r^2$.

Zhao et al. (2005) study involved the use of simulation, which indicated a number of multi-allelic pair-wise measures of LD – and $\chi^{2'}$ was the most reliable predictor of useable marker-QTL LD; that is, the measure of QTL variance that can be explained by the marker. We may want to quantify the extent of LD across a chromosome region that contains several markers, yet statistics like r^2 only consider two loci at a time. The chromosome segment homozygozity (CSH) is an alternative multi-locus definition of LD (Hayes et al., 2003). Consider an ancestral animal that lived many generations ago and has descendants now. The ancestor's chromosome is torn down with each generation until only little portions of chromosome that may be traced back to the common ancestor remain. By descent, these chromosomal regions are identical (otherwise called identical by descent, IBD). The likelihood that two chromosomal segments of the same size and location picked at random from the population originate from a common ancestor (i.e., IBD) without intervening recombination is the CSH. CSH refers to the length of a chromosomal segment, up to the entire chromosome length. The CSH cannot be determined directly from marker data but must be inferred from marker haplotypes for chromosomal segments.

Consider a chromosomal segment with marker locus A on the left end and marker locus B on the opposite end. Alleles A and B define the haplotype. Two of these segments are randomly selected from the population. The haplotype homozygosity (HH) is the likelihood that two haplotypes are identical by state (IBS). The two haplotypes can be IBS in one of two ways: one, either they descended from a common ancestor without intervening recombination and are thus identical by descent (IBD); or two, they are identical by state but not IBD. CSH is the likelihood of one. Given that the segment is not IBD, the likelihood of two is a function of the marker homozygotes. The haplotype homozygosity (HH) is calculated by adding the probabilities of one and two (Equation 7).

$$HH = CSH + \frac{(Hom_A - CSH)(Hom_B - CSH)}{1 - CSH} \quad (7)$$

where *HomA* and *HomB* are the homozygosities of marker A and marker B, respectively. When the haplotype homozygosities and individual marker homozygosities are observed from the data, this equation can be solved for CSH. The estimated haplotype homozygosity can be determined in a similar but more difficult manner for more than two markers.

Another advantage of employing multi-locus LD measurements over pair-wise measures is that they can be less variable. Two sampling mechanisms cause the variation in LD. The initial sampling process is based on finite population size and reflects the sampling of gametes to generate successive generations. The second sampling procedure is the selection of individuals from the population to be genotyped, which is determined by the sample size, n. The large variability of LD measurements is due to the first sampling step. Marker pairs located at different locations in the genome but separated by a comparable distance might have vastly varied r^2 values, especially if the marker separation is small. This is because an ancestral recombination between one set of markers but not the other may have occurred by accident.

Because they aggregate information across numerous loci in a time interval, multi-locus estimates of LD can

minimize variability by averaging some of the impacts of accidental recombinations. Hayes et al. (2003) used simulation to evaluate the variability of r^2 and CSH. They used a mutation-drift model with a constant *N* of 1000 to generate a chromosomal region of 10 cM containing 11 markers. They discovered that when at least four loci were included in the CS computation, CSH was less variable than r^2.

2.1. Origins of Linkage Disequilibrium in Livestock Populations

Migration, mutation, selection, a tiny finite population size, or other genetic processes can cause LD in a population. In an F2 QTL mapping experiment, LD is established between marker and QTL alleles by crossing two inbred lines; in an F2 QTL mapping experiment, LD is created between marker and QTL alleles by crossing two inbred lines.

The fundamental source of LD in livestock populations is widely thought to be finite population size. This is due to the fact that

i. most livestock populations have tiny effective population sizes, resulting in huge quantities of LD;
ii. LD caused by crossbreeding (migration) is substantial when crossing inbred lines but minimal when crossing breeds with similar gene frequencies, and it fades within a few generations (Goddard, 1991);
iii. mutations are likely to have occurred many generations ago; and
iv. while selection is most likely a major driver of LD, its impact is likely to be limited to specific genes, with little impact on the amount of LD 'averaged' across the genome.

2.2. LD Extent in Livestock and Human Populations

If LD is primarily caused by finite population size, it should be less severe in humans than in cattle, because the effective population size in people is around 10,000 (Kruglyak, 1999), whereas in livestock, effective population numbers might be as low as 100 (Riquet et al., 1999). The image is a little muddied by the fact that animal numbers have been substantially bigger, although the effective population size of Caucasians has been much smaller (following the out of Africa hypothesis). As a result, we should anticipate seeing that the r^2 values in livestock are significantly higher than in humans at long distances between markers, but the amount of LD is more equivalent at short distances. This is exactly what has been observed. Moderate LD ($r^2 \geq 0.2$ in humans, for example) often spans less than 5 kb (0.005 cM) depending on the group investigated (Dunning et al., 2000; Reich et al., 2001; Tenesa et al., 2007). In humans and cattle, however, very high levels of LD (e.g., $r^2 \geq 0.8$) only reach a short distance. The first whole-genome LD study in cattle, which used 284 microsatellite markers from 581 maternally inherited gametes in Dutch black and white dairy cows to quantify the extent and distribution of LD, was carried out, with high levels of LD extending over several tens of centimorgans (Farnir et al., 2000). LD in cattle has been confirmed in several following studies (Tenesa et al., 2003; Vallejo et al., 2003; Khatkar et al., 2006a; Odani et al., 2006). Only recently, a study in a large mildly selected cattle population from Western Africa conducted under an extensive breeding system revealed that LD extends over shorter distances than previous studies from developed countries, which was explained by increased selective pressure and/or an admixture process (Thévenon et al., 2007). All of these LD investigations used microsatellite loci that were highly informative but had a low locus density. With the conclusion of the bovine genome sequencing project, it is now possible to determine the extent of LD using dense single nucleotide polymorphism (SNP) marker maps, resulting in significantly higher resolution. SNP markers have minimal genotyping costs, in addition to their abundance in the genome (Snelling et al., 2005). (Hinds et al., 2005). Khatkar et al. (2006b) used SNP loci to generate a first- generation LD map of bovine chromosome 6 in Australian Holstein–Friesian cattle, and D′ to estimate the extent of LD. The distance over which LD is expected to be beneficial for association mapping was discovered to be 13.3 Mb, indicating that the range of LD in Holstein–Friesian dairy cow is broad. McKay et al. (2007) used 2670 SNP markers to build LD maps for eight cow breeds from the *Bos taurus* and *Bos indicus* subspecies, and found that the amount of LD (calculated using r^2) available for association analysis does not surpass 500 kb. The disparities in the degree of LD between McKay et al. (2007) and prior investigations were related to differences in LD reporting measures, notably D′ vs. r^2. Previous investigations have found that D′ overestimates the extent of LD (Ardlie et al., 2002; Ke et al., 2004), resulting in extensive LD at long intermarker distances (Farnir et al., 2000; Tenesa et al., 2003; Vallejo et al., 2003; Khatkar et al., 2006a; Odani et al., 2006). Du et al. (2007) used 4500 SNP markers genotyped in six lines of commercial pigs to determine the degree of LD in pigs. Because paternal haplotypes were over-represented in the population, only maternal haplotypes of commercial pigs were utilized to calculate r^2 between SNPs. According to the findings of their investigation, pigs may have significantly greater LD than cattle. The average value of r^2 for SNPs separated by 1 cM was roughly 0.2. In cattle, LD of this size barely extends 100 kb. The average r^2 in pigs at 100 kb was 0.371. Heifetz et al. (2005) investigated the degree of LD in several breeding chicken populations. They employed microsatellite markers and applied the statistics to determine the degree of LD. They discovered considerable LD over large distances in their populations. For example, 57% of marker pairs separated by 5-10 cM had an $\chi^{2`} \geq 0.2$ in one line of chickens and 28% in the other. Heifetz et al. (2005) pointed out that the lines they studied had small effective population sizes and were largely inbred, so the level of LD in other chicken populations with greater effective population sizes may

differ significantly. The extent of LD in domestic sheep was studied by McRae et al. (2002). Because they employed the D' parameter rather than the r^2 parameter, it's impossible to compare their findings to those of other species. They discovered that high levels of LD lasted for tens of centimorgans and then dropped as marker distance increased. They also looked at D' bias under various conditions and discovered that D' can be skewed when uncommon alleles are present. To establish the true extent of LD, they suggested using the statistical significance of LD in conjunction with coefficients such as D'.

