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Research Article
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THE EFFECT OF REARING SYSTEM ON PLUMAGE QUALITY AND FOOT-PAD DERMATITIS IN GUINEA FOWLS AND PHEASANTS

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Abstract: This study aims to determine welfare parameters such as foot-pad dermatitis (FPD) and plumage quality (PQ) scores at different slaughter ages in barn and free-range rearing systems for guinea fowl and pheasants. The birds randomly distributed with half of 200 Pheasant and 200 guinea fowl chicks were reared in both indoor and free-range systems. Game birds were examined for both FPD score and feather score (PQ) at 6, 12, 14, 16 and 18 weeks of age. The litter moisture content was also measured at 14, 16 and 18 weeks of the growing period. In guinea fowls, litter moisture content differed significantly according to the rearing system ($P<0.01$). In both game birds, gender differences were determined in wing feather quality for 6 weeks ($P>0.05$). In terms of FPD, there was no difference in guinea fowl according to the rearing system, gender and slaughter age, but there was a difference in pheasants according to the slaughter age. In guinea fowl, the head PART feather quality was lower than the barn system ($P<0.05$) and the lowest feather quality was found at 12 weeks of age in terms of slaughter age ($P<0.01$). It was determined that in terms of back, wing and tail feather quality of pheasants, those reared in closed system were lower ($P<0.01$). As a result, it was determined that guinea fowls had lower litter moisture in the free-range system, but this did not affect FPD score. On the other hand, it was found that FPD scores increased with age in pheasants. It was determined that free-range system was better in terms of head part feather quality in guinea fowls and back, wing and tail feathers were better in this system, similarly in pheasants. In terms of feather quality, a free-range system is recommended for better welfare for both species, especially pheasants.

Keywords: Game birds, Welfare, Rearing system, Pheasant, Guinea fowl

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1. Introduction

Although pheasants originate in Asia and guinea fowls in Africa, they spread to all continents due to their high adaptability. The breeding of game birds is a common practice in many European and American countries, and they make up a large proportion of game birds in these countries (Dahlgren, 1988; Nielsen, 2009; Jameel et al., 2022; Śmiecińska et al., 2022). These game birds are largely rearing in our country for the purpose of stocking the nature (Uçar and Sarıca, 2018). In some studies, data on the reproduction and growth performances of pheasants and guinea fowls were obtained and these studies give an idea about their potential (Yamak et al., 2016a; Yamak et al., 2018; Yamak et al., 2020; Boz et al., 2022). Welfare parameters such as PQ and FPD are important for the performance characteristics of poultry species. While most studies of poultry welfare have been conducted on broilers, laying hens and turkeys, studies on other species such as pheasants and guinea fowl are very limited (Nielsen, 2009).

FPD is characterized by necrotic and inflamed lesions ranging from superficial to deep on the surface of the foot-pad. Progressive deep inflammation can lead to chronic abscess and fibrosis of underlying structures (Greene et al., 1985). The thick epidermis of the foot-pad has a similar structure to scales, claws and beaks, but the keratin components of foot-pad are weaker and more sensitive due to thinner cell layers and the absence of keratin-bound calcium salts (Stettenheim, 1972). FPD is associated with decreased live weight and leg meat yield and increased carcass condemnations (Abraham et al., 2021). Factors such as ration content, litter type, stocking density, rearing system, age and litter moisture are also effective in FPD (Andrews and McPherson, 1963; Jensen et al., 1970; Harms et al., 1977; Dawkins et al., 2004; Bilgili et al., 2006; Buijs et al., 2009; Liebl et al., 2022).

The development of feathers in poultry is one of the most important physiological processes in the pre-breeding stage (Murphy, 1996). Naturally, chick plumage is such that it develops during the first weeks of life, while still under parental care ages. Feather structure is simpler in young birds because chicks often face a trade-off between investment in feather quality and rapid body growth (Butler et al., 2008). The higher PQ is likely to

increase thermoregulation and flight capabilities (Nilsson and Svensson, 1996; Swaddle et al., 1996). Factors similar to those that affect FPD affect PQ, and in addition, feather pecking is the most important factor in PQ (Brunberg et al., 2011; Bennewitz et al., 2014). When raising poultry species such as pheasants and guinea fowl in captivity or in large numbers in a field, the main obstacle is initiation of harmful pecking, which leads to a decline in their welfare (Rodenburg et al., 2013; Jensen, 2018). This causes serious economic losses on many farms (Draycott et al., 2002; Draycott et al., 2005). The main factor affecting harmful pecking in game birds is the size of rearing area (Kjaer, 2004). The smaller size of the group and the use of traditional and furnished cages are associated with lower levels of harmful pecking compared to the larger bird groups typical of free-range systems (Zimmerman et al., 2006; Lay Jr et al., 2011). Ranging outside for a longer period of time with free access severely reduces harmful pecking in birds (Bestman and Wagenaar, 2003; Leone et al., 2010). There is a difference in PQ between the sexes and a higher pecking was observed in male pheasant flocks (Zapletal et al., 2011). Various studies suggest that feather pecking in different rearing systems for poultry species will decrease if they are encouraged by foraging on the litter, grass-straw hanging from perforated baskets, or other objects that can be pecked (Homeyer, 1969; Nørgaard-Nielsen et al., 1993; Channing, 1998; Huber-Eicher and Wechsler, 1998; Wechsler and Huber-Eicher, 1998; Colton and Fraley, 2014; Coton et al., 2019).

There are many studies on FPD and PQ in species such as chicken and turkey, especially in broilers, but since the number of such studies is low in species such as pheasant and guinea fowl, our study is important in terms of being to the literature. This study aims to determine welfare parameters such as FPD and PQ scores at different slaughter ages in Barn and Free-Range Rearing Systems for Guinea Fowl and Pheasants.

2. Materials and Methods

2.1. Animal Material

All procedures were approved by the Ondokuz Mayis University Ethical Committee for Experimental Animals. Guinea fowl eggs were collected from the flock reared at the Turkish Ministry of Agriculture and Forestry Yozgat Breeding Station, and 200 day-old Guinea fowl keets were randomly selected for use in the experiment. Pheasant (*Phasianus colchicus*) eggs were collected from a flock reared at the Turkish Ministry of Agriculture and Forestry Samsun Breeding Station, and 200 day-old Pheasant chicks were randomly selected for use in the experiment.

2.2. Rearing System and Conditions

Guinea fowl keets were randomly allocated to pens belonging to either an indoor ("barn") or outdoor-access ("free-range") production system that was interspersed within windowed houses, with 4 pens per system and 25 keets per pen. Groups were formed in the same way in pheasant chicks. Pens (3.5 × 3.5 m) were separated and covered by 0.5 × 0.5 cm wire mesh to prevent birds from flying between pens. Each pen contained one round feeder and one round drinker. The indoor pen also contained an 8-cm layer of wood shavings used as litter, and no fresh litter was added during the production period. Heating was provided by infrared heaters, and economic white bulbs were used for lighting. A 24-hour light regime was applied during the first 3 days. Light was incrementally decreased to 20 h over d 3 to 14 and then remained constant until 6 wk, after which natural lighting (app. 14 h/d) was applied until slaughter. After 6 wk of age, birds in the outdoor free-range system were given 24-hour access to outdoor pens measuring 14 × 3.5 m through a single doorway measuring 50 × 90 cm. All birds were fed *ad libitum* with the same commercial layer chicken diet based on corn and soybean meal until 12 wk of age (19% Crude Protein and 11.72 MJ/kg Metabolisable Energy) and with layer chicken developer diet from 12 wk until the end of the experiment (16% Crude Protein and 11.30 MJ/kg Metabolisable Energy).

2.3. Litter Moisture

Litter moisture content was measured at Weeks 14, 16 and 18 using samples collected from 5 different points in each pen. Samples were oven-dried at 105°C until weight-loss stability was achieved, and dry matter (%) was recorded (Sarıca and Çam, 1998).

2.4. Plumage Quality (PQ)

Feathers of all pheasant and guinea fowls were scored individually at 6 weeks and slaughter ages of 12, 14, 16 and 18 weeks, with scores obtained from six (head, neck, breast, back, wings and tail) body parts (Yamak and Sarica, 2012) using a 4-point scoring system to rate plumage conditions, as follows: 4 = completely protected by feathers; 3 = local deterioration (up to 1/3 loss);2 = significant deterioration (between 1/3 to ½ loss) and 1 = bare skin (Tauson et al., 1984). First feather-change (moult) was also noted. The feather score was made as an indicator of PQ.

2.5. Foot pad Dermatitis (FPD)

FPD of all pheasant and guinea fowls were scored individually at 6 weeks and slaughter ages of 12, 14, 16 and 18 weeks. FPD incidence was evaluated on both left and right foot pads and webs using a 4-point scale, as follows: 0 = no lesions; 1 = lesions on < 25% of pads; 2 = lesions on 25%-50% of pads; 3 = lesions on 50%-75% of pads; 4 = lesions on > 75% of pads (Sarica and Yamak, 2010). FPD scores for pads and webs as well as total FPD scores are also given.

2.6. Statistical Analysis

Analysis of variance (ANOVA) was used to evaluate data. Factorial variance analysis of FPD scores and PQ, rearing system, age and gender was performed using the non-parametric Friedman's Test, with Kruskal-Wallis testing conducted for traits whose interactions were found significant. Differences among groups were examined using Duncan's multiple comparison test. All data analysis was performed using SPSS Software Version

20.0 licensed to Ondokuz Mayis University (SPSS Inc., Chicago, IL, USA).

3. Results

In guinea fowls, litter moisture content, or litter dry matter content, differed significantly according to the rearing system (P<0.01). However, no relationship was found between slaughter age and litter content (Table 1). Although a difference was found between rearing system and litter moisture content in guinea fowls, no difference was found in pheasants. Again, slaughter age was not effective on litter content in pheasants, similar to guinea fowls (Table 2).

General averages of FPD and PQ scores were given for guinea fowls (Table 3) and pheasants (Table 4) by sex, as they were reared in a barn system at the first 6 weeks of age. In both game birds, gender differences were determined in wing feather quality (P>0.05). Although the wing part feather quality score of males was found to be better in guinea fowls, it was found to be lower in male pheasants.

Effect of rearing system and slaughter age on FPD in Guinea Fowls and Pheasants are shown in Table 5 and Table 6, respectively. In terms of FPD, there was no difference in guinea fowl according to the rearing system, gender and slaughter age, but there was a difference in pheasants according to the slaughter age (FPD score increased as the slaughter age increased).

Table 1. Litter moisture content of guinea fowls at different rearing system and ages

Rearing System	Age (Week)	Moisture	Dry Matter
		%	
	14	11.63	88.37
FR	16	12.76	87.23
	18	12.79	87.21
	14	13.90	86.10
IN	16	14.20	85.80
	18	14.46	85.54
SEM		0.264	0.264
Effects			
Rearing System		**	**
FR		12.39[b]	87.61[a]
IN		14.18[a]	85.81[b]
Age		NS	NS
14		12.76	87.23
16		13.48	86.52
18		13.62	86.38
Rearing System x Age		NS	NS

FR= free-range system; IN= indoor system; SEM= standart error of mean; **= P<0.01.

Table 2. Litter moisture content of pheasants at different rearing system and ages

Rearing System	Age (Week)	Moisture	Dry Matter
		%	
	14	12.81	87.19
FR	16	15.72	84.28
	18	13.23	86.78
	14	14.02	85.98
IN	16	13.35	86.65
	18	14.82	85.18
SEM		0.474	0.474
Effects			
Rearing System		NS	NS
FR		13.92	86.08
IN		14.06	85.94
Age		NS	NS
14		13.42	86.58
16		14.53	85.47
18		14.02	85.97
Rearing System x Age		NS	NS

FR= free-range system; IN= indoor system; SEM= standart error of mean; ** P<0.01.

Table 3. FPD and PQ at week 6 in Guinea fowls [X±Sx (med:min-max)]

		PQ					
Gender	FPD	Head	Neck	Back	Wing	Tail	Breast
Male	0±0 (0:0-0)	4±0 (4:4-4)	4±0 (4:4-4)	3.93±0.24 (4:3-4)	3.76±0.04 (4:3-4)[a]	3.96±0.02 (4:3-4)	3.97±0.02 (4:3-4)
Female	0±0 (0:0-0)	4±0 (4:4-4)	4±0 (4:4-4)	3.90±0.03 (4:3-4)	3.62±0.10 (4:3-4)[b]	3.98±0.02 (4:3-4)	3.95±0.03 (4:2-4)
P	NS	NS	NS	NS	*	NS	NS

* P<0.05.

Table 4. FPD and PQ at week 6 in Pheasants [X±Sx (med:min-max)]

		PQ					
Gender	FPD	Head	Neck	Back	Wing	Tail	Breast
Male	0±0 (0:0-0)	0±0 (0:0-0)	0±0 (0:0-0)	2.59±0.13 (3:1-4)	3.32±0.06 (4:3-4)[b]	3.85±0.04 (4:3-4)	3.98±0.01 (4:3-4)
Female	0±0 (0:0-0)	0±0 (0:0-0)	0±0 (0:0-0)	2.63±0.14 (3:1-4)	3.49±0.06 (4:3-4)[a]	3.89±0.04 (4:3-4)	4±0 (4:4-4)
P	NS	NS	NS	NS	*	NS	NS

* P<0.05.

Table 5. Effect of rearing system and slaughter age on FPD in Guinea fowls [X±Sx (med:min-max)]

Rearing System	Slaughter Age (Week)	Gender	FPD
Free-Range	12	Male	0±0 (0-0)
		Female	0.03±0,02 (0:0-1)
	14	Male	0.03±0,02 (0:0-1)
		Female	0±0 (0:0-0)
	16	Male	0.02±0,02 (0:0-1)
		Female	0±0 (0:0-0)
	18	Male	0.06±0,03 (0:0-1)
		Female	0±0 (0:0-0)
Indoor	12	Male	0±0 (0:0-0)
		Female	0±0 (0:0-0)
	14	Male	0±0 (0:0-0)
		Female	0±0 (0:0-0)
	16	Male	0±0 (0:0-0)
		Female	0±0 (0:0-0)
	18	Male	0±0 (0:0-0)
		Female	0±0 (0:0-0)
Effects			
Rearing System			NS
		Free-range	0.02±0.01 (0:0-1)
		Indoor	0±0 (0:0-0)
Slaughter Age			NS
		12	0,01±0.01 (0:0-1)
		14	0.01±0.01 (0:0-1)
		16	0.01±0.01 (0:0-1)
		18	0.03±0.02 (0:0-1)
Gender			NS
		Male	0.02±0.01 (0:0-1)
		Female	0.01±0.01 (0:0-1)
Rearing System x Slaughter Age			NS
Rearing System x Gender			NS
Slaughter Age x Gender			NS
Rearing System x Slaughter Age x Gender			NS

Table 6. Effect of rearing system and slaughter age on FPD in Pheasants [X±Sx (med:min-max)]

Rearing System	Slaughter Age (Week)	Gender	FPD
Free-Range	12	Male	0±0 (0:0-0)
		Female	0±0 (0:0-0)
	14	Male	0±0 (0:0-0)
		Female	0±0 (0:0-0)
	16	Male	0.04±0.04 (0:0-1)
		Female	0.04±0.04 (0:0-1)
	18	Male	0.10±0.07 (0:0-1)
		Female	0.06±0.05 (0:0-1)
Indoor	12	Male	0±0 (0:0-0)
		Female	0±0 (0:0-0)
	14	Male	0±0 (0:0-0)
		Female	0±0 (0:0-0)
	16	Male	0±0 (0:0-0)
		Female	0±0 (0:0-0)
	18	Male	0.06±0.04 (0:0-1)
		Female	0±0 (0:0-0)
Effects			
Rearing System			NS
	Free-range		0.03±0.01 (0:0-1)
	Indoor		0.01±0.01 (0:0-1)
Slaughter Age			**
	12		0±0 (0:0-0)[a]
	14		0±0 (0:0-0)[a]
	16		0.02±0.01 (0:0-1)[a]
	18		0.05±0.02 (0:0-1)[b]
Gender			NS
	Male		0.02±0,01 (0:0-1)
	Female		0.01±0,01(0:0-1)
Rearing System x Slaughter Age			NS
Rearing System x Gender			NS
Slaughter Age x Gender			NS
Rearing System x Slaughter Age x Gender			NS

** P<0.01.

Effect of rearing system and slaughter age on PQ in Guinea Fowls and Pheasants are shown in Table 7 and Table 8, respectively. In guinea fowl, the head area feather quality was lower than the barn system (P<0.05) and the lowest feather quality was found at 12 weeks of age in terms of slaughter age (P<0.01). There was an interaction between head feather quality, rearing system and slaughter age (P<0.01), and slaughter age and gender (P<0.05). It was determined that in terms of back, wing and tail feather quality of pheasants, those reared in closed system were lower (P<0.01). It was determined that the feathering of the dorsal part differed according to the slaughter age and the females had lower feather quality in this part (P<0.01). In terms of back feathering, interaction (P<0.05) was determined between rearing system and slaughter age and rearing system and gender.

Table 7. Effect of rearing system and slaughter age on PQ in Guinea fowls [X±Sx (med:min-max)]

RS	SA	G	Head	Neck	Back	Wing	Tail	Breast
FR	12	M	3.96±0,02 (4:3-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
		F	3.95±0,04 (4:3-4)	3.97±0,03 (4:3-4)	3.97±0,03 (4:3-4)	3.97±0,03 (4:3-4)	4±0 (4:4-4)	4±0 (4:4-4)
	14	M	4±0 (4:4-4)	3.98±0,02 (4:3-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
		F	4±0 (4:4-4)	3.97±0,03 (4:3-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
	16	M	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
		F	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
	18	M	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
		F	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
IN	12	M	3.90±0,06 (4:2-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
		F	3.67±0,01 (4:1-4)	3.92±0,06 (4:2-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
	14	M	4±0 (4:4-4)	3.98±0,02 (4:3-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
		F	4±0 (4:4-4)	3.98±0,02 (4.3-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
	16	M	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
		F	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
	18	M	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
		F	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
Rearing System			*	NS	NS	NS	NS	NS
	FR		3.99±0,01 (4:3-4)[a]	3.99±0,01 (4:3-4)	3.99±0,01 (4:3-4)	3.99±0,01 (4:3-4)	4±0 (4:4-4)	4±0 (4:4-4)
	IN		3.93±0,02 (4:3-4)[b]	3.98±0,01 (4:3-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
Slaughter Age			**	NS	NS	NS	NS	NS
	12		3.87±0,03 (4:3-4)[a]	3.97±0,03 (4:1-4)	3.99±0,01 (4:3-4)	3.98±0,01 (4:3-4)	4±0 (4:4-4)	4±0 (4:4-4)
	14		4±0 (4:4-4)[b]	3.97±0,01 (4:3-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
	16		4±0 (4:4-4)[b]	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
	18		4±0 (4:4-4)[b]	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
Gender			*	NS	NS	NS	NS	NS
	Male		3.98±0,01 (4:2-4)	3.99±0,01 (4:3-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
	Female		3.93±0,02 (4:1-4)	3.97±0,01 (4:2-4)	3.99±0,01 (4:3-4)	3.99±0,01 (4:3-4)	4±0 (4:4-4)	4±0 (4:4-4)
RS x SA			**	NS	NS	NS	NS	NS
RS x G			NS	NS	NS	NS	NS	NS
SA x G			*	NS	NS	NS	NS	NS
RS x SA x G			NS	NS	NS	NS	NS	NS

RS= rearing system; SA= slaugter age; G= gender; M= male; F= female; FR= free-range; IN= indoor, **= P<0.01, *= P<0.05.

Table 8. Effect of rearing system and slaughter age on PQ in Pheasants [X±Sx (med:min-max)]

RS	SA	G	Head	Neck	Back	Wing	Tail	Breast
FR	12	M	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
		F	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
	14	M	4±0 (4:4-4)	4±0 (4:4-4)	3.89±0,06 (4:3-4)	4±0 (4:4-4)	3.96±0.03 (4:3-4)	4±0 (4:4-4)
		F	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
	16	M	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
		F	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
	18	M	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
		F	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)	4±0 (4:4-4)
IN	12	M	4±0 (4:4-4)	4±0 (4:4-4)	3.35±0.15 (4:1-4)	3.97±0.02 (4:3-4)	2.95±0.19 (3:1-4)	4±0 (4:4-4)
		F	4±0 (4:4-4)	4±0 (4:4-4)	3.55±0.12 (4:1-4)	4±0 (4:4-4)	2.80±0.19 (3:1-4	4±0 (4:4-4)
	14	M	4±0 (4:4-4)	4±0 (4:4-4)	3.23±0.09 (3:2-4)	3.95±0.04 (4:3-4)	2.97±0.16 (3:1-4)	4±0 (4:4-4)
		F	4±0 (4:4-4)	4±0 (4:4-4)	3.46±0.12 (4:1-4)	3.95±0.03 (4:3-4)	2.71±0.16 (3:1-4)	4±0 (4:4-4)
	16	M	4±0 (4:4-4)	4±0 (4:4-4)	3.61±0.09 (4:2-4)	3.94±0.04 (4:3-4)	3.30±0.14 (4:2-4)	4±0 (4:4-4)
		F	4±0 (4:4-4)	4±0 (4:4-4)	3.80±0.08 (4:2-4)	4±0 (4:4-4)	3.14±0.15 (3:1-4)	4±0 (4:4-4)
	18	M	4±0 (4:4-4)	4±0 (4:4-4)	3.56±0.10 (4:2-4)	3.97±0.03 (4:3-4)	3.03±0.16 (3:1-4)	4±0 (4:4-4)
		F	4±0 (4:4-4)	4±0 (4:4-4)	3.78±0.09 (4:2-4)	4±0 (4:4-4)	2.97±0.17 (3:1-4)	4±0 (4:4-4)
Rearing System			NS	NS	**	**	**	NS
	FR		4±0 (4:4-4)	4±0 (4:4-4)	3.98±0,01 (4:3-4)^{a}	4±0 (4:4-4)^{a}	3.99±0,01 (4:3-4)^{a}	4±0 (4:4-4)
	IN		4±0 (4:4-4)	4±0 (4:4-4)	3.53±0,04 (4:1-4)^{b}	3.97±0,01 (4:3-4)^{b}	2,97±0.06 (3:1-4)^{b}	4±0 (4:4-4)
Slaughter Age			NS	NS	**	NS	NS	NS
	12		4±0 (4:4-4)	4±0 (4:4-4)	3.67±0,06 (4:1-4) ^{ab}	3.99±0,07 (4:3-4)	3.33±0.09 (4:1-4)	4±0 (4:4-4)
	14		4±0 (4:4-4)	4±0 (4:4-4)	3.59±0,05 (4:1-4) ^{a}	3.97±0,01 (4:3-4)	3.30±0.08 (4:1-4)	4±0 (4:4-4)
	16		4±0 (4:4-4)	4±0 (4:4-4)	3.82±0,04 (4:2-4) ^{c}	3.98±0,01 (4:3-4)	3.52±0.07 (4:1-49	4±0 (4:4-4)
	18		4±0 (4:4-4)	4±0 (4:4-4)	3.79±0,04 (4:2-4) ^{bc}	3.99±0,01 (4:3-4)	3.37±0,09 (4:1-4)	4±0 (4:4-4)
Gender			NS	NS	**	NS	NS	NS
	Male		4±0 (4:4-4)	4±0 (4:4-4)	3.46±0,04 (4:1-4)^{b}	3.98±0,01 (4:3-4)	3.43±0,06 (4:1-4)	4±0 (4:4-4)
	Female		4±0 (4:4-4)	4±0 (4:4-4)	3.78±0,03 (4:1-4)^{a}	3.99±0,06 (4:3-4)	3.32±0,06 (4:1-4)	4±0 (4:4-4)
RS x SA			NS	NS	*	NS	NS	NS
RS x G			NS	NS	*	NS	NS	NS
SA x G			NS	NS	NS	NS	NS	NS
RS x SA x G			NS	NS	NS	NS	NS	NS

RS= rearing system; SA= slaugter age; G= gender; M= male; F= female; FR= free-range; IN= indoor, **= P<0.01, *= P<0.05.

4. Discussion

Ammonia evaporation from litter in poultry houses varies depending on the moisture and temperature content of the litter. As the temperature and humidity increase, ammonia release from the litter also increases (Miles et al., 2011). Ammonia emissions are known to be very sensitive to litter moisture content (Liu et al., 2007). The high release of ammonia from the litter causes irritation to the respiratory tract and skin in birds, but also causes foot-pad dermatitis, hock burns and breast blisters (Nairn and Watson, 1972; Martland, 1984; Nauaraj et al., 2006; Youssef et al., 2011). The litter content is an important factor affecting not only the FPD but also the feather (especially breast and abdomen area)

quality (Terčič et al., 2015). In our study, especially in guinea fowls, the litter moisture was found to be higher in the barn system than in the free-range system.

But this difference in moisture content was not reflected in FPD scores. It is reported that the amount of ammonia increases with the reused litter, and as a result, the FPD rates increase (Yamak, et al., 2016b), but this problem will disappear with adequate ventilation (Dawkins, et al., 2004). In a study on turkeys, it is emphasized that litter moisture should be kept below 30% to reduce the risk of FPD (Wu and Hocking, 2011). In our study, the fact that the litter moisture was quite low in both rearing systems explains the good FPD scores and the lack of difference between the systems (Table 1 and 2).

In a study conducted in broiler chickens, it was reported that litter moisture and FPD ratios increased as the fattening time increased (Eichner et al., 2007). In a study conducted in turkeys, it was stated that litter moisture is highly effective in causing FPD and it causes inflammation in young turkeys in a very short time, but the exact mechanism by which this occurs is unknown. Therefore, wet litter control is likely to be highly effective in reducing the severity and prevalence of FPD in commercial poultry flocks (Mayne et al., 2007). Because litter moisture is crucial to the control of FPD, a multifactorial approach to litter management will be necessary to strike a balance with the many other factors involved in poultry management (Taira et al., 2014). In a study about broilers, estimated prevalence of FPD ranged from 9.6 to 98.1% depending on the housing system used. Flocks with outdoor access (free-range and organic systems) have been reported to have a higher prevalence of FPD than those kept in completely enclosed systems (Pagazaurtundua and Warriss, 2006). In a study on geese, the incidence of FPD decreases when the animals are provided with a swimming pool (Liao et al., 2021). In another study on ducks, it is reported that the presence of a pool has a positive effect on foot pad cleaning and feather quality, similar to geese (Jones and Dawkins, 2010). In a study on turkeys, similar to our findings in guinea fowls, it was reported that the litter moisture was lower in the free-range system and accordingly, the FPD level was lower in the free-range system (Sarica and Yamak, 2010). However, according to our study results, it was determined that the rearing system did not affect FPD in both pheasants and guinea fowls (Table 5 and 6). FPD should be seen as an important animal welfare issue. Considering today's both traditional and organic poultry farming systems, it does not seem possible to completely prevent the formation of footpad lesions (Freihold et al., 2019). It is known that FPD severity increases with age in poultry species (Shepherd and Fairchild, 2010). However, in our study, while slaughter age and FPD score did not change in guinea fowls, FPD score increased as slaughter age increased in pheasants, similar to the literature.

Harmful feather pecking and cannibalism can have a serious impact on feather quality and therefore bird welfare (Petek et al., 2015). Genetics and age are known to be important factors in feather pecking. In addition, the most common causes are boredom, high light intensity, low humidity, restricted nutrition and perhaps nutritional deficiencies (Leeson and Walsh, 2004). Guinea Fowl and Pheasants attain their first young plumage at 4-5 weeks of age, and in especially pheasants at 20 weeks young males reach plumage that is almost unnoticeable from adult males (Westerskov, 1955). According to the results we obtained from guinea pigs, it is seen that they have the lowest feather score at the age of 12 weeks since the feather cover is not fully developed yet in terms of head area feathering (Table 7). Also, in terms of feathering in this part, it was determined that reared in the free-range system and male guinea fowls had better PQ than the barn system and females. In pheasants, unlike guinea fowls, females had a better feather score in terms of back feathering (Table 8). Again, it was observed that the feathers of this area were irregular but significantly different according to slaughter age. In terms of feathering in the back, wing and tail parts, it was determined that those reared in the free-range system had higher scores as in the guinea fowls. Similar to our study findings, Boz et al. (2017) also reported that geese reared in the free system were better in terms of wing and tail feathering. In a study on laying hens, it was reported that as the area per animal increases, both the feather quality and the yield are better (Sarica et al., 2008). In our study, it can be said that animals have a better PQ compared to the barn system, since they have more space in the free-range system and feel less boredom.

5. Conclusion

As a result, it was determined that guinea fowls had lower litter moisture in the free-range system, but this did not affect FPD score. On the other hand, it was found that FPD scores increased with age in pheasants. It was determined that free-range system was better in terms of head part feather quality in guinea fowls and back, wing and tail feathers were better in this system, similarly in pheasants. In terms of feather quality, a free-range system is recommended for better welfare for both species, especially pheasants.

Author Contributions

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

	A.U.	M.A.B.	M.S.
C	30	40	30
D	30	30	40
S			100
DCP	40	40	20
DAI	40	40	20
L	60	30	10
W	50	30	20
CR	10	40	50
SR	80	20	
PM	10	60	30
FA	10	20	70

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 experiment was conducted between May and August 2015 at the Ondokuz Mayis University Agricultural Faculty's Research Farm, Turkey. All procedures were approved by the Ondokuz Mayis University Ethical Committee for Experimental Animals (protocol code: 2015/55 and date: May 15, 2015). This study was conducted using material from previously published studies (Yamak et al., 2018; 2020).

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EVALUATION OF IN OVO CINNAMON, GINGER OR ANISE EXTRACT INJECTION ON BROILER HATCHING PERFORMANCE

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Abstract: Firstly, this study aimed to evaluate the effects on hatchability and relative weights of yolk, metabolic organ (heart, liver, breast muscle, and thigh muscle) and total digestive system (GUT) of in ovo feeding of cinnamon, ginger or anise extract. Secondly, it was investigated to determine the appropriate dose of cinnamon, ginger or anise extract in in ovo feeding. For this purpose, 480 fertilized broiler eggs were randomly distributed into 12 groups with four replicates as a factorial arrangement of three extracts (cinnamon, ginger, anise) x 4 doses (0, 3, 9, 12 mg/egg) to hatching trays. On the 18th day of incubation, 1 ml of 0.9% saline solution containing 0, 3, 9, 12 mg of cinnamon, ginger or anise extract was injected into the eggs with a 19 mm and 27-gauge needle. The cinnamon and ginger groups had higher hatchability, chick quality and proventriculus weight, while the anise group had higher thigh muscle weight. Chick weight was 12 mg/egg, chick quality was 0 and 3 mg/egg, breast muscle weight was 9 mg/egg, and liver, gizzard and GUT weights were higher at 0, 3 and 9 mg/egg in ovo extract doses. In ovo anise injection increased the number of non-pipped dead embryos. The interaction effect of factors on the hatchability and chick quality were found significant. The results of this study indicate that 9 mg/egg cinnamon, 12 mg/egg ginger, and 3 mg/egg anise extract can be used in in ovo injection without negative effects on the investigated parameters. The role of in ovo cinnamon, ginger or anise extract injection in broiler needs further research.

Keywords: In ovo, Extract, Hatchability, Cinnamon, Ginger, Anise, Chick quality

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1. Introduction

In recent years, the use of medicinal plants and their derivatives in combination with feeding practices has been adopted as an alternative to antibiotics due to the multiple beneficial effects on productivity, immunity, gut development and disease resistance in poultry nutrition (Pathak et al., 2016; Oke et al., 2017; Oke, 2018; Al-Ashoor and Al-Salhie, 2020; Al-Mosawy and Al-Salhie, 2021). A large number of studies have reported that phytobiotics have antimicrobial, anti-inflammatory and transcription modulation potential (Liu, 2004; Kikusato, 2021), have beneficial effects such as inhibiting and reducing pathogenic bacteria (Alcicek et al., 2004; Alshelmani et al., 2021) and can be used as a non-antibiotic growth promoter by virtue of their effects on reducing the inflammatory process, improving gastrointestinal function, increasing growth and production performance and modulating the immune system (Saeed et al., 2020; Kairalla et al., 2022a, 2022b). Among these phytobiotic plants, the effects on broiler growth and physiological responses of cinnamon (Ahmed et al., 2019; Mohammed and Amin, 2019), ginger (Qorbanpour et al., 2018; Daramola et al., 2020; Thomas et al., 2020; Gupta et al., 2021) and anise (Al-Kassie, 2008; Soltan et al., 2008) have been studied and the benefits on performance, immune response, feed digestibility, gut health, meat quality and some blood parameters have been demonstrated. Cinnamon contains various compounds such as sinnamaldehyde, eugenol and carvacrol which have biological activities such as medical treatment, anti-inflammatory, antimicrobial and antioxidant properties (Chang et al., 2013). Ginger contains various compounds and enzymes including gingerdiol, gingerol, gingerdion and shogaols which have antimicrobial, antioxidant and pharmacological effects (Ali et al., 2008; Zhao et al., 2011; Kairalla et al., 2022b). In addition, anise contains various compounds such as sesquiterpene (Wang et al., 2011) anethole, estragole, limonene, linalool and cis-anethole (Dzamic et al., 2009) which have biological activities such as digestive stimulant, antibacterial, antiviral, antifungal, anticancer and antioxidant properties (Mugnaini et al., 2012).

In recent years, in ovo feeding, which offers beneficial biochemical and physiological balances including improved oxidative protection to embryos, has become widespread due to the development of science, technology and breeding in poultry farming and its lower cost (Kadam et al., 2013; Kop-Bozbay et al., 2019; Karamik and Kop-Bozbay, 2020; Atan and Kop-Bozbay, 2021; Kop-Bozbay and Ocak, 2019, 2022). Given that the use of medicinal plants and their derivatives has been proven to have many benefits, in ovo feeding with

bioactive compounds can provide developments for the poultry industry by studying the effects of these compounds on post-hatching immune responses, antioxidant defense and performance as a strategy to improve the health and production performance of poultry. For this purpose, many phytogenic compounds have been tested (Morovat et al., 2016; Zarei et al., 2016; Faseleh Jahromi et al., 2017; Elsaadany, 2019; Ranjbar et al., 2019; Taha et al., 2019; Oladokun and Adewole 2020; Oke et al., 2021; Shehata et al., 2021). However, it is necessary to individually examine each of the numerous phytogenic compounds as alternatives to antibiotics to determine their effectiveness in poultry feeding. Although there are various studies in the literature on the supplementation of cinnamon, ginger or anise to animal rations, there is a lack of information regarding the effect of forementioned extracts at different doses in the same or different studies. For this purpose, in this study aimed, firstly, to evaluate the effects on hatchability and relative weights of yolk, metabolic organ (heart, liver, breast muscle, thigh muscle) and total digestive system (GUT) of in ovo feeding of cinnamon, ginger, or anise extract. Secondly, it was investigated to determine the appropriate dose of cinnamon, ginger or anise extract in in ovo feeding.

2. Material and Method

A total of 480 fertile chicken eggs were collected from a 36-week-old ROSS 308 breeders and were incubated under routine conditions (Çimuka T1280, Ankara, Türkiye).

The eggs were distributed with four replicates of 10 eggs each with an average egg weights as a factorial arrangement of three extracts (cinnamon, ginger, anise) x four doses (0, 3, 9, 12 mg/egg) to hatching trays. On the 18th day of incubation after the fertility control, 1 ml of 0.9% saline solution containing 0, 3, 9, 12 mg of cinnamon, ginger or anise extract was injected into the eggs with a 19 mm and 27-gauge needle. Plant extracts were obtained from a commercial company (Alfasol).

Within two hours of upon hatch all measurements were ascertained. The chicks were weighed and recorded. The hatchability and the number of pipped and non-pipped dead embryos were calculated according to Kop-Bozbay and Ocak (2019). The chick quality (Tona score; Tona et al., 2003) and relative asymmetry (Yalcin et al., 2005) were determined for four randomly selected chicks from each replication. One male and one female chick were selected from each replication to obtain samples from the digestive system and metabolic organs and were euthanized by cervical dislocation. The contents of each chick were opened and the yolk sac, heart, liver, muscles (breast and thigh) and digestive system were carefully removed from the abdominal cavity (Kop-Bozbay and Ocak, 2019) and were weighed and standardized according to live weight.

The collected data was analyzed using the GLM procedure in the SPSS statistical package (SPSS 17.0; SPSS Inc., Chicago, IL, USA). The effect of in ovo plant extract injection, dose and their interaction on all data were analyzed in a randomized block design as a factorial arrangement (3 x 4) of treatments.

3. Result

3.1. Effect of Extract

In ovo feeding with anise extract decreased the hatchability and chick quality compared to other herbal extracts while increased the number of pipped dead embryos ($P<0.05$, Table 1). According to Table 2 in ovo anise extract feeding, increased the relative thigh muscle weight compared to other herbal extracts and decreased the relative proventriculus weight compared to cinnamon extract ($P<0.05$).

3.2. Effect of Extract Dose

Table 1 shows that chick weight was affected by in ovo herbal extract feeding at different doses ($P<0.05$), while hatchability and chick quality were not affected ($P>0.05$). The highest chick weight was found at the 12mg/egg in ovo extract dose ($P<0.05$). The highest relative breast muscle weight was found at the 9mg/egg dose, while the lowest was found at the 0mg/mg dose ($P<0.05$, Table 2). The relative liver and gizzard weights at the 12 mg/egg dose were lower than the other doses and the relative GUT weight was lower than the 0 and 3mg/egg doses ($P<0.05$, Table 2).

3.3. Effect of Interactions

Table 1 shows that extract x dose interaction was observed for hatchability and tona score ($P<0.05$). Increasing the in ovo anise extract dose led to decrease the hatchability and tona score in the anise groups and only tona score in the cinnamon group.

Table 1. The influence of in ovo injection of herbal extracts on the weights of chick (g, CW) and hatchability traits

Extract	Dose	CW	Hatchability of*		EM*		Chick quality**	
			Set eggs	Fertile eggs	Pipped	Non-pipped	Tona score	Relative asymmetry
Cinnamon	Control	40.03	86.39	86.39^{ab}	2.78	10.75	99.50^{a}	1.12
	0.3%	38.93	85.00	89.44^{ab}	5.00	5.00	97.75^{ab}	1.70
	0.9%	39.79	85.00	89.44^{ab}	2.50	7.50	100.00^{a}	1.54
	1.2%	40.77	75.00	92.86^{a}	0.00	5.00	97.38a^{b}	1.03
Ginger	Control	39.92	86.95	88.89^{ab}	0.00	10.50	97.50^{ab}	1.258
	0.3%	39.53	82.50	93.75^{a}	0.00	5.00	98.75^{a}	1.46
	0.9%	39.44	80.00	93.75^{a}	2.50	2.50	99.25^{a}	1.15
	1.2%	40.34	85.00	97.22^{a}	2.50	0.00	99.75^{a}	1.65
Anise	Control	40.078	86.67	86.67^{ab}	2.78	10.50	99.25^{a}	0.95
	0.3%	39.42	85.00	92.22^{a}	7.50	0.00	98.63^{a}	1.20
	0.9%	40.28	62.50	74.11^{bc}	12.50	10.00	86.00^{c}	1.73
	1.2%	41.33	60.00	66.96^{c}	17.50	10.00	92.88^{b}	1.16
Extract								
	Cinnamon	39.88	82.85	89.53^{a}	2.57^{b}	7.06	98.66^{a}	1.35
	Ginger	39.81	83.6	93.40^{a}	1.25^{b}	4.50	98.81^{a}	1.38
	Anise	40.27	73.54	79.99^{b}	10.07^{a}	7.63	94.19^{b}	1.26
Dose								
	Control	40.01^{b}	86.67	87.31	1.85	10.58	98.75	1.11
	0.3%	39.30^{b}	84.17	91.81	4.17	3.33	98.38	1.46
	0.9%	39.84^{b}	75.83	85.77	5.83	6.67	95.08	1.47
	1.2%	40.81^{a}	73.33	85.68	6.67	5.00	96.68	1.28
SEM		0.146	2.209	1.758	1.157	1.003	0.635	0.089
Main effect of	Extract	0.301	0.093	0.002	0.002	0.364	0.001	0.860
	Dose	0.002	0.075	0.411	0.353	0.059	0.062	0.446
	Extract x Dose	0.834	0.341	0.039	0.253	0.354	0.000	0.502

^{a,b,c} Within a row, means with different superscripts differ significantly (P<0.05). EM= embryonic mortality, SEM= standard error of the mean. *The values are means of the four replicates (trays). **The values are means of the eight chicks.

Table 2. The influence of in ovo injection of herbal extracts on relative yolk-sac, metabolically organ, total gastrointestinal tract (GUT) and some digestive system segments weights (g/100 g live weight)

Extract	Dose	Yolk	Breast muscle	Thigh muscle	Heart	Liver	Gizzard	Proventriculus	GUT
Cinnamon	Control	9.66	3.24	11.19	0.77	2.42	4.80	0.90	13.76
	0.3%	9.26	3.36	11.32	0.77	2.29	4.53	0.87	13.46
	0.9%	11.41	6.02	11.57	0.87	2.42	4.54	1.00	13.49
	1.2%	12.20	4.72	11.38	0.75	1.99	3.84	0.84	11.31
Ginger	Control	11.66	3.36	10.65	0.67	2.16	4.59	0.91	12.99
	0.3%	12.68	3.87	11.08	0.77	2.18	4.48	0.80	13.04
	0.9%	11.97	4.31	10.67	0.81	2.39	4.49	0.87	12.68
	1.2%	11.01	3.40	10.79	0.77	1.99	4.31	0.85	12.27
Anise	Control	9.56	3.52	11.53	0.82	2.41	4.57	0.80	13.20
	0.3%	10.26	3.96	12.20	0.83	2.46	4.57	0.85	13.32
	0.9%	13.77	5.18	12.01	0.86	2.42	4.42	0.74	11.53
	1.2%	12.04	4.93	12.34	0.79	2.11	4.02	0.63	10.95
Extract									
	Cinnamon	10.63	4.33	11.37^{b}	0.79	2.28	4.43	0.90^{a}	13.01
	Ginger	11.83	3.73	10.80^{b}	0.76	2.18	4.47	0.86^{ab}	12.75
	Anise	11.41	4.40	12.02^{a}	0.83	2.35	4.39	0.75^{b}	12.25
Dose									
	Control	10.29	3.37^{c}	11.12	0.75	2.33^{a}	4.65^{a}	0.87	13.32^{a}
	0.3%	10.73	3.73^{bc}	11.53	0.79	2.31^{a}	4.53^{a}	0.84	13.27^{a}
	0.9%	12.38	5.17^{a}	11.42	0.85	2.41^{a}	4.48^{a}	0.87	12.57^{ab}
	1.2%	11.75	4.35^{ab}	11.50	0.77	2.03^{b}	4.06^{b}	0.77	11.51^{b}
SEM		0.402	0.187	0.134	0.015	0.049	0.068	0.024	0.209
Main effect of	Extract	0.479	0.191	0.001	0.159	0.356	0.892	0.032	0.256
	Dose	0.261	0.002	0.628	0.111	0.044	0.016	0.384	0.004
	Extract x Dose	0.541	0.384	0.965	0.821	0.967	0.825	0.584	0.542

^{a,b,c}Within a row, means with different superscripts differ significantly (P<0.05). SEM= standard error of the mean. The values are means of the four chicks.

4. Discussion

The embryonic development and post-hatch performance of poultry can be manipulated by applied various nutrients (carbohydrates, amino acids, etc.), phytochemicals, vaccines, and immune system stimulators to eggs using the in ovo technique (Kop-Bozbay et al., 2019; Kop-Bozbay and Ocak, 2019; Hajati et al., 2021; El-Kholy et al., 2021). The application of phytochemicals in in ovo feeding technique has also been shown to have the potential to support these features (Moghaddam et al., 2014; Morovat et al., 2016; Faseleh Jahromi et al., 2017; N'nanle et al., 2017; Khaligh et al., 2018; Al-Shammari et al., 2019; Elsaadany, 2019; Ranjbar et al., 2019; Araujo et al., 2020; El-Kholy et al., 2021; Hajati et al., 2021). These studies demonstrated that phytochemicals can improve antioxidant defense and immunity. However, these effects are influenced by a variety of factors, such as the chemical composition of the extract, extraction method, dose and in ovo injection technique. In the current study, cinnamon and ginger extracts were found to have increased the hatchability by 12-17% compared to the anise group. This result may be attributed to the safrole content of anise. Indeed, the pipped rate was higher than the other groups and the chick quality was lower, which supports this conclusion. Ebrahimnezhad et al. (2011), emphasized that the negative effects of in ovo feeding may be caused by the allergenic properties of the substance used.

In the current study, although the extract factor had no effect in in ovo feeding, the dose (12mg/egg) caused an increase in the hatching weight of the chicks. This increase may be due to the enhanced antioxidant status of the embryos with increasing dose. In addition, plant extracts with high antioxidant content used in our study may have reduced oxidative stress during incubation and thus protected the muscles from oxidative damage (Choi et al., 2016). As a result, this led to an increase in hatchling weight, as well as the relative metabolic organ and GUT weights. Relative thigh muscle weight in the anise group was highest which may be explained by the increase in pipped rate, that is, the survival of strong embryos. When the relative breast muscle, liver and gizzard weights were evaluated together, the most appropriate dose was found to be 3 or 9 mg/egg. This effect may be due to the beneficial effects of phytochemicals on the digestive system, due to their antimicrobial and antioxidant effects (Valenzuela-Grijalva et al., 2017; Yang et al., 2019). When all these physiological changes are evaluated together, it can be said that animals can perform better after hatching.

5. Conclusions

In this study, two important findings were obtained. Firstly, the study has shed light on the usability of cinnamon, ginger or anise extracts in in ovo feeding. Secondly, it has been shown that the use of 9 mg/egg cinnamon, 12 mg/egg ginger and 3 mg/egg anise extract in in ovo feeding can be used without negatively affecting the parameters studied. However, the role of in ovo cinnamon, ginger or anise extract injection in broiler needs further research on the health and performance of chickens.

Author Contributions

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

	C.K.B	B.G.
C	100	
D	100	
S	100	
DCP	50	50
DAI	50	50
L	40	60
W	50	50
CR	50	50
SR	100	
PM	100	
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

The authors confirm that all the procedures with animals were approved by the Local Ethics Committee of Animal Experiments of the Eskişehir Osmangazi University (protocol code: HAYDEK-880/2021, date: January 15, 2021).

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IMPACTS OF CLIMATE CHANGE AND POPULATION GROWTH ON FOOD SECURITY IN NIGERIA

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Abstract: Food production in Nigeria has not matched with the rate of population growth leading to reduction of national food independence and self-reliance. As a result, Nigeria is facing serious food insecurity. Therefore, this research examined the impacts of climate change and population growth on food security in Nigeria. Annual time-series of food security (proxy of food production index), annual rainfall, annual temperature, population growth rate, urban population rate and agricultural land used from 1980 to 2019 were used. The research used Augmented Dickey-Fuller (ADF) unit root test, Vector Autoregressive (VAR) model, Johansen Cointegration test and Vector Error Correlation Models (VECM) were used to analyze the data. ADF unit root test result shows that all variables were completely stationary at the first different orders I(1) at both at intercept and intercept with trend at level of significance of 1%. Three (3) lags were conclusively selects as the optimum lag in the VAR model. The result of the estimation indicates that the Johansen cointegration shows an existence of long-run relationship among the variables used in the study. The result of the VECM estimation shows that rainfall, temperatures, population growth rate and agricultural land used were negatively significantly related to food security at various levels of significance (1%, 5% and 10%). At the long-run all the variables were adversely related to food security in Nigeria. The coefficient of multiple determinations (R^2) indicates about 91%, the adjusted R^2 of 0.86 was obtained and Durbin-Watson of 2.1 was obtained which implies that the tools were good fit to estimates the data. Decomposition of variance shows dwindling in food security. The research therefore recommends public enlightenment campaign on birth control; and appropriate climate change adaptation methods should be adopted to enhance food security in Nigeria among others.

Keywords: Agricultural land, Climate change, Food security, Nigeria, Population growth

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1. Introduction

Food insecurity and hunger are increasing globally, with above 30 percent of the worldwide population presently facing food insecurity (Sibanda and Mwamakamba, 2021). Food and Agriculture Organization of United Nation FAO (2021) reported that about 927.6 million people suffered food insecurity in diverse and several periods in 2020. Out of this, about 346.6 million signified 37.3% in Africa people suffered food insecurity (Xie et al., 2021). Coronavirus (COVID-19) pandemic has added to the prevailing causes of food insecurity, climate change, unstable economic factors, undernourishment, and population growth rate in most countries. Nations that are experiencing food insecurity are mostly susceptible to climate change and adaptation strategies capacities are limited (Ntiamoah et al., 2022). Climate change is contributing largely to the effect of food security in Sub-Saharan African. Nigeria's food insecurity has been increased by climate change such as prolonged drought, increase in temperatures, erratic rainfall patterns, carbon emissions (CO_2), and flood among others.

Nigeria has an enormous capacity to attain and reach food security with her absolute excessive natural endowment and human resources. In spite of this, Nigeria has a nation is not capable and proficient to feed its populaces owing to various difficulties and setbacks distressing agricultural sector productivity. Foremost amongst these difficulties are climate change and rapid population growth. The rapid population growth remains one of the key difficulties confronting foods security in Nigeria. However, agricultural productivity has increased, yet, it has not overtaken population growth. According to United Nations as cited in Pontianus and Oruonye (2021), Nigeria population will be about 440 million by 2050, with five live births per woman and a population growth rate of 3.2 percent per annual. This means that there will be more populaces to feed in Nigeria. Hence, if agricultural production is not outpaced the rapid population growth rate in the country, it is certain it might lead to critical food crisis in years to come, coupled with climate change impacts (severe drought and intense flooding) on agricultural production. The impacts of this climate change include reduction in land availability for food production, high cost farmland procurement, devastation of crops and livestock and subsequently, decrease in food production. It is in

contrast to this scenery that this research is planned. Therefore, this research examined the impacts of climate change and population growth on food security in Nigeria empirically.

Climate change is the variations that can be ascribed to the direct and indirect activity of human that alters the components of the atmosphere globally which is observed over some considerable periods (UNFCCC, 2011). These variations in climatic factors extremities may possibly impact the food chain; from the point of production on the farm activities to consumption. Extreme weather factors such as floods, droughts, storms and fires impact negatively on food security.

The United Nations' Food and Agricultural Organization describes food security as "when all the people at all times, have physical, social and economic access to sufficient, safe and nutritious food for an active and healthy life" individuals in households are the hub of concern against hunger and poverty. Four scopes of food security according to Bajagai (2013) are:

Food availability: This is related to adequate in both quantity and quality of food supply via national agricultural production and/ or import, distribution, and marketing.

Food accessibility: Refers to individuals or households have sufficient economic resources to afford apt and suitable quantity of quality food (devoid of stealing, scavenging, emergency food supply, or other surviving systems).

Food utilization: This comprises food preparation, household-level food distribution, clean water and hygiene, as well as good health care systems.

Food stability: This scope means ability to acquire or get food at all times. Hence, nations cannot be regarded food secured until there is availability, accessibility, adequate utilization stipulations, and stability of food for the majorities.

Population is the aggregate number of residents comprising a specific race, group or class in an identified area (AbdulRahaman, 2013). Population growth is the change in the proportions of population due to natural increases (AbdulRahaman, 2013) both by birth and immigrants, it is determined in absolute and relative terms. While, population growth rate is the mean yearly percentage change in the population, which is influenced by birth and death rate, and the balance between immigrants and emigrants in a particular nation within a definite period (Mundi Index, 2021). Hence, population growth rate is a key factor in determining food security, greenhouse gases (GHGs) emission, and other infrastructures stability of a nation. Increase in population growth and rural-urban migration are distressing domestic agricultural productivity which is the backbone of securing food in the country.

2. Efforts in Achieving Food Security in Nigeria

In view of the importance of the status of food security in sustainable development goals, and the role of food security in eradicating hunger and malnutrition which wear away human capacity and decreases labour supply for economy productivity; Nigeria government as a matter of urgency as diving into programmes, which hope to facilitate the alleviation of hunger and poverty in the country. Some of these programmes embarked in Nigeria according to Osu (2017) includes the following: Operation Feed the nation (OFN) which aim at sensitizing Nigerian on food production; River Basin Development Authority (RBDA) equipped with regard of increasing fish production, control of flood, and water pollution as well as erosion in the country specially in the Southern part of the country; Agricultural Development Project (ADP) equipped towards increasing the technical and economic productivity and profitability of the small-scaler farmers; Green Revolution geared to alleviate hunger; The National Special Food Security Programme geared towards spreading the adoption of relevance innovations to the farmers at minimum cost; The National Fadama Development project (that's FADAMA I, II and III) equipped towards reducing the distresses and misfortunes of the farmers to empower and facilitate them to achieved their maximum potential profit in agricultural production operations. All these programmes were inaugurated to achieve food security and economic growth in Nigeria. Regardless of the impressive and creditable objectives of all these programmes, it could not achieve it expectation because of the impact of climate change, population growth rate and other socio-factors.

3. Theoretical Framework

The theoretical behind this study is based on Malthusian population theory. Reverend Thomas Malthus (1798) published his theory on population titled, *"An Essay on the principle of population."* Malthus in his classical work postulated that population have a propensity to exceed the means of sustenance, that's incessant increase in population growth will result in economy standstill if necessary and immediate measures are not adopted to curb this (Aidi et al., 2016; Pontianus and Oruonye, 2021). He claimed that population increased in a geometrical succession (such as 2,4,8,16,32,64…) while food production increased in arithmetical succession (such as 2,4,6,8,12,14…), this disequilibrium will negatively affect food security and economic growth. Consequently, Malthus argued that at the proper and right time there would be food supply deficiency resulting in reduction in the living standard, poverty as well as misery (Oladimeji, 2017), since farmland reduces as poor or deficient quality farmlands are planted as a result of rapid increase in population density. This opinion was substantiated and upheld by scholars such

as Coale and Hoover (1958) and Ehilich (1968) as cited in Pontianus and Oruonye (2021) who investigated and remarked that rapid increase in population growth has the propensity to impact economy adversely since it could devastate any stimulated reaction by technological advancement, food production and capital accumulation. Malthus theory was based on population pessimistic.

Based on the hypothesizes and proposes of Malthus theory, he recommended positive and precautionary curbs (control). The positive curbs include rise in death rates due to wars, scarcity of food, hunger, pandemics among others. For precautionary curbs, Malthus promoted and endorsed decrease in the birth rates through pure and modest restrictions. By pure and modest restrictions, he advocated birth control methods like marrying late and restriction by married couples. Being a clergyman, he did not agree with unnatural birth control methods such as using contraceptives drugs, abortions among others. Malthus was viewed as a prophet of tragedy in the western economy due to his projections which falls short of prospect. However, in Sub-Saharan African countries, the certainties of Malthus's projections cannot be condemned and criticized. Albeit Nigeria population has not increased at geometric rate as proposed by Malthus, ultimately population growth rate has been enormous (Ewugi and Yakubu, 2012). The vice predicted by Malthus's theory is obvious and distinct in Nigerian economy and persist chasing Nigerians, thus the brutal, agitation and restlessness experience in the nation, which has resulted in scores of deaths. Domestic food production continually falls behind the food requirements of the population rate of the nation; this has led to huge importation of food which is destructive to national balance of payment. Despite the fact that there has not been dire and grave starvation in Nigeria over the past years, yet reports show that food security has been supported by food importation to feed rapid population growth in the country. The government report as at 1985, which assess the food condition in Nigeria reported that there was hardly a single food commodity in which the country can states categorically to be independent and self-reliant (Asua, 2016). This pathetic fact mirrors the condition of Nigeria even presently; that is to say, Nigeria's ever-increasing population growing rate cannot provide sufficient food to feed her populace.

Some of the research carried out on the relationship between food security, climate change and population growth include Kumar and Sharmar (2013) who carried out their studies on the impact of climate variation on food security in rural India discovered that climate variation impact negatively on food security. Josephson, Ricker-Gilbert and Florax (2014) examined the relationship between rural population growth and agricultural intensification and production in Ethiopia. Their result shows a significant relationship between population growth and farm sizes and reduction in agricultural productivity. Zewdie (2014) stated categorically that climate change has harmful impacts on food stability in Sub-Sahara Africa. Mahrous (2019) found out that temperature has negative influence on food security whereas rainfall and increase in cereal crops planting has a positive effect on food security in the East African Community (EAC) region under the five countries considered for the study.

Osuafor and Nnorom (2014), Ladan (2014), Mbah, Ezeano and Saror (2016), and Idumah et al. (2016) found out that climate change adversely affects food security in Nigeria negatively. Ahungwa et al. (2019); and Osu (2017) established that rapid population growth negatively affecting food production in Nigeria. While Oladimeji (2017) confirmed the Malthusian population theory in his research on the relationship between population growth and rice production in Nigeria, and found out that population was increasing exponentially whereas rice production was increasing arithmetically in Nigeria.

From the empirical studies in Nigeria, most of the studies focused on either the impact of population growth rate or climate change in relation to food security in Nigeria. Some research examined only one crop, and some used only one State or Region for their studies. Therefore, to fill the knowledge gaps, this research incorporated the impacts of both climate change and population growth on food security in Nigeria, by using national-wide data to examine the impact of climate change and population growth on food security in Nigeria.

4. Materials and Methods

The study used yearly time-series data from 1980 to 2019. The data were collected from FAO, World Bank, and Macro trends database (2022). The mathematical model function is specified thus (equation 1);

$$FPI = f(RF, Temp, PGR, UPR, ALU) \quad (1)$$

Where; FPI is food production index (a proxy for food security; covers food crops that are edible and contain nutrients); RF is rainfall (mm); Temp is temperatures (^oC); PGR is population growth rate; UPR is urbanization population rate (used as control variable); and ALU is the agricultural land used (km^2).

Ordinary Least Square OLS is expressed thus (equation 2);

$$FPI = \beta_0+\beta_1 RF+\beta_2 Temp+\beta_3 PGR+\beta_4 UPR+\beta_5 ALU+e \quad (2)$$

Where;
β_0= Regression constant
$\beta_1 - \beta_5$= Coefficients of independent variables
e = Error term

In logarithm form as (equation 3);

$$LnFPI = \beta_0 + \beta_1 LnRF + \beta_2 LnTemp + \beta_3 LnPGR +$$

$\beta_4 LnUPR + \beta_5 LnALU + e$ (3)

Where; Ln is natural logarithm

In accordance with the assumption that the data used is time-series and stationary. In order to prevail over the non-stationarity incident and other limiting factors related time-series analysis, Augmented Dickey-Fuller (ADF) unit root test was utilized to test the stationarity of the data. In accordance with the properties of time series, it is very essential to carry out the unit root test on the series in the Vector Autoregressive (VAR) model (Salako, Lawrence, Aremu and Egbekunle, 2015).

Johansen Cointegration test has been ascertained to be consistent and dependable (Salako et al., 2015) and it is adopted in this study. In accordance with the result of ADF unit root test, Johansen cointegration test was utilized to test the long-run relationship and the vector error correlation models (VECM) was utilized to estimates the short-run variability of the data to equilibrium trend. The data were analyzed using gretl software. The VECM model can be expressed as (equation 4);

$\Delta y_t = \Pi y_{t-1} + \sum_{i=1}^{k-1} \Gamma_i \Delta y_{t-1} + \mu + \in_t$ (4)

Where;

Δy_{t-1} = First difference operator of (n x 1) vector of the n variables

y_{t-1} = Lagged values of Y_t

Δ = Difference operator

Π = (n x n) coefficient matrix

Γ = (n x (k-1)) matrix of short-run coefficients

μ = error term

$\in_t$ = (n x 1) vector of white noise disturbances

The fundamental theory of the Johansen cointegration test is that if the coefficient matrix (Π) has decreased rank (r < n), this could be disintegrated into a matrix (n x r) of the loading coefficients alpha (α) and a matrix (n x r) of cointegrating vectors beta (β) such that coefficient matrix becomes products of alpha and beta, that's Π = αβ (Idumah et al., 2016). The cointegrating rank (r) is the number of the cointegrating relationships. The coefficients of alpha (α) specify the cointegration relationships in the individual equations of the system in the model and the rate of the adjustment to disequilibrium, whereas the cointegrating vectors beta (β) indicates the long-run equilibrium relationship.

Idumah et al. (2016) described two likelihood ratio tests specifically they are Trace and maximum Eigen value statistic tests which are utilized to ascertain the number of cointegrating equations specified by the cointegration rank (r). The trace statistic test equation specified thus (equation 5);

$Trace = -T \sum_p \ln (1 - \sigma i)$ (5)

The Trace test examines the null hypothesis that there are most rank (r) cointegrating vectors in contrast to the alternative hypothesis that there are ranks (r) or additional cointegrating vectors (Idumah et al., 2016). The Maximum Eigen-value (Lmax) examines the null hypothesis that there are more r cointegrating vectors in contrast to the alternative of r + 1 cointegrating vector and expressed as (equation 6);

$Lmax = -T.\ln(1\gamma r + 1)$ (6)

A priori theoretical expectations

$LnFPI = \beta_0 + \beta_1 LnRF + \beta_2 LnTemp + \beta_3 LnPGR + \beta_4 LnUPR + \beta_5 LnALU + e$

($\beta_1 < 0, + \beta_2 < 0, \beta_3 < 0, \beta_4 < 0, \beta_5 < 0$)

5. Results and Discussion

Non-stationarity data may generate unauthentic and specious regression; consequently the result may perhaps be misleading the end users and policy-makers. As a result, it is rational and sagacious to ascertain the stationarity of the data. The stationarity test result of the Augmented Dickey-Fuller (ADF) unit root test for the time-series variables data employed in the estimation are presented in Table 1.

Table 1 shows the stationarity test result of the ADF unit root test of the variables. The existence of unit root signifies that the variables are non-stationary. LnFPI, LnPGR and LnALU variables were non-stationary at their own levels I(0) both at intercept and intercept with trend, but are completely stationary at the first different orders at both at intercept and intercept with trend. While LnRF, LnTemp and LnUPR were both stationary at their own levels and at first different orders at both intercept and intercept with trend. All tested at level of significance of 1%. This implies that the variables are of the mixed order zero and one orders, i.e *I(0)* and *I(1)*. Thus, the null hypothesis of possessing a unit root is therefore rejected and accepted the alternative hypothesis.

Table 1. Stationarity: Augmented Dickey-Fuller (ADF) unit root test

Variables	Level				First Difference			
	t-value	*p*-value	OI	Remarks	*t*-value	*p*-value	OI	Remarks
Intercept:								
LnFPI	-1.7019	0.4225	I(0)	NS	-8.7571	0.0000	I(1)	S
LnRF	-5.9741	0.0000	I(0)	S	-9.7271	0.0000	I(1)	S
LnTemp	-3.3269	0.02032	I(0)	S	-8.4256	0.0000	I(1)	S
LnPGR	-0.7453	0.8231	I(0)	NS	-3.7404	0.0072	I(1)	S
LnUPR	-4.3534	0.0013	I(0)	S	-10.4095	0.0000	I(1)	S
LnALU	-2.2177	0.2036	I(0)	NS	-5.6583	0.0000	I(1)	S
Intercept and trend:								
LnFPI	-1.0705	0.9212	I(0)	NS	-9.7158	0.0000	I(1)	S
LnRF	-5.7905	0.0001	I(0)	S	-9.6971	0.0000	I(1)	S
LnTemp	-4.8780	0.0017	I(0)	S	-8.3212	0.0000	I(1)	S
LnPGR	-1.1402	0.9087	I(0)	NS	-3.6688	0.0371	I(1)	S
LnUPR	-3.8364	0.0250	I(0)	S	-11.1656	0.0000	I(1)	S
LnALU	-1.2986	0.8732	I(0)	NS	-7.0537	0.0000	I(1)	S

OI= order of integration; NS = non stationarity; S = stationarity; I(0) = zero level; I(1) = first different

Table 2 above shows the estimation of the VAR model for decisive of an optimal lag orders. The asterisks (*) above signify the best values of the respective information criteria level of significance at 5%. Akaike Information Criterion AIC (-39.8859) and Hannan-Quinn Criterion HQC (-38.1371) called for three (3) lags, exclusive of Bayesian Information Criterion BIC (-36.0360) that called for one lag. Therefore, this study conclusively selects three (3) lags as the optimum lag in the model.

The results of Johansen Cointegration test in Table 3, shows that Trace test and Lmax test indicates 3 cointegrating equations respectively at 5% level. This means that the null hypothesis of not having a cointegrating equation (that's r = 0) is rejected and the alternative hypothesis of possessing three (3) cointegrating equations (r = 2) is accepted. This indicates that the Johansen cointegration result shows the existence of a long-run relationship among the variables used in the research. This finding agrees with Idumah et al. (2016) who reported cointegrating equation in their research on climate change and food production in Nigeria between 1975 and 2010.

The presence of cointegrating relationship between the dependent and explanatory variables as shown by Johansen cointegration test demanded the assessment of the short-run dynamic contrast between the variables in the cointegrating relationship equation by assessing the error correlation model.

Table 2. Vector Autoregressive (VAR) model lag order selection criteria

Lags	Loglik	P(LR)	AIC	BIC	HQC
1	705.29259	N/A	-37.9024	-36.0360*	-37.2581
2	741.54846	0.0003	-37.9171	-34.4509	-36.7205
3	812.00372	0.0000	-39.8859*	-34.8199	-38.1371*

Table 3. Johansen Cointegration results

Rank = r	Eigen value	Trace test	*P-value*	Lmax test	*P-value*
r = 0*	0.8301	173.49	0.0000	62.035	0.0000
r ≤ 1*	0.7729	111.45	0.0000	51.885	0.0000
r ≤ 2*	0.6207	59.568	0.0022	33.933	0.0047
r ≤ 3	0.3566	25.634	0.1441	15.434	0.2705
r ≤ 4	0.2128	10.200	0.2705	8.3746	0.3498
r ≤ 5	0.0508	1.8253	0.1767	1.8253	0.1767

* Indicates rejection of the hypothesis at the 5% level

Table 4 shows the Vector Error Correction Models (VECM) result contains the short-run estimate and diagnostics measurements. Rainfall lagged by one period (LnRF (-1)) coefficient is positive and significant at 5% relating with food security (proxy of FPI – food production index). At lagged two periods (LnRF (-2)) rainfall coefficient is also positive and significant at 10%, while the long-run the coefficient is negative (-2.7576). This indicates that rainfall will have negative impact on food security at long-run in Nigeria. However, coefficient of LnRF (rainfall) that is positive in the short-run and negative in the long-run and significant at 1% and 10% in the short-run. This implies and proofs that the vagaries of change in the climatic of rainfall will have negative impacts on food security; availability, accessibility, utilization and stability in Nigeria both presently and in the future. This result agrees with Idumah et al. (2016) who reported negative and positive coefficient of rainfall at long-run and short-run respectively affecting agricultural output in Nigeria.

Table 4. Vector Error Correction Models (VECM) of food security (PFI)

Variables	Coefficient	Std. Error	t-ratio	p-value
Const	-1.13795	0.241794	−4.706	0.0001***
ΔLnRF(-1)	0.872805	0.368989	2.365	0.0277**
ΔLnRF(-2)	0.372304	0.187892	1.981	0.0608*
ΔLnTemp(-1)	7.66926	1.48921	5.150	0.0000***
ΔLnTemp(-2)	3.56993	1.17803	3.030	0.0064***
ΔLnPGR(-1)	−29.3567	19.5126	−1.504	0.1473
ΔLnPGR(-2)	−72.4525	18.1278	−3.997	0.0007***
ΔLnUPR(-1)	1.88965	6.39658	0.2954	0.7706
ΔLnUPR(-2)	5.54598	6.54922	0.8468	0.4066
ΔLnALU(-1)	−3.15678	1.19590	−2.640	0.0153**
ΔLnALU(-2)	−1.39756	0.774330	−1.805	0.0855*
EC1	−2.75762	0.490033	−5.627	0.0000***
EC2	−8.08636	1.71979	−4.702	0.0001***
Mean dependent var	0.006962	S.D. dependent var		0.199354
Sum squared resid	0.118725	S.E. of regression		0.075190
R-squared	0.912135	Adjusted R-squared		0.857743
Rho	−0.055787	Durbin-Watson		2.111077

***, **, * Significant at 1%, 5% and 10% respectively.

Temperature lagged by one period (LnTemp (-1)) and two periods (LnTemp (-2)) has a positive coefficient and significant at 1% respectively in relationship with food security, with negative coefficient (-0.0637) in the long-run. This implies that temperature has a significant impact on food security in Nigeria. Therefore, the variables of climate factor of rainfall and temperature employed in the research proved that climate change has a significant harmful impact on food security in Nigeria.

The coefficient of population growth rate lagged at one period (LnPGR (-1)) has a negative but insignificant relationship with food security, while at lagged of two periods (LnPGR (-2)) is negatively significant at 1% relating to food security, with negative coefficient (-0.0637) in the long-run. The implication of this is that, if the rapid population growth rates continues unchecked or control it has a capacity to throw the country into food crisis as stated in Malthus theory in literature. On the other hand, urbanization population growth rate at both one (LnUPR (-1)) and two lagged periods (LnUPR (-2)) are positive and insignificant related to food security at short-run. This implies that urbanization population growth is an essential component in the development of any nation economy growth; it provides labour-force needed for production of goods and services to enhance and achieve economic growth and development, as well as a vital determinant capacity of a nation investment. This result agrees with Pontianus and Oruonye (2021) who stated that increase in population growth is a crucial dynamic in national economic development, and nation's benefit from its rapid population growth is a function of such nation's quality of her human capital prowess. At long-run the coefficient of urbanization population growth rate shows negative (-0.0103) relationship with food security. This maybe has a result that most urban dwellers do not engaged in agricultural activities that will results in food security, this may have happened as a result of migration from rural areas to urban areas in search of green pastures for livelihood of Nigerians.

The lagged period of agricultural land used at one (LnALU (-1)) and at (LnALU (-2)) were negatively significant at 5% and 10% respectively, with negative coefficient (-0.0081) at long-run related to food security. The implication of this short-run negative but

insignificant related to food security could be that the farmland is not maximally utilized for production of food needed to feed many mouths as a result of rapid population growth and rural-urban migration in Nigeria. This could lead to food crisis, hunger and malnutrition; if the agricultural land is not maximally utilized and the migration to urban areas goes on unchecked.

The error correction (EC) coefficient EC1 and EC2 indicates a negative signed, this implies that the error correction is rightly signed. The coefficient of EC1 and EC2 were negative and significant at 1% respectively. This substantiating the existence of long-run relationship between dependent and explanatory variables (food security with climate change, population growth rate), *ceteris paribus*. This shows the rate of adjustment to the previous year short-run disequilibrium from long-run equilibrium value of the independent variables; this reveals that the food security in Nigeria is a consequence of rainfall, temperatures, population growth rate, urban population growth rate and agricultural land used in the same way as other tropical countries.

The coefficient of multiple determinations (R^2) is 0.912135. This indicates that about 91% of the overall variations in the dependent variable FPI (food security) was jointly explained by the action of all the independent variables. The adjusted R^2 is 0.857743 implying that decrease in the degree of freedom as a consequence of the including additional independent variable may not alter significantly the goodness of fit of the regression level and also shows the reliability of the variables included in the model. Durbin-Watson test is use to examines the null hypothesis that the linear regression residuals of any time-series data are uncorrelated. Durbin-Watson estimation coefficient (2.1) was obtained; this indicates a negative serial correlation, this falls within the acceptable value (1.50 - 2.50). The implication of this negative autocorrelation is that the variable under consideration has influence on itself over time. For instance, if there is food insecurity in the previous year, there is a greater likelihood that there will be stability in food security in the preceding year or *vice versa*. This can be proved in the variance decomposition as sown in Table 5 as well.

Table 5. Variance decomposition of food security (PFI)

Period	Std. Error	LnPFI	LnRF	LnTemp	LnPGR	LnUPR	LnALU
1	0.0363	100.000	0.0000	0.0000	0.0000	0.0000	0.0000
2	0.0382	90.0877	0.3512	7.5339	0.3539	0.0107	1.6626
3	0.0550	87.9908	4.3789	5.1570	0.4897	0.5674	1.4161
4	0.0559	86.0033	6.1886	5.0477	0.5488	0.5735	1.6382
5	0.0640	85.5527	6.6141	5.5583	0.4452	0.5692	1.2605
6	0.0654	85.3706	6.4479	5.9433	0.4306	0.5990	1.2087
7	0.0701	86.0779	6.0761	5.7067	0.3929	0.6733	1.0730
8	0.0719	86.1311	5.7843	6.0166	0.3779	0.6675	1.0226
9	0.0755	86.3891	5.5212	6.0923	0.3496	0.7130	0.9348
10	0.0776	86.6132	5.2356	6.2102	0.3313	0.7231	0.8867

The decomposition of variance signifies the extent of information of each variable impact to the other variables in the autoregression. It shows to what extent the forecast error variance of each variable can be clarified by the exogenous shocks to the other variables. Table 5 shows the variance decomposition of the shocks received by the PFI (food security) to its component bases.

In the short-run for instance, in the period 3, shock to RF (rainfall) and Temp (temperatures) estimated to be about 4.38% and 5.16% respectively of the variation in PFI (food security). Whereas, the result shows that in the short-run the shock to PFI itself estimated to be about 87.99% deviations in its variability and flux. In the long-run, at period 10 the shock to RF and Temp estimated to be about 5.24% and 6.21% respectively variation in PFI, while the long-run shock to PFI itself was about 86.61%.

4. Conclusion

This research examined the impacts of climate change and population growth on food security in Nigeria. Augmented Dickey-Fuller (ADF) unit root test was utilized to test the stationarity of the data, Vector Autoregressive (VAR) model, Johansen cointegration test was utilized to test the long-run relationship and the vector error correlation models (VECM) was utilized to estimates the short-run trend. ADF unit root test result shows that all variables were completely stationary at the first different orders at both at intercept and intercept with trend. VAR model shows that Akaike Information Criterion AIC and Hannan-Quinn Criterion HQC were three (3) lags, hence, three (3) lags were conclusively selects as the optimum lag in the model.

The result of Johansen cointegration shows the existence of a long-run relationship among the variables used in this research. VECM result shows that climatic factors used in this research (rainfall and temperatures) has a

negative and significant effects at lagged period of one and two respectively related to food security in Nigeria both at present and in the future in the short-run. Population growth rate is also negative and significant at lagged two periods, while urban population growth has positive effect on food security in the short-run. Since most of the urban dwellers did not engage in farming activities. The lagged one and two periods of agricultural land used were negatively significant related to food security. Hence, agricultural land used also has adverse effect on food security as most agricultural land are cleared for urban development and other social activities. The decomposition of variance signifies the extent of information of each variable impacts to the other variables in the autoregression. The research therefore, recommends that:

i. Since rapid population growth rate in itself is not a problem to the nation, migration from rural areas to urban areas should be controlled by providing social amenities needed for their comfort in the rural areas; at such it will lead to more land for cultivation which will eventually lead to more harvesting then food security. Similarly, to curb the adverse of urban population on food security, there should be public enlightenment campaign to educate Nigerians on birth control through family planning methods.

ii. To minimize the negative effect of climate change on food security, modern irrigation systems can be adopted to solve the problem of erratic rainfall pattern uncertainty in cropping systems. This will enhance crop productivity in such areas with such difficulties. While, drought resistant crop seed and seedling varieties should be grown while livestock with resistant to diseases should be rear for maximum productivity.

iii. For agricultural land use good soil management should be adopted, efficient manure application and agricultural waste management should be adopted, good agricultural land management techniques (like agroforestry, tillage maintenance, restoration and reintegration of degraded crop and pasture land) and indiscriminate bush burning and deforestation should be stopped and backed up with legal laws. Although, it will be challenging for most Nigerian farmers to adapt to the climate change because of their poverty levels without government aids. Hence, government can expedite the rate of adaptation through some measures like agricultural insurance, distribution of improved seed crop varieties, provision of timely information on climatic factors and practical production advice to farmers.

Author Contributions

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

	A.R.A.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

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

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

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

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HAEMATOLOGICAL AND SERUM BIOCHEMICAL INDICES OF WEST AFRICAN DWARF RAMS FED ENSILED ELEPHANT GRASS AND GMELINA ARBOREA LEAVES

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Abstract: In a study to evaluate the haematological parameters and serum indices of rams fed with ensiled Elephant grass and *Gmelina arborea* leaves, 16 WAD rams were randomly assigned to four dietary treatments for 4 weeks. Each treatment was replicated thrice in a Completely Randomized Design (CRD). The data obtained were subjected to analysis of variance (ANOVA) using procedure of SAS (2002); where analysis indicated significant difference, the significant means were compared using the Least Significant Difference method. Water and feed were given ad-libitum. Major parameters measured included: Red Blood Cell (RBC), Packed Cell Volume (PCV), White blood Cell (WBC), Hemoglobin, lymphocytes, neutrophils, monocytes, Total Protein (TP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), albumin, glucose, urea, creatinine, cholesterol, High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL). The four dietary treatments were 80% Elephant grass+ 20% cassava peels+0% Gamhar (T1), 60% Elephant grass+ 20% cassava peels+20% Gamhar (T2), 20% Elephant grass+ 20% cassava peels+60% Gamhar (T3), and 0% Elephant grass+ 20% cassava peels+80% Gamhar (T4). Except for red blood cell (RBC), white blood cell (WBC), monocytes, creatinine, urea and LDL, all the haematological and biochemical parameters measured were significantly ($p<0.05$) different across the dietary treatments. It was observed that the ensiled diets offered to the rams did not have deleterious effect on the haematological and serum biochemical indices as the values registered across the dietary treatments falls within the normal ranges. It was concluded that all four test diets were suitable for dry season ram feeding and that *Gmelina arborea* could be included in ram diets up to 80% without any harmful effect on their haematological and serum biochemical profile.