3. Conclusion

QTL mapping can now be done using linkage disequilibrium. The population level connections between markers and QTL are used in linkage disequilibrium (LD) mapping of QTL. Because there are little pieces of chromosome in the current population that are descended from the same common ancestor, these relationships occur. These chromosome segments with no intervening recombination will have identical marker alleles or haplotypes, and if there is a QTL inside the chromosome segment, they will have identical QTL alleles. The genome-wide association test with single marker regression is the simplest of the QTL mapping procedures that take advantage of LD. Due to the availability of tens of thousands of single nucleotide polymorphism (SNP) markers in cattle, pigs, chickens, and sheep soon, doing trials to map QTL in genome-wide scans using LD has recently become practical.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	G.A.I.	M.O.	U.Ş.
C	10	80	10
D	20	80	
S			100
DCP	50	50	
DAI		100	
L	20	80	
W	20	60	20
CR	30	40	30
SR	30	40	30
PM	40	30	30

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

Conflict of Interest

The authors declare that there is no conflict of interest.

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EFFECTS OF *Phyllocoptruta oleivora* (ASHMEAD) ON FRUIT YIELD, QUALITY AND ECONOMIC VALUE IN CITRUS PRODUCTION

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Abstract: Citrus, which represents important species cultivated such as orange, tangerine, lemon, grapefruit and bitter orange and is one of the most important species in the field of fruit growing, is a fruit species with high economic value cultivated in the world and in Türkiye. However, there are diseases, pests and weed species that have negative effects on the yield, quality and economic value of citrus during the production process. The pest *Phyllocoptruta oleivora* (Ashmead) or citrus rust mite (CRM) causes great losses in terms of yield, quality and economic value in citrus fruits grown intensively in Türkiye and its surroundings as well as all over the world. In this study, the effects of CRM pest on the yield, quality and economic value of citrus fruits were investigated in citrus production. In the study where the literature review method was used, the data set consists of articles, bulletins, journals belonging to scientific studies on the subject; publications of academic institutions and organizations; studies of experts on the subject; studies, published information and documents conducted by public and private institutions and organizations with authority on the subject; and information obtained from units operating in the field and involved in the agricultural production process. According to the study findings, CRM damages the leaves and fruits of citrus fruits, reduces tree productivity by 30% and fruit productivity by 2.6-65%. Physical quality characteristics of fruit reduce fruit volume (weight, length, and diameter) by 12.5-25% and increase rind thickness by 13.95-23.81. Fruit chemical quality characteristics reduce fruit juice by 22.68-32.69%, Brix/Acid value by 9.22-27.56; increase Brix value by 4.23-16.36 and acid value by 14.66-80.82. CRM reduces the market value of citrus fruits by impairing the quality of 87% of the total marketable fruit. Damages caused by CRM affect tree productivity (30%), fruit productivity (15%), the quality of total marketable fruits by 87%, thus causing losses in market value and finally, causing a cost of $ 47 per acre in pest control, thus causing losses in total economic value of the fruit. As a result, CRM causes a decrease in fruit yield, fruit quality and fruit economic value in citrus. According to the study findings, prevention of this pest will increase the economic benefit from agricultural production.

Keywords: Citrus, Citrus rut mite, Product yield, Product quality, Product economic value

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1. Introduction

Citrus fruits, which are members of the *Aurantroideae* subfamily of the *Rutaceae* family and are one of the most produced fruits in the world and Türkiye, create significant added value to the world and Türkiye economy in terms of meeting the nutritional needs of people and the raw material needs of agriculture-related industries, as well as the employment it creates. (Turgutoğlu et al., 2023). As the most cultivated fruit species in more than 130 countries in the world (Gill et al., 2023), citrus fruits are a very important agricultural product for the world and Turkish economy due to their high nutritional, market and economic added value and being the main raw material of many industrial branches (Greenhalgh, 2023).

The varieties of citrus fruits with high economic value such as orange, tangerine, lemon and grapefruit, which are the most commercially produced in the world and in Türkiye (Oral and Akpınar, 2015), there are also varieties with less commercial importance compared to other varieties such as bitter orange, kumquat, citron, shaddock and bergamot (Aygören, 2023).

Citrus fruits, which are an important part of healthy and balanced diets, may vary depending on the variety; contain dietary fiber, pectins, essential oils, minerals, approximately 75-90% water, 6-9% sugar (Santiago et al., 2020). Citrus fruits are consumed daily as fresh fruit and a significant portion, approximately 80%, is used as raw material in the food industry, and approximately 40 million tons of organic waste is generated in these productions and consumption processes (Khan et al., 2021). These wastes, especially the fruit peel, seeds and fruit juice wastes that constitute 50% of the fresh weight and are rich in valuable compounds (Santiago et al., 2020), are re-evaluated and have created a new recycling industry branch and market that produces economic added value as raw material or consumer goods for sectors such as chemistry, medicine and cosmetics (Khan et al., 2021).

According to FAO (2022) data for 2022, a total of 76,410,037.46 tons of orange production and a total of 44,179,830.73 tons of tangerine production were realized in the world (Table 1).

When ten-year orange production data in the world are examined, it is seen that orange production increased with the increase in productivity per unit area on approximately the same area until 2018, and it followed a stable course between 2018-2022, whereas tangerine production and production areas have been following an increasing course since 2017 (Table 1). According to FAO (2022) data for 2022 in Türkiye, a total of 1,322,000.00 t of orange production was carried out in an area of 49,536.00 hectares, and a total of 1,865,000.00 t of tangerine production was carried out in an area of 67,854.00 hectares (Table 2).

In recent years, while a decrease has been observed in orange production areas and quantities in Türkiye, a continuous increase has been observed in tangerine production areas and quantities (Table 2). In the world, two other citrus varieties that have significant economic value in commercial terms, lemon and grapefruit, produced 21,529,604.13 tons of lemon and 9,761,754.88 tons of grapefruit in 2022 (Table 3).

In the last decade, a continuous increase has been observed in the production areas and quantities of lemon and grapefruit in the world. In Türkiye, 1,323,000.00 t of lemon and 198,000.00 t of grapefruit were produced in 2022 (Table 4).

As in the world, in Türkiye, a continuous increase in lemon production areas and production has been observed in the last decade, while a general decrease in grapefruit production areas and quantity has been observed (Table 4).

The transformation of resources consumed in the production of citrus fruits and other production processes into products that will obtain the highest benefit, the preservation of these products and their evaluation by processing them as waste and redirecting them to the production process are sustainable activities in the agricultural production process in terms of protecting, improving and developing the existence of nature, humans and other living beings.

There are pests, diseases and weeds (Gonçalves, 2018; Soares et al., 2021) that cause significant damage to the citrus tree and fruit, causing product loss and major economic losses in the citrus production processes (Gonçalves et al., 2018; Jaouad et al, 2020).