Keywords: Haematology, Serum, Indices, Dietary treatment, West African Dwarf rams

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1. Introduction

Small ruminants such as rams play important role in the livestock subsector of the Nigerian agricultural economy (Lakpini et al., 2002). The West African dwarf rams are well adapted to the environment i.e. West African humid zone and trypanotolerant.

Decline in nutritive value of vegetation resulting from senescence combine to make it difficult for livestock to meet their nutritional requirement during the dry season. Such a situation has long been recognized to result in cyclic body weight gain in the rainy season and weight loss in the dry season (Annor et al., 2007). To break this cycle, animal nutritionists have recommended feed supplementation. However, the use of staple cereal, grains as supplements leads to competition between humans and animals and increases the cost of feed supplementation, making supplementation unprofitable or unsustainable, especially in poor communities. The need, therefore, exists to find reliable and sustainable sources for feed supplementation with the view to helping to improve the profitability of livestock production during periods of inadequate and/or poor-quality herbage supply.

According to Ranjhan, 2001 crop residues (straws and stovers) and agro-industrial by-products will remain important sources of feed for livestock production.

Madubuike and Ekenyem (2006) reported that haematological and serum chemistry assay in livestock could indicate the physiological response of livestock to their nutrition. Esonu et al. (2001) had earlier come to the same conclusion that haematological constituents reflect the physiological responsiveness of the animal to its internal and external environment.

Blood is an important index of physiological and pathological changes in an organism (Mitruka and Rawnshey, 1977). The primary function of blood is to transport oxygen from respiratory organs to body cells (Duke, 1975) distributing nutrients and enzymes to cells and carrying away waste products thereby maintaining homeostasis of the internal environment (Bentrick, 1974). The various functions of blood are carried out by the individual and collective actions of its constituents-

the haematological and biochemical components (Akinmutimi, 2004).

Haematological tests have been widely used for the diagnosis of various diseases and nutritional status of animal. The information gained from the blood parameters would substantiate the physical examination and together with medical history provide excellent basis for medical judgment (Schalm et al., 1975).

In general, blood examination is performed for several reasons as a screening procedure to assess general health (Jain, 1993). Glucose, cholesterol, calcium, total protein, alkaline phosphates, uric acid, sodium, potassium, chloride levels are diagnostic values for diabetes mellitus, liver disease, hyperparathyroidism, chronic hepatopathy and liver disease, gout, kidney disease, chronic diarrhea and dehydration respectively. It had been reported that biochemical changes because of toxins have effects on haematological parameters (Karnish, 2003).

A quantifiable variation was reported in blood parameters due to management, feeding level, age, sex, health status, method of blood collection, haematological techniques used, diurnal and seasonal variations, ambient temperature, and physiological status (excrement, muscular exercise, time of sampling, water balance and transportation. (Schalm et al., 1975)

Nutrition, breed, sex, age, reproductive status, environmental factors, stress, and transportation are known to affect haematological and biochemical indices and thought to play major roles in the differences in haematological and biochemical parameters.

These differences have further underlined the need to establish appropriate physiological baseline values for various breeds of livestock in Nigeria, which could help in the realistic evaluation of the management practices, nutrition, and diagnosis of their health condition.

The objective of this work was, therefore, to evaluate the nutritional value of four diets formulated from locally available feedstuffs in Ejigbo district of Osun as feed for rams. The test diets were ensiled mixture of elephant grass and cassava peels with different levels of *Gmelina arborea* leaves. West African dwarf rams were used for the dietary testing and the data collected and evaluated included haematological and serum biochemical indices.

2. Materials and Methods

2.1. Experimental Location

The experiment was carried out at the Research site of Osun State University, Ejigbo campus, Osun State, Nigeria, located on the longitude $7^0 54' 0'' N$ $4^0 18' 54'' E$ and latitude $7.90000^0 N$ $4.31500^0 E$ and at an altitude 426M above sea level (EN.Wikipedia.org/wiki/ejigbo, 2011). Ejigbo is in the middle of 35km to Northeast of Iwo, 30km from Ogbomoso in the North and about 24km to East. The mean annual rainfall in Ejigbo is about 52.35mm and there is deviation from the mean value from year to year.

2.2. Experimental Materials

The materials used for the study are 16 juvenile West African Dwarf rams, jute bags, roll of nylon, roll of robe, plastic bowls and drums, cotton wool, methylated spirit, ice block, sand, Ethylene Diamine Tetra acetic Acid bottles.

2.3. Experimental Animals and Their Management

Sixteen West African dwarf rams of $1^{1/2}$ years were purchased from the rural settlers with an average live weight of 20-30kg. The animals were housed intensively in well-ventilated pens, in an open-sided house with corrugated aluminum roofing sheet and a concrete floor, which was washed, disinfected with Izal and covered with bedding material (wood shavings) before the arrival of the animals. The rams were given prophylactic treatments which consist of intramuscular application of oxytetracycline at the dosage of 1ml/10kg body weight of the animal. Fresh water was supplied ad-libitum.

Before the commencement of the experiment, the animals were left for a week to acclimatize to the new environment; the experimental units were treated against ectoparasites with 0.5ml/10kg body weight of Ivermectin.

2.4. Experimental Diet

Three test ingredients used are *Gmelina* leaves, Elephant grass and cassava peels; they were collected at their early stage in the morning and evening. The test ingredients were ensiled for 21 days. The ensiled mixture of Elephant grass, *Gmelina* leaves and Cassava peel was done at the following ratio T1 (80:0:20%), T2 (60:20:20%), T3 (20:60:20%), T4 (0:80:20%). Experimental units were subjected to the diets without concentrate feeding using a complete randomized design

2.5. Experimental Procedure

10mls of blood samples were collected from each of the animals via jugular vein puncture using syringes. 5mls of the collected blood samples was put into plastic tubes containing the anti-coagulant Ethylene Diamine Tetra Acetic Acid (EDTA) for the determination of haematological parameters i.e., the analysis for packed cell volume (PCV), hemoglobin (Hb), white blood cells (WBC), red blood cells (RBC), lymphocytes, neutrophils, and monocytes. The remaining 5mls of the blood samples was put into anti-coagulant free plastic tubes and allowed to coagulate at room temperature for subsequent biochemical analysis: serum protein, serum glucose, serum albumin, creatinine, urea, serum alanine transaminase (ALT) and serum aspartate transaminase (AST). The blood cholesterol levels were also analyzed including the HDL (High Density Lipoprotein) and the Low-Density Lipoprotein (LDL).

2.6. Data Collection and Analysis

Blood samples were taken from the rams before feeding via the jugular vein puncture between 07:00 and 09:30 h local time at the last day of each experimental period for haematological and blood biochemical assays. The blood samples were taken to the laboratory soon after collection in a sample holder placed in an ice chest. Two different test tubes were used to harvest blood from each of the rams. A plain test tube was used to collect blood to

obtain serum for the determination of blood glucose, total protein, albumin, urea, creatinine, AST, and ALT. The other test tube, which contained Ethylene Diamine Tetra Acetic Acid (EDTA) as anticoagulant, was used to analyze for Hemoglobin (Hb) concentration, Packed Cell Volume (PCV), White Blood Cell (WBC) count, RBC, lymphocyte, neutrophil, and monocytes.

2.7. Chemical Analysis

The packed cell volume percentages were measured for each blood sample in fresh ethylene diamine tetra acetic acid (EDTA) anticoagulant samples within 24hours of collection using the micro-hematocrit method. Hemoglobin concentration was also measured in fresh EDTA anticoagulant samples using the Sahl's (acid hematin) method. RBC was measured in fresh EDTA with the aid of Neubaur counting chamber (hemocytometer). Blood smears were used for total WBC counts. Differential relative and absolute counts were classified as lymphocytes, neutrophils, and monocytes.

Plasma glucose was measured using the enzymatic glucose oxidase method (Bauer et. al. 1974). Total serum protein was measured in serum for individual animal using the biuret method. Serum alanine transaminase and serum aspartate transaminase was analyzed spectrophotometrically by using commercially available diagnostic kits (Randovl Test Kits). Serum creatinine was determined using the principle of Jaffe reaction.

2.8. Statistical Analysis

Resulting haematological and biochemical data obtained from the samples was laid out as Completely Randomized Design and analyzed with one-way Analysis of Variance (ANOVA) using procedure of SAS (2002). The significant means were compared using the least significant different (LSD) method.

3. Results and Discussion

Haematological parameters of WAD rams fed ensiled Elephant grass and *Gmelina arborea* leaves is presented Table 1. The reference ranges of values were reported by Oyeyemi and Ajani (2014) for West Africa Dwarf (WAD) rams of 18–24 months, which weighed 20–25kg and stated to be within normal range. The observed haematological values show that except for Packed Cell Volume, Hemoglobin, lymphocyte, and monocytes where the mean values between the four diets significantly ($P<0.05$) differs, although the white blood cells count is not significant, the means on the same rows differs. All the other haematological parameters did not. Mean packed cell volume is highly significant $P\leq0.001$ and has the highest mean value in T4 and least in T1. However, these values were within the range of 21-35% reported for WAD goats by Daramola et al. (2005). The implication of this observed PCV values, going by the reports of Dargie and Allonby (1975), is that only the rams on T4 diet could probably have the high tendency for a return of PCV to normal value following an infection through compensatory accelerated production. This is because only the rams on this diet had values above the 32% PCV documented to be normal for circulatory system in sheep.

Table 1. Haematological parameters of WAD rams fed ensiled Elephant grass and *Gmelina arborea* leaves

Parameters	Reference values	T1	T2	T3	T4	SEM
RBC (10^6/mm^3)	9-15	9.83	9.62	9.94	10.30	1.47
PCV (%)	27-45	28.91	30.05	31.92^b	32.46^a	3.92***
WBC (10^6/mm^3)	4-12	6.88^b	7.53^a	7.02^a	7.75^a	0.80
Hb (g/dl)	9-15	8.79^c	10.01^b	10.69^b	11.71^a	3.63**
Lymphocytes (%)	40-75	53.63^c	54.05^c	55.86^b	57.01^a	4.90*
Neutrophils (%)	10-50	35.76^c	37.58^b	37.51^b	38.41^a	4.56**
Monocytes (%)	7-9	2.07	2.04	2.02	2.05	0.49

^{a,b,c} Means on the same row with different superscript are significantly ($P<0.05$) different. *$P\leq0.05$, **$P \leq 0.01$, ***$P \leq 0.001$. SEM= standard error of mean, RBC= red blood cell, PCV= packed cell volume, WBC= white blood cell, Hb= haemoglobin.

The hemoglobin concentration in the blood of the studied rams showed a similar pattern of variation as with PCV. Nevertheless, the hemoglobin range in this study fell within the range of 7-15g/dL reported by Daramola et al. (2005). However, higher in T4 than the value of 11.40g/dL reported for Red Sokoto goats (Tambuwal et al. 2002) and in cattle fed different levels of extracted rice bran (Singh et al., 2002). With the relatively higher Hb concentration observed in this study, the dietary treatments generally seem to be capable of supporting high oxygen carrying capacity blood in rams.

The values obtained in this study for lymphocytes and neutrophils fell within the broad range of 47-82% and 51.6% reported by Daramola et al. (2005) and Tambuwal et al. (2002) and 36.4% for lymphocytes and 17-52% neutrophils reported by the same authors respectively. These values are suggestive of a well-developed immune system in the WAD rams with such number of immune cells to proffer good health (Daramola et al., 2005). The result also implies that an increase in lymphocytes is associated with a decrease in neutrophils and vice versa (Lazzaro 2001).

White Blood Cell count obtained in this study at the end of the experiment though not significant increases across the dietary treatment row compared favorably with values within the range of 6.8-20.1×10^6/mm^3 reported by Daramola et al. 2005. WAD rams seem to possess a protective system providing a rapid and potent defense

against any infectious agent, and this is probably the physiological basis for the adaptation of these species in their ecological zone (Daramola et al., 2005).

Serum biochemical indices of WAD rams fed ensiled Elephant grass and *Gmelina arborea* leaves is presented Table 2. Serum biochemistry is a generalized medium of assessing the health status of animals. Aside from the values of urea and creatinine differences between the measured biochemical parameters were not significantly (P≥0.05) different between the diets. ALT and glucose values were highly significantly (P≤0.001) different between the dietary treatments. Serum proteins are important in osmotic regulation, immunity, and transport of several substances in the animal body (Jain, 1986). However, in this experiment, the dietary treatments differ more significantly in terms of their Total Protein levels in the serum of the rams. Besides, the significant difference (P≤0.01) between the diets may be related to the serum protein and to the amount of calories contained in the diet but to the availability of protein.

Urea and creatinine levels did not differ significantly between the diets in this study. This study reports high serum urea values across the diets. This may probably have been due to persistent hypoglycemia; catabolic activity is increased for gluconeogenesis, thus resulting in higher serum urea levels. Enzymes are protein catalysts present mostly in living cells and are constantly and rapidly degraded although, renewed by new synthesis. Normal enzyme level in serum reflects a balance between synthesis and their release, because of the different physiological processes in the body.

Transaminase enzymes are those mostly responsible for the synthesis of non-essential amino acids through the process known as transamination.

Serum levels of AST are significantly high under and morbid conditions involving injuries to large numbers of metabolically active cells. However, the result of this study suggests a contrary situation in this regard thus indicating the potential of the studied plant leaves in the feeding of rams. The monitored activities of transaminases enzymes did not vary widely between the diets. The relatively close mean values observed for transaminases could be an indication that the test diets did not differ in their effects on enzyme secretion mechanism.

Table 2. Serum biochemical indices of WAD rams fed ensiled Elephant grass and *Gmelina arborea* leaves

Parameters	T1	T2	T3	T4	SEM
TP (g/l)	6.32^{b}	6.38^{b}	7.04^{a}	7.22^{a}	1.52**
AST (UI/I)	77.37^{c}	79.48^{b}	81.62^{b}	83.92^{a}	5.30**
ALT (UI/I)	27.94^{b}	28.16^{b}	29.20^{a}	29.42^{a}	2.49***
Albumin (g/l)	2.65^{b}	2.59b	3.22^{a}	3.29^{a}	0.11**
Creatinine (mg/dl)	1.24	1.03	1.24	1.24	0.05
Glucose (mg/dl)	59.01^{a}	61.36^{a}	54.99^{b}	52.81^{b}	7.92***
Urea (mg/dl)	8.93	9.65	10.54	10.42	2.30

^{a,b,c} Means on the same row with different superscript are significantly (P<0.05) different. *P≤0.05, **P ≤ 0.01, ***P ≤ 0.001. SEM= standard error of mean, TP= total protein, AST= aspartate aminotransferase, ALT= alanine aminotransferase.

Cholesterol level of WAD rams fed ensiled Elephant grass and *Gmelina arborea* leaves is presented Table 3. The resulting cholesterol values shows that the cholesterol and high-density lipoprotein (HDL) mean values between the dietary treatments significantly differ (P<0.05) while the low-density lipoprotein (LDL) values did not. Mean HDL was highest in diet 4 and lowest in diet 1, however, the implication of the observed values by reports is that there is evidence for a protective effect for dietary fiber against atherosclerosis; a disease of the heart through an increase in the low-density lipoprotein and colon cancer probably through an increased rate of passage of feed residues through the gastro-intestinal tract.

Though, the increase in plasma concentration of HDL cholesterol may be because of polyphenols, which are involved in the regulation of lipid and glucose metabolism. According to some authors, this activates the PPAR-α receptor, with an increased stimulation effect in the liver of the expression of key proteins involved in the metabolism of HDL. It was reported that cholesterol concentration is influenced by the degree of stress.

Table 3. Cholesterol level of WAD rams fed ensiled Elephant grass and *Gmelina arborea* leaves

Parameter	T1	T2	T3	T4	SEM
Cholesterol (mg/dL)	63.06^{b}	64.33^{b}	68.88^{a}	69.93^{a}	4.41***
HDL (mg/dL)	50.58^{c}	54.13^{b}	54.50^{ab}	55.43	2.56***
LDL (mg/dL)	8.13	7.35	7.73	7.04	2.59

^{a,b,c} Means on the same row with different superscript are significantly (P<0.05) different. *P≤0.05, **P ≤ 0.01, ***P ≤ 0.001. SEM= standard error of mean, HDL= high density lipoprotein, LDL= low density lipoprotein.

4. Conclusion

All the haematological and biochemical parameters of WAD rams from this study fell within the normal range of values. It can be concluded that the ensiled Elephant grass and *Gmelina arborea* leaves can be used as dry season feed for WAD rams without any negative effect on the health status of the animals. More studies should be carried on the ensiled Elephant grass with *Gmelina arborea* using other ruminant animals such as goat and cattle.

Author Contributions

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

	O.A.	V.A.
C	50	50
D	50	50
S		100
DCP	70	30
DAI	40	60
L	90	10
W	100	
CR	20	80
SR	60	40
PM	80	20
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 declare no conflicts of interest. The funders had no role in the design of the study, in the collection, analyses, interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

Ethical Consideration

The experimental procedures used in the present study were reviewed and validated by the local Animal Care and Ethics Committee of Osun State University (protocol code: 2021/15 and date: January 27, 2021).

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CHANGES IN STOMATAL PROPERTIES OF SAFFLOWER CULTIVARS UNDER SALINITY

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Abstract: The stomatal characteristics in the leaves play a key role to adapt to several abiotic stresses such as drought, heat, and salinity. This study was conducted at the Seed Science and Technology Laboratory, Eskişehir Osmangazi University in 2022 in order to examine the abaxial and adaxial stomatal properties of 9 safflower cultivars (Dinçer 5-18-1, Remzibey-05, Balcı, Yekta, Linas, Olas, Olein, Safir, and Zirkon) under salt stress (100 mM NaCl). The density, width, length, size, and index of the stomata were measured. The data was analyzed by a two-factor factorial in completely randomized design. The results showed that significant differences for all stomatal features of the safflower cultivars were determined. The stomata density changed with safflower cultivars between 143 and 57 number mm^{-2} and stomata size was observed as 510-698 μm^2. The number of abaxial stomata was higher than the adaxial part of leaves and the stomatal density on the abaxial part of six safflower cultivars (Remzibey-05, Balcı, Yekta, Olas, Olein, and Safir) was decreased by salinity. In addition, abnormal stomata were observed in salt-affected cultivars of Dinçer 5-18-1, Remzibey-05, Yekta, Olein, and Zirkon. The stomata density mainly depended on genetic factors, suggesting that it should be used for separating safflower cultivars, but they declined considerably by salinity. It was concluded that stomatal properties should be considered to clarify the salt tolerance of safflower genotypes.

Keywords: *Carthamus tinctorius* L., Stomata density, Genotype, NaCl

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1. Introduction

Safflower (*Carthamus tinctorius* L.), an annual oilseed crop, is generally produced for edible oil content in seeds in the world. Also, its flowers with different colors are used for natural dye sources and spice (Silva et al., 2022). Due to the wide adaptation ability, safflower can be grown in a wide range of ecological conditions. It is a moderately salt-tolerant plant and produces sufficient seed yield under drought and heat conditions. For this reason, it is especially preferred in arid, semi-arid, and salt-infected areas where the other oilseed crops could not be grown in Türkiye (Kaya et al., 2019).

Salinity has hazardous effects on crop plants at every stage of their life cycle (Kumar et al., 2022) by enhancing the osmotic pressure of water in the soil, and the toxic ion effect, which causes an ionic imbalance in the plant tissues due to excessive Na^+ and Cl^- (Bresler et al., 2012). It leads to retardation in germination and emergence, irregular seedling establishment, and reduction in seed yield resulting from morphological and physiological disorders (Ergin et al., 2021a). Under salt stress, hormonal balance is destroyed, and photosynthesis and protein synthesis are reduced by decreasing nitrate intake (Hasanuzzaman et al., 2021). Moreover, stomatal structures and functions can be changed by salinity (Hedrich and Shabala, 2018). Because the stomata regulate gas exchange between plants and the environment, they are very important in adaptation to different environmental conditions (Ergin et al., 2021b). In wet, dry, cold, or warm conditions, the optimal parameters of the stomatal apparatus are different (Babosha et al., 2022). Additionally, the relationship between the resistance of plants to various environmental conditions and the stomatal characteristics was identified by several researchers (Reynolds-Henne et al., 2010; Hamani et al., 2021; Pitaloka et al., 2022). Mohamed et al. (2020) found that salt-tolerant rapeseed cultivars had fewer stomata than susceptible cultivars. On the other hand, detailed information on stomata movements in safflower under salinity stress has not been found in the literature. The objective of the present study was to investigate the stomatal characteristics and behaviors of some safflower cultivars newly registered in Türkiye under salt stress.

2. Materials and Methods

This study was carried out at the Seed Science and Technology Laboratory at Eskişehir Osmangazi University, Türkiye in 2021. The old and newly registered nine safflower cultivars by the Transitional

Zone Agricultural Research Institute-Eskişehir (Dinçer 5-18-1, Remzibey-05, Balcı and Yekta), Trakya Agricultural Research Institute-Edirne (Linas and Olas) and Isparta University of Applied Sciences, Faculty of Agriculture-Isparta (Olein, Safir and Zirkon) were used as material. The genotypic properties of the safflower cultivars were shown in Table 1.

Table 1. Some varietal properties of the investigated safflower cultivars

Cultivar	Spininess	Flower color	Oleic/linoleic	Registration date
Dinçer 5-18-1	Spineless	Red	Linoleic	1983
Remzibey-05	Spiny	Yellow	Linoleic	2005
Balcı	Spiny	Yellow	Linoleic	2011
Linas	Spiny	Orange	Linoleic	2013
Olas	Spiny	Yellow	Oleic	2015
Yekta	Spiny	Yellow	Linoleic	2019
Olein	Spiny	Orange	Oleic	2019
Safir	Spiny	Orange	Linoleic	2019
Zirkon	Spiny	Orange	Oleic	2019

The seeds of safflower cultivars were pre-germinated in petri dishes at 25°C for 24 h and the seeds with radicle protrusion were transferred to the vials filled with peat:perlit:vermiculite (3:1:1 v:v:v) mixture. Thirty plants from each cultivar were grown in a growth chamber with temperatures of 20°C/15°C day/night, respectively, and relative humidity was set in the range of 60 to 70%. They were divided into two groups. Control group plants were irrigated with distilled water and salt-stressed plants were watered with 100 mM NaCl. Twenty days after sowing, the plants were in the four leaves stage, and five healthy leaves from the first true leaf from each treatment were selected for stomatal measurements. Both lower (abaxial) and upper (adaxial) epidermal surfaces of the leaf were peeled off. Extracted samples were first soaked in acetone, then washed with distilled water and stained with acetocarmine dye. Three-five places of each sample were randomly specified, and the stomata observations were performed by 40× objective lens and 10× eyepieces under a light microscope system Zeiss Axio Scope A.1. Stomata number was counted in a 0.083 mm^2 area. Besides, an ocular micrometer calibrated using a stage micrometer was used to measure the stomata width and length.

The leaf stomata density was expressed as the number of stomata per unit leaf area (number of stomata mm^{-2}). Stomata size was also computed according to the following formula (Equation 1).

$$\text{Stomata size } (\mu m^2)=\pi\left(\frac{\text{Stomata width}}{2}\times\frac{\text{Stomata length}}{2}\right) \quad (1)$$

The stomata index was calculated as stomata length divided by stomata width (Çimen et al., 2016). Abnormal stomata were observed and classified by following the description of Mandal et al. (2012).

The experiment was analyzed by a two-factor completely randomized design with four replications using the MSTAT-C (Michigan State University, v. 2.10) statistical program. The means were separated by Duncan's multiple range test at $P<0.05$ level.

3. Results

Analysis of variance showed that significant differences among safflower cultivars were determined for stomata density. The number of abaxial stomata ranged from 143 to 72 number mm^{-2}, while the number of adaxial stomata varied from 135 to 57 number mm^{-2} (Table 2). Zirkon and Linas gave lower stomata numbers than the others and their stomata number did not decrease by salinity. Our results revealed that the stomata density was changed by safflower cultivars. Similar findings were reported by Ergin et al. (2021b) and Roudbari et al. (2012), who indicated that stomata density was a genetic characteristic and the genotypes possessed different stomata densities. Ergin et al. (2021b) observed higher stomata number per mm^{-2} in safflower cultivars Balcı, Dinçer 5-18-1, Linas, Olas, and Yekta than our results because they used the plants grown at later stages. Under salt stress, the stomata density on abaxial and adaxial leaf surfaces was significantly lower than in control plants. The highest decrease in stomata density on abaxial and adaxial leaves occurred in cv. Safir. Although the number of abaxial stomata of Dinçer 5-18-1 and Linas increased, both the abaxial and adaxial stomata number of cv. Zirkon increased when the salinity was applied.

Table 2. Changes in stomata density (number mm^{-2}) of safflower cultivars under saline (100 mM NaCl) and non-saline (control) conditions

Cultivars	Abaxial		Adaxial	
	Control	100 mM NaCl	Control	100 mM NaCl
Dinçer 5-18-1	102^{ef}	112de	90^{cd}	89^{cd}†
Remzibey-05	119^{cd}	72^{g}	74^{fgh}	63^{ıj}
Balcı	120^{cd}	72^{g}	84de	64^{ıj}
Yekta	105^{ef}	76^{g}	86^{cde}	70^{ghı}
Linas	99^{f}	101^{ef}	79^{ef}	57^{j}
Olas	143^{a}	100^{ef}	115^{b}	95^{c}
Olein	129^{bc}	93^{f}	87^{cde}	74^{fgh}
Safir	141^{ab}	74^{g}	135^{a}	66^{hıj}
Zirkon	95^{f}	98^{f}	62^{ıj}	75^{fg}
Mean	117^{a}	89^{b}	90^{a}	73^{b}
Analysis of Variance				
Salinity (A)	**		**	
Cultivar (B)	**		**	
A×B	**		**	

†Means followed by the same letter(s) are not significantly different at P<0.05. ** Significant at 1%.

The stomata images of the investigated safflower cultivars were displayed in Figure 1 and it can be easily understood that the number of abaxial stomata was reduced by salt stress. Also, we determined that the salinity caused a reduction in stomatal density on the abaxial part of 6 safflower cultivars. In previous studies, a decreased stomata density in salt-affected plants was reported by Hamani et al. (2021) in cotton, Çavusoğlu et al. (2007) in barley, El-Kady et al. (2021) in sugar beet, and Dikobe et al. (2021) in maize. As expected, the adaxial part of the leaves had a lower number of stomata than the abaxial surface.

The effect of salt stress on the width of the abaxial stomata was significant (Table 3). The stomata width on the adaxial part was not changed by salinity, while it was decreased in the abaxial. However, Remzibey-05, Yekta, and Safir had similar stomata width on the abaxial surface. Adaxial stomata width was narrowed in all cultivars except Dinçer 5-18-1, Remzibey-05, and Olein cultivars. To regulate water balance during salt stress, the plants reduce evaporation by closing the leaf stomata. Thus, variations in stomatal morphology and physiology can be considered the first defensive reactions or acclimation mechanisms against salinity (Kiani-Pouya et al., 2020; Yan et al., 2020). A decrease in stomatal width was recorded by Cavusoglu et al. (2007) in barley and Hamani et al. (2021) in cotton.

Under salt stress, the stomata length of safflower cultivars was significantly varied. The abaxial stomata length of cv. Yekta (33.6 µm) and the adaxial stomatal length of cv. Balcı (35.4 µm) were superior to the other cultivars (Table 4). Generally, the abaxial stomatal length was enhanced under salinity, while the adaxial stomatal length was decreased. Safflower cultivars Dinçer 5-18-1, Balcı, Olas, and Olein shortened their abaxial stomatal length under salt stress. However, the adaxial stomatal length of safflower cultivars was severely depressed by salt stress, while it was slightly increased in Remzibey-05, Olein, and Safir. These results are in agreement with the findings of Kiliç and Kahraman (2016), who determined a 24% reduction in stomata length of barley under salt stress. Roudbari et al. (2012) stated that there were limited significant changes in stomata length of 15 safflower genotypes, but the length of stomata was shortened by drought.

There was a significant difference in the interaction of salinity × cultivar for stomata size of abaxial and adaxial parts. Under salinity, Yekta showed a 14.8% increase in stomata size, while Dinçer 5-18-1 cultivar possessed 20.6% smaller stomata than the others (Table 5). The largest adaxial stomata size was measured in cv. Balcı (724 µm^2), but Yekta had the smallest stomata (470 µm^2). A clear negative correlation between the size of stomata and sensitivity to salinity in cotton by Munis et al. (2010) and in bean by Bray and Reid (2002) was reported. In our study, the negative and significant correlation between stomata density and size in control and salinity was calculated as r= -0.508** and r= -0.690**, respectively. This shows that increasing the stomata number caused a decrease in stomata size.

No significant changes were determined in the abaxial stomata index, and it was measured between 1.34 and 1.12 (Table 6). However, salinity led to a reduction in the stomatal index of the adaxial layer. The highest decrease was calculated in cv. Olein with 13%. These results agree with the findings of Bray and Reid (2002), who reported significant differences in the stomata index of bean.

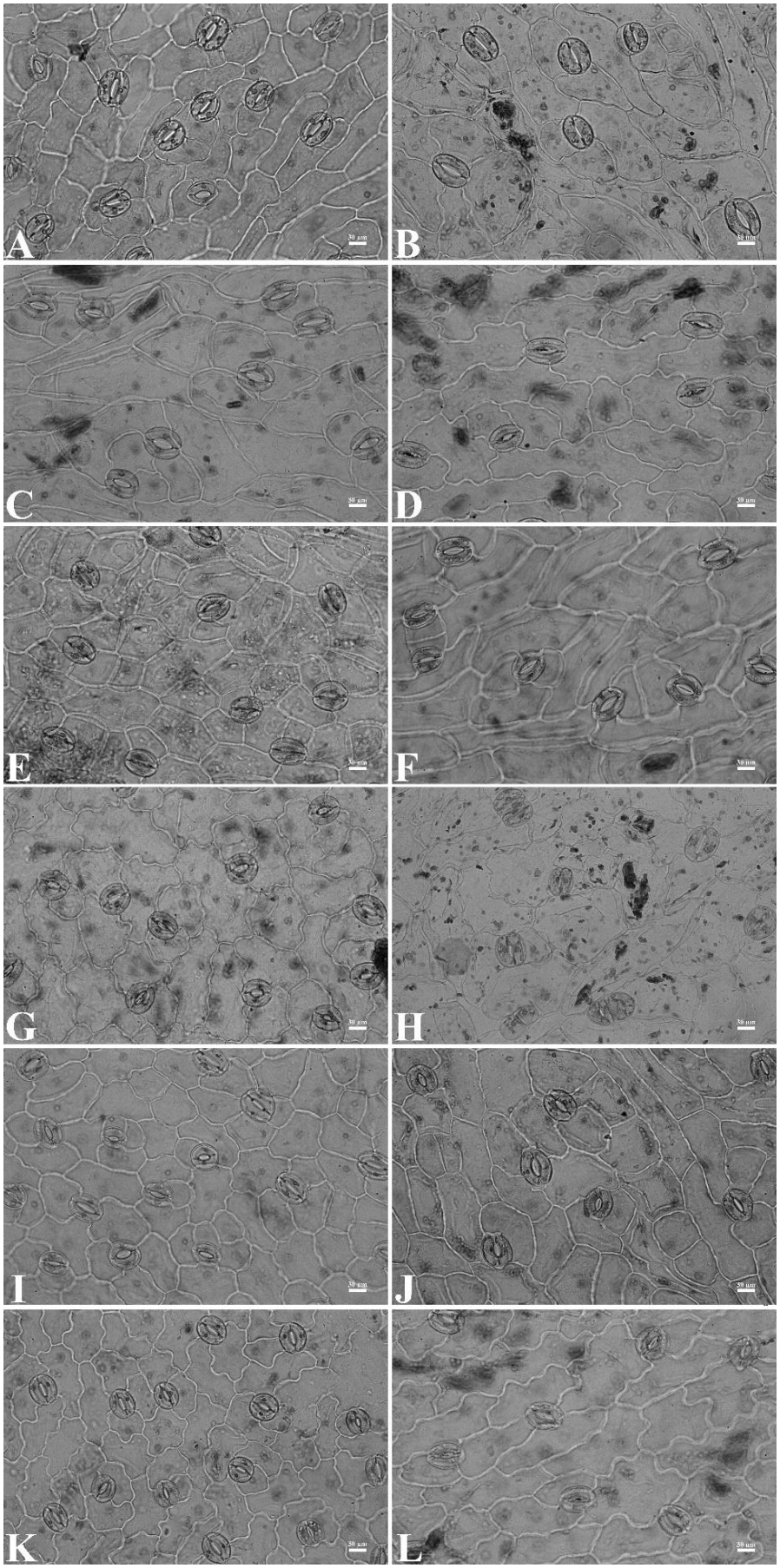

Figure 1. The abaxial stomata images of safflower cvs. Remzibey-05 (A), Balcı (C), Yekta (E), Olas (G), Olein (I), and Safir (K) in control on the left column, and salt-stressed cvs. Remzibey-05 (B), Balcı (D), Yekta (F), Olas (H), Olein (J), and Safir (L) on the right column.

Table 4. Changes in stomata length (µm) of safflower cultivars under saline (100 mM NaCl) and non-saline (control) conditions

Cultivars	Abaxial		Adaxial	
	Control	100 mM NaCl	Control	100 mM NaCl
Dinçer 5-18-1	32.4^{abc}	28.7^{f}	30.4^{def}	28.9^{fgh}†
Remzibey-05	30.9^{cde}	32.7^{ab}	28.0^{hı}	31.4^{bcd}
Balcı	32.5^{abc}	31.8^{b-e}	35.4^{a}	30.1^{def}
Yekta	30.6^{e}	33.6^{a}	31.3^{cd}	30.4^{de}
Linas	28.9^{f}	32.3^{a-d}	29.7^{efg}	29.2^{e-h}
Olas	28.9^{f}	28.4^{f}	32.6^{bc}	28.6^{ghı}
Olein	32.1^{a-e}	30.6^{e}	28.3^{ghı}	29.7^{efg}
Safir	28.7^{f}	31.0^{cde}	27.5^{ı}	29.7^{efg}
Zirkon	30.7^{de}	32.0^{a-e}	32.7^{b}	28.0^{hı}
Mean	30.6^{b}	31.2^{a}	30.6^{a}	29.6^{b}
Analysis of Variance				
Salinity (A)	**		**	
Cultivar (B)	**		**	
A×B	**		**	

†Means followed by the same letter (s) are not significantly different at P<0.05. **Significant at 1%.

Table 5. Changes in stomata size (µm^2) of safflower cultivars under saline (100 mM NaCl) and non-saline (control) conditions

Cultivars	Abaxial		Adaxial	
	Control	100 mM NaCl	Control	100 mM NaCl
Dinçer 5-18-1	698^{a}	554^{ghı}	558^{ef}	574de†
Remzibey-05	615^{c-f}	654^{abc}	510^{g}	632^{bc}
Balcı	686^{ab}	634^{b-f}	724^{a}	571^{e}
Yekta	578^{e-h}	664^{abc}	581de	564^{ef}
Linas	591^{d-h}	619^{c-f}	615^{bcd}	590de
Olas	543^{hı}	512^{ı}	644^{b}	513^{g}
Olein	613^{c-g}	581^{e-h}	470^{h}	599^{cde}
Safir	572^{fgh}	629^{b-f}	517^{g}	562^{ef}
Zirkon	651^{a-d}	639^{a-e}	647^{b}	527^{fg}
Mean	616	610	585^{a}	570^{b}
Analysis of Variance				
Salinity (A)	ns		*	
Cultivar (B)	**		**	
A×B	**		**	

†Means followed by the same letter (s) are not significantly different at P<0.05. *, **Significant at 5% and 1%, respectively.ns= non-significant.

Table 6. Changes in stomata index of safflower cultivars under saline (100 mM NaCl) and non-saline (control) conditions

Cultivars	Abaxial		Adaxial	
	Control	100 mM NaCl	Control	100 mM NaCl
Dinçer 5-18-1	1.18	1.17	1.30^{a-d}	1.15^{gh}†
Remzibey-05	1.22	1.29	1.21^{d-h}	1.23^{c-g}
Balcı	1.21	1.26	1.36^{a}	1.25^{b-f}
Yekta	1.28	1.34	1.33^{ab}	1.29^{a-d}
Linas	1.12	1.21	1.12^{h}	1.14^{h}
Olas	1.21	1.24	1.30^{a-d}	1.25^{b-e}
Olein	1.33	1.28	1.34^{a}	1.16^{fgh}
Safir	1.13	1.20	1.15^{gh}	1.23^{c-g}
Zirkon	1.14	1.26	1.31^{abc}	1.17^{e-h}
Mean	1.20^{b}	1.25^{a}	1.27^{a}	1.21^{b}
Analysis of Variance				
Salinity (A)	**		**	
Cultivar (B)	**		**	
A×B	ns		**	

†Means followed by the same letter (s) are not significantly different at P<0.05. **Significant at 1%. ns= non-significant.

In our study, abnormal stomata were monitored and it was determined that some cultivars had contiguous (twin) stomata under salt stress, while abnormal stomata in non-saline conditions were detected in 3 of 9 cultivars (Figure 2A-P). Dinçer 5-18-1, Remzibey-05, Yekta, Olein, and Zirkon cultivars had contiguous stomata only in the presence of saline stress. The contiguous stomata were observed in both control and salt-stressed plants of Linas, Olas, and Safir cultivars. As seen in Figure 2G, Linas had abnormal stomata in control plants. On the other hand, no contiguous stomata were determined in cv. Balcı, indicating that abnormal stomata were mainly determined by genetic factors and were secondarily affected by salinity. Abnormal stomatal patterning has been recorded in *Brassicaceae*, *Asteraceae, Crassulaceae*, *Iridaceae*, *Leguminosae*, *Sonneratiaceae*, and *Moracea*e (Gan et al., 2010; Khan et al., 2018; Choi et al., 2022). Abnormal stomata have been found in many plants grown in arid, salty, or otherwise adverse environments, similar abnormality was also detected in a halophyte, *Sonneratia alba* J. Smith (Gan et al., 2010; Khan et al., 2018).