Table 1. Total orange and tangerine production area, productivity and quantity in the world (FAOSTAT, 2022)

Years	Oranges			Tangerines, mandarins, clementines		
	Area harvested (ha)	Yield (g ha^{-1})	Production (t)	Area harvested (ha)	Yield (g ha^{-1})	Production (t)
2013	4,121,615.00	177,663.00	73,225,907.61	29,106,411.19	121,287.00	29,106,411.19
2014	4,183,444.00	173,359.00	72,523,714.97	31,275,648.83	130,776.00	31,275,648.83
2015	4,040,461.00	180,147.00	72,787,538.68	33,384,549.49	131,266.00	33,384,549.49
2016	3,987,898.00	183,668.00	73,244,874.27	32,436,221.04	129,217.00	32,436,221.04
2017	3,937,959.00	186,983.00	73,632,991.46	32,947,192.61	129,713.00	32,947,192.61
2018	3,855,676.00	190,566.00	73,476,061.68	34,484,495.96	128,652.00	34,484,495.96
2019	3,946,378.00	193,387.00	76,317,766.17	38,972,615.66	128,782.00	38,972,615.66
2020	3,971,167.00	193,089.00	76,678,733.66	39,227,438.50	129,453.00	39,227,438.50
2021	3,979,466.00	191,775.00	76,316,327.95	42,431,495.76	133,162.00	42,431,495.76
2022	3,976,571.00	192,151.00	76,410,037.46	44,179,830.73	132,121.00	44,179,830.73

Table 2. Total orange and tangerine production area, productivity and quantity in Türkiye (FAOSTAT, 2022)

Years	Oranges			Tangerines, mandarins, clementines		
	Area harvested (ha)	Yield (g ha^{-1})	Production (t)	Area harvested (ha)	Yield (g ha^{-1})	Production (t)
2013	54,759.00	325,290.00	1,781,258.00	38,693.00	243,513.00	942,226.00
2014	54,653.00	325,632.00	1,779,675.00	41,745.00	250,784.00	1,046,899.00
2015	54,298.00	334,598.00	1,816,798.00	43,506.00	265,794.00	1,156,365.00
2016	52,696.00	351,070.00	1,850,000.00	46,569.00	287,109.00	1,337,037.00
2017	51,340.00	379,821.00	1,950,000.00	50,699.00	305,818.00	1,550,469.00
2018	50,806.00	373,972.00	1,900,000.00	51,590.00	319,829.00	1,650,000.00
2019	75,112.00	226,329.00	1,700,000.00	53,554.00	261,418.00	1,400,000.00
2020	46,012.00	289,919.00	1,333,975.00	59,834.00	265,005.00	1,585,629.00
2021	48,177.00	361,583.00	1,742,000.00	60,720.00	299,572.00	1,819,000.00
2022	49,536.00	266,877.00	1,322,000.00	67,854.00	274,855.00	1,865,000.00

Table 3. Total lemon and grapefruit production area, productivity and quantity in the world (FAOSTAT, 2022)

Years	Lemons and limes			Pomelos and grapefruits		
	Area harvested (ha)	Yield (g ha^{-1})	Production (t)	Area harvested (ha)	Yield (g ha^{-1})	Production (t)
2013	1,001,265.00	154,041.00	15,423,634.13	322,213.00	263,579.00	8,492,832.66
2014	1,021,825.00	155,824.00	15,922,457.71	320,295.00	259,805.00	8,321,447.59
2015	1,061,057.00	160,069.00	16,984,227.57	356,483.00	249,196.00	8,883,428.36
2016	1,060,967.00	160,888.00	17,069,662.83	363,883.00	247,299.00	8,998,809.65
2017	1,106,884.00	157,521.00	17,435,737.19	348,016.00	249,260.00	8,674,638.92
2018	1,168,226.00	166,515.00	19,452,694.13	385,247.00	234,669.00	9,040,560.76
2019	1,254,892.00	157,544.00	19,770,009.38	371,621.00	255,396.00	9,491,035.44
2020	1,287,477.00	159,347.00	20,515,518.01	375,999.00	254,264.00	9,560,290.12
2021	1,349,775.00	159,632.00	21,546,659.85	381,703.00	254,176.00	9,701,955.92
2022	1,334,255.00	161,360.00	21,529,604.13	393,704.00	247,947.00	9,761,754.88

Table 4. Total lemon and grapefruit production area, productivity and quantity in Türkiye (FAOSTAT, 2022)

Years	Lemons and limes			Pomelos and grapefruits		
	Area Harvested (ha)	Yield (g ha^{-1})	Production (t)	Area Harvested (ha)	Yield (g ha^{-1})	Production (t)
2013	27,425.00	264,825.00	726,283.00	6,420.00	356,385.00	228,799.00
2014	27,665.00	262,147.00	725,230.00	6,388.00	359,353.00	229,555.00
2015	28,570.00	262,706.00	750,550.00	6,348.00	393,864.00	250,025.00
2016	30,033.00	283,222.00	850,600.00	6,155.00	411,243.00	253,120.00
2017	32,428.00	310,575.00	1,007,133.00	5,359.00	485,165.00	260,000.00
2018	35,911.00	306,313.00	1,100,000.00	5,182.00	482,439.00	250,000.00
2019	40,155.00	236,583.00	950,000.00	5,222.00	477,183.00	249,185.00
2020	46,935.00	253,226.00	1,188,517.00	5,052.00	471,124.00	238,012.00
2021	52,233.00	296,747.00	1,550,000.00	5,039.00	494,146.00	249,000.00
2022	55,246.00	239,474.00	1,323,000.00	4,982.00	397,431.00	198,000.00

One of the most important pests in citrus fruits is CRM which invades the branches, leaves and fruits of all kinds of citrus specie and causes significant damage to fruit yield, quality and economic value (Demard and Qureshi, 2020).

This study was carried out to investigate the effects of CRM on fruit quality traits (physical properties: weight, length, diameter and rind thickness; chemical properties: juice, Brix, acids and Brix/acid ratio) fruit yield and economic value in citrus fruits.

2. General Characteristics of CRM

CRM or silver rust mite was first reported on citrus in Florida in 1879 by entomologist William Harris Ashmead, who first discovered CRM (*Phyllocoptruta oleivora* Ashmead) on citrus and named it after him (Yothers and Mason, 1930). CRM has been found all over the world and is an important pest of citrus, especially in humid regions (McCoy and Lye, 1995), in many countries such as China, Brazil, America, Argentina, Australia, Egypt, and England (Demard and Qureshi, 2020).

CRM, which is thought to have first emerged in Southeast Asia, the homeland of citrus fruits, has the potential to reproduce in large numbers in a short time (; development from egg to egg in 7 days) under suitable growing conditions (Yothers and Mason, 1930; Hall and Simms, 2003; Demard and Qureshi, 2020), however, it has very few effective natural enemies to compensate for this (McCoy and Lye, 1995) and is difficult to detect visually because of its very small structure, posing a significant threat, especially to commercial citrus species (Ferragut et al., 2012).

The eggs of CRM, laid singly (20-30 per day during the summer season) (Beattie and Gellatley, 1983), have a smooth and transparent surface of yellow colour and are seen in groups without contact with each other in the pits on the surface of leaves and fruits (Knapp, 1994). The incubation period of CRM eggs, which is longer during the winter months when temperatures are low, is 3.01 days on average in May, June and July when temperatures are high, and they are found in very large numbers on citrus trees during these months and cause great damage by infecting green fruit (Yothers and Mason, 1930; Beattie and Gellatley, 1983; Sarada et al., 2018). Depending on the temperature, CRM can reproduce and develop very rapidly in the summer (7-10 days) as the temperature drops to a standstill in the winter months, with the reproduction rate and development time of the new generation (14 days or more) decreasing as the temperature drops (Yothers and Mason, 1930; Demard and Qureshi, 2020). Although CRM normally has a short lifespan of one week or less, up to three weeks (maximum 23 days), depending on conditions, it maintains its existence by reproducing very

quickly in very large numbers and reaching adulthood in a short time (Sarada et al., 2018).

CRM, which varies in colour from lemon yellow to yellow, light brown or brown, depending on the presence or absence of fungal disease and the stage of life, is usually yellow in Florida (Burditt et al., 1963). Although CRM is not very active when it emerges from the egg as a larva at the end of the incubation period, it begins to eat the epithermal cells on the surface of leaves and fruits and undergoes metamorphosis twice until it becomes an adult (Qureshi et al., 2023). On citrus trees, CRM is very difficult to detect when they are few in number (Demard and Qureshi, 2020), but when they are very numerous (several hundreds), each mite appears as a speck of dust with a dusty or powdery texture on new and fresh leaves, fruits and small shoots (Yothers and Msaon, 1930; Plantix, 2024). CRM is sensitive to temperature, which is a climate-related event, and as temperatures increase, more favourable environments are created for CRM reproduction and development (Ullah et al., 2022). Reproduction and development do not occur at low temperatures, and they cannot survive at very low temperatures (Ullah et al., 2022). Drought has a similar effect on CRM as temperature, and they cannot survive in extreme drought (Beattie and Gellatley, 1983), CRM is less or not found at all on leaves and fruits exposed to sunlight than on leaves and fruits that do not see sunlight (Futch et al., 2021). Although most of the CRM is washed away during long rainy periods, it is protected in the lower parts of citrus trees and spreads to every part of the tree immediately after rain, so it is not affected by water (Yothers and Msaon, 1930; Sarada et al., 2018). CRM move by jumping, albeit to a limited extent (Li-juan et al., 2000), and it is estimated that they migrate from the upper surfaces of fruits and leaves during the day to the lower surfaces at night, thus protecting themselves from heavy rainfall (Knapp, 1994; Prochemica, 2020). CRM is spread by agricultural control tools, wind, rain splash, insects (ants and spiders, etc.) and birds (Li-juan et al., 2000; Sarada et al., 2018). The CRM is most active in the summer months of April, May, June and July compared to the rest of the year (Demard and Qureshi, 2020), and the citrus species it is most commonly seen and damaged during this period are lemon, grapefruit, orange and tangerine, respectively (Yothers and Msaon, 1930; Beattie and Gellatley, 1983; Sarada et al., 2018).

The fruit peel, which is a natural packaging, protects the fruit flesh from pests, balances the gas exchange of the fruit with the environment, prevents the loss of fruit water and is a determining factor in the shelf life of the fruit from harvest to the end of the marketing process (Petracek, 2002). CRM reside in the peel of citrus fruits and feed on the epidermal cells of the peel, preventing the fruit from breathing in a way.

Typical descriptions of damage caused by CRM on citrus fruit peel have been made. The spots caused by CRM on orange fruit peel are classified according to the severity of damage caused by CRM and the time of fruit ripening (Sarada et al., 2018); when CRM damage is mild, it is called “golden”; If the CRM effect is moderate, the fruit is “black russet” when it is unripe; when CRM is severe and does not spread to the entire peel, this CRM damage is called “russet” (Yothers and Msaon, 1930; Demard and Qureshi, 2020); this CRM damage is called shark ridge in early lemon and grapefruit fruits (Yothers and Msaon, 1930; Knapp, 1994; Bayer, 2024).

3. Effect of CRM on Fruit Yield

Fruit yield, which is considered a kind of reward for the producer in the agricultural production process, is a factor that determines the income to be obtained in return for the labour and production costs incurred during a production period. The amount of citrus fruit produced (kg) and the product price determined under market conditions are the components of the income function. Therefore, it is not enough for the market price to be high, but the production amount must also be high enough to provide income and additional profit. While the extent of the damage caused by CRM to citrus fruits varies according to the level of infection of the fruits by CRM and the type and variety characteristics of the citrus, the damage caused by CRM to citrus fruits reduces fruit yield (Robles-Acosta et al., 2019). Table 5 shows the fruit yield loss rate due to CRM damage in scientific studies on damage caused by CRM in citrus.

In their study examining the effect of CRM on the quality traits of orange fruits in Valencia orange variety under Adana climate conditions, Satar et al. (2020) reported the weight of heavily infected fruit was 67.78% lower than that of uninfected fruit in their study in which they classified the damage caused by CRM in the citrus Valencia orange fruit according to three different levels of infection (light, medium and high bronze) (Table 5). Imbachi et al. (2012) investigated the damage caused by *Polyphagotarsonemus latus* (Banks) and CRM on Valencia variety orange fruits and found the fruit weight value to be 199.00 g in CRM infected orange fruits and reported that damage caused by CRM in citrus cultivation reduced fruit yield by 30%. Kalaisekar et al. (2003) reported that the fruit weight of CRM-infected fruits was 25% lower in orange and 17% lower in lemon compared to uninfected fruits in their study examining the effect of CRM on citrus varieties Sathgudi orange and Rangpur lemon. In their study on CRM, van Brussel (1975) reported that damage caused by CRM in citrus cultivation in Suriname reduced fruit yield on Duncan grapefruit by 25%. In a study conducted by Yothers (1918) on the use of spray methods in pest control in citrus fruits, it was reported that CRM infected orange and grapefruit fruits were 12.50% smaller than healthy fruits, thus fruit yield decreased by 12.5%. In their studies examining effect of the EM (effective microorganisms) on soil, leaves, CRM populations, fruit quality and yield of orange trees in the Pera sweet orange variety, Paschoal et al. (1994) reported that fruit yield in CRM infected orange was reduced by 2.6%.

Table 5. Effect of CRM on citrus fruit weight

	CRM Uninfected Weight (kg)	CRM İnfected Fruit Weight (kg)	*Change Decrease (%)	Citrus Variety & Source of Data
Orange	193.40 g	67.78	65	Valensiya (Satar et al., 2020)
Citrus	-	-	30	Citrus (Imbachi et al., 2012)
Orange	184.80	138.70	25	Sathgudi (Kalaisekar et al., 2003)
Lemon	68.40	56.50	17	Rangpur (Kalaisekar et al., 2003)
Grapefruit	-	-	25	Duncan (van Brussel, 1975)
Orange	151.20	147.30	2.6	Pera (Paschoal et al., 1994)
Citrus	-	-	12.5	Citrus (Yothers and Msaon, 1930)

*= calculation of change rate % = (healthy fruit value - infected fruit value) / healthy fruit value * 100.

The reason why the effect of CRM was very low in the study of Paschoal et al. (1994) can be explained by the fact that the EM, they used in their study, reduced the effect of CRM or the EM population became dominant and reduced the level of infection of CRM.

4. Effect of CRM on Fruit Quality

In the study, the effects of CRM on the physical quality traits (fruit weight, fruit length, fruit diameter and rind thickness) and chemical quality traits (juice content, brix, total acid and brix/acid ratio) of citrus fruits were investigated.

4.1. Physical Quality Parameters in Citrus Fruits

In the Citrus, fruit weight is an important physical quality parameter in terms of commercial evaluation of the fruit as well as creating a preliminary idea about the physical and chemical components present in the fruit volume (Robles-Acosta et al., 2019; Ruiz-Camacho et al., 2023). Kalaisekar et al. (2003) observed that CRM uninfected fruit weight was 184.80 g and infected fruit weight was 138.70 g in Sathgudi orange variety and uninfected fruit weight was 68.40 g and infected fruit weight was 56.50 g in Rangpur lemon variety (The change rate decrease was 24.95% in orange and 17.40% in lemon: Calculation of change rate % = (healthy fruit value - infected fruit value)/healthy fruit value *100). Sarada et al. (2018) reported that CRM reduced fruit weight by 25% in Duncan grapefruit variety in Suriname. Imbachi et al. (2012) investigated the damage caused by *Polyphagotarsonemus latus* (Banks) and CRM on Valencia variety orange fruits and found the fruit weight value to be 199.00 g in CRM infected orange fruits and reported that there was no relationship between CRM and the weight value.

Fruit diameter and height are important physical quality parameters in determining fruit shape index in terms of marketing and customer preference in citrus fruit. CRM causes fruit to fall before ripening, deformity and shrinkage of fruit shape, thus reducing fruit yield and quality (Yang et al., 1994; Puspitarini and Endarto, 2021). Yang et al. (1994) studied the effects of CRM damage on orange fruit growth and abscission in Hamlin orange cultivar, and divided the fruit infected level into five categories (0-19, 20-39, 40-59, 60-79, 80-100) and determined the fruit diameter growth of CRM as percentage. Yang et al. (1994) reported that fruit diameter growth values in the least infected fruits were 2.6% and in the most infected fruits were 1.7% as of December 17, in their observations starting in January 1992. Satar et al. (2020) reported that the fruit diameter of Valencia orange cultivar was 69.84 mm in non-CRM infected fruits and 58.14 mm in CRM heavily infected fruits (Change rate decrease 16.75%). Kalaisekar et al. (2003) observed that fruit diameter in CRM uninfected orange and lemon fruits was 68.00 mm and 49 mm, respectively; and fruit diameter in CRM infected orange and lemon fruits was 57.00 mm and 40.00 mm, respectively (Change rate decrease 16.18% and 18.37%, respectively). Sarada et al. (2018) reported that damage caused by CRM can reduce fruit volume by 25%.