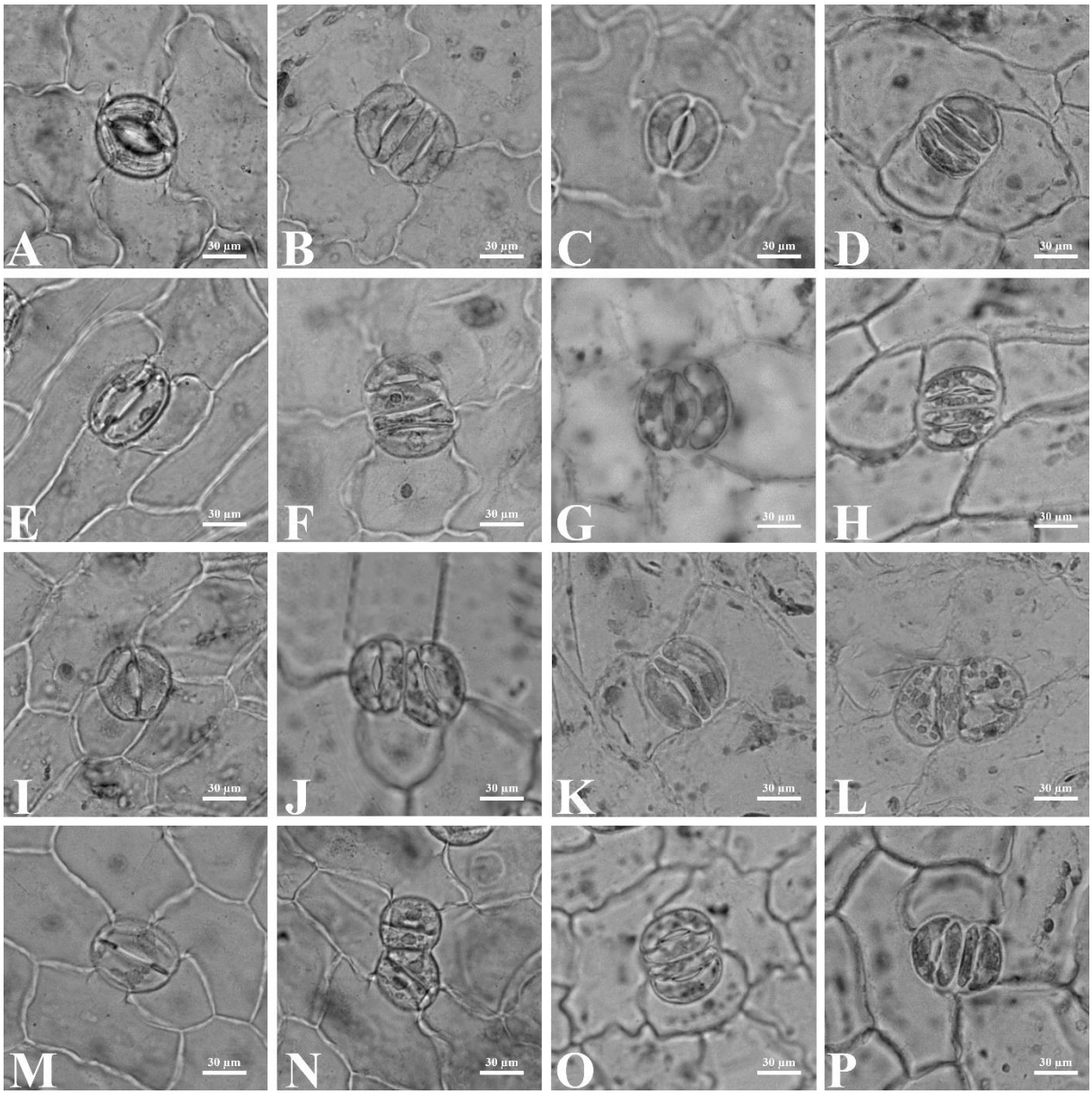

Figure 2. The abaxial contiguous stomata images of cvs. Dinçer 5-18-1 (A), Remzibey-05 (E), Yekta (I), Olein (M), Zirkon (C), Linas (G), Olas (K), and Safir (O) in control on first and third columns, and salt-stressed cvs. Dinçer 5-18-1 (B), Remzibey-05 (F), Yekta (J), Olein (N), Zirkon (D), Linas (H), Olas (L), and Safir (P) on the second and fourth columns.

4. Conclusion

The stomatal characteristics of the plants may vary with genetic and environmental factors. Therefore, determining the stomatal characteristics is very important to classify the adaptation level of plant varieties to adverse soil and climatic conditions. This study showed significant differences in stomata number and size of safflower cultivars; moreover, the stomata densities in cvs. Remzibey-05, Balcı, Yekta, Olas, Olein, and Safir were reduced by salinity. The number of

stomata of safflower cultivars with low stomata density in control did not significantly change by salinity, considering that low stomata number is a hopeful indicator for tolerance to salinity in safflower. Salt stress altered the stomata size with significant reductions in cvs. Dinçer 5-18-1, Balcı, Olas, Olein, and Zirkon. However, abnormal stomata were observed in five cultivars exposed to salt stress. This study provides evidence that stomata anatomy may give useful clues for the salinity tolerance of safflower cultivars, so the stomata observations should be evaluated in further studies on salinity.

Author Contributions

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

	N.E.	M.D.K.
C	50	50
D	50	50
S	30	70
DCP	80	20
DAI	70	30
L	50	50
W	50	50
CR	30	70
SR	80	20
PM	50	50
FA	20	80

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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EVALUATION OF NEUTRAL ELECTROLYZED WATER AS A POTENTIAL FIG PROCESSING SURFACES SANITIZER IN THE FIG INDUSTRY

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Abstract: In this study, the antimicrobial activity of neutral electrolyzed water (NEW) on *Bacillus cereus* forming endospore, *Escherichia coli* and, toxin producer *Aspergillus flavus* and *Penicillium expansum* was determined both on the surface of steel plates in the presence of organic matter artificially inoculated and in cell suspensions. Also, the antimicrobial efficiency of NEW was compared to that of Sodium hypochlorite (NaClO). Experiments were carried out at room temperature (22 °C). 1% sodium hypochlorite solution (with 531 ppm free chlorine), and different concentrations of NEW, 5% (with 63 ppm free chlorine), 10% (with 120 ppm free chlorine), and 15% (with 187 ppm free chlorine) were used for the comparison. Cell suspensions and stainless-steel plates inoculated with a final 10% liquid fig solution were treated with NEW and NaClO for 0 (untreated, control), 15, 30, and 60 seconds. Then, viable cell counts both in cell suspensions and on the inoculated stainless-steel plates were determined. It was determined that there were significant differences ($P<0.05$) in the decrease in the number of microorganisms depending on the application time and free chlorine concentration. The reduction ratios (%) in cell suspensions after 60 seconds of treatment with NEW ranged from 48.8 to 100 for *E. coli*, 11.39 – 32.23 for *B. cereus* and, 31.12 – 100 for *A. flavus*. The reduction ratio for *P. expansum* was %100 for all concentrations of NEW after 60 sec. After 60 seconds application of 1% NaClO to the cell suspensions, the reduction ratios (%) were determined to be 29.56, 23.48, 39.19 and 69.92 for *E. coli, B. cereus*, *A. flavus* and *P. expansum*, respectively. However, in the experiments performed after inoculation of microorganisms and sterile 10% liquid fig solution on the surface of steel plates, it was observed that microorganisms showed greater resistance to NEW and 1% NaClO compared to direct application to the cell suspension. The reduction ratios (%) on the surface of steel plates after 60 seconds of treatment with NEW ranged from 17.66 to 40.07 for *E. coli*, 23.93–31.77 for *B. cereus*, 10,91–30,91 for *A. flavus* and, 49.77–64.85 for *P. expansum*. After 60 seconds application of 1% NaClO on the surface of steel plates, the reduction ratios (%) were 19.38, 11.70, 7.5 and 46.52 for *E. coli, B. cereus, A. flavus* and *P. expansum*, respectively. The results of this study showed that 15% NEW can be used as a strong bactericide and fungicide against endospore-forming bacteria and toxin-producing fungi. Also, 15% NEW is more effective than 1% NaClO in cleaning the surfaces used for fig processing. Therefore, NEW also can be a good alternative to commonly used disinfectants. This is the first report on the use of NEW as a fungicide and bactericide on fig processing surfaces in the fig industry.

Keywords: Neutral electrolyzed water, Fig industry, Endospore-forming bacteria, *E. coli, Aspergillus flavus, Penicillium expansum*

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1. Introduction

Fig (*Ficus carica* L.), named after Caria, an ancient settlement in the Aegean Region, is a fruit belonging to the Moraceae (mulberry) family. The fig, which homeland is Türkiye, spread to Syria and Palestine, and then to China and India. Türkiye ranks first in the world in fig production and export and exported 20 thousand tons of fresh figs and 57 thousand tons of dried figs in 2019/2020.

During the production of dried figs in Türkiye, the figs are dried on trays in a greenhouse or under sunlight (Aksoy, 1997). Since figs are exposed to outdoor air for a long time on the ground during the drying process, the risk of contamination with pests and pathogens is quite high (Flaishman et al., 2008). It has been determined that dried figs are contaminated with aflatoxin and ochratoxin A during the preparation, processing, storage, and distribution stages for marketing (Tosun and Delen, 1998). It has been determined that *Aspergillus* species such as *Aspergillus flavus* and *Aspergillus parasiticus* cause aflatoxin formation, and many saprophytic fungi species such as especially *A. niger* and *Penicillium* species cause ochratoxin A formation in dried figs (Heperkan, 2006). Studies on dried figs have shown the presence of *Bacillus cereus* and *Escherichia coli* contaminations in numbers ranging from 10^7 to 10^8 cfu per gram, except for fungi (Akbas and Ozdemir, 2008).

The amounts of aflatoxins detected in export products are very high in the records of the Rapid Alert System for

Food and Feed (RASFF). For example, the amounts of aflatoxins detected in products such as hazelnuts, dried figs and peanuts exported in 2017 are much higher than the amounts allowed by Türkiye's legislation. In Türkiye, 137 lots in 2017 and 110 lots in 2018 of food products were returned due to aflatoxin residues (RASFF, 2017; 2018). These data show that current food safety standards and regulations are insufficient. Therefore, the development of an effective disinfectant in killing pathogenic microorganisms in food production and agriculture is one of the most critical food safety steps for the principles of Hazard Analysis Critical Control Points (HACCP). The presence and proliferation of pathogenic microorganisms in food establishments and health institutions pose great risks to public health (CDC, 2013). The use of chemical agents as disinfectants in food and food processing areas is the most economical and common method to reduce the number of microorganisms that cause foodborne disease risk to acceptable levels (Brasil, 2007). Approved food disinfectants must be safe for use on food contact surfaces, require no rinsing after disinfection, and be free of dyes and fragrances (Gaulin et al., 2011). However, only a few products are allowed for use in food production areas. In addition, there is limited information on the fight against mycotoxin-producing species. Fungicides such as difenoconazole, azoxystrobin, thiabendazole, and copper oxychloride are effective, but over time, fungi develop resistance to these fungicides (Vasquez-Lopez et al., 2021). The most known fungicides, on the other hand, are not environmentally friendly and also leave residues. Hydrogen peroxide (H_2O_2) is an alternative sanitizing agent approved as safe by Food and Drug Administration (FDA), but it has been determined to cause phytotoxicity in both sweet cherry fruit and stem (Sehirli et al., 2020). Since chemical disinfectants used in food and food processing areas have left residues, more sustainable alternatives such as electrolyzed water (EW, electrolytically generated hypochlorous acid or electrochemically activated (ECA) water) have begun to be investigated (Ovissipour et al., 2015). In recent years, EW containing hypochlorous acid (HOCl) has been recommended as a new and promising sanitizer and cleaning agent. In the literature, it has been stated that there are 5 different types of EW according to the pH value. These are neutral EW (pH 7–8), acidic EW (pH 2–3), alkaline EW (pH 10–13), slightly alkaline EW (pH 8–10), and slightly acidic EW (pH 5–6.5) (Rahman et al., 2016). The three germicidal factors of pH, ORP, and available chlorine concentration (ACC), which includes chlorine (Cl_2), HOCl, and OCl, are used to classify the sterilizing methods of EOW. In the EW solutions, $^{\cdot}OH$ and HOCl both show powerful antibacterial properties and antioxidant actions that support oxidative stability. Neutral EW (NEW) is stated to be the most promising among these types. NEW contain several compounds such as hypochlorous acid (HOCl), hypochlorite ions (ClO), chlorine dioxide (ClO_2), and ozone (O_3). NEW predominantly contains HOCl. HOCl compounds are more active in microbial cell wall penetration and oxidative attacks than ClO^- (Veasey and Muriana, 2016). HOCl infiltrates the membranes of germ cells and produces OH-, which functions as antimicrobial agents through oxidation.

Chlorine loss of NEW (pH 7; ORP 800–900 mV) is not as fast as that of acid EW due to Cl_2 volatilization (Guentzel et al., 2010) and, NEW causes fewer health problems and has less cytotoxic secondary effects compared to acid EW and sodium hypochlorite (NaOCl) (Wang et al., 2007). The usability of EAW in food production, disinfection of food processing and non-food contact processing surfaces has been investigated by many researchers (Guentzel et al., 2010). NEW is a disinfectant that can be used safely because it has fewer adverse effects on workers' hands, food processing surfaces and human health compared to chlorine gas (Al-Haq et al., 2005). It has been determined that it has the potential to be used successfully after harvest due to its strong effect, low production cost, not producing harmful by-products or leaving residues, and being accepted for use in organic production (Villarreal-Barajas et al., 2022).

Stainless steel is the most commonly used material for food production surfaces in the food industry. Ayebah and Hung (2005) reported that there was no negative effect of EW water on stainless steel surfaces for 8-days.

Although there are many studies focused on the antibacterial properties of NEW, studies focusing on its antifungal activity are not many. Some studies reported that NEW shown promising effects on *Aspergillus* spores isolated from peanut seeds (Xiong et al., 2010), on *Botrytis cinerea* and *Monilia fructicola* (Guentzel et al., 2010), and, on Fusarium isolated from cereals (Audenaert et al., 2012). In the same available chlorine concentration level, NEW contains more $^{\cdot}OH$ than acid EW. The $^{\cdot}OH$ is an important fungicidal factor that damages the cellular normal function. The oxidative molecules in NEW destroy microbiological organisms' nucleic acids and enzymes, the cells finally die (Xiong et al., 2010).

This study aims to determine the antimicrobial efficacy of NEW (63, 120, and 187 ppm free chlorine) compared to NaClO (531 ppm free chlorine) on *B. cereus, E. coli*, toxin producer *A. flavus* and *P. expansum* in a short time like 15, 30 and 60 seconds both on the surface of steel plates inoculated organic matter (dry fig) and in the cell suspensions.

2. Materials and Methods

2.1. Preparation of Microorganism Cultures

To prepare the suspensions of *B. cereus* ATCC 11778 and *E. coli* ATCC 35218, these bacteria were inoculated on Nutrient Agar (NA, Merck Ltd., Germany) and after incubation at 37°C for 24 h, several colonies of these bacteria were transferred to the tubes containing 10 mL of NaCl solution (0.9%, w v^{-1}) with the sterile inoculation loop and finally the tubes were vortexed using a thermal

mixer. The final cell concentration was adjusted to 10^7 log CFU mL^{-1}. To confirm the number of bacteria in each culture, 0.1 mL portions of appropriately diluted culture were plated on NA. After incubation at 37°C overnight, the number of viable cells was counted and reported as log10 CFU g^{-1} sample. The prepared bacterial cultures were used in the experiments (Zang et al., 2019).

P. expansum and *A. flavus*, isolated from fig fruits, were cultured on Potato Dextrose Agar plates (PDA, Merck Ltd., Germany) at 25°C for one week. After one week incubation at 25°C, the spores of *P. expansum* and *A. flavus* were collected and suspended in sterile Ringer's solution. After filtering through eight layers of sterile cheese-cloth, the spores were counted and adjusted to a final concentration of 10^7 cells per mL (Spadaro et al., 2002).

2.2. Inoculation

50 g of dried figs were homogenized in sterile distilled water with a blender (Bosch MSM66150) and then, the final volume was completed to 250 mL with sterile distilled water. The prepared 20% liquid dried fig solution was sterilized in an autoclave (Hirayama HG-133) at 121 °C for 20 minutes. 1mL samples from the sterile liquid dried fig solution, which was shaken well, were mixed with 1mL of bacteria and fungal cultures (approximately 10^7 CFU mL^{-1}). Thus, the final ratio of each liquid-dried fig solution became 10% (Zang et al., 2019).

Stainless steel plates (6.0 x 15.0 cm) were purchased from a commercial source. Before inoculation, they were sterilized in an autoclave (Hirayama HG-133) at 121 °C for 20 min after washing thoroughly with tap water. After sterilization, they were dried in a biosafety cabinet for 30 minutes. 0.1 mL of a solution of figs inoculated with microorganisms was spread over a 5 x 5 cm area of the dried stainless steel plates. Then, the inoculated plates were left to dry for 30 minutes at room temperature in a biosafety cabinet so that bacteria and fungi could adhere completely to the surface. Each application was carried out in three parallels and two replications.

2.3. Preparation of NEW

NEW was obtained by electrolysis of a mixture of NaCl (20 g L^{-1}) and tap water using a Stel-10H-120-01 generator (Stel - 10H- 120-01, Russia) at 40.0 V, 9.0 A and a rate of 250 mL 22 sec^{-1}. NEW dilutions were prepared by using sterile tap water, prepared by autoclaving at 121 °C for 15 min, at rates of 5, 10 and 15%. Analytical indices (Oxidation Reduction Potential (ORR), pH and available chlorine concentration (ACC)) of the treated solutions were measured immediately after 5, 10 and 15% NEW preparation. The pH was measured with a pH meter (HI 2211-02, HANNA, USA), and ORP was measured with an ORP meter (HI98120, Hanna, USA). The pH meter was calibrated using commercial standard buffers at pH 4.0 and 7.0 (Merck Ltd., Germany). The ACC was measured based on the iodometric method reported by Dychdala (1983).

2.4. Direct NEW Treatment on Pure Cultures

0.9 mL of 1% NaHOCl or NEW (5, 10 and 15%) was transferred to a sterile tube. 0.1 mL of bacteria and mould cultures were added to the tubes. The cultures were treated with disinfectants for 15, 30 and 60 seconds. Another 0.1 mL liquid was mixed with sterile distilled water as the control. After incubation, 9 mL of neutralizer (0.5% $Na_2S_2O_3$) was added to each tube and the activity of disinfectants was terminated. After a 5-minute neutralization period, viable bacterial counts were determined by the dilution method. To determine viable cell numbers, NA was used for *B. cereus* ATCC 11778 and *E. coli* ATCC 35218 while PDA was used for *A. flavus* and *P. expansum*. Nutrient agar was incubated at 37 °C for 24 hours while PDA was incubated at 25 °C for 3-5 days (Messer et al., 2000). The sterilization rate was calculated using the below equation.

Sterilization rate (%) = 100 (Mc-MT)/Mc

Where, Mc is the total number of microbial colonies before disinfection, CFU mL^{-1}; MT is the total number of microbial colonies after disinfection, CFU mL^{-1}.

Each application was carried out in three parallels and two replications. The average values of the outcomes were presented.

2.5. NEW Treatment on Inoculated Steel Plates and Microbiological Analysis

1% NaHOCl (with 531 ppm free chlorine), and different concentrations of NEW, 5% (with 63 ppm free chlorine), 10% (with 120 ppm free chlorine), and 15% (with 187 ppm free chlorine) and sterile physiological water as a control were sprayed separately on the inoculated stainless steel plates. After different application times of 15, 30 and 60 seconds, the samples collected from the surface by wiping 20 times with sterile swabs were transferred to tubes containing 9 mL neutralizer (0.5% $Na_2S_2O_3$) for microbial analysis. After the tubes were thoroughly mixed with a vortex device at 1500 rpm, the numbers of viable microorganisms were determined by the dilution method. To determine viable cell numbers, NA was used for *B. cereus* ATCC 11778 and *E. coli* ATCC 35218 while PDA was used for *A. flavus* and *P. expansum*. NA was incubated at 37 °C for 24 hours while PDA was incubated at 25 °C for 3-5 days (Messer et al., 2000). The inactivation rate was calculated using the above equation. Each application was carried out in three parallels and two replications. The average values of the outcomes were presented.

2.6. Statistical Analysis

Each application was of complete randomized design and carried out in three parallels and two replications. Results were analyzed by One-way ANOVA using the LSD test ($P<0.05$) to determine differences in the efficiencies of disinfectants on microbial inactivation. The statistical analyses were performed with the statistical program JMP Pro 11.

3. Results and Discussion

For consumers, food safety is very important. A very important step before bringing food to market is cleaning and sterilization. If food processing surfaces are not cleaned and disinfected effectively, microbial contamination can cause food-borne health problems. Several factors complicate or limit the reduction of microbial load and the application of chemical food disinfectants, such as the nature, species, and initial number of any residual microorganisms on the surface, the nature of the organic and inorganic content present on the surface, the effective dose of the disinfectant, the contact time, presence of chemical residues after disinfection, corrosion on food contact surfaces. Therfore, alternatives to traditional disinfectants have been investigated (Wang et al., 2016).

In recent years, a lot of research has been conducted on electrolyzed water. Most of the studies with EW have focused on gram-positive and negative bacteria that do not form endospores in food contamination (Al-Qadiri et al., 2016; Ovissipour et al., 2015). But, Al-Qadiri et al., (2019) examined the antimicrobial activity of NEW against endospore-forming *Bacillus cereus* and *Clostridium perfringens* in cell suspensions laboratory inoculated fresh produce and polypropylene cutting board surfaces.

This study might be the first study to show the efficacy of NEW with low ACC concentrations on endospores and toxin-forming microorganisms in such a short time on dried fig-contaminated surfaces

In our study, it was investigated the effectiveness of NEW containing very low concentrations of ACC (63 -187 ppm) (Table 1) in very short periods (15-60 seconds) against toxin-producing moulds (*A. flavus* and *P. expansum*) as well as gram-negative bacteria (*E. coli* ATCC 35218) and gram-positive endospore-forming bacteria (*B. cereus* ATCC 11778) both in pure cultures and on surfaces containing organic matter.

Table 1. Concentration and physicochemical properties of NEW used in the efficacy test

Concentration (%)	pH	ORP (mV)	ACC (ppm)
5	7.64	850	63
10	7.62	865	120
15	7.6	880	187

NEW application showed a broad spectrum effect on the microorganisms, which was used in experiments (Table 2 and 3). It was observed that there were significant differences in the decrease in the number of bacteria and fungi depending on the application time, presence of organic matter and concentration. The maximum inactivation effect was obtained after 60 seconds of application of NEW containing 187 ppm ACC. In the first 15 seconds of application of NEW (5, 10 and 15%) in the cell suspensions, the extents of reductions (%) were 8.95, 25.29, and 28.39 for *E. coli*, 6.78, 7.47, and 32.23 for *B. cereus*, 7.03, 31.12, and 35.25 for *A. flavus*, 63.36 , 100 and 100 for *P. expansum*, respectively. The extents of reductions (%) of sodium hypochlorite containing 531 ppm ACC in the same duration were 18.8 for *E. coli*, 9.86 for *B. cereus*, 35.10 for *A. flavus* and 59.14 for *P. expansum*, respectively. 60 seconds application of NEW has a significantly ($P<0.05$) high inhibitory effect when compared with its 15 and 30 seconds application results. The extents of reductions (%) of NEW for 60 seconds were 48.8, 100 and 100 for *E. coli*, 11.39, 13.37 and 32.23 for *B. cereus*, 7.12, 36.52 and 100 for *A. flavus* and 100, 100 and 100 for *P. expansum*, respectively. In the same duration, the extents of reductions (%) of sodium hypochlorite containing 531 ppm ACC were 29.56 for *E. coli*, 23.48 for *B. cereus*, 39.19 for *A. flavus* and 69.92 for *P. expansum*, respectively. Considering the results of the study, it is seen that sodium hypochlorite, which contains 2.84 times more ACC (531 ppm), does not have as much inactivation effect as 15% NEW (187 ppm ACC). In addition, it is seen that there are clear differences in the resistance of spore-forming bacteria and non-spore-forming bacteria. Kim et al., (2000) showed that within 60 seconds, acid EW (pH 2.5, ORP of 1123 mV, and 10 mg L^{-1} free available chlorine (FAC)) caused a reduction of 10 log CFU mL^{-1} in *E. coli* O157:H7 and a reduction of 3 log CFU mL^{-1} in spore-forming *B. cereus* which was more resistant.

Al-Qadiri et al., (2019) reported that after a minute application of NEW containing 120 mg L^{-1} FAC on *B. cereus* in the cell suspension, the number of spore-forming *B. cereus* decreased from 5.85 to 3.72 log 10 CFU ml^{-1}, that is, NEW had an inhibiting effect of 36% in the number of spore-forming bacteria. In our study, the inhibition effect of NEW containing 187 ppm ACC on *B. cereus* within 1 minute was 32.23%. It has also been confirmed in previous studies that endospore-forming bacteria are less sensitive to chemicals such as EW and nisin than non-spore-forming bacteria (Kim et al., 2000; Al-Qadiri et al., 2019).

When the rate of microbial inactivation of NEW on the inoculated steel surfaces (Table 3) compares with that of cell suspensions (Table 2), it is seen that the cells in the cell suspensions are more sensitive to NEW. As the reduction ratios (%) on the surface of steel plates after 60 seconds of treatment with NEW ranged from 17.66 to 40.07 for *E. coli*, 23.93–31.77 for *B. cereus*, 10.91–30.91 for *A. flavus* and, 49.77–64.85 for *P. expansum*, the reduction ratios (%) by 1% NaClO were 19.38, 11.70, 7.5 and 46.52 for *E. coli*, *B. cereus*, *A. flavus,* and *P. expansum*, respectively (Table 3). The results of the study showed that the presence of organic matter on the surface has

negative effects on reducing the microbial load and the antimicrobial activity of NEW increased as the ACC concentration increased (Table 2 and 3).

Al-Qadiri et al., (2016) investigated the efficiency of NEW (ORP = 805 mV, pH = 6.6, and ACC = 200 mg L-1) on 5 different non spore-forming bacteria (*Staphylococcus aureus, E. coli* O157:H7, *S. typhimirium, L. monocytogenes* and *C. jejuni*) inoculated on the surfaces of wooden and polypropylene food cutting boards. The results of the study showed that the contact time, surface properties and the presence of organic matter on the surface have positive or negative effects on reducing the microbial load. Al-Qadiri et al., (2019), in another study, examined the antimicrobial activity of NEW (pH = 6.6, ORP = 805 mV, and ACC= 120 mg L-1) on two different endospore-forming bacteria (*B. cereus* and *C. perfringens*) inoculated on the surface of a polypropylene cutting board. They found that a 5-minutes application of NEW caused a decrease of 2.33 log CFU / 100 cm^2 in the number of *B. cereus* and a decrease of 3.06 log CFU / 100 cm^2 in the number of *C. perfringens*. When we compare the two studies conducted on the surface of food cutting boards by Al-Qadiri et al., in 2016 and 2019, it is seen that NEW is more effective on non-spore-forming bacteria than spore-forming bacteria. In our study, on stainless steel surfaces inoculated with 10% dried fig solution, 15% NEW inactivated *E. coli* at a rate of 40.07% within 60 seconds, while inactivating spore-forming *B. cereus* at a rate of 31.77% (Table 3). These data are similar to the literature.

Table 2. Sterilization effect of NEW and NaClO in cell suspensions*

Disinfectant	Disinfectant concentration (%)	ACC (ppm)	Treatment solution time (sec.)	Inactivation Rate (%)			
				E.coli ATCC 35218	*B.cereus* ATCC 11778	*A.flavus*	*P. expansum*
NEW	5	63	15	8.95^{i}	6.78^{f}	7.03^{g}	63.36^{d}
			30	13.92^{h}	7.29^{f}	8.39^{g}	69.36^{bc}
			60	48.80^{b}	11.39^{de}	7.12^{g}	100.00^{a}
	10	120	15	25.29^{e}	7.47^{f}	31.12^{f}	100.00^{a}
			30	100.00^{a}	9.79^{e}	33.30^{ef}	100.00^{a}
			60	100.00^{a}	13.37^{cd}	36.52^{cde}	100.00^{a}
	15	187	15	28.39^{d}	32.23^{a}	35.25^{de}	100.00^{a}
			30	100.00^{a}	32.23^{a}	42.98^{b}	100.00^{a}
			60	100.00^{a}	32.23^{a}	100.00^{a}	100.00^{a}
NaClO	1	531	15	18.80^{g}	9.86^{e}	35.10^{de}	59.14^{e}
			30	22.57^{f}	14.59^{c}	37.69^{cd}	66.11^{cd}
			60	29.56^{c}	23.48^{b}	39.19^{bc}	69.92^{b}

$^{a-i}$ Indicate the differences in the columns.

*It was grouped in the Ninety-five Percent Confidence Interval with the LSD Test. In random blocks, the project was carried out according to the factorial trial design (2 factors- 1. Factor disinfectants, 2. Factor application time). Since the data are percent values, the square root transformation was applied.

Table 3. Sterilization effect of NEW and NaClO on the surface of steel plates in the presence of organic matter artificially inoculated with a final 10% liquid dried fig solution

Disinfectant	Disinfectant concentration (%)	ACC (ppm)	Treatment solution time (sec.)	Inactivation Rate (%)			
				E.coli ATCC 35218	*B.cereus* ATCC 11778	*A.flavus*	*P. expansum*
NEW	5	63	15	2.13^{e}	18.60	10.27	40.71^{g}
			30	7.65^{d}	18.42	10.39	41.40^{g}
			60	17.66^{c}	23.93	10.91	49.77^{de}
	10	120	15	8.55^{d}	15.81	16.09	51.18^{d}
			30	32.46^{b}	19.51	17.70	52.66^{cd}
			60	53.36^{a}	25.13	18.39	57.19^{b}
	15	187	15	17.53^{c}	26.25	27.62	51.43^{d}
			30	36.12^{b}	27.82	25.78	55.93^{bc}
			60	40.07^{b}	31.77	30.91	64.85^{a}
NaClO	1	531	15	15.87^{c}	9.325	8.32	46.37^{ef}
			30	18.74^{c}	10.63	7.87	45.28^{f}
			60	19.38 ^{c}	11.70	7.50	46.52^{ef}

$^{a-g}$ Indicate the differences in the columns.

*It was grouped in the Ninety-five Percent Confidence Interval with the LSD Test. In random blocks, the project was carried out according to the factorial trial design (2 factors- 1. Factor disinfectants, 2. Factor application time). Since the data are percent values, the square root transformation was applied.

The presence of mycotoxin-producing fungi in food processing and packaging areas is a matter of concern. They first contaminate food products and then produce toxins. To avoid these downsides, food processing areas, and equipment must be sanitized with an effective antifungal agent using an effective hygiene procedure (Lemos et al., 2020). Sodium hypochlorite is the most commonly used agent in the food industry in Brazil as a sanitizer. It is quite popular in the food industry worldwide. When used alone against mycotoxin-producing fungi in the food industry, sodium hypochlorite is ineffective due to fungal spores (Menegaro et al., 2016). The results of our study showed that 15% NEW (187 ppm ACC) against Sodium hypochlorite containing 531 ppm ACC was more effective on fungal spores (Tables 2 and 3).

Buck et al. (2002) showed that *A. flavus* and *A. niger* spores (5×10^6 conidia mL^{-1}) were completely eliminated within 30 seconds by acid EW (54-56 ppm ACC). Xiong et al., (2010) demonstrated that NEW was more effective than acid EW in the inhibition of *A. flavus* spores. It was determined by Yamaner et al., (2016) that 5% NEW at 50°C caused a reduction of 5.54 log CFU ml^{-1} in the number of *A. flavus* spores and a reduction of 7 log CFU ml^{-1} in the number of *P. expansum* spores in one minute. It is seen that *P. expansum* spores are more sensitive to NEW compared to *A. flavus* spores. These results show parallelism with the data of our study (Tables 2 and 3).

Lemos et al., (2020) determined that the effectiveness of acid (pH 2.67) and basic (pH 11.29) electrolyzed water (ACC 121 ppm) on toxigenic *Aspergillus* species was low (reduction rate of *Aspergillus* species < 3 log CFU g^{-1}). In our study, although a 100% inactivation rate was reached in 60 seconds when 15% NEW was applied directly to *A. flavus* and *P. expansum* spores, the inactivation rates were 30.91% for *A. flavus* and 64.85% for *P. expansum* in the presence of organic material (10% dried fig liquid) on the stainless steel surface under the same conditions. When 1% NaClO was applied to stainless steel surfaces for 60 seconds, it caused an inactivation of 7.5% in the number of *A. flavus* and 46.52% in the number of *P. expansum*. Therefore, NEW is a very good potential disinfectant compared to NaClO in the presence of organic matter, especially in the food industry.

Preventing fungal contaminations and minimizing cross-contaminations before and after harvest is vital (Al-Haq et al., 2005). The use of NEW, a new and promising disinfectant and cleaning agent, in surface cleaning minimizes corrosion and reduces the negative risks associated with the use of conventional decontaminating agents (Guentzel, 2008). It has also become popular in the food industry in recent years (Villarreal-Barajas et al., 2022).

Electrolyzed water is widely used as an antibacterial agent in many fields such as agriculture, animal husbandry, food sanitation, medicine, etc. EW has been approved for use as a food sanitizer in Russia, Japan and China. The fact that EW is 100% organic, non-toxic, and low-cost makes its use widespread in both households and industrial applications. The results of this study showed that NEW can be used effectively in the disinfection of fig processing surfaces in the fig industry.

4. Conclusion

Fig infection by toxigenic fungi reported in several studies. Sanitation of food processing surfaces is very important in preventing cross-contamination. But, no study has been conducted related to the use of electrolyzed water treatments as a fungicide and bactericide on fig processing surfaces in the fig industry. This is the first study in this field. The results of this study showed that 15% NEW can be used effectively in the disinfection of fig processing surfaces in the fig industry.

Author Contributions

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

	Ç. Y.	R.K.
C	100	
D	100	
S	80	20
DCP	90	10
DAI	50	50
L	80	20
W	100	
CR	90	10
SR	100	
PM	80	20
FA	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, FA= funding acquisition.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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 POLLINATOR CHARACTERISTICS OF SOME HAZELNUT GENOTYPES

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Abstract: This research was carried out in Karasu, Kocaali and Arifiye districts of Sakarya Province to determine the suitable pollinators for Çakıldak hazelnut cultivar (*Corylus avellana*) in 2021-2022. In the study, 27 genotypes that were late male flowering, formed a large amount of catkins, had high pollen quality, had round nut shape and short husk length were examined. Pollen viability was detected according to 2, 3, 5,-Triphenyl Tetrazolium Chloride (TTC). Pollen viability of the genotypes was ranged from 22.3% to 93.7% according to the TTC method. According to the agar method, pollen germination rate was determined between 15.6% and 78.1% at 20% sucrose concentration containing 1% agar. 89% of the genotypes were round, 11% were in the oblong nut group. It was determined that most of them amount of male inflorescences (catkins) depending on the tree crown volume and age. T-22KRS02, T-22KRS03, T-22KRS07, T-22KRS08, T-22KRS09, T-22KRS10, T-22KRS11, T-22KCL11, T-22KCL14, T-22KCL16 and Mincane were selected to be evaluated in the second phase of the study.

Keywords: Climate change, *Corylus avellana*, Dichogamy, Pollinator, Yield, Quality

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1. Introduction

Hazelnut *(Corylus avellana* L.) is a fruit species cultivated in temperate climate zones, whose production area, amount and trade continue to increase day by day. Türkiye constitutes 72.3% of the world hazelnut production area with a production area of 734,538 hectares. With a production of 665,000 tons, it is the most important hazelnut producer country, realizing 62% of the world hazelnut production. Türkiye is followed by Italy, USA, Azerbaijan and Chile. Hazelnut (*Corylus avellana* L.) is monoecious, self and cross-compatible and wind-pollinated. Incompatibility in hazelnut is sporophytic-type controlled by a single locus and S alleles (Thompson, 1979). As a result of the studies carried out to the present day, 33 S alleles have been determined (Mehlenbacher, 2014). It was determined that allele genes in the pistil were co-dominant and those in the pollen were dominant or co-dominant (Mehlenbacher ve Thompson, 1988).

Investigation of the periodic biological events affected by the environment, especially the temperature changes caused by weather and climate in plants, is called phenology (Crepinsek et al., 2012). Bud break, harvest, male and female flowering time are phenologically important stages in hazelnut cultivation. Unlike many temperate fruit species, hazelnuts bloom in winter. Male and female flowers may vary depending on the variety, altitude and year (Beyhan, 2000). Although hazelnut cultivars are partially self-incompatible, studies have shown that cross pollination increases nut set and nut quality (Balık and Beyhan 2019a; Balık and Beyhan 2019b; Balık and Beyhan 2020; Balık and Beyhan 2021; Fatahi et al., 2014; Hosseinpour et al., 2015; Javadi and Gheshlaghi, 2006). Cross pollination is required for high yield in hazelnut and at least two pollinators are recommended. Pollinators should not show incompatibility with the main cultivar, pollen quality should be high, pollen distribution time should be as long as possible (Hampson et al., 1992).

There are two basic approaches in terms of nut set in hazelnut. The first is pollen-stigma compatibility, and the other is phenological male and female flowering at the same time. In recent years, as a result of the adverse effects of climate changes, high temperatures in the Black Sea Region in autumn and winter cause male flowers to bloom much earlier in hazelnut cultivars, and female and male flowering do not coincide enough to provide sufficient nut set (Balık and Beyhan, 2019a; Beyhan and Marangoz, 2007). Cross-pollination is necessary due to dichogamy and incompatibility in hazelnut. Male flowering is usually in January in Turkish hazelnut cultivars. Due to global warming, it is noteworthy that in recent years, male flowering has taken place earlier and may be as early as 1 month. However, pollination does not occur during this period as female flowers have not receptive yet. Therefore, there is a need for pollinators that bloom during the period when female flowers are receptive. In hazelnut, it is desired that the pollinators

bloom as late as possible and the pollen distribution takes place in a long period. In addition, pollinators should have high viability and germination rates and should not show incompatibility (Beyhan, 2000; Balık and Beyhan, 2019b).

In this study, the potential of genotypes with high pollen quality, which do not show incompatibility, flowering at the same time with the main cultivars, as pollinator Karasu, Kocaali and Arifiye districts of Sakarya was investigated.

2. Material and Methods

The plant material of the study consists of 27 genotypes selected in Karasu, Kocaali and Arifiye districts of Sakarya province (Table 1).