In citrus, fruit height is an important component of the fruit shape index in terms of marketing processes and customer preferences. Yothers (1918) reported in his study that orange and grapefruit fruits infected with CRM developed 12.50% less than in uninfected fruits. Kalaisekar et al. (2003) observed that the fruit length in CRM uninfected orange and lemon fruits was 66.00 mm and 47 mm, respectively; and in CRM infected orange and lemon fruits, the fruit length was 54.00 mm and 38.00 mm, respectively (Change rate decrease 18.18% and 19.35%, respectively). Satar et al. (2020) reported fruit height as 70.89 mm in CRM uninfected fruits and 59.19 mm in the most heavily infected fruits (Change rate decrease %16.50).

Peel thickness in citrus fruits is an important physical quality parameter in terms of the ripening, marketing processes and shelf life durability of the fruit. Peel thickness varies according to the citrus type and market needs, for example, thick peel is desired for fresh consumption and thin peel for juice. The peels of citrus fruits, which consist of 25% peel (Shan, 2016), have turned into a new industry branch (Shan, 2016) in the waste conversion process due to the important functional components they contain (essential oil, pectin, carotenoids, hesperidin and limonene) and are processed in the waste conversion process and used as raw materials for the chemical and pharmaceutical industries (Olife and Mohammed, 2021). CRM damages the fruit skin cells, disrupts the characteristic structure and colour

of the fruit, prevents the development of the fruit, and thus can significantly reduce the marketability and economic value of the fruit (Cartwright and Browning, 1988; Chávez-Dulanto et al., 2018). Kalaisekar et al. (2003) reported the fruit peel thickness of Sathgudi orange variety as 4.30 mm in non-CRM infected fruits, 4.90 mm in heavily CRM infected fruits, and 2.10 mm and 2.60 mm in Rangpur lemon variety, respectively (Change rate increase 13.95% in orange and 23.81% in lemon).

In general, CRM feeds on the epidermal cells on the green branches, leaves and fruits of all varieties and species of citrus trees with its piercing & sucking mouth structures (Futch et al., 2021), causing these cells on the surface of fresh branches and leaves and in the peel of the fruits to lose their ability to photosynthesize (Afzal et al., 2021; Garrido et al., 2023; Roth-Nebelsick and Krause, 2023). CRM has negative effects on fruit volume (weight, height, diameter and rind thickness), one of these negative effects is that in citrus fruits infected with CRM, the water loss rate is 3 times higher than in non-infected fruits, depending on the fruit and environmental conditions, reported by Allen (1978) in a study on the Valencia orange variety. According to Imbachi et al (2012), this can cause a 30% decrease in plant fruit yield. According to scientific study data, CRM reduces the physical quality characteristics, weight, length, diameter and peel thickness, of citrus fruits that are moderately and heavily infected.

4.2. Chemical Quality Parameters in Citrus Fruits

Consumer preferences for juice, one of the intrinsic quality attributes of the citrus fruits, have strong economic importance in determining marketing processes (Rodríguez et al., 2016). Yang (2016) reported that the juice content in citrus fruits was between 40-45%, while EL-Gioushy et al. (2018) reported that the juice content of Washington Navel orange was between 41.99-44.99%. Kalaisekar et al. (2003) observed that the juice ratio in orange and lemon was 62.10 mL and 28.30 mL in CRM uninfected fruits and 41.80 mL and 20.10 mL in CRM infected fruits, respectively (Change rate decrease 32.69% in orange and 28.98% in lemon). Satar et al. (2020) reported the juice ratio of CRM in uninfected fruits of the Valencia orange variety as 49.96% and the juice ratio of the most heavily infected fruits as 38.63% (Change rate decrease %22.68).

Brix value is one of the fruit juice quality indicators used to evaluate fruit juice quality by determining the amount of water-soluble solids content as a measure of ripeness, flavour and sweetness level (Koubaa et al., 2018). Imbachi et al. (2012) observed that the Brix value was 9.70 and that CRM damaged the outer surface of the fruit, but according to the Pearson correlation test result (-0.29; 0.22), there was no significant relationship between the damage caused by CRM and the Brix values. Yothers and Mason (1930) determined the average Brix value of oranges in seven measurements taken at certain intervals from November 1 to December 30 as 10.64% in CRM-uninfected fruits and 11.09% in CRM-infected fruits (Change rate increase 4.23%). Paschoal et al. (1994) observed a Brix value of 10.70 in the control group and a Brix value of 11.30 in the EMPS (effective microorganisms applied to soil and to citrus trees) application where the CRM population was high (Change rate increase 5.60%). Kalaisekar et al. (2003) observed that the brix value in orange fruits without CRM was 11.00, in fruits with CRM infection the brix value was 12.80, and in lemon fruits it was 10.70 and 11.30, respectively (Change rate increase 16.36% in orange, 10.28% in lemon).

Acidity is an important fruit juice quality indicator (Kraus and Popek, 2013) used to determine the process from unripe fruit to full maturity and to determine fruit juice quality (Bartholomew and Sinclair, 1943). Satar et al. observed that the titratable acid value in orange fruits of the Valencia orange variety was 0.73% in CRM-infected fruits and 1.32% in CRM-infected fruits (Change rate increase 80.82). Yothers and Mason (1930) determined the average citric acid value in oranges as 1.16% in CRM-uninfected fruits and 1.33% in CRM-infected fruits (Change rate increase 14.66%). Paschoal et al. (1994) observed the citric acid value as 1.30% in the Pera sweet orange variety in CRM uninfected fruits (control group) and 1.20% in the EMPS application where the CRM population was highest (Change rate decrease 8.33%). Satar et al. and Yothers and Mason findings that CRM affects acidity values, acidity values in CRM infected fruits are higher than in CRM uninfected fruits. Contrary to these findings, Paschoal et al. (1994) observed that titratable acidity value (8.33%) was higher in CRM uninfected orange fruits than in CRM infected fruits.

Brix/acid ratio, one of the fruit juice quality indicators, is used to determine the balance between sweetness and sourness in fruit taste, as the sugar content increases and the acidity decreases as the fruit ripens, and to determine the degree of ripening of the orange fruit (Kaur et al., 2023). Yothers and Mason (1930) determined the average Brix/acid ratio value in oranges as 9.22% in CRM-uninfected fruits and 8.37% in CRM-infected fruits (Change rate decrease 9.22%). Satar et al. (2020) observed that the Brix/titratable acid value in the Valencia orange variety was 14.84% in CRM-uninfected fruits and 10.75% in CRM-infected fruits (Change rate decrease 27.56%). Kalaisekar et al. (2003) reported Brix/acid ratio values in CRM uninfected orange and lemon fruits as 12.79% and 11.38%, respectively; Brix/acid ratio values in CRM infected fruits as 16.20% and 13.56%, respectively (Change rate increase 26.66% in orange and 19.14% in lemon). Paschoal et al. (1994) observed that the brix/acid ratio value in the Pera sweet orange variety was 8.40% in the CRM-uninfected fruits (control group) and 9.70% in the EMPS application where the CRM population was the highest (Change rate increase 15.48%). While the Brix/acid ratio values of Yothers and Mason, Satar et al. confirmed the data in scientific studies on CRM (Knapp, 1994; Sarada et al., 2018; Robles-Acosta et al., 2019; Demard and Qureshi,

2020), on the contrary, Paschoal et al. observed that the Brix/acid ratio values were higher in CRM infected fruits. This difference can be explained by the EM used in the study of Paschoal et al. or by the change in the citrus variety characteristic, water-soluble solids content and acid values of the fruit juice depending on time during fruit ripening (Yang, 2016; Hussain et al., 2017).

According to scientific study data, CRM reduces the chemical quality characteristics of citrus fruits infected at moderate and heavy levels, respectively, by decreasing the juice value, increasing the Brix and acid values and decreasing the Brix/acidity ratio.

5. Effect of CRM on Fruit Economic Value

The economic value losses caused by CRM in citrus fruit production can be listed under three headings: damage to the citrus tree and fruit, market price, and control costs.

Firstly, yield loss in the tree and fruit; Imbachi et al. reported that the biggest effect of CRM on low fruit yield is the damage it causes to the fruit surface, and that production losses due to the damage it causes to the upper leaves may be approximately 30% due to the decrease in the photosynthetic capacity of the citrus tree. The studies conducted by Yothers and Mason (1930) and McCoy and Albrigo (1975), it was reported that CRM damages the epidermal cells in the fruit pod, prevents the physical and chemical development of the fruit and causes a decrease in the yield and quality of the product and causes significant economic losses. Excessive water loss in fruits heavily infected by CRM causes three times more water loss in CRM citrus fruits, the fruit peel cannot fulfil its function, the fruit volume and weight decrease; the acidity value and water-soluble solid amount increase in the fruit with reduced juice volume and all these effects are reflected in the aesthetic appearance of the fruit, as a result, the market value of the fruit whose aesthetic structure is damaged decreases (Robles-Acosta et al., 2019).

Secondly, loss in market value; the damage caused by CRM to the fruit peel reduces the fruit quality and therefore the market value of the fruit, and accordingly, it was determined that 13% of the citrus fruits grown in Florida during the 1915-16 growing season were first class, 41% second class and 46% third class (Yothers, 1918). Sarada et al. (2018) reported that CRM reduced fruit yield by 40% in Duncan grapefruit variety in Suriname. This rate is the amount of decrease in fruit weight caused by CRM by 25%, and 15% is the rate of fruit loss that falls to the ground before it is harvested due to CRM (van Brussel, 1975).

Finally, CRM control costs; Van Leeuwen et al. (2015) reported in their study analysing the global acaricide market that the market for combating mites was approximately 900 million € according to sales records of chemicals used to control mites in 2013. Childers (2011) compared the control methods used by producers with foliar spray programs containing HMO, especially in the control of CRM, and reported that the annual cost of chemical control of mites by citrus growers in Florida in the late 1990s was 171 million US dollars. In a study conducted by Pimentel (2002) on human population growth and biodiversity decline, it was reported that the annual import cost of pesticides used to control arthropod pests in the USA was approximately 20 billion dollars. According to 1977 data, the cost of controlling mites in citrus is $47 per acre. The total cost of control in Florida, United States, is approximately $40 million (Boyd, 1978).

As a result, CRM causes economic losses in citrus trees with moderate or severe infection by reducing tree productivity (30%), fruit yield (2-65%), market value (affecting the market value of 87% of the total fruit amount) and increasing production costs (costs incurred to control CRM are $47 per acre).

6. Conclusion

This literature review was conducted to investigate the effects of damages caused by CRM in citrus on fruit yield, fruit quality traits and economic value of the fruit. According to the findings of the scientific study, CRM reduces fruit yield by 2.6 - 65% depending on the level of infection and citrus variety. Similarly, it reduces fruit physical quality traits in the range of fruit volume (weight, length and diameter) by approximately 17.40 - 25.00% and increases rind thickness by 13.95 - 23.81%. In the chemical properties of fruits heavily infected with CRM, it was observed that fruit juice decreased by 22.68 - 32.69% and Brix/acid ratio by 9.22 - 27.56%, Brix value increased by 4.23 - 16.36% and acidity value increased by 14.66 - 80.82%. In citrus, the damages caused by CRM to citrus trees are 30% reduction in tree productivity, 40% reduction in fruit yield due to damages it causes to fruit (15% of fruit falling before ripening, 25% of damage to fruit at the harvest stage), 87% reduction in the quality of marketable fruit quantity and causing it to be sold at a lower market price, and finally, the cost of controlling CRM ($47 per acre) are the economic losses caused by CRM in the economic value of the fruit. Scientific studies support that CRM pest significantly affects fruit yield, fruit quality characteristics and fruit economic value in citrus cultivation. As a result, measures should be taken against CRM pest and more research should be done on this subject in order to reduce agricultural production losses and preserve its economic value.

Author Contributions

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

	H.S.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
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 author declare that there is no conflict of interest.

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SOME REPRODUCTIVE DEFECTS IN FARM ANIMALS

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Abstract: The importance of animal products for human health and development cannot be ignored. It is important for the sustainability and profitability of the enterprises that the animals raised do not have problems in terms of reproduction, as well as in terms of meeting the demand for animal products regularly. Depending on the type of animals used in production, the number of offspring obtained in a production period varies according to the reproductive activities of the animals and the care and management methods of adding the obtained offspring to the economy. In terms of reproductive efficiency in animal production enterprises, few or many reproductive problems seen in animals do not completely prevent the realization of reproductive performance at the optimal level. The formation of a new individual in farm animals covers the process until birth, such as the formation of gamete cells, fertilization, embryo implantation and fetal development. At the same time, the postnatal factors that take place from the birth of the new individual to its entry into the economy are also part of this process. Depending on the animal species, rates of embryo losses, genetic defects, losses due to disease and losses due to care and feeding errors can vary. The care that owners take in the selection of animals and the care and management methods they apply to them is decisive in reducing possible losses, obtaining more offspring and ensuring the profitability and sustainability of the business. In this review, it is aimed to explain the problems such as embryo losses, fetal losses and loss of offspring during and after birth and the precautions that can be taken against these problems in the process from the beginning of the formation of a new individual in livestock breeding until the new individual is brought into the economy.

Keywords: Reproductive disorders, Embryo losses, Care-management errors, Offspring losses

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1. Introduction

The reproductive health of farm animals is one of the cornerstones of the livestock sector. The reproductive health of farm animals is important for the sustainability and profitability of farms and for meeting the demand for animal products regularly. However, reproductive defects are a serious problem that is frequently encountered in farm animals and can occur for various reasons. Reproductive defects reduce the reproductive efficiency of animals, decrease the survival rate of offspring and may adversely affect their general health status. This situation causes economic losses in farm enterprises and threatens animal welfare.

Reproduction in farm animals is a critical trait for sustainability, product continuity, business profitability and animal breeding studies. As a result of increasing the amount of product obtained per unit animal, reproductive results have started to experience disruptions.

In animal production, the main objective is to ensure that reproductive activities are carried out regularly and smoothly, that each gamete cell, which has the potential to be born alive as a new individual, develops without loss and is brought to the economy. In order to achieve this goal, losses should be avoided or minimized at all stages of reproduction and from the birth of a healthy individual until it is brought into the economy. When reproductive losses are analyzed, it is reported that the highest rate (20%-50%) occurs during the acceptance of the embryo by the maternal (Dixon et al., 2007; Abdel-Mageed and Abd El-Gawad, 2015; Campanile et al., 2021). In developing reproductive biotechnologies, it is reported that losses in in-vitro embryo production are reported to 60%-70%, and pregnancy rates in in-vitro produced embryos vary between 10%- 40% (Ealy et al., 2019).

Embryo losses prior to maternal acceptance vary between species and, within species, between breeds. The lack of satisfactory progress in reducing pre-implantation embryo losses is due to a poor understanding of the functioning of this period (Campanile et al., 2021).

Reproductive defects can be caused by genetic factors (Berry et al., 2017; 2018), environmental factors, inadequate care and feeding conditions and infections (Ali et al., 2015; Akbarinejad and Robert, 2024). Genetic factors include structural abnormalities and hereditary diseases. Environmental factors that cause reproductive defects include hygienic conditions of the environment, climate change and stress factors. Nutrition is an important factor that directly affects reproductive health.

Nutritional deficiency can impair the functions of reproductive organs and reduce reproductive performance. Infections, on the other hand, can cause reproductive disorders and infertility by directly affecting the reproductive organs (Ali et al., 2015; Akbarinejad and Robert, 2024).