Table 1. Location Information of Genotypes

No	Genotypes	District	Village	Altitude (m)
1	T-22KRS01	Karasu	Kuyumculu	50
2	T-22KRS02	Karasu	Kuyumculu	81
3	T-22KRS03	Karasu	Kuyumculu	65
4	T-22KRS04	Karasu	Salıkkaya	200
5	T-22KRS05	Karasu	Salıkkaya	232
6	T-22KRS06	Karasu	Salıkkaya-Kirazlı	249
7	T-22KRS07	Karasu	Kirazlı	160
8	T-22KRS08	Karasu	Kirazlı	220
9	T-22KRS09	Karasu	Kirazlı	206
10	T-22KRS10	Karasu	Kirazlı	195
11	T-22KRS11	Karasu	Kirazlı	190
12	T-22KCL03	Kocaali	Gümüşoluk-Demiraçma	103
13	T-22KCL04	Kocaali	Gümüşoluk	202
14	T-22KCL05	Kocaali	Gümüşoluk	211
15	T-22KCL06	Kocaali	Açmabaşı	120
16	T-22KCL07	Kocaali	Açmabaşı	175
17	T-22KCL08	Kocaali	Açmabaşı	209
18	T-22KCL11	Kocaali	Kızılüzüm	658
19	T-22KCL12	Kocaali	Kestanepınarı	382
20	T-22KCL13	Kocaali	Kestanepınarı	385
21	T-22KCL14	Kocaali	Chamber of Agriculture	160
22	T-22KCL15	Kocaali	Açmabaşı	156
23	T-22KCL16	Kocaali	Açmabaşı	150
24	Allahverdi	Arifiye	SUBU Faculty of Agriculture Research Center	30
25	Kalınkara	Kocaali	Chamber of Agriculture	160
26	Mincane	Kocaali	Chamber of Agriculture	160
27	Okay 28	Arifiye	SUBU Faculty of Agriculture Research Center	30

In the selection of genotypes, it was taken as a criterion that the male flowering was later than the standard cultivars. Since January 2022, phenology has been followed in both districts on the basis of cultivar and altitude, and genotypes that still continue to bloom after the end of male flowering in standard cultivars have been determined. Catkins were taken from the genotypes and viability and germination tests were performed on the pollen obtained after they were kept at room temperature for 24 hours. While 2, 3, 5,-Triphenyl Tetrazolium Chloride (TTC) method was applied in pollen viability test (Figure 1), pollen germination was determined at 20% sucrose concentration containing 1% agar compared to agar method (Figure 2).

Figure 1. Pollen viability test in hazelnut (viable pollen and dead pollen).

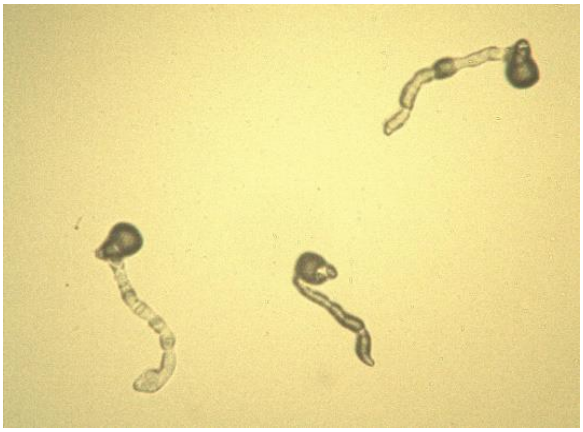

Figure 2. View of the pollen germination in hazelnut (20% sucrose solution).

In addition, the husk and nut characteristics of the genotypes were also evaluated. Evaluation of genotypes was made according to Weighed Rating Method (Table 2).

TTC method was used to determine pollen viability. 2 h after the application of TTC test, which was carried out in daylight, counting under the light microscope (Zeiss Axiolab 5, 40×) was made, and the red-stained pollen were considered as alive and the unstained ones as dead.

Agar method was used to determine of the pollen germination (Beyhan and Odabaş, 1997). It was kept in the test cabinet (Microtest Mit 500) at 20 °C for 36 h under 65% relative humidity conditions. Pollens with a length of pollen tube greater than the diameter of pollen were considered germinated under the light microscope (Zeiss Axiolab 5, 40×).

Phenological characters were determined according to Çalışkan and Çetiner (1997). Genotypes are grouped as less, medium, and high in male inflorescences, taking into account the size of the crown, age, and growth pattern.

Table 2. Properties evaluated in the Weighed Rating Method

Traits	Relative Score	Categories	Categories Range	Point
Pollen viability (%)	25	Very high	79<	9
		High	65-78	7
		Medium	51-64	5
		Low	37-50	3
		Very low	<36	1
Pollen germination (%)	15	Very high	66<	9
		High	53-65	7
		Medium	41-52	5
		Low	28-40	3
		Very low	<27	1
Amount of catkins	25	More		5
		Maedium		3
		Low		1
Nut shape	25	Globular		5
		Pointed		3
		Cylindirical		1
Husk length	10	Taller than nut		1
		Equal to nut		3
		Shorter than nut		5

3. Results and Discussion

The pollen viability of the genotypes was determined between 22.3% and 93.7% (Table 3). Most of the genotypes are included in the wild hazelnut population and it was planted in orchards due to its pollinating properties. Due to the low yield and nut quality of these genotypes, it is seen that cultural management processes such as pruning and fertilization are not applied. It is considered that the lack of cultural practices and the wide genetic variation cause the difference in pollen viability rates of genotypes to be high. As a matter of fact, Beyhan (2000) stated that pollen quality in hazelnut can vary depending on the variety, year, cultural practices and ecology. Beyhan and Odabaş (1995) determined pollen viability rate as 89% in Tombul, 78% in Palaz, 72% in Çakıldak, 76% in Sivri and 88% in Kalınkara. Balık and Beyhan (2019c) determined that pollen viability rate in ranged from 1.61% (Yassı Badem) to 71.03% (Allahverdi).

Pollen germination rate of genotypes was determined between 15.6-78.1% (Table 3). Beyhan and Odabaş (1995) determined pollen germination rates of Turkish hazelnut cultivars between 27-76% in pollen germination tests performed at different sucrose concentrations. In the same study, pollen germination rates were determined as 69% in Tombul, 49% in Palaz, 60% in Çakıldak, 63% in Sivri and 52% in Kalınkara at 25% sucrose concentration, where pollen germination rates were the highest. The pollen germination rates determined in the research were similar to the literature. However, it was noteworthy that pollen germination rates of genotypes were lower than pollen viability rates. Fatahi et al. (2014) emphasized that pollen quality was changed to according to cultivars, and pollen germination rates in the examined cultivars were determined above 60%. Stösser et al. (1996) reported that pollen germination tests performed in vitro, as well as climatic conditions, may be affected by pollen collection time and pollen storage conditions. Moore and Janick (1983) emphasized that pollen density, germination density and pH were effective on pollen germination rate under in vitro conditions. Mert (2009) determined that pollen germination rate increased as the temperature increased in the walnut cultivars. Beyhan and Odabaş (1995) determined that not all pollen that was determined to be viable germinated and this situation was more pronounced in some cultivars. It was emphasized that it would be more accurate to determine the pollen quality of cultivars by viability test, because variable external factors such as ambient humidity, temperature and the properties of the substances used as substrate were effective in pollen germination. When pollen viability test results were compared with germination results, it was seen that higher rates were obtained in viability tests. On the other hand, it was stated that there was a strong relationship between pollen viability and germination rate (Novara et al., 2017).

Table 3. Pollen viability and germination of genotypes, amount of catkins, nut shape, husk length values and total weighted rating scores.

G	PV (%)	Point	PG (%)	Point	AC	Point	Nut shape	Point	Husk length	Point	TWRS	Evaluation
T-22KRS01	93.5	9	51.6	5	Much	5	Cylindrical	1	Short	5	500	
T-22KRS02	86.7	9	63.9	7	Much	5	Globular	5	Tall	1	590	Chosen
T-22KRS03	89.7	9	41.3	5	Much	5	Globular	5	Short	5	600	Chosen
T-22KRS04	92	9	36	3	Much	5	Globular	5	Equal	3	550	
T-22KRS05	60.8	5	42.6	5	Much	5	Globular	5	Equal	3	480	
T-22KRS06	76.8	7	29.5	3	Much	5	Globular	5	Tall	1	480	
T-22KRS07	71.3	7	72.3	9	Much	5	Globular	5	Tall	1	570	Chosen
T-22KRS08	89.7	9	58.9	7	Much	5	Globular	5	Tall	1	590	Chosen
T-22KRS09	85	9	57	7	Much	5	Globular	5	Tall	1	590	Chosen
T-22KRS10	93.7	9	65.4	7	Much	5	Globular	5	Tall	1	590	Chosen
T-22KRS11	93.7	9	59.7	7	Much	5	Globular	5	Tall	1	590	Chosen
T-22KCL03	90.3	9	36.9	3	Much	5	Cylindrical	1	Short	5	470	
T-22KCL04	65	7	52.4	5	Much	5	Cylindrical	1	Tall	1	410	
T-22KCL05	78.3	7	57.6	7	Much	5	Globular	5	Equal	3	560	
T-22KCL06	90.7	9	30.5	3	Much	5	Globular	5	Tall	1	530	
T-22KCL07	73.3	7	58.4	7	Much	5	Globular	5	Tall	1	540	
T-22KCL08	70	7	54.5	7	Much	5	Globular	5	Tall	1	540	
T-22KCL11	84	9	53.4	7	Much	5	Globular	5	Short	5	630	Chosen
T-22KCL12	91.7	9	34.2	3	Much	5	Globular	5	Equal	3	550	
T-22KCL13	77.3	7	32.4	3	Much	5	Globular	5	Short	5	520	
T-22KCL14	87	9	78.1	9	Much	5	Globular	5	Tall	1	620	Chosen
T-22KCL15	43	3	28.3	3	Much	5	Globular	5	Tall	1	380	
T-22KCL16	92.3	9	71.7	9	Much	5	Globular	5	Equal	3	640	Chosen
Allahverdi	81.3	9	70.8	9	Much	3	Globular	5	Tall	1	570	
Kalınkara	22.3	1	15.6	1	Much	3	Globular	5	Tall	1	300	
Mincane	88.7	9	75.8	9	Medium	3	Globular	5	Tall	1	570	Chosen
Okay 28	43.7	3	67.3	9	Few	1	Globular	5	Tall	1	370	

G= genotypes, PV= pollen viability, AC= amount of catkins, PG= pollen germination, TWRS= total weighted rating scores

Male inflorescences in hazelnuts begin to appear on the ends and sides of seasonal shoots in July. There are 150-200 male flowers in catkins. On the other hand, more than one catkin may form from one bud. There are 4 male organs in each bract leaf of catkins. There are 2 anthers in each of the male organs. Cross pollination is required for high nut set in hazelnut. At least two pollinator cultivars are recommended. Pollinators should not show incompatibility, produce a large number of catkins, pollen viability should be high, pollen shading should be as long as possible (Hampson et al., 1992). In our study, it was observed that although the genotypes were grown in different ecological conditions, they formed quite a lot of catkins depending on the plant age and tree volume (Figure 3). However, the fact that these genotypes have a high amount of catkins does not mean that they are ideal pollinators.

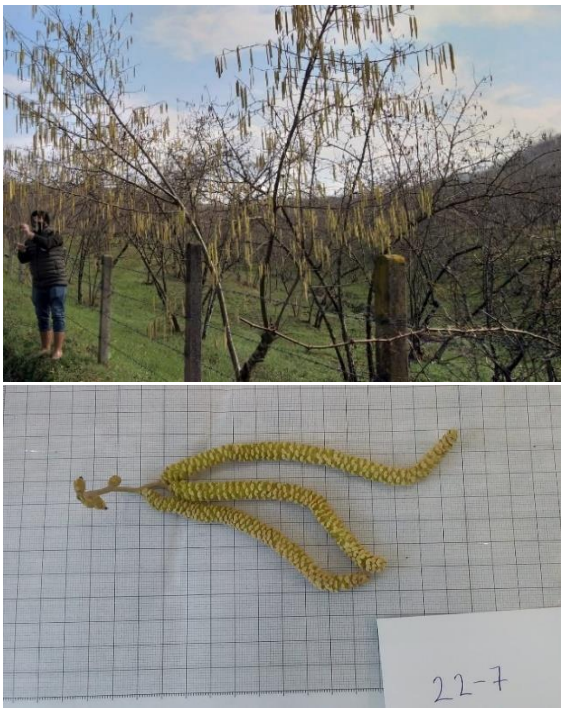

Figure 3. Plant and catkins image of pollinator genotypes

It has been determined that the genotypes are in two groups as the nut shapes are cylindrical and globular (Figure 4). Turkish hazelnut cultivars are divided into three groups according to nut shape: globular (Ex:

Tombul, Palaz, Çakıldak, Kalınkara), conical (Ex: Acı, Sivri) and cylindrical (Ex: Yuvarlak Badem, Yassı Badem) (Balık et al., 2016). Balık (2018), determined that pollinators cause a change in nut shape in hazelnut. Çetiner et al. (1984) stated that Sivri and İncekara cultivars, which provide the highest level of nut set, but it may cause deterioration of homogeneity and product quality in orchards due to the sharp nut shape. In addition, it has been stated that the nut shape may deteriorate in case of an increase in the number of nuts in cluster, which is a type of hazelnut and can change depending on climatic conditions and maintenance conditions (Balık et al. 2014). Owais (2014) reported that pollinators cause a significant change in kernel shape in almonds. On the other hand, it has been noted that self-pollination in almonds causes irregular fruit shape (Graselly and Olivier, 1988; Torre Grossa et al., 1994) and abortive kernel (Torre Grossa et al., 1994).

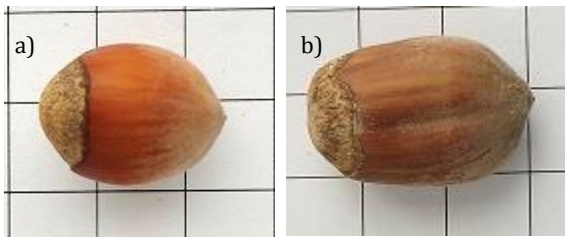

Figure 4. Globular (a) and cylindrical (b) nut shape in Turkish hazelnut cultivar (Balık et al., 2016).

Husk image of pollinator genotypes is presented in Figure 5. The husk length in hazelnut was compared with the nut and grouped into three categories as shorter than the nut, equal to the nut and longer than the nut (UPOV, 2022). While the husk length was longer than the nut in 17 of the genotypes examined, it was determined that the husk and nut were equal in length in 5 genotypes, and the husk length was shorter than the nut in 5 genotypes. It is seen that the cultivars grown especially in countries where mechanical harvesting is applied have a short and loose husk structure that surrounds the nut. Turkish hazelnut cultivars, especially due to the topographic structure of the Eastern Black Sea Region, where they were cultivated for the first time, have resulted in the surviving of the varieties in which the husk is long and tightly wraps the nut, as a result of a conscious selection (Balık et al., 2021).

Figure 5. Husk image of pollinator genotypes

4. Conclusions

This research was carried out to determine the ideal pollinators for Çakıldak cultivar, which was found to have pollination problems due to climate change. For this purpose, 27 genotypes that were still in the male flowering stage despite the completion of male flowering in standard hazelnut cultivars were examined. Pollen viability of genotypes was determined as 22.3-93.7%, pollen germination between 15.6-78.1%. It is considered that the variation in pollen viability and germination rates is due to the different altitude, vertical and ecological conditions in the locations where the genotypes are determined, and the genetic variation is high due to the fact that wild hazelnut types are included in the seedling population.

The changes in the nut and kernel characteristics of the pollinators in hazelnut are called xenia and metaxenia (Balık, 2018). Since pollinators cause changes in nut shape, the genotypes examined in our research were also evaluated according to nutshape and was scored with 25 relative points in the weighted grading method. 89% of the genotypes examined were globular and 11% were in the cylindrical nut group. Globular shaped hazelnuts are preferred in the world hazelnut markets.

In hazelnut cultivation, it is expected that pollinators do not incompatibility and dichogamy, have a globular nut shape, high pollen quality, as well as producing a large amount of catkins every year. It was determined that most of the genotypes examined in our study formed a 'many' amount of catkins depending on the tree crown volume and age.

The origin of hazelnut is Anatolia and the majority of production is done in the Black Sea Region. Hazelnut cultivars, which have been produced for thousands of years, have reached the present stage as a result of selection. The rough and sloping land of the Black Sea Region has caused the producers to care about the cultivars with a long husk, which tightly surrounds the fruit, and the selection of these cultivars has caused the production to become widespread. For this reason, Turkish hazelnut cultivars have long husk characteristics that tightly wrap the nut. However, these characteristics of the cultivars make mechanical harvesting difficulty. Harvest labor, which has a 40% share in the hazelnut production cost, is getting harder every year due to the migration of the young population to the cities, the aging of the population in the villages, and the high cost of recruiting harvest workers from other regions and abroad. For this reason, it is necessary to establish the orchards with a training system and cultivars suitable for mechanical harvest. It is important to use pollinators with short husk in terms of suitability for mechanical harvesting.

Çakıldak is the most widely grown hazelnut cultivar in Karasu and Kocaali district. The cultivation rate of Çakıldak in these districts is 60% on average, and the high kernel ratio and yield and the fact that it is not damaged by late spring frosts due to late bud burst

causes Çakıldak to become widespread rapidly. For this reason, efficient and high quality production of Çakıldak is very important in terms of producer comfort. It has been determined that the pollinators are not included in the orchards established with the Çakıldak, the male and female flowering times differ due to global warming, and the lack of pollination due to the increase in the dichogamy degree causes yield losses.

As a result of this research carried out in order to reduce the yield loss due to the lack of pollination in Çakıldak; among the genotypes with high pollen quality, high amount of catkins, short husk length and globular nut shape, the total weighted rating score is 570 and above T-22KRS02, T-22KRS03, T-22KRS07, T-22KRS08, T-22KRS09, T-22KRS10, T-22KRS11, T-22KCL11, T-22KCL14, T-22KCL16 and Mincane were selected for evaluation in the second phase of the investigation.

Whether the genotypes are suitable pollinators for Çakıldak should be examined by following controlled hybridizations (nut set) and development of the pollen tube in style (incompatibility) under fluorescence microscope. Promising pollinator cultivar candidates identified as a result of these studies should be registered, a large number of plants should be produced with tissue culture, and the use of producers should be expanded.

Author Contributions

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

	H.İ.B	T.D	Ö.B
C	50	25	25
D	50	25	25
S	50	25	25
DCP	100		
DAI	50	25	25
L	50	25	25
W	50	25	25
CR	50	25	25
SR	50	25	25
PM	80	10	10
FA	50	25	25

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

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

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PURIFICATION AND CHARACTERIZATION OF GLUTATHIONE REDUCTASE ENZYME FROM *Arum Maculatum* LEAF

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Abstract: *Arum* species grow in temperate and Mediterranean climates and have been used for hundreds of years for food and medicinal purposes, although they are highly toxic if not cooked using proper techniques. Glutathione reductase (GR) is a member of the pyridine nucleotide disulfide oxidoreductase family of flavoenzymes that catalyzes the reduction of glutathione disulfide (GSSG) to reduced GSH using NADPH or NADH. In this study, GR enzyme was characterized by partial purification processes including homogenate preparation, ammonium sulfate precipitation and dialysis from the leaf of *Arum maculatum* plant. The highest enzyme activity was found at 40-60% saturation range. Optimum ionic strength, pH and substrate concentration were investigated for GR enzyme from *A. maculatum* leaf. As a result of the study, these values were found to be 150 mM potassium phosphate buffer, pH 7.00, and 0.18 mM, respectively. The GR enzyme was partially purified from the leaf of the *A. maculatum* with a specific activity of 1.640 EU mg^{-1} in 34.9% yield, 1.108-fold. This study is the first study in terms of purification and characterization of GR enzyme from *A. maculatum* leaf.

Keywords: Glutathione reductase, *Arum maculatum*, Purification, Enzyme, Characterization

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1. Introduction

Glutathione reductase [NADPH:oxidized-glutathione oxidoreductase, EC 1.8.1.7: GR] is a member of the pyridine-nucleotide disulfide oxidoreductase family of flavoenzymes (Meister and Anderson, 1983). This enzyme catalyzes the reduction of glutathione disulfide (GSSG) to reduced glutathione (γ-L-glutamyl-Lcysteinyl glycine; GSH) using NADPH or NADH as a reducing agent. Thus, GR maintains the reduced glutathione/oxidized glutathione (GSH/GSSG) ratio in the cell environment (Şentürk et al., 2009). GSH, which is present in both prokaryotic and eukaryotic cells, is a crucial thiol that protects the cell against the harmful effects of oxidized molecules, thanks to the -SH groups in its tripeptide structure, and it constitutes a large part of the intracellular free sulfhydryl groups (Alscher, 1989). It is also involved in DNA and protein synthesis, detoxification of some metabolic end products and drugs, transport of amino acids, and breaking the disulfide structures of some proteins, such as insulin, which contain disulfide bonds (Couto et al., 2016; Çakmak et al., 2011; Townsend et al., 2003). Moreover, GSH is an abundant metabolite in plants that can protect enzyme thiol groups and is also known to be involved in signal transduction and protect plant cells from oxidative damage caused by reactive oxygen species (ROS) (Foyer and Noctor, 2005; Liu et al., 2020). Due to these critical tasks, metabolic disorders occur as a result of the decrease in the concentration of GSH in the cell. GR activity has been associated with reactive oxygen species generated by abiotic stresses such as salinity, drought, UV radiation, high light intensity, heavy metals and herbicides (Kaur et al., 2022; Romero-Puertas et al., 2006). GR is a very critical enzyme in cellular redox balance, as it can maintain the GSH/GSSG ratio to protect plant cells from oxidative damage by reactive oxygen species (Gill et al., 2013; Liu et al., 2020; Şentürk and Şentürk, 2020).

Arum maculatum is a perennial herb with glossy green leaves that look like arrowheads at the ends of long stems and whose roots form tubers (Gibernau et al., 2004). *Arum* species, which grow in temperate and Mediterranean climates and are known by common local names such as nivik, tirsik, snakeshead, adder's root, lords and ladies, devils and angels, cuckoo-pint, have been used for food and medicinal purposes for hundreds of years, although they are highly poisonous if not cooked with appropriate techniques (Ceylan and Sahingoz, 2022; Dayisoylu, 2010; Raju et al., 2018; Yurt et al., 2019). Compounds such as gum, mucilage, starch, glycoside saponin and an alkaloid (conicine) have been reported in its fresh leaves and tubers (Baytop, 1999). In addition, a high rate of protein 56.93% was identified in *A. maculatum* leaves (Ali, 2008). Alcoholic macerate of *A.*

maculatum leaves is used as antirheumatic and antineuralgic in Lebanon (Marc et al., 2008), also it is reported that the infusion in the form of compresses is used in the treatment of rheumatism and gout, anti-inflammatory, digestive, gastrointestinal and respiratory tract, joint pain, hemorrhoids, liver diseases, lung diseases (Tetik et al., 2013). Previous studies have shown that plant extracts of *A. maculatum* prepared with chemicals such as petroleum ether and methanol result in antimicrobial activity (Çolak et al., 2009; Farahmandfar et al., 2019; Uzun et al., 2004).

To date, GRs from various plants have been purified and characterized, including *Zea mays* (Mahan and Burke, 1987), *Oryza sativa* (Wu et al., 2013), *Pisum sativum* (Madamanchi et al., 1992) and Scots pine needles (Wingsle, 1989). To the best of our knowledge, no previous study has been reported in the literature on the GR enzyme from the *A. maculatum* plant. Therefore, in this study, it was aimed to partially purify GR enzyme from *A. maculatum* leaves and to determine some of its characteristic properties.

2. Materials and Methods

2.1. Materials

Ethylenediaminetetraacetic acid (EDTA), tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), ammonium sulfate ($(NH4)_2SO_4$), potassium phosphate (KH_2PO_4), dithiothreitol (DTT), oxidized glutathione (GSSG), β- nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH), sodium hydroxide, coomassie brilliant blue G-250, polyvinylpyrrolidone (PVP) and bovine serum albumin (BSA) were analytically graded and obtained from Sigma Chemical Co., MO, USA.

2.2. Preparation of Homogenate

In the study, fresh leaf tissues of *A. maculatum* plant were used for partial purification of the GR enzyme. In order to determine the enzyme activity, 3 g of tissue samples were ground in a porcelain mortar with the help of liquid nitrogen, and then homogenized in 30 ml of 50 mM Tris-HCl / 2 mM EDTA (pH 7.8) buffer containing 5% (w/v) PVP and 1mM DTT. After a short vortexing process, it was centrifuged (Hermle Z 326 K, Hermle Labortechnink, Wehingen, Germany) at 15,000 x*g* for 30 min at 4 °C. The supernatant was separated from the precipitate using filter paper and used in further analysis.

2.3. Ammonium sulfate precipitation and dialysis

In order to the purification of GR enzyme from the homogenate prepared from *A. maculatum* leaves, ammonium sulfate precipitation processes were carried out separately at intervals of 0-40% and 40-60%. For the precipitation of ammonium sulfate, solid $(NH_4)_2SO_4$ was added very slowly to the homogenate at 4°C on the cooled magnetic stirrer. In each precipitation process, the homogenates were centrifuged at 5,000 x*g* for 15 minutes. The supernatant was taken into a separate beaker and the precipitates were dissolved with a sufficient amount of 150 mM Tris (pH 7.8) buffer and enzyme activities were measured both in the supernatant and in the precipitate. By repeating the procedures at all ammonium sulfate precipitation intervals, the activity measurement was carried out, and the range in which the enzyme was active was determined. Dialysis was used to desalinate the protein solution. For this purpose, the mixture obtained as a result of ammonium sulfate precipitation was placed in a dialysis bag and dialyzed for 2 hours by changing it against 10 mM Tris (pH: 7.8) buffer twice (Smith et al., 1988). Dialysis was carried out on a magnetic stirrer at 4 °C. The product obtained at the end of dialysis was stored at -20 °C to be used in the following analysis.

2.4. Activity Assay of GR Enzyme

Enzyme activity was measured spectrophotometrically using the modified method of Carlberg and Mannervik (1975) at 25 °C with a Shimadzu UV-1800 spectrometer (Shimadzu Corporation, Shimadzu, Japan). In this method, the activity was determined by the decreasing amount of NADPH due to the oxidation of NADPH in the presence of GSSG. The enzyme activity assay was carried out in a one ml reaction mixture consisting of assay buffer (50 mM Tris-HCl, pH 7.0), 2 mM GSSG, 2 mM NADPH and 25 µl sample. Spectrophotometric measurements were made in the kinetics rate program at 340 nm for 3 minutes. In the calculation of the enzyme unit, the molar extinction coefficient (ε) of NADPH at 340 nm was used as 6.22 mM^{-1} cm^{-1}. Enzyme activity was expressed as specific activity (EU mg $protein^{-1}$).

2.5. Protein Determination

Protein contents of all samples were determined spectrophotometrically at 595 nm according to Bradford (1976) method using BSA containing 1 mg protein ml^{-1} as a standard.

2.6. Characterization Studies

2.6.1. Optimum ionic strength determination

In order to determine the optimum ionic strength, the partially purified *A. maculatum* GR enzyme activity was measured using different concentrations of Tris-HCl (pH 7.0) and K-phosphate (pH 7.0) buffers, in the range from 5 to 250 mM (Tekman et al., 2008).

2.6.2. Optimum pH determination

For the optimum pH characterization, buffers were prepared in the range of pH 5.0 - 10.0 using the buffer concentration showing the highest activity and enzyme activity measurements were carried out (Tekman et al., 2008).

2.6.3. Optimum substrate concentration determination

The optimum substrate concentration was determined by using the buffer and pH with the highest activity in the previous steps. Activity measurements were performed using NADPH substrate in the 0.02 - 0.4 mM cuvette concentration range (Tekman et al., 2008).

3. Results and Discussion

Within the scope of this study, *A. maculatum* leaves GR enzyme was first partially purified and characterized. The purification procedure was carried out by

preparation of the homogenate, ammonium sulfate precipitation and dialysis. In the homogenate prepared for the partial purification of the GR enzyme from the leaf tissue of *A. maculatum*, the highest activity was obtained at 40-60% ammonium sulfate saturation, as a result of the activity measurements performed after the ammonium sulfate precipitations at the previously mentioned intervals. In previous studies, the ammonium sulfate precipitation range of GR enzyme purified from soybean seed (Bilir, 2017), human and bovine erythrocytes (Erat et al., 2003; Senturk et al., 2008, 2009), sheep brain (Kocaoğlu et al., 2019) and sheep liver (Ulusu et al., 2005) was found to be 40-60%, 30-70%, 35-55% and 0-60%, respectively. In our study, ammonium sulfate precipitation range for GR, which was partially purified for the first time from *A. maculatum*, was found to be compatible with the literature. Afterward, dialysis was applied and the activity measurement was repeated. GR was purified from leaf tissues of *A. maculatum* in 34.9% yield and 1.108 purification coefficient with a specific activity of 1.64 EU mg^{-1} (Table 1.).

Table 1. The summary of purification steps for *A. maculatum* GR

Purification step	Activity (EU/mL)	Total volume (mL)	Protein (µg/mL)	Total activity (EU)	Total protein (µg)	Specific activity (EU/mg)	Yeild (%)	Purification factor
Homogenate	0.280	25	0.194	7.00	4.852	1.479	100	1
ASPD	0.407	6	0.248	2.44	1.490	1.640	34.9	1.108

ASPD= ammonium sulfate precipitation and dialysis

In order to determine the most suitable ionic strength for GR enzyme activity, activity measurements were performed in Phosphate and Tris buffers at different concentrations and the obtained values are given in Figure 1. According to the results obtained, it was determined that the most suitable ionic strength for the *A. maculatum* leaf tissue GR enzyme was 150 mM KH_2PO_4 buffer. In order to determine the optimum pH, the selected 150 mM KH_2PO_4 buffer was prepared at different pH levels and GR activities were determined spectrophotometrically. The data obtained are shown in Figure 2. The optimum pH value for the GR enzyme of *A. maculatum* leaf tissue was determined as pH 7.0 in 150 mM KH_2PO_4 buffer. In a study by Lascano et al. (2001), it was reported that the optimum activity of chloroplastic GR of the wheat plant they purified was at pH 8.0 and an ionic strength between 60 and 100 mM. GR obtained from the plant *Larix kaempferi* has been reported to have an optimum pH ranging from 7.0 to 9.0 (Wang Xin, 2013). GRs purified from *Pisum sativum* have been reported to have optimum activity at pH 7.8 (Madamanchi et al., 1992). In addition, the optimum pH of the GR purified from the seed of the soybean plant was determined as pH 8.5 (Bilir, 2017). Moreover, it has been observed that GR optimum pH values purified from different organisms such as fish, sheep, chicken, humans and turtles are in similar ranges such as 6.5 - 8.5 (Acan and Tezcan, 1989; Erat et al., 2005; Ogus and Ozer, 1998; Tekman et al., 2008; Willmore and Storey, 2007). The optimum concentration of substrate for the GR enzyme activity obtained from the leaf of *A. maculatum* was determined by the activity measurements using the optimum buffer solution at the optimum pH value containing different concentrations of NADPH (Figure 3). According to the results obtained, it was observed that the most suitable substrate concentration for the *A. maculatum* GR enzyme was 0.18 mM NADPH in 150 mM phosphate buffer (pH 7.0).

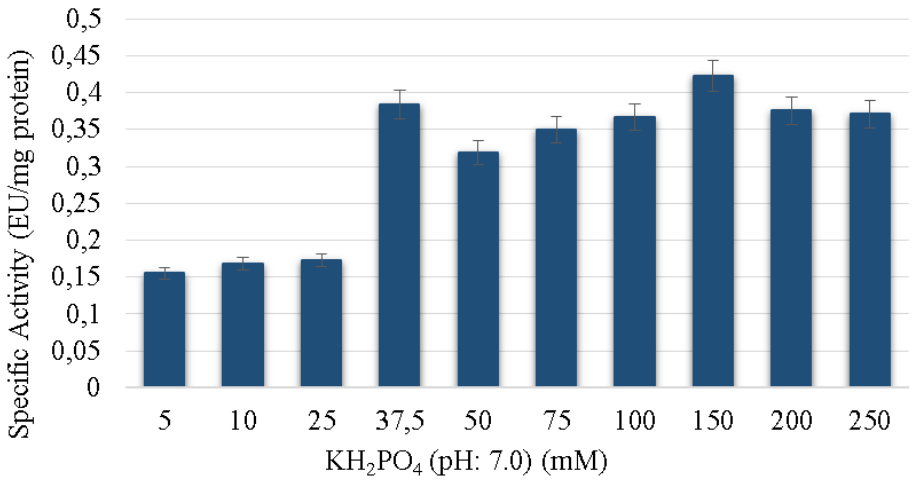

Figure 1. Specific activity – [ionic strength] graph for *A. maculatum* GR enzyme using K-phosphate buffer solutions at different concentrations.

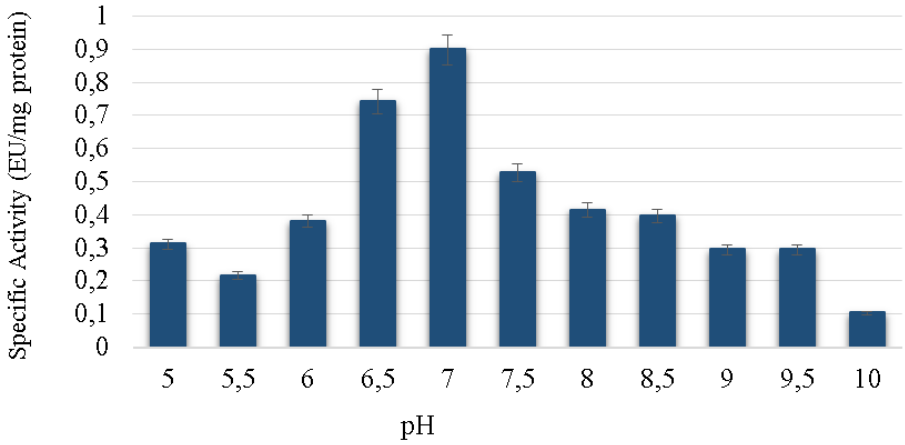

Figure 2. Specific activity – [pH] graph for determination of optimum pH for GR enzyme from *A. maculatum*.

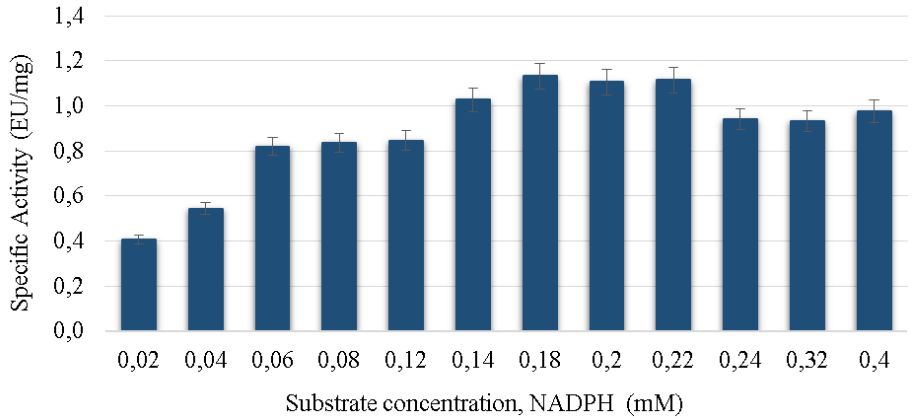

Figure 3. Specific activity – [Substrate concentration] graph for *A. maculatum* GR enzyme using NADPH solutions at different concentrations.

4. Conclusion

This study is the first research on the partial purification and characterization of the GR enzyme from the leaf of *A. maculatum*, which has been reported to have positive effects on health. To the best of our knowledge, no previous studies have reported on the purification of GR from A. maculatum. As a result of the study carried out using the leaf of the *A. maculatum* plant, the GR enzyme was obtained at a saturation of 40-60% ammonium sulfate. With the studies carried out for the characterization of the partially purified enzyme, it was determined that the optimum ionic strength was 150 mM potassium phosphate buffer, the optimum pH value was 7.0 and the optimum substrate concentration was 0.18 mM. It is thought that the results obtained will be a guide for future studies on the GR enzyme, which is of great metabolic importance.

Author Contributions

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

	G.B.	M.S.	D.E.
C	30	20	50
D			100
S			100
DCP	30	20	50
DAI	30	20	50
L	30	20	50
W	40	10	50
CR			100
SR			100
PM	40		60
FA			100

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

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

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Research Article
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CHARACTERIZATION OF GUAIACOL PEROXIDASE ENZYME FROM CARAMBOLA FRUIT

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Abstract: Carambola is a fruit grown in tropical and subtropical regions of the world. Natural antioxidants including vitamin C, carotenoids, and certain phenolic substances are abundant in carambola fruit. As antioxidants support health by acting as nutraceutical and functional food additives, they help preserve food by preventing oxidation processes. The oxidation of various organic or inorganic substrates by hydrogen peroxide or organic peroxides as terminal oxidants is a process in which peroxidase, which is abundantly present in fruits and vegetables, participates. In this study, guaiacol peroxidase enzyme from carambola fruit was partially purified and characterized. Purification procedure made up the homogenate preparation, ammonium sulfate precipitation, and dialysis. After purification, optimum ionic strength, pH and substrate concentration were investigated. These values were determined as 200 mM Tris, pH: 7.5, 7.5 mM H_2O_2 and 15 mM guaiacol for carambola fruit guaiacol peroxidase enzyme, respectively.

Keywords: Carambola, Enzyme, Characterization, Peroxidase, Purification

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1. Introduction

Nutritional content of fruits is one of the most important factors affecting the customer demand for the product and the development of the market value of these products. The promotion and living campaign for a healthy lifestyle by health institutions has increased the awareness of the significance of customers' dietary needs (O'Dougherty et al., 2006; Barrett et al., 2010; Lampila et al., 2009). Fruits and vegetables, which have important health benefits, play an important role in reducing the incidence of various diseases due to these properties (Park et al., 2003; Marnewick et al., 2011; Franzini et al., 2012; Rautiainen et al., 2012).

Carambola (*Averrhoa carambola* L.) is a tropical and subtropical fruit that is widely grown (Muthu et al., 2016). *Averrhoa carambola* (Figure 1) is a woody plant from the Oxalidaceae family that is native to India, Indonesia, the Philippines, Malaysia, Vietnam, Sri Lanka, and Bangladesh (Manda et al., 2012). Its fruits and leaves are frequently utilized in Ayurvedic medicine to treat a variety of diseases (Dasgupta et al., 2013). Because of its unusual form, it is historically known as "kamrakh" and more popularly known as star fruit. Malaysia is a significant producer of carambola in the globe (Zainudin et al, 2014). Carambola has both antioxidant capacity and high nutritional value (Leong and Shui, 2002; Shui et al., 2004; Isabelle et al., 2010; Shofian et al., 2011). The fruit contains proanthocyanins, which mainly act as antioxidants and play an important role for the immune system in defending against cancer, reactive oxygen species (ROS) damage and lipid peroxidation, as well as helping to remove toxins from the body (Ikram et al., 2009). In studies on different fruit varieties of Carambola, it was determined that the antioxidant potential changed (Zainudin et al., 2013). In addition, it has been found that different fruits such as olive, orange, tomato and pear jujube show different antioxidant properties among different fruits with varying antioxidant properties (Huang et al., 2007; Castrejón et al., 2008; Ilahy et al., 2011; Wu et al., 2012).

Figure 1. Carambola (*Averrhoa carambola* L.) fruit (Herath et al., 2021).

ROS, an essential component of aerobic life, are formed when photosynthetic organisms release molecular oxygen directly into the atmosphere (Gupta et al., 2017; Taverne et al., 2018). Although reactive oxygen species are a natural component of a cell metabolism, when the

ratio of ROS created to ROS scavenged is out of balance, the detrimental effects of these species (Olson and Straub, 2015; Sewelam et al., 2016; Sengul et al., 2021).