The formation of the new individual in livestock includes processes starting from the production of gametes and continuing until parturition, such as fertilization, embryo implantation and fetal development (Uju and Unniappan, 2024). At the same time, postnatal factors, which continue until the parturition of the new individual and its introduction into the economy, are also part of this process. Depending on the animal species, rates of embryo losses, genetic defects, losses due to disease and losses due to care and feeding errors can vary (Dixon et al., 2007; Abdel-Mageed and Abd El-Gawad, 2015; Ealy et al., 2019; Campanile et al., 2021). The care of the owners in the selection of the animals and the care and management methods they apply to them is decisive in reducing possible losses, obtaining more offspring and ensuring the profitability and sustainability of the farm.

In this review, it is aimed to present the problems such as embryo losses, fetal losses, and loss of offspring during and after birth and the measures that can be taken against these problems in the process from the beginning of the formation of a new individual until it is brought into the economy.

2. Female Reproductive System and Offspring Yield

The formation of a new individual in farm animals begins with the coming together of mature female and male gamete cells in a suitable environment. The production of mature female and male gamete cells takes place in healthy individuals that reach sexual maturity and do not have anatomical and physiological problems in the reproductive system (Lea and England, 2019). In normal production systems, errors in the care and feeding conditions of male and female individuals affect sexual maturity and cause a decrease in the number of offspring to be obtained from an individual for a lifetime. In the process following the fusion of gametes (fertilization, embryo), the primary responsibility for the survival, development and birth of the new individual depends on the female individual who carries it as a zygote, embryo and fetus (Lonergan et al., 2023). Here, the possible situations that can be encountered in the process starting from the gonad structures responsible for the production of gametes related to each sex and until the age of the offspring's economicization after birth will be evaluated.

2.1. Dysfunction of the Ovaries

Ovarian dysfunctions in farm animals are important health problems affecting the reproductive ability of female animals. These disorders reduce fertility rates by preventing the production of sufficient and healthy eggs from the ovaries. Ovarian dysfunctions can occur as a result of the combined effects of one or more of several causes such as disease, infection (Davis, 2019), nutritional deficiencies and imbalances (Ali et al., 2015), including temperature increase due to climate change (Chen et al., 2021).

2.1.1. Ovarian cysts

Ovarian cysts are a common ovarian disorder in farm animals. These cysts are fluid-filled sacs that grow abnormally in the ovary and adversely affect the reproductive cycle. Clinical signs vary depending on the number of cysts and the degree of luteinization. Ovarian cysts are more common in dairy cattle and reproductive problems in dairy cattle triple when milk production doubles (Lonergan et al., 2023; Steeneveld et al., 2024). Ovarian cysts are rare in sheep and goats. Ovarian cysts cause impaired ovarian function due to disruption of the hormonal mechanism (Viana et al., 2021), frequent and irregular estrus and constant desire to mate in animals (Kaymakçı, 2002; Roy et al., 2024).

2.1.2. Ovarian hypoplasia

Ovarian hypoplasia is a condition in which the ovaries are smaller and underdeveloped than normal. Ovarian hypoplasia can be found in the ovaries of farm animals, either unilaterally or bilaterally. Depending on the degree of hypoplasia and whether it is unilateral or bilateral, the animal may be infertile or sterile. This is a serious condition that negatively affects the animal's reproductive capacity and general health. It may occur due to genetic factors, embryo development, hormonal imbalances and nutritional deficiencies (Kaymakçı, 2002; 2016; Rhoads, 2023; Akbarinejad and Robert, 2024).

2.1.3. Freemartinismus

In twin or triplet pregnancies, when one of the offspring is male and the other female, various developmental anomalies can be observed in the female offspring's reproductive organs. This is due to the more rapid development of the male offspring's testicles and the hormonal effect on the development of the female offspring's ovaries and other reproductive tracts. Female offspring in this situation are called freemartins (Kaymakçı, 2016; Bozkurt et al., 2024).

Freemartinismus is especially common in cattle, but similar conditions can also be seen in other farm animals (Özhan et al., 2012; Kaymakçı, 2016). The incidence of freemartinism in sheep ranges from 0.23% to 1.23% (Davis, 2019).

2.2. Fertilization Disorders

Fertilization is the first phase of reproduction and takes place when the sperm cell and the egg cell successfully unite. Disorders that occur in this process are serious problems that negatively affect reproductive efficiency and lead to economic losses (Hafez and Hafez, 2000; Lonergan et al., 2023; Steeneveld et al., 2024). Fertilization disorders are generally divided into two main categories: failed fertilization and abnormal fertilization.

Fertilization failure can be caused by the death of the egg before it meets the sperm, structural and functional

abnormalities in the egg, poor sperm quality or obstruction of the oviduct. Abnormal fertilization, on the other hand, can result from a variety of causes that interfere with the normal fertilization process and prevent the development of a healthy embryo (Hafez and Hafez, 2000).

2.2.1. White heifer disease

White Heifer Disease is an anatomical defect characterized by various degrees of developmental delay of the Müller ducts (Ishiyama et al., 2019). This condition manifests itself in anomalies such as a perforated or partially closed hymen, absence of the cervix or the cranial part of the vagina. In addition, other developmental disorders such as unilateral development of the uterus can be observed (Kaymakçı, 2002; Ishiyama et al., 2019). In a study conducted with Holstein cattle, it was reported that defects related to Müller duct (fusion, obstruction) development occurred at a level of 2.09% (Ishiyama et al., 2019).

2.2.2. Failure of oviduct development

It is defined as a developmental disorder related to the reproductive system in farm animals. It has been observed that some or all of the oviduct of animals in this condition does not develop (Kaymakçı, 2002; Ishiyama et al., 2019). As a result of breeding efforts for high milk yield in dairy cattle, it has been reported that problems related to ovarian tract development deficiency have increased (Mee, 2012; Ishiyama et al., 2019; Steeneveld et al., 2024). This result reveals the necessity to implement production models that provide a balance between productivity increase, health and welfare in animals (Galioto et al., 2017; Segerkvist et al., 2020; Whatford et al., 2022).

2.2.3. Vulva atresia

The vulva is markedly reduced in size, creating an impediment to copulation. This usually makes it impossible for even pregnant individuals to give birth. Such animals cannot give birth normally even if they become pregnant (Kaymakçı, 2002).

2.2.4. Delay of ovulation

In normally cycling animals, the time of ovulation can be altered due to many factors such as temperature stress (Rhoads, 2023), sudden changes in nutrition, dehydration, disease and sudden hormonal imbalance. Such situations can lead to failure of inseminations. Delayed ovulation is mostly hormonal and is due to insufficient secretion of LH. In some cases, oval-bursal adhesions may completely cover the ovary and prevent ovulation (Kaymakçı, 2002; Rhoads, 2023).

2.3. Embryonic Losses and Fetal Losses

Most reproductive defects in farm animals result in pregnancy loss. Pregnancy loss can be divided into embryonic and fetal (Hafez and Hafez, 2000).

Embryonic death is one of the important phenomena that usually cause temporary infertility in farm animals (Kaymakçı, 2002). Embryonic death refers to the death of fertilized eggs and embryos until the end of implantation. In farm animals, approximately 25% to 40% of embryos are lost depending on the species (Hafez and Hafez, 2000). In cattle, up to 50% of embryos are lost in the first 7 days of pregnancy; however, failure of implantation of the conceptus and defects in placentation can cause up to 20% additional pregnancy losses between days 20 and 60 (Davenport et al., 2023). These embryonic losses are often unrecognized by breeders and the dead embryo is usually resorbed by the organism and absorbed into the body (Hafez and Hafez, 2000).

The fetal period refers to the period during which the embryo implants and continues to develop in the uterus until it leaves the uterus at birth. Losses that occur during this period occur at a later stage than embryo losses and are usually characterized as miscarriage or premature birth. Fetal losses can significantly reduce the reproductive efficiency of animals and cause serious economic losses on farms. Fetal mortality is lower than embryonic losses and varies between 3-5% depending on the species (Çam et al., 1998; Dixon et al., 2007; Abdel-Mageed and Abd El-Gawad, 2015; Koyuncu and Duymaz, 2017; Ealy et al., 2019; Campanile et al., 2021).