Endogenous antioxidant enzymes can scavenge reactive oxygen species (ROS). Antioxidant enzymes like as superoxide dismutase, catalase, and peroxidases are well recognized for preventing intracellular ROS production and lipid peroxidation (Gelen et al., 2021).

Peroxidases (EC 1.11.1.7) are heme-containing enzymes that may decrease hydrogen peroxide while also oxidizing another substrate (Manu et al., 2009). Peroxidase (POD), an oxidoreductase, has been widely employed as a component of reagents for clinical diagnosis and numerous scientific investigations. Peroxidase has been attributed physiological tasks such as indole-3-acetic acid metabolism, lignification, cross-linking of cell wall polymers, suberin production, and infection resistance (Veitch, 2004). Peroxidases are present in a wide range of organisms, including mammals, bacteria, fungus, and the majority of green plants (Sakharov et al, 2000). Plant peroxidases play crucial physiological roles, such as defending against pathogens and stress (Twala et al., 2020), regulating cell wall damage (Wakamatsu and Takahama, 1993), taking part in the removal of hydrogen peroxide, and protecting against unwanted discoloration (Ashie et al, 1996; López-Serrano and Barceló, 1996).

In order to reveal the importance of POD enzyme in plant defense system, we aimed, for the first time, to partially purify the enzyme from carambola fruit, to determine new potential natural antioxidant sources and optimum ionic strength, pH and substrate amount.

2. Material and Methods

2.1. Chemicals

The chemicals used in the purification process were supplied by Sigma-Aldrich (St. Louis, Mo, USA). The other chemicals used were supplied by Merck (Darmstadt, Germany).

2.2. Homogenate Preparation and Enzyme Analysis

The fruit was obtained from the plant *Averrhoa carambola*. It was brought in small pieces and homogenized through crushing with liquid nitrogen (approximately -196°C). The powdered sample was mixed with 100 mM Tris (2-Amino-2-(hydroxymethyl)-1, 3-propanediol) buffer and then centrifuged at 4°C, 15.000 xg. After centrifugation, the supernatant was filtered, and the enzyme's activity was evaluated. The activity was measured with a Shimadzu UV-1800 spectrophotometer at 470 nm (Şişecioğlu et al., 2010).

Hydrogen peroxide (H_2O_2) and guaiacol was used as the substrate for POD. 100 µl of H_2O_2 and 100 µl guaiacol in 0.1 M Tris buffer (pH 7.0) was added in the cuvette. In order to start the enzymatic reaction, 100 µl of supernatant containing POD enzyme was added to the cuvette. The remaining 1 ml of the solution was then filled with distilled water. The reaction's absorbance value was measured in a spectrophotometer for 3 minutes at 470 nm.

2.3. Ammonium Sulfate Precipitation and Dialysis

The obtained homogenate was precipitated with ammonium sulfate. The homogenate was then tested at various solid ammonium sulfate at regular intervals ranging from 0 to 100% salt content. The precipitate was sufficiently dissolved with 0.1 M Tris. Dialysis was applied to remove salts from the protein content. The obtained sample was placed in the dialysis bag, passed through the appropriate buffer and mixed slowly. As tiny molecules flowed through the membrane during this application, the buffer outside the membrane was altered several times until the osmotic pressure was controlled (Smith et al., 1988).

2.4. Kinetic Parameters for The Enzyme's Characterization

In the enzyme characterization study, pH and substrate in different ranges, in addition to, ionic strength parameters in different buffers and ranges were investigated.

3. Result and Discussion

In this study, POD enzyme from the fruit of the carambola plant was partially purified and characterized. The high antioxidant content of carambola fruit and the positive effects of its use in human health reveal the importance of our study. Peroxidase has been found in a variety of species, including bacteria, fungus, and higher plants. It has also been isolated, sequenced, and described (Passardi et al., 2007; Welinder, 1992).

Characterization study is important for determination and selection of optimum values for POD enzyme and antioxidant properties obtained from carambola fruit. Many plant peroxidases, especially those from horseradish (*Armoracia sp*.), are well recognized for their structures, substrate specificities, and kinetic characteristics (Al-Senaidy and Ismael, 2011). In this study, it is of great importance to determine the optimum values for the importance of the POD enzyme obtained from the carambola plant and its antioxidant properties. In addition to the importance of the antioxidant content of the Carambola fruit, POD characterisation is important for the enzyme and the plant.

For the first time, POD enzyme from Carambola fruit was partly purified and characterized in this work. After the homogenized sample, the precipitate saturation of the enzyme with solid ammonium sulfate $(NH_4)_2SO_4$ was determined in the range of 60-80%. This result demonstrates that the functioning during the purification protocol period is consistent with previous research results and will serve as an example for future studies. In order to determine the optimum ionic strength optimization, both tris and potassium phosphate buffer optimization measurements were carried out.

Optimum ionic strength optimization experiments were evaluated in the range of 10 mM to 400 mM Tris buffer and determined as 200 mM Tris (Figure 2). In order to find the optimum pH, measurements were made between

pH 6.5 and 8.5 and the optimum pH was found to be 7.5 (Figure 3). Moreover, the optimal substrate concentration for H_2O_2 was determined to be 7.5 mM H_2O_2 after being evaluated between 2.5 and 15 mM (Figure 4). In addition, the optimum substrate concentration for the other substrate, guaiacol, was evaluated between 5 and 30 mM, and 15 mM guaiacol was found to be the optimal substrate concentration (Figure 5).

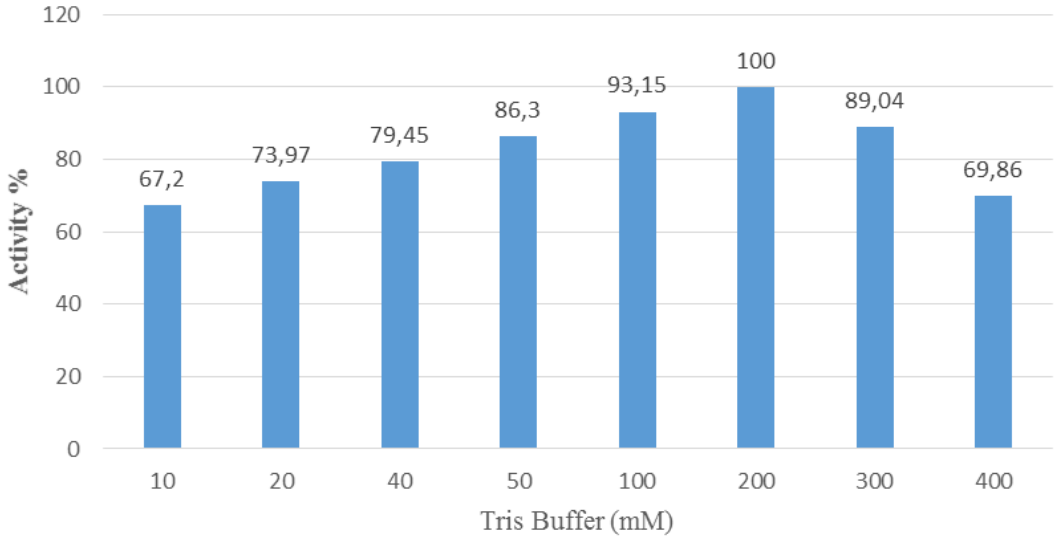

Figure 2. Activity measurements of carambola fruit guaiacol peroxidase enzyme optimal ionic strength TRIS buffer.

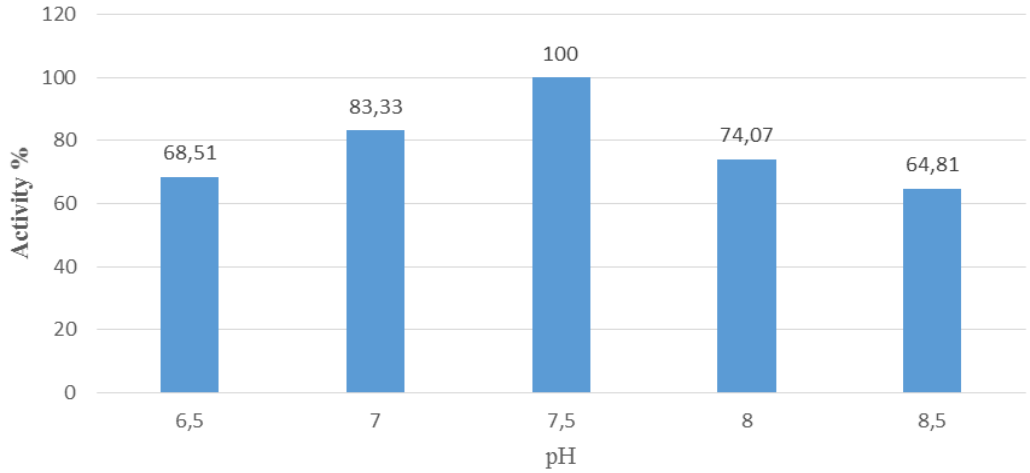

Figure 3. Activity measurements of carambola fruit guaiacol peroxidase enzyme optimal pH value Tris (200 mM) buffer

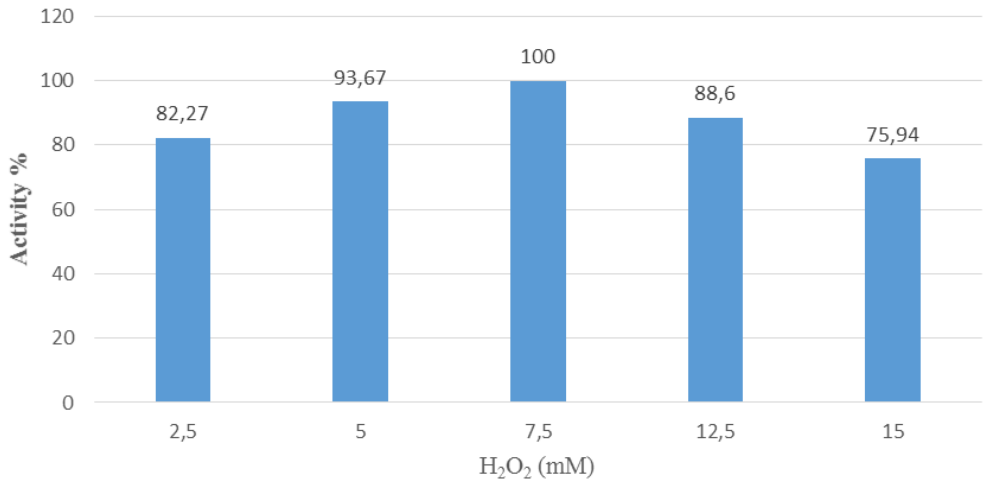

Figure 4. Carambola fruit guaiacol peroxidase enzyme activity measurements in buffer at 200 mM TRIS (pH=7.5) for optimum concentration of H_2O_2 substrate

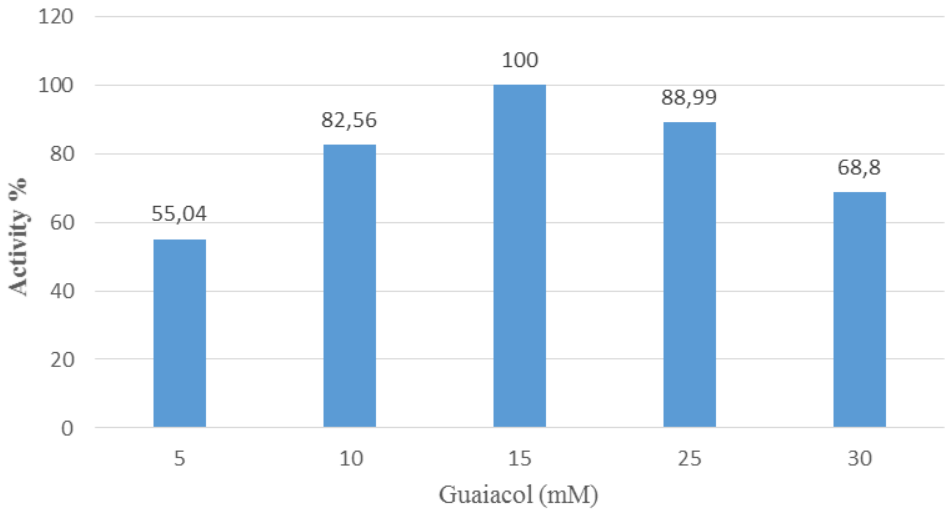

Figure 5. Carambola fruit guaiacol peroxidase enzyme activity measurements in buffer at 200 mM TRIS (pH=7.5) for optimum concentration of guaiacol substrate

Many researches on the characterisation of POD and other antioxidant enzymes have been published in the literature, with comparable results to ours. In a study by Aghelan and Shariat (2015), POD characterization was performed from leaf tissue of Rosemary (*Rosmarinus officinalis L.*) plant. Optimum pH, temperature and ionic strength values were found in 6.0, 40 °C and 0.3 M sodium phosphate buffer, respectively.

Loukili et al. (1999) tomato plant internode POD characterization study, optimum pH and temperature were found to be 5.0 and 55 °C, respectively. Bursal et al. (2013) POD enzyme characterization study of chard leaves the optimum temperature, pH and ionic strength were found to be 40 °C, 5.5 and 25 mM, respectively. In another study conducted by Koksal et al. (2012) on the characterization of POD from sweet gourd, optimum pH, optimum ionic strength and optimum temperature measurements were made and the values were found as 7.2, 50 °C, 0.4 M, respectively.

In a study by Al-Senaidy and Ismael (2011), the optimum pH and temperature of the palm leaf POD enzyme were determined and the optimum values were found to be 5.5 and 55 °C, respectively. POD enzyme partial purification and characterization study by Mafulul et al. (2018) in the *Calotropis procera* leaves optimum pH and temperature values were found to be 6.0 and 50 °C, respectively. The range of POD enzyme ammonium sulfate was found to be 40-80% in lettuce stems by Hu et al. (2012). In addition, optimum temperature and pH values were found to be 5.0 and 45 °C (Hu et al., 2012).

Erdem et al. (2015) evaluated optimal pH, ionic strength, and temperature parameters in their POD characterisation research using white cabbage (*Brassica Oleracea var. capitata f. alba*). It was observed that the values were 6.5, 0.1 M KH_2PO_4, and 30 °C, respectively. Lavery et al. (2010) determined optimal pH, ionic strength, substrate, and temperature parameters in Horseradish (*Armoracia rusticana*) roots POD characterisation investigation. The results obtained are 7.0, 50 mM KH_2PO_4, 0.5 and 0.3 mM (guaiacol and H_2O_2) and 30 °C, respectively. In a study by Maciel et al. (2007) studies on optimum pH, substrate and temperature were carried out in the study of POD enzyme characterization from *Copaifera langsdorffii* leaves. The values obtained as a result of the measurements are 6.0, 0.04 and 0.39 mM (guaiacol and H_2O_2) and 35 °C, respectively.

POD enzyme was isolated from many tissues of both plant and mammalian animals and characterization tests were performed as observed in our study and other studies. We believe that the findings of our investigation will be useful in future plant and POD enzyme purification and characterisation studies.

4. Conclusions

As a consequence, POD enzyme was isolated from carambola fruit. This is the first study to demonstrate the characterization and partial purification of the POD enzyme of carambola in the fruit part, which is an important plant with high economic and antioxidant value. These findings will help encourage the consumption of the plant and its antioxidant benefits, as well as the preference for carambola fruit properties.

Author Contributions

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

	Ö.T	M.Y	D.E
C	40	20	40
D	40	20	40
S	20	10	70
DCP	40	20	40
DAI	40	20	40
L	30	20	50
W	30	20	50
CR	30	10	60
SR	40	20	40
PM	20	20	60
FA	40	20	40

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

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

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Research Article
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DETERMINING REGRESSION MODELS FOR PHOTOSYNTHESIS AND STOMATAL RESISTANCE AS AFFECTED BY TEMPERATURE AND LIGHT INTENSITY IN TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) AND EGGPLANT (*SOLANUM MELONGENA* L.) GROWN IN GLASSHOUSES

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Abstract: This study was carried out to examine the relationships between net leaf photosynthesis and temperature and light intensity, between stomatal resistance and temperature and light intensity in tomato and aubergine grown with a range temperature from 10 to 30 ˚C and different light intensities from 3 to 7 MJm^{-2} d^{-1}. The study was carried out in a six-compartment greenhouse (size 4 m * 8 m), the temperature of which can be controlled by air conditioning, on tomato and eggplant plants. Each of the six greenhouse compartments was set to have maximum temperatures of 10, 12, 16, 18, 20 and 24 ˚C. Commercial varieties named "Counter" for tomato and "Bonica" for eggplant were used. "Fisons M2" commercial compost was used in all growing media and nutrient was applied equally. In the study, different sowing and planting dates were applied to benefit from natural light conditions (between 3 and 7 $MJm^{-2}d^{-1}$). Average temperature in each compartment was recorded using a 'Combine' data logger at 15 minute intervals. A porometer (Delta-T device, MT -3) was used to measure the stomatal resistance of tomato and eggplant leaves. The stomatal resistance measurements of the plants were made at the same time of the day (between 11.00-13.00) at 15-day intervals at the top, middle and lower levels of the crown of four different plants in different environmental conditions. In tomato, leaf photosynthesis increased curvilinearly with temperatures up to about 20.5 ˚C at low light intensity and declined at higher temperatures. The highest photosynthesis was obtained from the plants grown at a temperature of 22.5 ˚C and 7 $MJm^{-2}d^{-1}$ light intensity. The lowest photosynthesis was at 10 ˚C and 3 $MJm^{-2}d^{-1}$. In aubergine, at low light intensities, net photosynthesis increased curvilinearly up to 23 ˚C while it increased up to 20 ˚C at high light intensities and declined at higher temperatures. Maximum net leaf photosynthesis was found to be greater in tomato than aubergine.

Keywords: Light intensity, Photosynthesis, Stomatal resistance, Temperature, Tomato

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1. Introduction

Interest in the response of crop photosynthesis to temperature and light is growing in eco-physiological research and practical agriculture (Tuzet et al., 2003; Uzun, 2006). Light limitation on photosynthetic productivity of all crops is of great importance. This phenomenon is especially important for glasshouse crops since the amount of light that plants receive is reduced by 30% or more by the glasshouse structure, and whereas other environmental factors, namely temperature, CO_2 concentration, mineral nutrients and water can be supplemented and controlled at economically optimal levels, supplementary artificial lighting is not commercially worthwhile (Jovanovic and Annandale, 2000).

Photosynthetic light response curves have been studied for tomato over the last twenty years to determine optimum light requirements of the plants and their adaptability to different environmental conditions (Acock et al., 1978; Cockshull, et al., 1992; Prusinkiewicz, 2004). The net photosynthetic rate of tomato crop canopies under semi-commercial glasshouse is almost directly proportional to light flux density at least up to 200 W m^{-2} (Atherton, 1986). For example, in aubergine, after reaching a light saturated value, a decline photosynthesis at higher values was reported to be due to closure of the stomates and also to a reduced CO_2 gradient (Bertin and Heuvelink, 1993; Uzun, 1996).

It has also been reported that the optimum temperature for photosynthesis adapts according to the temperature at which the plant is grown (Dayan, et al., 1993; Heuvelink and Bertin, 1994; Uzun, 1996). In tomato, a decline in photosynthesis at temperatures above 30 ˚C was reported to be because of higher stomatal resistance

(Seligman, 1990). Furthermore, a linear relationship was found between photosynthesis under saturating CO_2 and irradiance and temperature until the optimum temperature is reached. Temperature during growth also influences the rate of decline of photosynthesis with plant age (Acock, 1991; Uzun, 2006). Stomatal resistance to CO_2 was also reported to decline according to the leaf position on the stem of tomato. Leaf nitrogen content has also been reported to affect leaf net photosynthesis (Acock et al., 1978).

Since environmental preconditioning such as light intensity, temperature and water stress may affect gas exchange in the greenhouse, an attempt was made to study the effect of a wide range of light integral and temperature on adaptation of photosynthesis (Dorais, et al., 1991; Pearson, et al., 1994; Grimstad, 1995). Previous studies have tended to include a rather narrow temperature range for tomatoes, and particularly very little is known regarding photosynthesis and its adaptation to environment in aubergine. The aim of this study was to investigate the net photosynthetic rate in both tomato and aubergine grown with different daily mean light integral at various temperatures and to find relationships between photosynthesis, stomotal resistance, light intensity and temperature.

2. Materials and Methods

This study was carried out in the spring growing season in Ondokuz Mayıs University Faculty of Agriculture research and application greenhouses in 2005. Actual mean temperature measurements in the growing compartments according to experiment number are presented Table 1. A series of detailed experiments on tomato and aubergine were carried out in a suite of controlled temperature glasshouse compartments (size 4 m* 8 m). Six greenhouse compartments were used to give maximum set temperatures of 10, 12, 16, 18, 20 and 24 ˚C. The temperature in the compartments was controlled by the air conditioner and it was adjusted to provide automatic heat when the temperature in each compartment fell below the set point, and when the temperature rose 4 °C above the set temperature, the ventilations were operated automatically.

Table 1. Actual mean temperature measurements in the growing compartments according to experiment number.

Experiments		
1	2	3
11.4	15.2	16.1
14.3	15.4	17.1
16.4	17.5	18.0
19.8	21.3	22.1
22.5	22.8	23.1
25.3	26.0	25.8

Seeds of tomato cv. "Counter" and aubergine cv. "Bonica" were sown in modular seed trays containing 228 cells (2 cm x 2 cm) filled with comme rcial peat based compost (Fison F2). The seed trays were maintained in a glasshouse compartment maintained at a temperature of 22 ˚C. After the seedlings emerged, the seed trays were placed in a different compartment maintained at 20 ˚C. Sowing, transplanting and planting dates for each trial were performed in order to obtain natural light conditions (between 3 and 7 $MJm^{-2}d^{-1}$) during experiments.

Three weeks after emergence, plants were pricked out into 15 cm plastic pots containing Fisons M2 commercial compost. After the plants reached the fourth true leaf stage they were planted into Fisons growbags, containing a peat compost, spaced at a distance of 50 cm between the rows and 35 cm in rows. Plants were irrigated on four occasions each day with a standard nutrient feed (Sangral SS 112), diluted to give a concentration of 0.2 g l^{-1} nitrogen, 0.2 g l^{-1} phosphorous and 0.4 g l^{-1} potassium, equivalent to a conductivity of 1600 ms cm^{-2} and pH of 6.5, using a trickle irrigation system.

The mean temperature in each compartment was recorded at intervals of 15 minutes using a 'Combine' data logger (Uzun, 1996). Photosynthetically active radiation (PAR) was recorded using a tube solarimeter (Uzun. 1996). The tube solarimeter was connected to a data logger (Skye, Model 3).

A porometer (Delta-T instrument, MT -3) was employed to measure stomatal resistance of tomato and aubergine leaves. Measurements were taken at 15 day intervals at three different levels of the canopy of four different plants grown under different environmental conditions. Before every measurement, the instrument was calibrated by using a calibration plate. Care was taken to measure stomatal resistance at the same time of day (between 11.00 am and 13.00 pm) for every measurement. The aim of measuring stomatal resistance of both crops was to investigate the changes in stomatal resistance according to different light and temperature environments studied in the present study and consequently to find relationships between stomatal resistance, leaf net photosynthesis, temperature and light for both crops.

Measurements of CO_2 assimilation were carried out using an open system, portable infrared gas analyser system (Analytical Development Co. Ltd, model LCA3). The whole system consists of four units, an Infrared Gas

Analyser (IRGA), a leaf chamber with a lamp unit to supply artificial light, an air supply unit and a data-processor/logger. The air flow rate was adjusted to 400 ml $min.^{-1}$ for both tomato and aubergine. The air intake was placed outside the glasshouse to minimise disturbances in CO_2 concentrations caused by local factors. The leaf was placed into the leaf chamber with an area of 6.2 cm^2. Measurements were taken at three different levels of the canopy, namely top, middle and bottom and carried out at three week intervals throughout ontogeny of both crops. After every measurement of leaf, 30 seconds was allowed to elapse between two measurements in order to allow net photosynthesis to reach a new equilibrium. Randomly selected leaves from top, middle and bottom of the plants were used in the measurements for all light levels.

Multiple regression analysis was performed with Microsoft EXCEL following the procedure of Gomez and Gomez (1984). Curve fitting processes were continued until the least sum of squares of residuals was obtained. Fitted planes from multiple regression analysis were shown on 3-D graphs using the 'Slide Write' computer package Version 2.0.

3. Results

In order to determine the overall effect of temperature and light on photosynthesis, multi-regression analysis were carried out by plotting net photosynthesis of tomato and aubergine leaves from different parts of the plant canopy, namely top, middle and bottom, against temperature and light values at which the plants were grown and the following equations were obtained for tomato (equation 1) and aubergine (equation 2).

$$P = -19.2 + 23.68*T + 0.67*L - 0.06*T^2 \quad (1)$$

SE (3.79)*** (0.44)*** (0.16)*** (0.01)***

$r^2 = 0.91$*** for tomato.

$$P = -42.86 + 3.12*T + 7.26*L - 0.07*T^2 - 0.65*L^2 \quad (2)$$

SE (9.25)*** (0.87)*** (1.31)*** (0.02)*** (0.13)***

$r^2 = 0.89$*** for aubergine.

As seen from the equations above, most of the variation in leaf net photosynthesis, 91 % for tomato and 89 % for aubergine was explained by light integral and temperature. Although there was a positive linear effect of light intensity on net photosynthesis in tomato as well as a curvilinear effect of temperature, both light intensity and temperature had curvilinear effects on photosynthesis in aubergine. Utilising from Equation 1 and 2, the following figures (Figure 1a and b) showing the effect of light intensity and temperature on leaf net photosynthesis of tomato (Figure 1a) and aubergine (Figure 1b) were obtained.

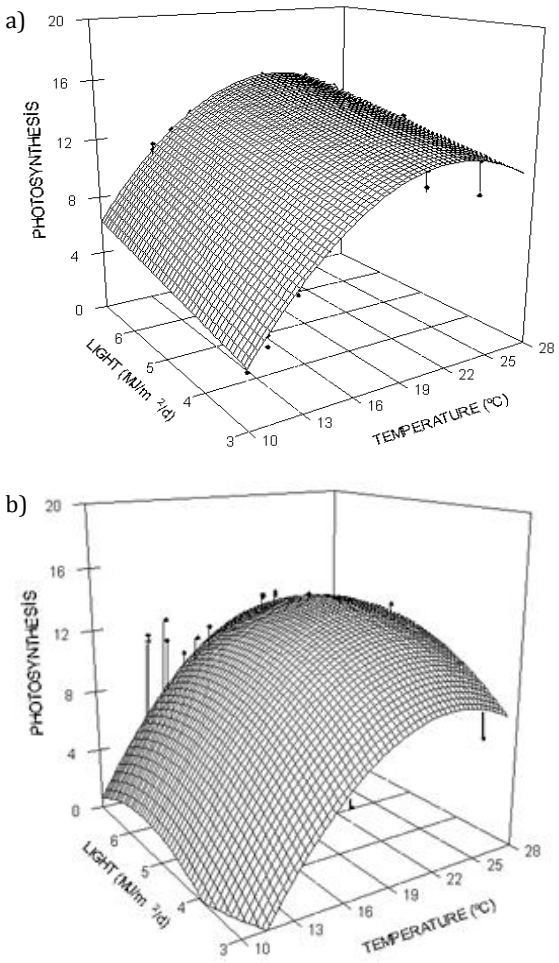

Figure 1. The response of leaf photosynthesis (μmol CO_2 $m^{-2}s^{-1}$) for (a) tomato and (b) aubergine leaves from different parts of plant canopy (top, middle, and bottom) averaged over growing period of 80 days after planting and grown with different light intensities (MJ $m^{-2}d^{-1}$) and temperatures (˚C).

As seen in Figure 1a and b, in general, the leaf net photosynthesis increased as temperature increased throughout the temperature range investigated at all daily mean light integrals and declined at the highest temperatures for both tomato and aubergine.

In tomato, leaf photosynthesis increased curvilinearly with temperatures up to about 20.5 ˚C at low light intensity and declined at higher temperatures. Therefore, it can be said that optimum temperatures for photosynthesis increased as light intensity increased (Figure 1a). The highest photosynthesis was obtained from the plants grown at a temperature of 22.5 ˚C and 7 $MJm^{-2}d^{-1}$ light intensity. The lowest photosynthesis was at 10 ˚C and 3 $MJm^{-2}d^{-1}$.

In aubergine, at low light intensities, net photosynthesis increased curvilinearly up to 23 ˚C while it increased up to 20 ˚C at high light intensities and declined at higher temperatures. Unlike tomato, optimum temperatures for net photosynthesis increased curvilinearly with light intensity. Maximum net leaf photosynthesis was found to

be greater in tomato than aubergine (Figure 1a and b).

In order to determine the overall effect of temperature and light on stomatal resistance, multi-regression analysis were carried out by plotting stomatal resistance against temperature and light and the following equations were obtained for tomato (equation 3) and aubergine (equation 4).

$SR = 11.97 – 0.19*T – 0.0029*L2*T$ (3)

SE (0.85)*** (0.057)** (0.00085)**

$r^2 = 0.81$*** for tomato.

$SR = 19.02 – 0.32*T – 0.0042*L2*T$ (4)

SE (1.09)*** (0.057)*** (0.00062)***

$r^2 = 0.90$*** for aubergine.

As seen in Equation 3 and 4, most of the variations in stomatal resistance was explained by temperature and light intensity for both tomato (r^2=0.81) and aubergine (r^2=0.90).

For both crops, increasing light intensities resulted in lower stomatal resistance such as there was a curvilinear decline in stomatal resistance with increasing light intensities (Figure 2a and b). However, the decline in stomatal resistance for both tomato and aubergine leaves was found to be sharper with higher temperatures compared to lower temperatures. Stomatal resistance was reduced by increasing temperatures for both crops. A similar response of stomatal resistance to temperature as to light intensity was found in both crops such as the increase in stomatal resistance with decreasing temperatures was sharper at high light intensities (Figure 2a and b). The highest stomatal resistance was obtained from the plant leaves grown at the lowest light and temperature regimes for both tomato and aubergine. The lowest stomatal resistance was from the plant leaves grown with the highest temperature and light intensities examined in the study.

Figures 3 a and b show the relationship between stomatal resistance and net leaf photosynthesis in both tomato and aubergine respectively. As seen from the figures, there was marked tendency that increasing stomatal resistance resulted in lower net leaf photosynthesis in both tomato and aubergine.

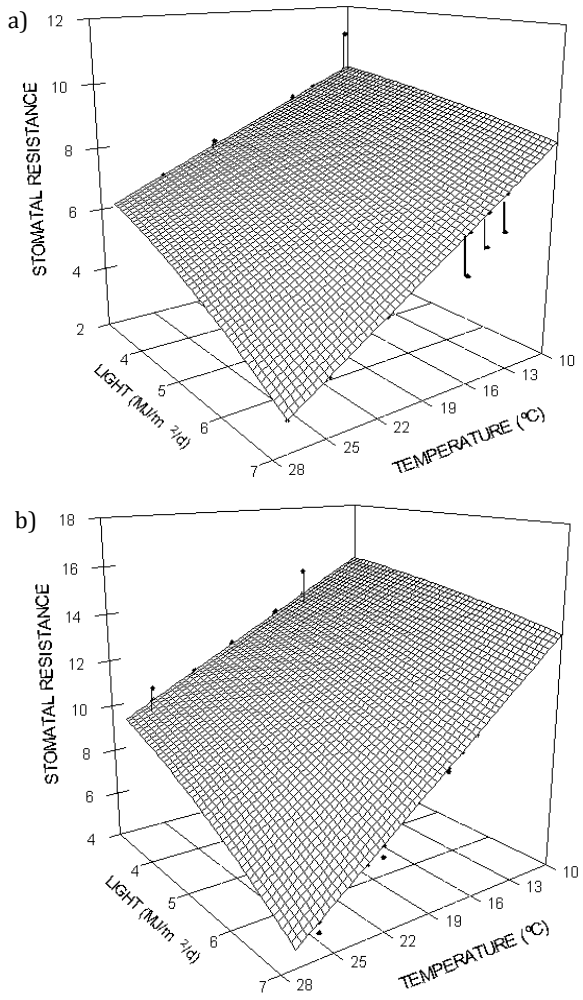

Figure 2. The response of stomatal resistance (s cm^{-1}) for (a) tomato and (b) aubergine leaves from different parts of plant canopy (top, middle and bottom) averaged over growing period of 80 days after planting and grown with different light intensities (MJ $m^{-2}d^{-1}$) and temperatures (°C).

4. Discussion

In this study, net photosynthesis and stomatal resistance of the leaves of tomato and aubergine grown with three different daily mean light integrals and different mean temperatures were investigated and the interrelations of these parameters was examined.

The present study revealed that maximum net leaf photosynthesis declined with time after sowing and more rapidly with increased daily mean light integral for both tomato and aubergine (Figures 3 a and b). This may be due a gradual degradation of all chloroplast number and reduced chlorophyll content which results in declining net photosynthesis with time (Uzun, 1996).

Long term maximum leaf net photosynthesis increased for both tomato and aubergine grown with higher daily mean light integrals. Many other studies have shown that the leaves of plants grown under high light levels have faster rates of carbon fixation than leaves of plants grown under low light levels (Acock et al. 1978; Özkaraman 2004) also indicated that maximum net photosynthesis per unit leaf from a tomato plant grown at 80 W m^{-2} was approximately twice that of a leaf from a plant grown at 20 W m^{-2}.

Stomatal resistance increased significantly with time at all daily mean light integrals and temperatures. However, the increase in stomatal resistance with time for both tomato and aubergine was less marked as daily mean light integral increased. There was also a significant decline in stomatal resistance with increasing temperature for tomato and aubergine. Bar-Tsur et al. (1985) also reported a decrease in stomatal resistance in tomato up to 25 ˚C and an increase above 35 ˚C. In this

study, stomatal resistance in the leaves of both tomato and aubergine grown with higher daily mean light integrals was lower than those of the plants grown with lower daily mean light integrals. The effect of light can be explained as an indirect effect via a lowering of the CO_2 concentration in the chloroplast of the leaf by photosynthesis since increased photosynthetic rate through increasing light intensity can cause lower internal CO_2 concentrations in the leaf leading to lower stomatal resistance (Acock et al., 1978).

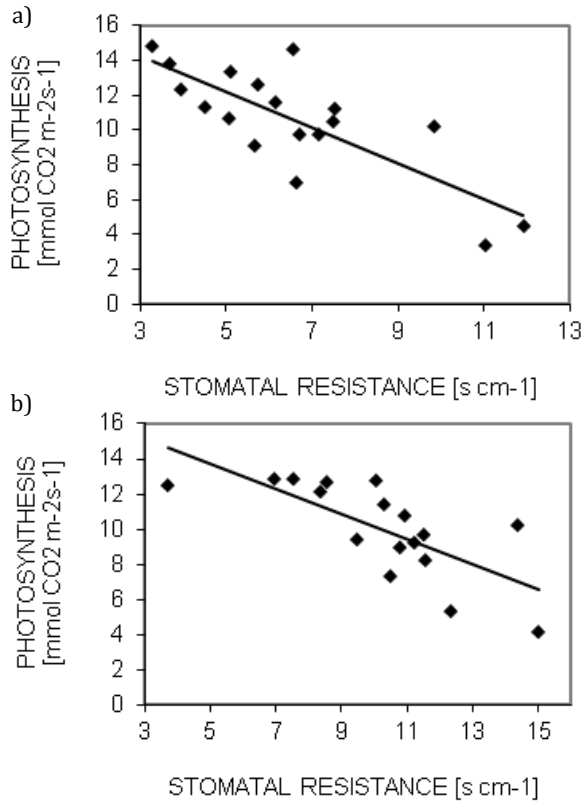

Figure 3. The relationship between mean canopy photosynthesis (μmol CO_2 $m^{-2}s^{-1}$) and stomatal resistance (s cm^{-1}) of (a) tomato and (b) aubergine grown with different light intensities (MJ $m^{-2}d^{-1}$) and temperatures (°C).

The effect of temperature on stomatal resistance may be as a result of its influence on leaf water stress, since it would be expected that increasing temperature result in higher stomatal resistance due to the thinner leaves produced under these conditions (Rand and Cooke, 1980). Stomatal resistance also decline from the bottom of the plants to the top for tomato and aubergine. Acock et al. (1978) reported that leaf resistance of tomato leaves from the uppermost leaf layer was smaller than those from the lowest layer which had been exposed to lower light than the upper leaves.

In the present study, it was found that net photosynthesis tented to increase with increasing leaf nitrogen content in both tomato and aubergine. The relationship between leaf nitrogen content and photosynthesis was not implemented since data the present study did not include different nitrogen levels at constant temperature. It has been reported that there is a strong relationship between the leaf nitrogen content and leaf photosynthesis (Novoa and Loomis, 1981; Evans, 1989). Changes in nitrogen content reflects changes in protein content and about half of the proteins within the leaf tissues are directly associated with photosynthesis and this explains the close relationship that exists between the rate of leaf photosynthesis and nitrogen concentration (Evans,1989). A similar but less clear relationship between net photosynthesis and leaf nitrogen content was shown here. It has also been reported that the photosynthetic capacity of leaves is greatly reduced when plants suffer nitrogen deficiency. In a wide variety of plants, there is a positive correlation between photosynthetic capacity and leaf nitrogen content, expressed either on a dry weight basis or an area basis (Brunetti et al., 2013) since leaf growth is sensitive to nitrogen supply, and leaf expansion rate increases as nitrogen supply increases (Novoa and Loomis, 1981).

Net leaf photosynthesis did however decline very significantly with increasing stomatal resistance in both tomato and aubergine. Many studies have shown that net photosynthesis decline with increasing stomatal resistance (Jones and Sutherland 1991).

The variation in nitrogen in the canopy is proportional to the light transmission. However, irradiance is not the only factor affecting the nitrogen distribution within the leaf canopy, leaf age increases from the top leaves to the bottom and that could also generate a gradient of leaf nitrogen content through the and the relative effects of these two factors, irradiance and age, on the leaf nitrogen distribution is unknown (Pozo and Dennett, 1991).

In the present study, analysis on changes in leaf net photosynthesis, stomatal resistance with daily mean light integral temperature showed that there was a negative relationship between stomatal resistance and net leaf photosynthesis in both tomato and aubergine.

Author Contributions

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

	F.Ö.
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.

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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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THE EFFECT OF URSOLIC ACID ADDITION INTO HİGH-ENERGY LAYING HEN DIET ON PERFORMANCE, EGG QUALITY PARAMETERS, SERUM LIPID PROFILE AND LIVER FAT RATE

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Abstract: This study was conducted to determine the effect of ursolic acid (UA) at different ratios (0, 0.5, 1 and 1.5%) supplementation into high-energy laying hen diet on performance, egg quality parameters, serum lipid profile, some liver enzymes and liver fat ratio.
A total of 120 Lohman LSL laying hens, 70 weeks old, were used in present study. The animals were divided into 5 groups and each group consisted of six subgroups. In the experiment, the control group was fed with basal feed, and the treatment groups were fed with high-energy (HE) diets including 0, 0.5, 1 and 1.5% UA, respectively. Experiment lasted for 8 weeks. Egg yield decreased in high energy feed groups except HE + 1.5% UA group. Egg weight was found to be highest in the HE + 1.5% UA group. Addition of UA into feed improved the feed conversion ratio (FCR). It was determined that liver fat ratio was higher in the group fed with HE feed ($P<0.01$) than other groups fed with diets including UA, but the addition of UA decreased the liver fat rate significantly. The addition of UA to feed increased blood plasma MDA and NEFA values, and decreased GSH and GPx values ($P<0.01$). The addition of 1.5% UA to high-energy feed increased ALT and total cholesterol, while lowering glucose. The highest VLDL, TG and LDL values were found for YE + 0% UA and YE + 1.5% UA groups. Conclusion, high-energy feed adversely affected performance values and liver fat ratio, but the addition of ursolic acid improved FCR and decreased liver fat ratio. Positive effects of ursolic acid have been seen, but more studies are needed.

Keywords: Fatty liver, Ursolic acid, Non-esterified fatty acid, Feed conversion ratio, Hypolipidemic

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1. Introduction

In parallel with the rapidly increasing world population, the demand for eggs is increasing day by day. In order to meet this increasing demand, it has been inevitable to make conventional animal production. More production depends on increasing the number of animals as well as good care and feeding. Therefore, it is inevitable to have more animals per unit area. The most advantageous system in laying hen is cage breeding.

However, in laying hens raised in cages, the restriction in the movement area and the high energy value of the feeds may cause fatty liver. Fatty liver syndrome is one of the important causes of death in commercial layers (Leeson, 2007). In many studies conducted in the past years, it has been reported that fatty liver syndrome is detected at a higher rate in animals housed in cages than those raised on the ground (Butler, 1976; Shini et al., 2006; Squires and Leeson, 1988).

Fatty liver is a disease characterized by an accumulation of fat in the abdominal cavity and liver. Excessive fatty liver adversely affects animal health and causes a significant decrease in egg production. Although there is no known method for the treatment of fatty liver syndrome, its development could be prevented by balanced nutrition techniques. One of them is ursolic acid. Phytosterolic ursolic acid is in the pentacyclic triterpenes group of triterpenes and is found in free or glycoside structure in plants, vegetables and fruits such as apple, basil, olive, oregano, rosemary and thyme (Babalola and Shode, 2013; Mendes Leal, 2012; Ikeda et al., 2008). In several recent studies on mice and rats, it has been reported that the addition of ursolic acid to feed has a hypolipidemic and hypoglycemic effect. It has been proven by studies that ursolic acid regulates lipid metabolism and has a protective effect on the liver (Azevedo et al., 2010; Liu et al., 1995).

Research to date has shown that older laying hens are more vulnerable to internal and external stimuli than younger hens, and that bioactive additives can have improving effects on chickens' performance and physiological function (Jiang et al., 2020; Liu et al., 2020). As a result of our research, no study was found that examined the effect of ursolic acid on fatty liver syndrome in layer hens.

In this study, it was aimed to determine the effect of high energy feed and ursolic acid on performance, egg quality criteria, mortality rate, some antioxidant enzymes and liver fat ratio in laying hens

2. Materials and Methods

In the study, 120 Lohmann (LSL) white laying hens of 70 weeks were weighed and divided into five groups depending on chance, and each group consisted of six subgroups. Chickens were placed in 4-storey battery-type cages (60 * 59 * 61 cm) with 24 animals in each group, 6 replications and 4 animals in each repetition. First group (control group) was fed basal diet (Table 1), and the treatment groups were fed with high-energy (3020 kcal / kg ME) diets including 0, 0.5, 1 and 1.5% ursolic acid, respectively. The feed and water were given to animals as ad-libitum during the 8 weeks' experimental period. Ursolic acid (99.9% purity) was obtained from a commercial company.

Table 1. Composition of feeds used in the trial (%)

Item	Basal diet (control)	High energy diet
Corn 8.5	63	64.17
Soybean meal 44-46	16.39	12.50
Corn gluten 60	8.48	10.64
Limestone	9.68	7.65
DCP 18	1.44	1.44
Soybean oil	0.17	2.68
Vitamin-Mineral mixture[1]	0.25	0.25
Salt	0.22	0.33
Sodium bicarbonate	0.16	0.16
L-Lysine	0.11	0.10
D-L Methionine %99	0.10	0.08
Calculated composition (%)		
Dry matter	88.41	88.54
Crude protein	17.52	17.20
Ether extracte	2.20	4.84
Crude ash	11.87	10.35
Crude fiber	2.78	2.57
D Methionine	0.38	0.38
Methionine	0.40	0.41
Lysine	0.76	0.70
ME Kkal/Kg	2726	3000
Analysed composition (%)		
Dry matter	88.78	88.37
Crude protein	17.12	16.92
Ether extracte	2.43	5.03
Crude ash	11.24	11.09
Crude fiber	3.18	2.91

[1]Per kg diet added 12 000 IU vitamin A; 2500 IU vitamin D3; 30 IU vitamin E; 4 mg vitamin K3; 3 mg vitamin B1; 6 mg vitamin B2; 30 mg niasin; 10 mg calcium D-pantothenate; 5 mg vitamin B6; 0.015 mg vitamin B12; 1 mg folic acid; 0.050 mg D-biotin; 50 mg vitamin C; 300 mg choline chloride; 80 mg manganase; 60 mg iron; 60 mg zinc; 5 mg copper; 0.5 mg cobalt; 0.2 mg iodine; 0.15 mg selenium.

The chemical composition of the diets used in the study was determined according to the Weende analysis method (Kutlu, 2008). As performance values in the study, daily feed consumption, feed conversion rate (kg feed/ kg egg), egg weight and egg production were determined by measurements made every two weeks. The numbers of the animals that died during the trial were recorded daily.

Egg quality criteria (shell thickness, breaking strength, white ratio, yellow ratio, shell ratio, shape index and Haugh unit) were performed every two weeks on one randomly selected egg sample from each subgroup.

At the end of the experiment, blood samples taken from the sub-wing vein of 6 animals from each group into heparinized tubes were centrifuged at 3000 rpm for 10 minutes and their plasma was extracted and stored at -80 ° C for examination. MDA level in plasma (Yoshioka et al., 1979), SOD activity (Sun et al., 1988), GSH level (Tietze, 1969), GPx activity (Matkovics et al., 1988) CAT activity (Goth, 1991), TP levels (Lowry, 1951) and NEFA levels (Biont Chicken NEFA ELISA Kit, Cat No: YLA0179CH) were measured with Biotek Elisa Reader (Bio Tek µQuant MQX200 Elisa reader / USA). TP levels were used to calculate the SOD and GPx activity. Plasma glucose, cholesterol, VLDL, LDL, HDL, AST, ALT and TG values were analyzed in a special laboratory.

At the end of the experiment, 6 animals from each group were slaughtered and their livers were removed and their wet weights were determined. Then the livers were dried at 105 °C and their dry weight was determined.

Later, samples were taken from dried livers and their fat percentage was determined (Kutlu 2008).

2.1. Statistical Analyses

Performance values, egg quality criteria, some blood parameters and antioxidant enzyme values variance analysis were performed by the General Linear Model procedure, and the importance controls of the important data were performed using the SPSS 17 package program. Mortality was determined by the X^2 independence test. Differences between groups were foun d by Duncan multiple comparison test (Düzgüneş et al., 1983).

3. Results and Discussion

The effect of adding different levels of ursolic acid to high energy feeds on feed consumption, egg production, egg weight and feed conversion ratio is given in Table 2. It was determined that there were no significant differences in feed consumption between the groups. It found that egg production decreased significantly ($P<0.05$) in HE + 0% UA, HE + 0.5% UA and HE + 1% UA groups. The highest egg weight was found only in YE + 1.5% UA group. The best feed injury rate was seen in control, HE + 1% UA and HE + 1.5% UA groups. There was no significant difference between the groups in terms of mortality.

Table 2. Effects of high energy feed and ursolic acid supplements on performance values

Groups	Feed Intake (g)	Egg Production (%)	Egg Weight (g)	Feed Convertion Ratio (g:g)	Mortality (%)
Control	111.87	79.80[a]	60.42[b]	2.39[c]	4.2
HE+ 0 % UA	111.18	73.44[b]	61.35[b]	2.56[b]	20.8
HE+ 0.5 % UA	118.11	73.59[b]	61.96[b]	2.90[a]	20.8
HE+1 % UA	110.63	75.26[b]	61.55[b]	2.38[c]	8.3
HE+ 1.5 %UA	113.22	77.32[a]	63.80[a]	2.35[c]	8.3
SE	2.45	1.51	0.34	0.091	X^2=5.22
P	ns	*	*	*	ns

[a, b] The averages shown with different letters in the same column are different from each other. HE= high energy, UA= ursolic acid, Control= basal fed group, HE + 0% UA: High energy fed group, HE + 0.5% UA: High energy fed + 0.5% ursolic acid, HE + 1%, UA= high energy fed + 1% ursolic acid, HE + 1.5% UA: High energy + 1.5% ursolic acid, SE= standard error, NS= not significant, *P<0.05.

Some researchers observed that the feed consumption of laying hens fed a high energy diet decreased compared to the control group (Harms et al., 2000; Jiang et al., 2013; Valkonen et al., 2008; Yousefi et al., 2005; Zhang et al., 2008). Contrary to these reports, Grobas et al. (1999) found that feed consumption of laying hens fed with feed containing 2680 kcal / kg ME was higher than those containing 2810 kcal / kg ME. Plavnik et al. (1997) reported that as dietary energy increases, feed intake decreases. One of the main reasons for this is that energy content plays a key role in controlling feed intake (McNab and Boorman, 2002).

It has been reported that there are large economic losses due not only to animal deaths but also to reduced egg production due to fatty liver syndrome in caiged-raised chickens (Squires and Leeson, 1988). The metabolic activity of the liver is quite high in poultry, especially during egg production where lipogenesis is stimulated (Nesheim and Ivy, 1970). Butler (1976) reported that animals with fatty liver syndrome may experience sudden decreases in egg production. Smilarly, in many previous studies (Hansen and Walzem, 1993; Julian, 2005; Thomson et al., 2003), it was reported that egg productivity decreased suddenly due to fatty liver syndrome. It has been reported that as dietary energy increases, feed consumption decreases and thus egg production decreases (Plavnik et al., 1997). In this study, it was determined that egg production decreased in high energy groups, but there was no negative change in egg production in the group where 1.5% ursolic acid was added to high energy feed. However, Grobas et al. (1999) reported that there is no significant difference between egg yields of animals fed with feed containing 2680 and 2810 kcal / kg ME. Similarly, Rozenboim et al. (2016) reported that high energy feed does not affect egg yield.

In the present study, it was determined that the addition of 1.5% ursolic acid to a high-energy diet increased egg weight compared to the control group. In previous studies, some of the researchers reported that egg weight was not affected by the energy content of the feed (Summers and Leeson, 1993; Keshavarz and Nakajima, 1995; Grobas et al., 1999; Mathlouthi et al., 2002; Valkonen et al., 2008; Zhang et al., 2008), some reported significant increases in egg weight (Marsden et al., 1987; Peguri and Coon, 1991).

In the current study, it was observed that high energy feed negatively affected the ratio of feed conversion, but the feed conversion ratio values in the groups that added 1% and 1.5% ursolic acid to the feed were similar to the control group.

Unlike this study, Grobas et al. (1999) reported that the group fed with high energy feed had a better feed conversion value. However, in another study conducted on laying hens fed with feed containing different levels of energy, it was reported that there was no significant difference between the groups in terms of feed efficiency (Zhang et al., 2008).

Leeson, (2007) reported that fatty liver syndrome causes

significant mortality in commercial layer flocks. Shini et al. (2006) reported that 74% of the cause of death in laying hens in cages in Australia was caused by fatty liver syndrome. Similar to the current study, Valkonen et al. (2008) found that the mortality rate in laying hens fed with high energy feed was higher than the control group, but the difference was not found to be significant.

The effects of ursolic acid addition to high energy feed on egg quality criteria are given in Table 3. No significant difference was detected between groups in terms of shell breaking strength, shell thickness, ratio of egg shell, yolk, albumen and Hough units' values. Similar to this study, Valkonen et al. (2008) reported that the energy value of the feed does not affect eggshell breaking strength, egg shell ratio, yolk ratio, albumen ratio and Haugh unit.

Table 3. Effects of high energy feed and ursolic acid supplements on egg quality of the laying hens

Groups	SBS (kg cm²)	ST (µm)	Egg shell (%)	Yolk (%)	Albumen (%)	Hough units
Control	3.27	0.459	12.42	30.22	57.27	81.97
HE+ 0 % UA	3.01	0.414	11.94	31.38	56.63	83.69
HE+ 0.5 % UA	3.16	0.434	12.84	30.34	56.81	78.32
HE+1 % UA	3.01	0.413	12.83	30.99	56.17	82.90
HE+ 1.5 %UA	3.32	0.450	12.94	30.56	56.49	80.95
SE	0.14	0.006	0.13	0.19	0.24	0.64
P	ns	ns	ns	ns	ns	ns

[a, b] The averages shown with different letters in the same column are different from each other. HE= high energy, UA= ursolic acid, Control= basal fed group, HE + 0%, UA= high energy fed group, HE + 0.5% UA= high energy fed + 0.5% ursolic acid, HE + 1% UA= high energy fed + 1% ursolic acid, HE + 1.5% UA= high energy + 1.5% ursolic acid, SBS= shell breaking strength, ST= shell thickness SE= standard error, NS= not significant

In many previous studies, it has been reported that high energy feed has no effect on egg shell thickness (Yousefi et al., 2005), yolk weight (Keshavarz and Nakajima, 1995) and albumen weight (Keshavarz and Nakajima, 1995; Grobas et al., 2001). However, Whitehead et al. (1991) reported that the addition of fat to the ration increases the albumen ratio. The liver is a central organ for lipid metabolism. The liver synthesizes cholesterol and triglyceride and produces lipoproteins. Generally, the hepatic lipid content is low (wet liver contains less than 5% fat of its weight) and fatty liver syndrome occurs when the liver lipid stores exceed this value.

The results of the wet weight, dry weight and fat ratio of the livers are given in Table 4. When Table 4 was examined, it was determined that using high energy feed had a significant effect ($P<0.05$) on the wet weight and dry weight of the liver and the lowest wet and dry liver weight was in the control group. However, it was observed that the liver wet weight was lower in the groups that added ursolic acid to the food compared to the YE + 0% UA group. It was determined that there was a significant difference ($P<0.01$) between the groups in terms of liver fat ratio on the basis of dry matter, and the group YE + 0% UA had the highest fat ratio.

Table 4. Wet weight (g), dry weight (g) and fat ratio (%) of liver

Groups	Wet weight of liver (g)	Dry weight og liver (g)	Fat ratio of liver % (DM)
Control	20.32[c]	6.05[b]	27.43[c]
HE+ 0 % UA	39.13[a]	14.12[a]	48.26[a]
HE+ 0.5 % UA	35.27[b]	11.60[a]	30.49[c]
HE+1 % UA	28.18[b]	11.78[a]	33.03[c]
HE+ 1.5 %UA	34.91[b]	13.93[a]	40.89[b]
SE	2.32	1.18	2.62
P	*	*	**

[a, b] The averages shown with different letters in the same column are different from each other. HE= high energy, UA= ursolic acid, Control= basal fed group, HE + 0% UA= high energy fed group, HE + 0.5% UA= high energy fed + 0.5% ursolic acid, HE + 1% UA= high energy fed + 1% ursolic acid, HE + 1.5% UA= high energy + 1.5% ursolic acid, SE= standard error, *P<0.05, **P<0.01.

However, it was observed that the liver age weight was lower in the groups in which ursolic acid was added to the high-energy diet than the group without ursolic acid (YE + 0% UA). It was determined that there was a significant difference ($P<0.01$) between the groups in terms of liver fat ratio on the basis of dry matter, and the group YE + 0% UA had the highest fat ratio.

Ivy and Nesheim, (1973) reported that liver fat ratio exceeds 40% of dry weight and can even reach up to 70% in fatty liver. In the present study, it was determined that the liver fat ratio was 56.8% higher in the group fed with high energy feed (YE + 0% UA) compared to the control group.

Akkılıç and Tanyolaç, (1975) reported that both liver weight and liver fat ratio increased when feed containing high levels of energy was given to the laying hens raised

in the cage system. Similar to this study, many studies conducted in previous years reported that the liver fat ratio increased with the increase in the energy value of the feed (Splitgerber et al., 1969; Jensen et al., 1970).

Rozenboim et al. (2016) reported that the liver fat ratio was not affected by the diet in young animals in laying hens fed a high-fat diet, but the liver fat ratio in older animals was lower in than in the control group. Jia et al. (2015) investigated the effects of adding 50 and 200 mg / kg ursolic acid to a high-energy diet on the liver in mice, and found that the liver fat ratio decreased significantly in the group that added 200 mg ursolic acid compared to the high-energy feed group without ursolic acid.

Previous studies have reported that ursolic acid has an anti-obesity effect by decreasing lipid accumulation in adipose tissues. According to these studies, ursolic acid acts as a phosphodiesterase inhibitor that increases lipolysis in adipocytes (Jia et al., 2011; Kim et al., 2009; Rao et al., 2011). Jayaprakasam et al. (2006) reported that the addition of ursolic acid to a high-fat diet reduced the amount of liver fat in mice.

As summarized above, some studies have suggested that liver fat is primarily due to increased lipogenesis rather than dietary lipids, while others have suggested that the condition may be due to excess energy. In this study, it was concluded that the fat in the liver developed due to the high energy in the diet. Hovewer, it was determined that the addition of ursolic acid to the diet significantly reduced the liver fat rate. This situation can be explained by the hypolipidemic effect of ursolic acid.

Differences among groups were found to be significant in terms of MDA, GSH, SOD, CAT, GPx and NEFA (Table 5). The highest MDA value was found in HE + 1% UA group. It was determined that the addition of ursolic acid to the ration significantly increased the amount of GSH ($P<0.01$). Superoxide dismutase (SOD) and catalase (CAT) values were significantly lower in the HE + 1% UA and HE + 1.5% UA groups. The lowest GPx value was detected in the YE + 1% UA group. Non esterated fatty acids (NEFA) concentrations increased significantly ($P<0.01$) in the high energy feed groups and the highest value was found in the YE + 1% UA group.

Table 5. Non-esterified fatty acids (NEFA) and some enzyme activity of liver of laying hen

Groups	MDA (nmol/L)	GSH (nmol/L)	SOD (U/L)	CAT (KU/L)	GPx (U/L)	NEFA (ng/L)
Control	7.79^{d}	2.33^{a}	57.43^{a}	146.65^{a}	1.46^{a}	0.219^{c}
HE+ 0 % UA	7.56^{d}	2.47^{a}	59.05^{a}	151.41^{a}	1.48^{a}	0.299^{b}
HE+ 0.5 % UA	10.15^{c}	1.92^{b}	57.20^{a}	139.21^{a}	1.40^{b}	0.262^{b}
HE+1 % UA	18.37^{a}	1.62^{c}	52.79^{b}	113.20^{b}	1.22^{d}	0.465^{a}
HE+ 1.5 %UA	15.93^{b}	1.77^{bc}	53.07^{b}	120.24^{b}	1.38^{c}	0.306^{b}
SE	1.18	0.09	0.82	4.23	0.02	0.02
P	**	**	*	**	**	**

$^{a, b}$ The averages shown with different letters in the same column are different from each other. HE= high energy, UA= ursolic acid, Control= basal fed group, HE + 0% UA: High energy fed group, HE + 0.5% UA= high energy fed + 0.5% ursolic acid, HE + 1% UA= high energy fed + 1% ursolic acid, HE + 1.5% UA= high energy + 1.5% ursolic acid, SE= standard error, MDA= malondialdehyde, GSH= glutathione, SOD= superoxide dismutase, CAT= catalase, GPx= glutathione peroxidase, NEFA= non-esterified fatty acids, **P<0.01.

Similar to this study, Yang et al. (2017) observed that serum MDA and NEFA levels increased in laying hens fed with high energy and protein feed. Sundaresan et al. (2014), in their study on mice that induced fatty liver syndrome with a high-energy diet, reported that free fatty acid levels increased significantly in mice fed high-energy ration compared to the control group, and the addition of ursolic acid to the diet significantly reduced these values.

Li et al. (2014) found that ursolic acid supplementation significantly lowered serum NEFA levels and increased SOD, MDA, CAT and GSH-PX values in mice with high-fat diet obesity. Researchers have suggested that the addition of ursolic acid increases the levels of b-hydroxybutyrate in the blood and, based on this result, ursolic acid may increase the oxidation of free fatty acids.

Average values of some plasma parameters are given in Table 6. As can be seen from Table 6, AST and HDL values were not affected by the treatment. Plasma VLDL, triglyceride and LDL values were significantly higher ($P<0.01$) in the HE + 0% UA and HE + 1.5% UA groups. The highest ALT and total cholesterol values were found in the HE + 1.5% UA group. Plasma glucose ratio decreased significantly ($P<0.05$) in the HE + 1.5% UA group.

In the present study, it was determined that the VLDL, TG and LDL values increased significantly in the high energy feed group, but the addition of 0.5% and 1% ursolic acid in the high energy feed decreased these rates, while the addition of 1.5% ursolic acid did not affect them.

Jia et al. (2015) reported that 200 mg of ursolic acid supplementation decreased plasma triglyceride and VLDL concentrations, increased HDL concentration, and did not affect the total cholesterol ratio in mice fed a high-fat diet. The high estrogen concentration in laying hens also promotes liver-transported TG synthesis in the form of VLDL (Zhu et al., 2013).

Table 6. Some blood plasma biochemistry parameters of laying hen

Groups	VLDL mg/dl	ALT U/L	AST U/L	Glucose mg/dl	Total cholesterol mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl
Control	71.66[b]	3.00[b]	244.66	260.66[a]	124.00[b]	142.50[b]	46.00	89.00[b]
HE+ 0 % UA	329.50[a]	7.00[b]	265.66	248.00[a]	135.00[b]	1567.00[a]	42.66	206.50[a]
HE+ 0.5 % UA	52.00[b]	2.33[b]	186.50	264.00[a]	113.00[b]	258.50[b]	44.33	93.00[b]
HE+1 % UA	67.33[b]	2.00[b]	252.00	278.66[a]	115.00[b]	337.66[b]	53.66	105.56[b]
HE+ 1.5 %UA	337.66[a]	17.50[a]	236.00	235.66[b]	278.50[a]	1687.66[a]	45.66	183.00[a]
SE	39.07	1.92	15.53	5.73	19.25	199.98	3.38	24.85
P	**	*	ns	*	**	**	ns	**

[a, b] The averages shown with different letters in the same column are different from each other. HE= high energy, UA= ursolic acid, Control= basal fed group, HE + 0% UA= high energy fed group, HE + 0.5% UA= high energy fed + 0.5% ursolic acid, HE + 1% UA= high energy fed + 1% ursolic acid, HE + 1.5% UA= high energy + 1.5% ursolic acid, VLDL= very low density lipoprotein, ALT= alanine aminotransferase, AST= aspartate aminotransferase, TG= triglyceride, HDL= high density lipoprotein, LD= low density lipoprotein, SE= standard error, *P<0.05, **P<0.01, NS= not significant

High-fat diet increases the amount of free fatty acids in plasma and leads to triglyceride accumulation in the liver (Yki-Järvinen, 2005). The liver plays a central role in maintaining systemic lipid homeostasis. Lipid balance is maintained by the regulation of lipogenesis and lipid oxidation, which are regulated by the cooperative effect of various enzymes and transcription factors found in the liver (Yki-Järvinen, 2005).

Peroxisome proliferator activated receptor (PPAR) is a major regulator of genes involved in fatty acid transport and utilization in the liver and mitochondrial and peroxisomal fatty acid-oxidation (Aoyama et al., 1998; Reddy, 2001). Activation of PPAR with synthetic or natural compounds increases cellular fatty acid uptake and subsequent oxidation rate (Motojima et al., 1998). Jia et al. (2011) reported that ursolic acid activates the nuclear receptor of the PPAR, and there is a decrease in lipid accumulations in hepatocytes through gelation of PPAR-responsive genes in hepatic lipid metabolism. Therefore, administration of PPAR agonists simultaneously improves lipid and glucose metabolism, decreases both plasma and hepatic triglyceride accumulation, increases glucose tolerance, and increases HDL cholesterol concentrations (Harano et al., 2006; Nakajima et al., 2009).

Yang et al. (2017) observed that the serum triglyceride, total cholesterol and LDL-cholesterol ratio increased significantly, while the HDL-cholesterol ratio decreased slightly in laying hens fed with high-energy feed. Jayaprakasam et al. (2006) reported that the addition of ursolic acid to a high-fat diet reduced serum triglyceride levels in mice.

Sundaresan et al. (2014) reported that ALT and AST values were significantly higher in mice fed with a high-energy diet with fatty liver syndrome compared to the control group, and ursolic acid added to the diet significantly reduced these values.

In the present study, it was determined that the addition of 1.5% ursolic acid to high-energy food significantly lowered the plasma glucose ratio. Jayaprakasam et al. (2006) reported that a high-fat diet increased glucose levels in mice, while the addition of ursolic acid significantly reduced this value. The same researchers stated that the passage of glucose into the blood is delayed by ursolic acid.

Due to the lack of a study on ursolic acid on laying hens and also the lack of up-to-date studies on fatty liver in laying hens in recent years, this study has not been discussed sufficiently.

4. Conclusion

In conclusion, it was determined that fatty liver syndrome occurs in laying hens fed with high energy feed. While 1.5% ursolic acid addition to high-energy diet positively affected performance values, it was determined that 0.5 and 1% ursolic acid supplementation significantly reduced liver fat ratio and plasma TG, LDL and VLDL values. As a result, it was concluded that the addition of ursolic acid to the feeds was successful in overcoming the diseases that may occur due to lipid metabolism in laying hens, as well as improving the performance values. In addition, this study will be a source for future studies on fatty liver disease.

Author Contributions

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

	F.P	Ş.C.B.A
C	50	50
D	50	50
S	50	50
DCP	50	50
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

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

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

The research protocol was approved and applied in accordance with the Animal Ethics Committee Guidelines of Atatürk University (protocol code: 2018/61 and date: 15 April 2018).

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GENERAL SITUATION, PROBLEMS AND SUGGESTED SOLUTIONS FOR GOOSE BREEDING IN KÜTAHYA PROVINCE

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Abstract: This study was carried out in order to reveal the current situation of goose breeding in extensive and semi-intensive conditions in Kütahya province and to determine the important problems encountered in breeding. The material of the study consisted of the survey data obtained from 125 goose producers in the villages of Merkez, Altıntaş, Aslanapa, Çavdarhisar and Tavşanlı districts of Kütahya province where goose breeding is intense. According to the research findings, it was determined that the average period of goose breeding of farmers in the province of Kütahya was 1-10 years. It was determined that the number of breeding male geese per farm was 1-5 and the number of breeding female geese was 3-20 (M/F:1/3-5/20). It was determined that 56.8% of the goose shelters were made of briquette or brick material. 88.8% of the breeders stated that gooselings were released to the pasture when they were 1-2 weeks old. Although the rate of not taking any precautions against diseases was 84.0%, the rate of those who stated that they did not experience any loss was 75.2%. As a result, it was determined that the structure and problems of goose breeding in Kütahya were similar to the country in general and the production was mostly done to meet the meat needs of the family. It can be stated that the main problems of the producers are feed costs, inadequacies in care and feeding, breedings with low-yielding domestic breeds, difficulties in the supply of breeding animals, and problems in marketing.

Keywords: Kütahya province, Goose breeding, Problems, Solution proposals

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1. Introduction

Goose breeding is generally located in areas with cold climatic conditions. Unlike other poultry, feed materials with high cellulose content are maintained as an important alternative livestock activity, as they have the ability to digest grasses. Goose breeding is applied in East and Southeast Asian countries and Eastern European countries in the world (Boz et al., 2014). The share of goose breeding in total poultry production is very low all over the world. The low egg production of the geese and the long slaughtering period have a significant effect on this situation. In addition, hot and dry climatic conditions make cultivation impossible (Şengül and Yeter, 2020). Despite the mentioned negativity, goose breeding has been increasing its importance among alternative livestock activities that attract attention all over the world in recent years. Goose breeding is mostly done for meat in line with the demands of consumers, liver, and feathers are in demand in European countries. In Türkiye, goose breeding is common in rural areas at the level of small family businesses and consists of 10-15 geese herds. Generally, goose breeding is carried out in order to meet the animal protein needs of the family, and the leftover production is sold in local markets and contributes to the family economy. In Türkiye, especially in Kars and Ardahan in Northeast Anatolia; goose breeding is more common in Muş, Van, Ağrı in Eastern Anatolia, in Yozgat and surrounding provinces in Central Anatolia, in Samsun and Çorum in the Black Sea, and in Kütahya, Afyonkarahisar, and Uşak in the Inner Aegean compared to other provinces. The mentioned provinces are very suitable for goose breeding in terms of climatic conditions and draw attention as an important livestock activity in rural areas. In the Aegean Region, as in other provinces, the traditional extensive production system has been adopted. Geese are grazed in the pasture for up to 1-1.5 months before slaughter, and they are fed with grains such as corn, wheat, and barley as well as bread and food scraps as supplementary feeding. It has been observed that the use of factory feed is at very low levels (Akın and Çelen, 2020). When the goose is mentioned, Kars and its region usually come to mind in Türkiye. In the Kars region, geese are either cooked in the tandoor or dried and made ready for consumption in case of need in the future. In other regions, the goose has a special place and importance. It has been observed that the socio-cultural structure of the region is effective in the consumption of goose meat and consumption takes place according to various cooking techniques. The goose tiridi, known as goose hanging, has an important place in Samsun and received a geographical indication in 2011 as "Samsun Kaz Tiridi" (Canbolat and Çakıroğlu, 2015). It

has been stated that goose meat is used in Kütahya style roast beef, priest stew, casserole, dry meatballs, and "kaz tiridi" in the Aegean Region, and goose meat is used in local dishes called "paçik" and veiled in Afyonkarahisar (Ceylan and Öz, 2018; Anonymous, 2019a; Anonymous, 2019b; Kızıldemir, 2019; Akın and Çelen, 2020). Boz (2017) stated that goose meat is used extensively in the preparation of "ara-aşı (arabaşı)" in the Yozgat region, and it is preferred in making "bulgur pilaf, gılnış," roast and goose meatballs. As in all livestock activities in Türkiye, feed costs are the biggest problem in sustainable livestock breeding. In addition, as a result of the loss of qualifications of many agricultural lands, livestock activities become increasingly difficult and producers have to withdraw from the sector. According to TUIK 2022 data, there has been a decrease in all livestock activities and product amounts in Türkiye compared to the previous year. According to 2021, it was indicated that laying hen production decreased from 120 million to 110 million, broiler production decreased from 270 million to 251 million, turkey production decreased from 4.7 million to 3.6 million, goose production decreased from 1.4 million to 1.3 million, and duck presence decreased from 500 thousand to 400 thousand (TUIK, 2023a; TUIK, 2023b). In the last 10 years Kütahya and its districts, the Aegean Region, and the total geese presence in Türkiye are shown in Tables 1, 2, and 3 (TUIK, 2023a). The geese presence in the region continued to increase periodically every year, from 68,000 in the first 5 years. While the goose population of the region increased by 40% to 96,000 in 2017, it increased from 102,000 to 104,000 by 2020 in the second 5-year period, and then decreased to 85,000 at the end of 2022, with a decrease of 18% compared to 2021. Afyonkarahisar, Kütahya, and Uşak have an important place in goose breeding in the Aegean region. In the first 5-year period covering the years 2013-2017, Afyonkarahisar ranked first in the region with around 30,000 geese, and the share of geese in the region (SGR) was around 40%. As of 2017, Kütahya ranked first with a goose production exceeding 44,000 (SGR 45%). On the other hand, Uşak doubled the number of geese (SGR 4%) from 3.000 as of 2017 and exceeded 6.000. In the second 5-year period covering the years 2018-2022, Kütahya decreased from 42,000 geese to 33,000 as of 2022, while Afyonkarahisar decreased from 32,000 to 21,000. In this period, Uşak increased from 9,000 units to 23,000 units as of 2020 (SGR 22%), then decreased to 18,000 units (SGR 18%) and then to 12,000 units by 2022 (SGR 14%). In Kütahya, goose breeding is concentrated in Altıntaş, Aslanapa, Merkez, Çavdarhisar, and Tavşanlı districts. In the last 10 years, covering the years 2013-2022, 5 districts met 93-95% of the total goose production. This study has tried to present information about the existence and share of geese in the Aegean Province of Türkiye, the demographic characteristics of breeders, goose breeding activities, problems and solutions to the problems.

Table 1. Türkiye geese production amounts for the last 10 years (TUIK, 2023a)

Region	Years									
	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
İstanbul TR1	3013	3025	2571	2428	2072	2177	3455	8294	3390	6552
West Marmara	32596	36130	37164	37997	39883	41478	41621	41207	42791	40558
Aegean TR3	68666	72463	73410	76791	96340	102739	104784	104239	101654	84886
East Marmara	30960	29966	30791	31227	36289	41837	48652	59079	63973	53367
West Anatolia TR5	22189	25210	25934	28292	33336	35023	37879	44737	45050	38992
Mediterranean TR6	17102	15776	17858	18937	29328	37041	45800	47211	48903	40510
Middle Anatolia TR7	52026	50332	52845	59704	67849	74354	82343	98065	130936	121132
West Black Sea TR8	51584	59210	66749	71027	85407	143037	116671	123381	115582	102275
East Black Sea TR9	891	1325	962	1281	1636	2385	6869	11189	10556	10253
Northeast Anatolia TRA	297818	432142	366648	426678	388849	403425	471099	474022	668351	690692
Middle East Anatolia TRB	52026	50332	52845	59704	67849	74354	82343	98065	130936	121132
Southeast Anatolia TRC	67819	63506	57431	58467	74119	74664	73518	162800	105566	103843
Total	75528	91199	85069	93335	97838	108019	115704	137396	147756	138550

Table 2. Aegean Region geese production amounts for the last 10 years (TUIK, 2023a)

Aegean Region	Years									
	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Afyonkarahisa	3094	3213	3213	3308	29568	32534	34835	27743	30460	21407
Aydın	2374	2674	2717	2836	3214	4330	3125	3135	3037	3032
Denizli	1837	2822	2531	3455	4683	4723	5421	5201	5676	5537
İzmir	1979	2641	2953	3522	4030	4554	4515	4862	4412	4041
Kütahya	2394	2467	2473	2508	44427	42211	42321	33742	34394	33539
Manisa	1327	1455	1594	1421	1732	2011	2109	3261	2703	2680
Muğla	2835	3055	3217	3169	2656	3526	3518	3099	2307	2338
Uşak	3430	3011	3720	4215	6020	8850	8940	23196	18665	12312
Total	6866	7246	7341	7679	96340	102739	104784	104239	101654	84886

Table 3. Goose production amounts of Kütahya province and its districts for the last 10 years (TUIK, 2023a)*

Kütahya Districts	Years									
	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Merkez	1505	1500	1500	1700	2000	2000	2050	1840	1760	1843
Altıntaş	5240	5460	6000	6350	25010	25062	25650	24550	24555	25000
Aslanapa	15000	15000	14750	14500	14830	12357	11792	4100	4500	3500
Çavdarhisar	350	340	380	370	380	670	720	920	985	990
Tavşanlı	390	575	610	591	750	884	850	809	848	832
Simav	540	849	475	480	475	226	250	330	280	205
Dumlupınar	400	400	430	475	455	450	405	122	115	120
Gediz	210	215	220	230	220	200	199	200	620	362
Hisarcık	165	147	145	150	60	60	60	57	60	55
Domaniç	80	80	75	80	75	60	80	200	50	60
Emet	25	80	116	126	115	183	190	453	454	412
Pazarlar	35	15	13	10	15	20	15	45	46	40
Şaphane	0*	14	24	35	42	39	60	116	121	120
Total	23940	24675	24738	25087	44427	42211	42321	33742	34394	33539

*The number of geese in the relevant year for the Şaphane District was stated as "0" by TUIK.

2. Materials and Methods

The study was created from the survey data made with the goose producers in the villages where goose breeding is carried out in Altıntaş, Aslanapa, Merkez, Çavdarhisar and Tavşanlı districts of Kütahya, according to 2022 TUIK data. The questionnaire forms used in the study were prepared by making use of the previously arranged questionnaires on zootechnics and agricultural management. While determining the sample size of the study, a grouped one-stage random probability sampling method based on population ratios was used (Alkan and Eren, 2019; Şengül and Yeter, 2020). In determining the sample size, the following formula (equation 1), which was used in limited societies as reported by Karasar (1994), was used.

$$n=(z^2*N*p*q)/(N*d^2+z^2*p*q) \quad (1)$$

here; n: Sample volume, z: "Z" table value corresponding to 95% significance level, N: Number of main masses, p: The probability of occurrence of the investigated event in the main mass is taken as 50%, q: The probability that the investigated event will not occur (1-p), d: Accepted margin of error (In this study, margin of error was taken as 5%).

According to this, it was determined that a survey should be conducted with 125 enterprises with equality and one-on-one interviews were made by going to the enterprises. 70 surveys were conducted in Altıntaş, 25 in Aslanapa, 20 in Merkez district, and 5 each in Çavdarhisar and Tavşanlı. In the study, the average number, age, of the geese, feeding of the geese, egg production, egg yield, goose breeding, infrastructure opportunities, shelters, slaughter time and slaughter age, marketing methods of goose products, besides, the advantages and disadvantages of goose breeding were investigated. The data of the study were evaluated in the SPSS 16.0 package program and expressed as descriptive statistics and percentage values.

3. Results and Discussion

The socio-demographic characteristics of the breeders who participated in the survey in the study area are shown in Table 4, the reasons for breeding goose, the breeding times, the presence of geese and their desire to increase are shown in Table 5.