2.4. Perinatal and Postnatal Losses of Offspring

2.4.1. Losses of offspring during the perinatal period

Perinatal losses are defined as the death of offspring shortly before birth, during birth or within 7 days of birth. Perinatal losses, including stillbirths, account for a large proportion of losses between birth and weaning. Offspring mortality during birth can be as high as 30%, and 80-90% of deaths occur within the first 7 days after birth (Hafez and Hafez, 2000; Celi and Bush, 2010; Koyuncu and Duymaz, 2017; İder and Ertürk, 2023). Offspring losses during and after birth on farms are an indicator of animal welfare problems and represent a significant economic loss (Koyuncu and Duymaz, 2017).

The birth process is a stage that carries vital risks for both maternal animal and offspring. Complications that occur during parturition can lead to the loss of the female animal during parturition or stillbirth of the offspring. This can vary depending on the difficulty of labor, the structure of the birth canal, the size of the offspring or the general health of the female animal.

Difficult parturition (dystocia) in animals is defined as a situation in which parturition does not take place within a certain, species specific period of time, is delayed or cannot take place without any intervention (Atasever et al., 2017). Dystocia can be caused by fetal and maternal factors.

Fetal dystocia can be caused by various reasons such as fetopelvic incompatibility, offspring position problems, offspring abnormalities, birth canal problems, maternal health problems, inadequate delivery assistance and multiple litters (Hafez and Hafez, 2000).

Maternal dystocia in farm animals is a condition in which the birth process becomes difficult due to functional disorders of the maternal birth canal or reproductive system during labor. This can be caused by factors such as narrowing of the maternal birth canal, inadequate contractions, birth position problems, genital infections

or uterine abnormalities (Hafez and Hafez, 2000; Jacobson et al., 2020).

Postpartum litter losses are often closely associated with low or high condition scores, poor maternal behavior, inadequate colostrum intake, infectious diseases and environmental factors. Against these factors, the breeder is more likely to intervene in offspring survival (Çam et al., 1998; Hafez and Hafez, 2000; Celi and Bush, 2010; İder and Ertürk, 2023). The pre-weaning mortality rate after live birth varies worldwide between 8% and 30% in lambs and 11.5% to 37% in kids (İder and Ertürk, 2023).

2.4.2. Postnatal offspring losses

Postnatal offspring losses are deaths from the end of the perinatal period until weaning (Koyuncu and Duymaz, 2017). The losses of offspring during this period are usually caused by factors such as postnatal trauma, environmental conditions, inadequate colostrum intake, poor maternal behavior, infectious diseases and malnutrition.

3. Male Reproductive Defects

Male reproductive efficiency in farm animals is associated with several phenomena. These are sperm production, sperm viability and fertilization capacity, sexual desire and mating ability. Infertile males can be easily detected, but males with low reproductive efficiency can pose serious problems and cause economic losses for breeders and the artificial insemination industry (Hafez and Hafez, 2000).

Male reproductive defects in farm animals are caused by various genetic, anatomical and environmental factors that can significantly reduce fertility. These disorders negatively affect not only animal welfare but also production efficiency.

3.1. Cryptorchidism

It is a condition in which one or both testicles do not descend to their normal position but remain in the abdomen. It is a hereditary defect seen in farm animals. This condition negatively affects spermatozoite production and can lead to infertility (Hafez and Hafez, 2000; Olğaç and Sabuncular, 2023).

Since body temperature prevents the formation of viable spermatozoites, an animal with both testes in the abdominal cavity is infertile. If one of the testes has descended into the scrotum, this male animal is capable of fertilization. It has been observed that sexual desire persists in animals with cryptorchidism even if both testes have not descended into the scrotum (Kaymakçı, 2016).

3.2. Testicular Hypoplasia

It is a condition in which the testicles do not reach normal size or do not develop fully. Testicular hypoplasia, which is a congenital defect, is seen in all farm animals, especially in bulls of various breeds. This condition may cause a decrease in sperm production or infertility (Kaymakçı, 2016; Hafez and Hafez, 2000).

3.3. Testicular Degeneration and Atrophy

They are common reproductive disorders in farm animals and can negatively affect the fertility capacity of the animal. The germinal epithelial cells in the testis are highly sensitive to various factors such as temperature, infection and trauma. Due to this sensitivity, testicular degeneration and atrophy can be seen in male animals in different sizes according to their etiologies (Watt, 1972; Roberts, 1986; Ladds, 1993). Depending on etiology, testicular degeneration may be mild or severe, focal or diffuse, unilateral or bilateral. Depending on the duration, severity and type of this condition, degeneration may have temporary or permanent negative effects on reproductive functions (Watt, 1972; McEntee, 1990; Younguist, 1997).

3.4. Impotence

Impotence refers to the situation in which spermatogenesis takes place in some farm animals, but the testicles do not produce enough testosterone so that the animals show no desire to mate. This situation manifests itself in the animals avoiding the search for females in heat or remaining indifferent to them (Pickett et al., 1977; Hafez and Hafez, 2000; Kaymakçı, 2016).

4. Conclusion

Reproductive health in livestock is of paramount importance for the sustainability and profitability of the livestock sector. Genetic, environmental and nutritional reproductive defects can adversely affect male and female reproductive efficiency and cause embryo, fetal and offspring losses. The causes and effects of reproductive defects can differ at each stage of this process.

Fertilization is the first stage of the reproductive process, resulting from the successful union of spermatozoon and oocyte. However, poor sperm quality, inadequate sperm motility or reduced sperm count in male animals and ovulation problems and reduced fertilization ability of the oocyte in female animals can negatively affect this process. These problems can be caused by genetic factors, hormonal imbalances, infections or environmental stressors. In particular, extreme temperatures, nutritional deficiencies or poor care conditions can negatively affect fertilization success. In order to prevent fertilization problems, it is necessary to optimize the nutritional and care conditions of the animals and to perform genetic evaluation and reproductive health checks regularly.

After fertilization, embryo development begins, but various defects and losses can occur during this process. One of the most common causes of embryo loss is genetic disorders. In addition, hormonal imbalances, infections and nutritional deficiencies can also lead to embryo loss. In order to reduce embryo loss in farm animals, infections affecting reproductive health should be controlled, animals should be properly fed and protected against stress.

The fetal period refers to the period during which the embryo continues to develop in the womb. Losses that occur during this period occur at a later stage than

embryo losses and usually manifest as miscarriage or premature birth. Fetal losses can be caused by genetic abnormalities, nutritional deficiencies, infections, trauma or hormonal imbalances. It is common in farm animals and can be associated with uterine infections or nutritional deficiencies during pregnancy. To prevent fetal loss, close monitoring of animals during pregnancy, appropriate care and nutrition programs, and prevention and treatment of infections are essential.

Parturition is a life-threatening stage for both maternal and offspring. In order to prevent losses during birth, it is necessary to closely monitor the birth process and intervene when necessary. Veterinary supervision plays a major role in reducing the risks. In addition, prenatal care can be effective in preparing the animal for delivery and preventing complications.

The postpartum period is a critical period for the health of the newborn offspring and the maternal. Protection of newborn offspring in the postpartum period can be achieved by strengthening their immune system and proper nutrition. Therefore, careful postnatal care and monitoring the health of the maternal play a critical role in preventing losses.

As a result, careful and informed management of all stages of the reproductive process in farm animals is essential to protect animal welfare and minimize production losses. By optimizing reproductive health, minimizing genetic and environmental risks, improving nutrition and care conditions, losses before and after birth can be significantly reduced. In this way, the sustainability of farms can be ensured by improving animal welfare, while at the same time the profitability and productivity of enterprises can be maintained in the long term.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	Ö.F.Y.	M.A.Ç.
C	50	50
D	50	50
S	50	50
DCP	50	50
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50

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 declare that there is no conflict of interest.

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