Table 4. The socio-demographic characteristics of the goose breeders

Age	Family (n)	R.F. (%)	Education	Family (n)	R.F. (%)	Number of individuals	Family (n)	R.F. (%)
18-39	26	20.8	Illiterate	6	48	1-3	41	32.8
40-59	72	57.6	Primary	62	49.6	4-6	68	54.4
60-80	23	18.4	Secondary	29	23.2	≥7	16	12.8
>80	4	3.2	High	17	13.6	-		
-	-	-	University	11	8.8	-		
Total	125			125				

n= number of families surveyed, R.F.= relative frequency

Table 5. Distribution of goose producers according to their breeders activities

Breeding Reason	Family (n)	Share in investigated family (%)
Addition to Livelihood	33	26.4
Meat Need-Consumption Habit	77	61.6
Hobby	9	7.2
No other income	6	4.8
Breeding Times (year)		
0-5	22	17.6
6-10	58	46.4
11-20	33	26.4
21-30	8	6.4
>30	4	3.2
Number of geese (number)		
1-10	19	15.2
11-20	73	58.4
21-50	22	17.6
51-100	8	6.4
>100	3	2.4
Desire to increase the presence of goose (number)		
No	14	11.2
Yes (11-20)	35	28
Yes (21-50)	57	45.6
Yes (51-100)	12	9.6
Yes (>100)	7	5.6
Person Responsible for Care and Feeding		
Myself	82	65.6
Wife/husband	14	11.2
Mother/Father	11	8.8
Kids and the whole family	16	12.8
Goose herder	2	1.6
Poultry Presence Other than Goose		
None	4	3.2
Chicken	92	73.6
Turkey	12	9.6
Duck	9	7.2
Quail, partridge, and other animals	8	6.4

While the age of 20.8% of the goose breeders in Kütahya was 18-39, 57.6% of them were 40-59 years old. This situation is promising for the future when goose breeding is carried out by the young population in Kütahya. According to Boz et al. (2014), 58% of breeders are 40-59 years old and 23% are 20-39 years old. Demir et al. (2013) mean age is 41.9. Alkan and Eren (2019) state that 49.67% of them are 40-59 years old, 30.46% are 60-80 years old. Şengül and Yeter (2020) state that 42.8% of breeders are younger than 40 years old, 26.7% are 50 years old and above. While the rate of households with 1-6 people was determined as 87.2%, it was seen that the education level of 49.6% was primary school, that of 23.2% was secondary school and that of 22.4% was high school and university. In previous studies, the number of households and education level were as follows: In Ağrı 56.29% 4-6 people, 48.34% primary school, in Yozgat 86% 1-6 people, 75.5% primary school-secondary school, in Muş % of breeders It was stated that 89.5% of them were at primary-secondary school, and 75% of them in Ardahan were at primary school level (Boz et al., 2014; Demir et al., 2013; Alkan and Eren, 2019; Şengül and Yeter, 2020).

While 61.6% of the breeders stated that they carried out goose breeding to meet the meat needs of the family, 31.2% stated that they contributed to their livelihood and did not have any other income. While 64% of goose breeders in the province stated that they had been playing an active role in goose production for 1-10 years, it can be stated that goose breeding is a relatively new alternative livestock activity in Kütahya compared to other provinces. In a study conducted by Şengül and Yeter (2020), the average rearing period in Muş was 17 years and 38.2% of the respondents stated that this period was 20 years or more. %64 of goose breeders stated that they were engaged in goose breeding in order to satisfy the meat need of the family and %11 of them indicated that they did goose breeding to generate income. While this period was reported as 18.6 years in Ardahan, 79.3% of them stated that goose breeding was an important source of income, 48% of the breeders in Yozgat had been breeding goose for less than 10 years and 85.5%. It was reported that they did breeding as a consumption habit, 63.58% of them had been breeding geese for 1-10 years and 64.9% of them were producing as a consumption habit (Demir et al., 2013; Boz et al., 2014; Alkan and Eren, 2020). It was observed that 65.6% of the breeders were themselves interested in the care and management of geese in Kütahya, they raised an average of 11-20 geese in a year, 73.6% of them raised hens other than geese and 88.8% of breeders wanted to increase the number of geese.

Alkan and Eren (2019) stated that 71.52% of the breeders raised non-goose chickens and 85.43% of them raised geese in addition to other livestock activities. While 73.51% of them wanted to increase the presence of geese and generally in the care and feeding of geese, they stated that women and children took an active role.

While 64% of the breeders kept an average of 1-5/3-20 male/female (M/F) breeder geese in their hands, 22.4% of them did not have breeding geese. 50.4% of them got their goslings by breeding/hatching. It was determined that nearly 90.4% of the breeders raised domestic breed geese and 62% of them preferred the variegated and white varieties. While the rate of breeders who did not make supplemental feeding was 11.2%, 36.8% of those who did supplemental feed used corn, 24% wheat, 18.4% barley, and others, respectively, using bread and food scraps. In general, 88.8% of the gosling in the province were taken to pasture within the first two weeks. While 15.2% of those did not use any equipment, 84.8% stated that they used at least one equipment. Alkan and Eren (2020) in their study in Ağrı, found that breeders kept 4-6 breeding goose, obtained goslings and breeders from hatching, almost all of them preferred the domestic goose breed and the variegated variety was more popular. It was stated that two-three weeks-old goslings were then released in pasture. In a study conducted in Ardahan, it was stated that geese were generally fed on pasture, 88.8% of them used barley for supplemental feeding, while wheat, barley and corn were preferred as supplementary feeding in Yozgat, and bread and leftovers were also evaluated (Demir et al., 2013; Boz et al., 2014). While it was seen that 1-15 eggs were taken from a goose on average in a year in Kütahya, the rate of those who stated that they received 26+ eggs was 8%. The breeders who stated that they received a high number of eggs were observed to have used high yielding breeds like Chinese, Linda and Mast. In Kütahya, the number of brood/chick, breeding geese, breeding supply and selection, keeping time in breeding and breeding egg price are shown in Table 6.

While an average of 21-50 eggs were incubated in the province, the number of hatched chicks was found to be 11-30 and the hatchability for Kütahya province was found to be 55-60%. While the ratio of those who provided breeding geese from their own resources was 58.8%, the ratio of those who provided them from the neighbors and local animal market was 38.2%. 67% of them stated that they considered body size and egg production in the selection of breeding geese. While the rate of those who kept breeding geese for 1-6 years was 89.7%, it was determined that the rate of those who kept them for 7-8+ years was 10.3%. While 64% of the producers stated that the prices of eggs were between 20-40 TL, the ratio of those who indicated they did not buy or sell eggs was 22.4%. Boz et al. (2014) stated that the average egg production was 11, the number of chicks obtained from hatching was 8, the hatchability was 73%, the average retention period of the breeders was 2 years, and the breeder male/female ratio was 1/3. In a study conducted in Kırşehir, the average number of eggs per farm was 53.13, the number of chicks was 45.11, the brood male/female ratio was 1.14/4.83, and the breeding period was 2-12 years.

Table 6. Number of hatching eggs and chicks, number of breeding geese, breeding geese supply and selection, period of keeping in breeding and breeding egg price

Hatching egg (E) / Chick (C)	Family (n)	Share in investigated family (%)
1-20 E / 0-10 C	25	20.0
21-30 E / 11-20 C	24	19.2
31-50 E / 21-30 C	52	41.6
51-100 E / 31-70 C	16	12.8
>100 E / >70 C	8	6.4
Number of breeding geese (M/F)		
Not has breeder geese	28	22.4
1-3 M / 3-10 F	49	39.2
4-5 M / 11-20 F	31	24.8
6-10 M / 21-50 F	14	11.2
>10 M / >50 F	3	2.4
Breeding geese supply		
From own resources	57	58.8
Neighbors	32	33.0
Animal markets	5	5.2
Other provinces	3	3.1
Breeding selection		
Randomly	17	17.5
Size/Body	23	23.7
Egg yield	42	43.3
Feather color	6	6.2
Race	9	9.3
Period of keeping in breeding (year)		
1-2	18	18.6
3-4	38	39.2
5-6	31	32
≥7-8	10	10.3
Breeding egg price (TL)		
No buying or selling	28	22.4
20-30	35	28.0
31-40	45	36.0
>40	17	13.6

It was announced that the rate of those who prioritized egg production was 35% and the rate of those who prioritized egg production was 30% (Taşkın et al., 2017). Slaughter time, slaughter age, live and carcass weight, plucking method and feather usage situation, place of sale, shape and price of goose are shown in Table 7. 40.8% of the breeders stated that they slaughtered geese in December-January, at the age of 10-12 months (48%), with a body weight of 4-7 kg (58.4%) and that they obtained an average of 3-5 kg of carcasses (52%, 8). While 62.4% preferred the wet method for feather plucking, the rate of those who stated that they discarded the hair without making any use of it was 70.4%. In the study conducted in Yozgat, it was stated that geese were slaughtered in October, November and December, while some breeders carried out slaughter in January-February. In this study, it was stated that the slaughter age was 8 months, the carcass weight was 3.7 kg on average, the feathers were removed by the wet method (96%), the feathers were used in making quilt pillows by breeders only of 2.5%. 77% of the breeders consumed the geese fresh without waiting (Boz et al., 2014).

In order for the goose feathers, which are extremely valuable and have high economic value, to be evaluated, it is urgently necessary to bring feathers to the economy by establishing various organizations affiliated to the Municipality, Ministry of Agriculture and Forestry and feather collecting units. 85.6% of the goose breeders sell the geese that they produce as live or carcasses. The rate of those who sells them to neighbors and to local markets in the village is 86.4%, and the rate of those who state that they earn 300-450TL from an average live goose is 72.4%.

Table 7. Slaughter time, slaughter age, live and carcass weight, feather plucking method and feather usage situation, place of sale, type and price of goose

Slaughter time		Family (n)		Share in investigated family (%)	
October-November		34		27.2	
December- January		51		40.8	
February-March		26		20.8	
Other months		14		11.2	
Slaughter age (month)					
6-9		32		25.6	
10-12		28		22.4	
13-15		35		28.0	
16-18		25		20.0	
≥19		5		4.0	
Live weight / carcass weight (kg)					
Do not know	Do not know	32	34	25.6	27.2
2-3	2-2,5	5	3	4.0	2.4
4-5	3-4	25	25	20.0	20.0
6-7	4,5-5	48	41	38.4	32.8
≥7	≥5	15	22	12.0	17.6
Feather plucking method					
Dry plucking		18		14.4	
Wet plucking		78		62.4	
Dry or wet plucking		29		23.2	
Feather usage situation					
Throwing		88		70.4	
Pillow/quilt making		24		19.2	
Selling to trader		13		10.4	
Place of sale					
No sale		9		7.2	
Neighbor / friends in the village		63		50.4	
Local animal markets		45		36.0	
Web / social media		8		6.4	
Sale type					
No sale		9		7.2	
Live		80		64.0	
Carcass		27		21.6	
Customer Request (Live/carcass/piece)		9		7.2	
Sale price (TL)					
200-300		21		18.1	
301-400		58		50.0	
401-450		26		22.4	
451-500		7		6.0	
>500		4		3.4	

While 44.8% of the producers stated that they would continue to see goose breeding as a profitable business, 24% stated that they would continue out of habit even though they could not see it as a profitable business. 36% of the respondents stated that they prefered to consume goose meat by frying, 18.4% boiling, 9.6% using it in local dishes. Şengül and Yeter (2020) stated that in Muş, 55.2% of live geese were generally sold in the city center and 44.8% in villages, while Taşkın et al., (2017) stated that the highest sales by breeders were in local markets (% 40), it was stated that sales were then made to the merchant (25%) and the immediate environment (15%).

In response to the question "Do the geese have a special shelter, is disinfection applied?", it was found that 66.4%

of the breeders answered Yes, 84% of them struggled with their own means in adverse conditions such as illness, and only 16% received support from veterinarians and Agricultural Organizations. In general, it was observed that the losses occurred in the first week after hatching (13.6%). Şengül and Yeter (2020) stated that goose shelters in Muş were 50 m2 in size on average, and that the shelters were made of materials like briquettes, wood, etc. While 67% of the breeders reported that they did not take any precautions against diseases, they stated that very few of the geese died. Boz et al. (2014) reported that breeders kept the geese in the same shelter with other animals, 61.5% did not apply any disinfection, and 98.5% stated that animals never got sick. 46.4% of breeders reported the advantages of goose breeding because geese were compatible with pasture and more resistant to diseases than other poultry, while 31.2% reported the advantages of goose breeding as it met the meat needs of the family and created additional income. In response to the question "What do you think are the biggest problems and difficulties you face in breeding?", 46.4% drew attention to high feed costs, 19.2% to low egg production, 8.8% to the difficulties experienced in the supply of breeding animals. Similarly, in response to the questions "What do you think is necessary for the development of goose breeding in our province, region and country?, what are the deficiencies? , what are your demands against the problems you experience? ", 44% of the breeders drew attention to the problems of advertisement, promotion and marketing of the products obtained from geese. While the rate of those who requested to breed with high-yielding breeds was 27.2%, 21.6% of the breeders stated that they needed a slaughterhouse, feather plucking machines and cold storage. Taşkın et al. (2017) reported that 50% of breeders stated that geese were easy to sell and resistant to diseases as an advantage. 40% of breeders stated that they cared about goose breeding in terms of meeting meat consumption and that the geese were compatible with the pasture as an advantage. 50% of the breeders considered high feed prices and low egg production of geese as problems among the difficulties and difficulties they faced. Researchers reported that 20% of the producers declared that they gave harm to the farmland of the geese. As a result of this study, it was seen that goose breeders expect support especially in terms of high feed costs and breeding animal supply.

4. Conclusion

Kütahya province is the 1st province in terms of the presence of geese in the Aegean region, but the number of geese, which was 42,000 in 2019, decreased to 33,000 at the end of 2022. This study has also shown that high feed costs are the most important problem for the sustainability of animal husbandry in Türkiye. In addition, "Goose Products, Collection and Sales Units, etc" should be established within the Municipality and Agricultural Organizations for the supply of breeding animals and the sale of goose products. These units can be provided to support the breeders in the marketing of the products. Considering the goose production potential of Kütahya, the scope of the goose incentives stated by the Ministry of Agriculture and Forestry in "Supporting Economic Investments Based on Agriculture within the Scope of Rural Development Supports 2022-2023 Application Period, Communiqué No: 2022/24" is quite limited. In the relevant communiqué, it is stated that "applications for new facilities in 81 provinces, completion of partially made investments, capacity increase and technology renewal and/or modernization" will be taken into consideration only for turkey and goose breeding. In the continuation of the Communiqué, there is the statement "No grant support is given for breeding eggs and/or egg production in goose breeding" (Anonymous, 2023). However, our breeders reported that they had the most problems in the supply of breeding eggs and breeding animals. Expanding the scope of the goose incentive will provide an opportunity for preventing the losses in our goose stock and for increasing our goose presence again in the future.

Author Contributions

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

	Y.A.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

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

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

This study was conducted within the scope of the decision of Uşak University Scientific Research and Publication Ethics Committee (protocol code: 2023/03-11 and date: 27 April 2023).

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THE INVESTIGATION OF THE USE RED BEET POWDER (*BETA VULGARIS*) AS A NATURAL COLORANT ON COLOR AND ANTIOXIDANT PROPERTIES OF HEAT TREATED SUCUK

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Abstract: The purpose of this study was to assess red beet as a natural colorant in heat treated sucuks and to investigate the effect of red beet on some quality characteristics of heat treated sucuks during 30 d of cold storage. Red beet was prepared as a powder and a substitute with sodium nitrite (nitrite 50 and 100 ppm) at 1% levels in heat treated sucuks. Both treatment and storage periods had significant (P<0.01) effects on moisture, pH, aw, residual nitrite, free fatty acids (FFA), 2-hiobabituric acid reactive substance (TBARS), total mesophilic aerobic bacteria (TMAB), *Enterobacteriaceae*, yeasts-moulds and color (L*, a* and b* values) of heat treated sucuks. The red beet powder has an antioxidant effects on sucuk samples. Red beet powder caused a decrease in L* value compared with the control sample with nitrite added. As a result, red beet powder can be used as an alternative to synthetic colorants in some meat products or as a reduction of nitrite.

Keywords: Sucuk, Red beet powder, Color properties, Lipid hydrolysis, Nitrite

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1. Introduction

The most important dried fermented meat product in Türkiye is sausage (sucuk). In the production of sucuk, red meat, water buffalo meat, veal, sheep tails and some chemical (nitrites, nitrate, salt, sugar) are used together with many different seasoning (black and red pepper, garlic, cumin). Starter culture is used depending on demand. In traditional sucuk production, heating process is not applied at the end of ripening process (Gökalp et al., 1999; Gençcelep et al., 2007). Heat treatment is preferred to ensure quality and microbial safety of meat products. Changes have been made in the sucuk production process over the last four decades and although heat application is not part of traditional production, it has begun to be applied to products at the end of ripening time (Ercoşkun et al., 2010). Heat treatment in sucuk production eliminates pathogens, prolongs shelf life and reduces training period and price (Ercoşkun et al., 2010).

Consumer concerns about artificial colorants used in meat products and the many side effects of these colorants on health have led to searches for the use of natural colorants in meat products. Owing to the powerful phenolic compounds contained in red beetroots, it has found use in many industrial and domestic food products (Sucu and Yıldız Turp, 2018; Posthuma et al., 2018 and Schopfer et al., 2022). The most effective factor for buyers to buy meat products is color. The bright red color of the meat products is regarded as an indication of freshness by the consumers (Barbut, 2001; Martinez et al., 2006a). The most important additive used in the production of meat products is nitrite salts. Nitrite restricts the development of certain microorganisms, *Clostridium botulinum* and *Listeria monocytogenes*, and contributes to the improvement of the product color and flavor. However, there is a growing concern about the use of nitrite in meat products because of the formation of N-nitroso carcinogens from amines and amides (Sang-Keun et al., 2014).

Long storage has a disadvantage in terms of stability of meat color. Red beet root (*Beta vulgaris*) is a very abundant source of betalain pigment group betacyanins (red) and betaxanthins (yellow). Betalaines are colorants containing water soluble nitrogen which are responsible for the red, violet (betacyanins class) and yellow (betaxanthins class) colors found in many flowers, fruits, vegetables and plants (Socaciu, 2008; Lee and Jin 2012). Red beet root is a good source of natural coloring matter and antioxidant substances. In addition, betalaines are more resistant to pH than anthocyanins used in food to give red purple color. Kujala et al. (2002) also determined many phenolic compounds in red beet root. Red beet root powder can be used to enhance the color of a lot of meat products (cooked, smoked, semidried and

fermented sausages) (Martinez et al., 2006a; Lee and Jin 2012). In addition, red beet is used as coloring material instead of nitrite, but betalain does not have antimicrobial properties. Moreover, because of the presence of nitrite naturally in the red beet composition, it contributes to the production of more nitrite in the food as a source of nitrite when added to the products (Socaciu, 2008). Most researchers have focused on the purification of betalaines from red beet and the investigation of phenolic compounds. A small number of work have known about the stability of betalain in the ingredient of colored foods. There is a few research into the added of red beet powder in meat products (Martinez et al., 2006b; Kujala et al., 2002; Lee and Jin, 2012; Sang-Keun et al., 2014 and Hwang et al., 2017). The aim of this research was to identify red beet powder as a natural antioxidant and colorant in heat treated sucuk and to research the influence of red beet powder on microbiological quality and chemical properties during 30 days of refrigerator storage period.

2. Materials and Methods

2.1. Production of Heat-Treated Sucuk

The ground meat (the back of cattle) was separated into five batches, which were mixed with the subsequent formulations: (1) control (C) (no nitrite and red beet powder), (2) nitrite (N) (100 ppm), (3) red beet powder (P) (1%), (4) red beet powder and nitrite (NP1) (1% and 50ppm, respectively) and (5) red beet powder and nitrite (NP2) (1% and 100 ppm, respectively); all the batches contained 1500 g minced meat, 500 g fat (beef meat fat), 36 g NaCl, 50g garlic, 72 g red pepper, 36 g cummin, 2 g black pepper, 1 g dextrose, 7 g dipolyphosphate, 1.4 g ascorbic acid and 2.5 g glikono delta lakton. Each batter was stuffed into dried natural casing (Ø35 mm; Delarom Aroma ve Gıda Katkıları, İstanbul, Türkiye) using a stuffing machine (Seydelmann KG, Stuttgart, Germany). The sucuk samples were exposed to heat treatment (63°C in the core of the samples in 15 min.) and cooled directly to 5°C with chilly water shower. Sucuks were storage 4±1°C in refregerator for 30 days. Sucuks were produced by Köytaş meat product (Sungurlu, Çorum, and Türkiye). Sucuks were brought to the laboratory by storage 4±1°Cimmediately after production.

2.2. Preparation of Red Beet Powder

Roots of red beet (*Beta vulgaris*) were purchased in a local market in Samsun, Türkiye. The roots were washed, peeled, and chopped into small pieces. The minced roots were then frozen at -20 °C. Chopped roots was later put into a freeze dryer (Freezone 12 plus, Labconco, Kansas City, MO, USA). After about 24-30 hours freeze drying, the dried product was ground using a hammer mill (TEKPA, Laboratory mill, Ankara, Türkiye). The final moisture content of the red beet powder (RBP) was determined to be 10.90±0.5%.

2.3. Physical and Chemical Analysis

Proximate analysis of meat and sucuk were analyzed according to the AOAC (2009). The homogenized meat and sucuk compound were determined as follows: crude protein, using the Kjeldahl method (Barros et al., 2007); crude fat, using the Soxhlet method (SOXTEC System, NUVE, Ankara, Türkiye); moisture by oven-drying to constant weight at 105±2 0C. pH value was measured by using a pH meter (Lab Star pH; Schott LTD 6880, Germany). The free fatty acids (FFA) were determined according to AOAC (2009). Thiobarbituric acid reactive substances (TBARS) test was performed according to Lemon (1975). The amount of residual nitrite was found according to the method of Tauchmann (1987). The water activity was determined using Aqua LAB Water Activity (Dew Point Water Activity Meter 4TE, Pullman, WA, USA). All measurements were duplicated.

2.4. Color Measurement

The color of sucuk was found using a reflectance Hunter's Lab. (ColorFlex EZ User's Manual Reston, Virginia, USA). L*(lightness), a*(redness), b*(yellowness) color values were measured. Measurements were taken from six different points on the surface color of the samples during storage (0, 15 and 30 days) and central part of the cut surface of the two slices (3.6-cm diameter) of two sucuks. The mean of the six measurements were taken for each L*, a* and b* values (Soyer et al., 2005).

2.5. Microbiological Analysis

A sample of 25 grams of sucuk was taken under aseptic conditions and homogenized in a blender (Waring 80011S, Torrington, CT, USA) sterile physiological saline (0.85% NaCl). The number of total aerobic mesophilic bacteria was determined on Plate Count Agar (PCA; Merck) incubated at 37°C for 48 h, while moulds-yeasts on Potato Dextrose Agar (PDA; Merck) incubated at 25°Cfor 5 days. *Enterobacteriaceae* were cultured on Violet Red Bile Glucose Agar (VRBGA; Merck) incubated at 30°Cfor 24 h. (Rödel et al., 1975; Baumgart, 1986).

2.6. Statistical Analysis

The result of analyses, which depend on RBP and nitrite levels and storage time, were analyzed according to a completely randomized design with two replicates. Exemplification was made by selecting of sucuk randomly, 0, 15 and 30 days. All data were subjected to variance analyses and the differences between means were evaluated by Duncan's multiple range test (significance $P<0.01$) using the SPSS statistic program (Chicago, IL) (2011). The results of statistical analysis are shown as mean values ± standard deviation in tables (SPSS, 2011).

3. Results and Discussion

The compositions of fresh meat+fat mixture are pH, protein, fat, moisture, 6.40, 19.65%, 31.50%, 47.67%, respectively. Red beet powder (RBP) analysis results are pH, moisture, L*, a*, b*, 5.50, 10.90%, 27.26, 29.53 and -0.53, respectively. Kerr and Varner (2019) found that the freeze-dried beet powders had the moisture contents 8.10 %. Hwang et al. (2017) found that pH values fermented red beet extract 4.65. Antigo et al. (2018) showed that freeze-dried beet extracts had less

degradation and longer shelf-life than spray-dried samples. Nemzer et al. (2011) found the lowest levels of total betalains in spray-dried extracts (0.24–0.46%) followed by air-dried extracts (0.56–0.59%), and with freeze-dried products having the most (0.89–1.26%). The lightness of sample values ranged from 24.80 to 29.33. The lightness of the raw beets prior to dehydration was much lower, at 19.79. This indicates that samples got darker from the removal of water during dehydration. The L* values did not differ based on the temperatures at which samples were dried at, with values of 25.74, 25.64, and 24.80 for samples dried at 75, 85, and 95 °C (Kerr and Varner, 2019). The results determined in the study are similar to the literature values.

The results of chemical composition of sucuk at the beginning of storage are control (C), 100 ppm nitrite (N), 1% red beet powder (P), 50 ppm nitrite+1% RBP (NP1) and 100 ppm nitrite+1% RBP (NP2), protein %, 17.38±0.50, 16.78±0.51, 15.98±0.45, 16.09±0.48, 16.49±0.39, respectively and fat %, 27.99±0.68, 27.95±0.75, 26.31±0.49, 26.39±0.64 and 26.47±0.54, respectively. It was determined that the treatment had no effect on the protein and fat content of the sucuks (P> 0.05).

3.1. Chemical Analysis

The chemical analysis results of the heat-treated sucuks in the study are given in Table 1. The application, storage time and interaction had very important effects on the pH (P<0.01) of samples (Table 1 and Figure 1). A significant (P<0.01) lower pH value characterized the sucuks with beetroot powder. This result may be explained by the low pH value of beetroot powder besides other components of beetroot.

Table 1. Overall affect of treatment and storage period on the pH, moisture, aw, FFA, TBARS, residual nitrite and microbiological counts of sucuk (values are means± SD)

	pH	Moisture %	a_w	Resudial Nitrite (ppm)	FFA g oleic acid/100 g	TBARS g MDA/kg	TMAB Counts (log CFU/g)	Enterobacteriae Counts (log CFU/g)	Moulds-Yeast Count (log CFU/g)
Treatment									
C	6.44±0.06^{a}	48.29±4.48^{b}	0.94±0.01^{b}	2.06±0.52^{c}	1.22±0.34^{b}	0.54±0.05^{b}	5.68±0.33^{b}	2.33±0.37^{b}	2.02±1.56^{b}
N	6.45±0.19^{a}	51.97±3.65^{a}	0.96±0.00^{a}	7.00±2.03^{b}	1.38±0.47^{a}	0.54±0.41^{b}	5.08±1.45^{c}	2.97±0.28^{a}	2.79±1.44^{a}
P	6.37±0.10^{b}	41.61±4.03^{d}	0.94±0.01^{b}	7.02±1.88^{b}	1.00±0.14^{c}	0.55±0.38^{b}	5.49±0.40^{b}	1.94±0.68^{c}	2.81±2.27^{a}
NP1	6.42±0.11^{a}	42.83±5.57^{c}	0.92±0.02^{c}	9.01±2.58^{a}	1.00±0.19^{c}	0.65±0.07^{a}	5.85±0.74^{a}	1.90±0.59^{c}	2.89±2.27^{a}
NP2	6.15±0.19^{c}	43.50±3.25^{c}	0.94±0.00^{b}	8.67±2.51^{a}	1.41±0.31^{a}	0.56±0.05^{b}	5.84±1.91^{a}	2.08±0.51^{bc}	2.76±1.70^{a}
Significance	**	**	**	**	**	**	**	**	**
Storage period									
0d	6.37±0.07^{b}	54.49±1.29^{a}	0.95±0.01^{a}	4.63±1.45^{b}	0.83±0.10^{b}	0.56±0,02^{ab}	4.33±1.71^{b}	2.11±0.65^{b}	0.78±1.01^{c}
15d	6.34±0.20^{b}	45.66±6.97^{b}	0.95±0.01^{a}	7.66±3.76^{a}	1.41±0.25^{a}	0.60±0,06^{a}	4.67±1.08^{b}	1.93±0.58^{b}	3.67±0.34^{b}
30d	6.39±0.21^{a}	40.97±6.69^{c}	0.92±0.01^{b}	7.96±2.89^{a}	1.37±0.26^{a}	0.54±0,08^{b}	5.97±0.54^{a}	2.69±0.34^{a}	4.72±0.98^{a}
Significance	**	**	**	**	**	**	**	**	**
TxS	**	**	**	**	**	**	**	**	**

C= control; N 100 ppm nitrite; P= % 1 red beet powder; NP= 150 ppm nitrite + %1 red beet powder; NP_2= 100 ppm nitrite + %1 red beet powder. $^{a-d}$ Any two means in the same column having the same letters in the same section are not significantly different at P>0.05, **P<0.01, NS= not significant; SD= standard deviation

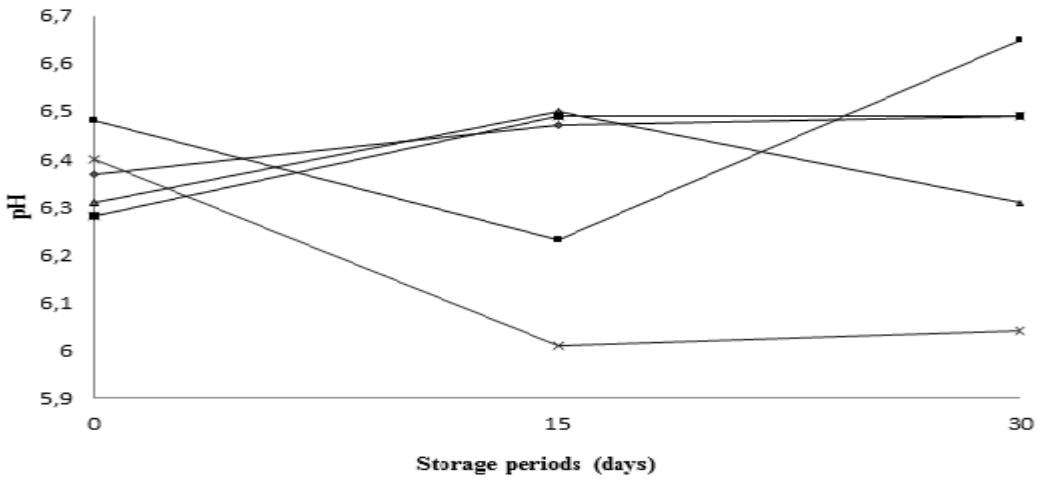

Figure 1. The effect of the interaction between treatments x storage period on pH values.
Control (–♦–); 100ppm nitrite (–■–); 1% RBP (–▲–); 50ppmNitrite+1% RBP (–☒–); 100ppm Nitrite+1% RBP (-x-).

The starting pH values of all sucuk tested was approximately determined 6.4. The pH values of all sucuk groups were not found below 6.0 during the storage period. pH is a significant factor in the control of microbiological growing in sausages (Buncic et al., 1993; Teodorovic et al., 1994). Therefore, the pH value of sucuks did not have a preventing effect on microbial growth (Table 1). Sang-Keun et al. (2014) pH of

emulsified sausage of red beet added was found 6.31. Aykın-Dinçer et al. (2020) found the pH value of sucuk to be 6.49 after heat treatment. These researchers suggest that these pH results might be attributed to the greater degree of water loss and lactic acid formation in sausages during storage. The aw values decreased during the storage time; at the starting of the ripening, with a mean value of 0.95±0.01, and 0.92±0.01 at the end of storage period (Table 1). The moisture of sucuk decreased with the adding of red beet powder regardless of the added level (1.0%). The water content of red beet powder is much lower than that of sucuk, and as the beet ratio increases, the amount of water in the product decreases. Moisture decreased during the storage time depending of drying (Table 1).

The quantity of residual nitrite in the sucuks reduces after the heat treatment and it slightly increased during the storage time. Treatments with added red beet and nitrite had higher residual nitrite values at treatments of compared to control ($P<0.01$). As expected, all nitrite-added treatments were showed high values of residual nitrite, varying from 4.58 to 5.81 ppm at 0 d of storage. These values increased to 4.63-7.96 ppm to 0 and 30 days depending of drying. In this study, the joining of 1% red beet powder in sucuk contained residual nitrite (Table 1). It is accepted that vegetables, including red beet root, are well sources of nitrite because of their nitrate content (Sebranek and Bacus 2007). Socaciu (2008) reported that red beet has high nitrate levels. There are found almost the same residual nitrite level in the other groups except for control. The same increasing residual nitrite, as shown by Sindelar et al. (2007) and Sang-Keun et al. (2014) was determined in the present study; however, nitrite and red beet powder-added treatments (N and NP 2) did not demonstrated the similar trends. Residual nitrite contents of salami-type sausages containing different levels of nitrite reduced to similar levels at the beginning of the ripening period and detected as 1.4 and 1.9 mg/kg at the end of the ripening period (Sammet et al., 2006). According to Xi et al. (2012), the residual nitrite amount was about 75% of the initial concentrations after the production process and ranged between 4 mg/kg and 10 mg/kg by the end of the storage period. Depending on the type of processed meat, the processing conditions, the presence of sodium ascorbate, and other factors (myoglobin, nonheme proteins, and lipids), the added nitrite can react with many components in the matrix. Thus, the analytical detection of nitrite or nitrate content does not reflect the initially added preservative (Cassens, 1997; Jiménez-Colmenero and Solana, 2009).

Sucu and Yıldız-Turp (2018) As a result of their studies using nitrite and red beet powder in sucuk, found that there was no significant difference between the residual nitrite contents of the samples at the end of the storage period. Feifei et al. (2022) suggest that in the industrial application stage, it will be possible to reduce the amount of nitrite used when natural colorants are mixed with nitrite.

Lipid oxidation is one of the most important changes during meat products storage and production. It may change the color, aroma, flavor, texture, and nutritive values of meat products (Tarladgis et al., 1960). The results of the FFA amounts determined during the storage in the sucuk samples are given in Table 1. There was a significant ($P<0.01$) difference between the control group and the other groups in terms of FFA values determined in sucuks (Table 1). This level was determined to be at lower quantity in samples P and NP1 than in the control, N and NP2 samples. The FFA levels were significantly affected by storage period ($P<0.01$). The level of FFA in fat correlates with the lipolytic activity of lipases, the microbial metabolic process, and oxidative reactions that work on the FFA released in lipolysis. The prevention of hydroperoxide decomposition by antioxidants are critical in protect food quality (Maillard et al., 1996). These reactions are clearly related to both the crude material used to prepare sucuk and the production process (Toldra, 1994). In whole the groups, there was a developing increase of the FFA production over time, but some differences determined among the treatments (Figure 2).

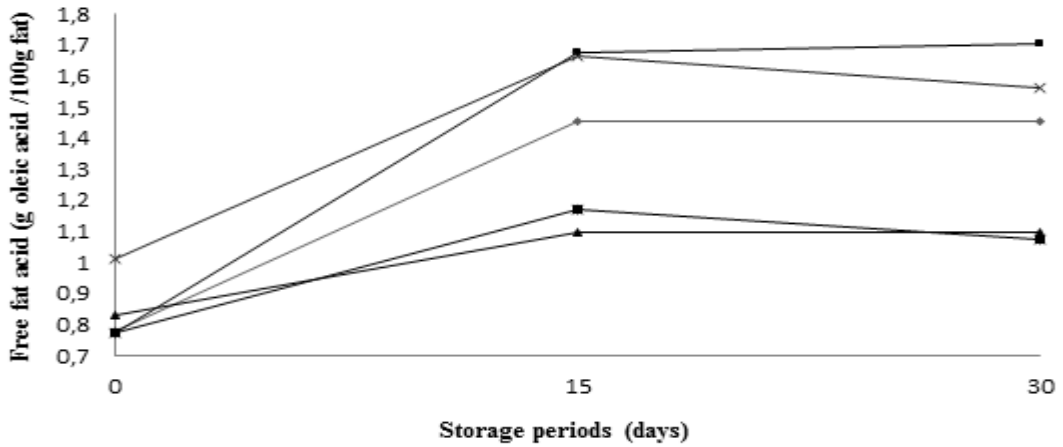

Figure 2. The effect of the interaction between treatments x storage period on ffa values. Control (–♦–); 100ppm nitrite (–■–); 1% RBP (–▲–); 50ppmNitrite+1% RBP (–☒–); 100ppm Nitrite+1% RBP (-x-).

In the sucuk with RBP had found lower FFA values. RBP is regarded as a well source of antioxidants and natural colorants because of the phenolic compounds and betalains that are existing in red beet (Sang-Keun et al., 2014; Ravichandran et al., 2012; Ravichandran et al., 2013). Betalains may exhibit strong antioxidant activity in biological environments (Socaciu, 2008). These results showed that the red beet powder may have an antioxidant effects on sucuk samples in this study.

The results of the thiobarbituric acid reagent (TBARS) analysis were not affected by the red beet powder added to the sucuks ($P>0.05$) but, TBARS was significantly affected by treatments and storage period ($P<0.01$). The utmost mean of TBARS level was found NP1 group (Table 1). An effect from red beet powder was not found big differences among treatments during the storage time except for 15 d when the addition of red beet powder increased TBARS levels ($P<0.01$).

According to Tarladgis et al. (1960), the acceptable limits of TBARS value of cooked meat products during storage is 0.5–1.0 mg MD/kg. Kohsaka (1975) have reported that malondialdehyde concentration of 0.5 mg MD/kg is a threshold value for rancidity perception by consumers. These results suggested that TBARS values less than 1.0 mg MD/kg does not indicate rancidity. The use of beetroot powder would have been expected to bigger exhibition an antioxidant activity due to betalain content. However, TBARS values of red beet powder were determined as almost the same results in comparison with control and during the storage days TBARS values were lower than the limit (< 1.0 mg/kg) (Tarladgis et al., 1960). As noted by many authors (Goulas and Kontominas 2007; Maqsood and Benjakul 2010), the TBARS value of meat products tending to decrease towards the end of storage time is attributed to the interaction of these unstable low molecular weight compounds with organic acids. These degradation products cannot be determined by the TBARS test. This effect of red beet supplementation on the TBARS value of sausage was expected because red beet contains betalains that show antioxidant and radical scavenging activities (Sang-Keun et al. 2014; Escribano et al., 1998; Georgiev et al., 2010).

However, the addition of 1.0% of red beet powder determined too small to influence of lipid oxidation of heat treatment sucuk. The amount of nitrite and beetroot powder used in the study affected the free fatty acid level and this amount increased during the storage period. Heat treatment caused oxidation of oxidizable fatty acids and increased the amount of TBARS. Based on this threshold value and our results were showing that TBARS values of treatments added with red beet powder did not exceed 0.65 mg MD/kg by the end of the storage period. These results were in accordance with study indicating that the TBARS values of fermented sausages with freeze-dried leek powder used as a nitrate source are higher than those of control group sausages with nitrite thus indicating the necessity of adding nitrites to sausages with freeze-dried leek powder (Tsoukalas et al., 2011).

More specifically, studies are needed to determine the chemical reactions between betalains and meat components and the effects of betalains on physical changes in meat product during processing.

3.2. Microbiological Analysis

The microbiological analysis of heat treatment sucuks with red beet powder and nitrite substituted are given in Table 1. The use of RBP and storage period and interaction had very important effects ($P<0.01$) on total mesophilic aerobic bacteria (TMAB), moulds/yeasts and *Enterobacteriaceae* counts (Table 1). While the total number of mesophilic aerobic bacteria (TMAB) decreased in the N group samples on the 30th day of storage, it increased in the samples from the other groups. On the 30th day of storage, *Enterobacteriaceae* and mold-yeast numbers in all samples increased compared to the initial numbers. The TMAB numbers in the N and control groups were lower than the RBP added groups. TMAB numbers reached almost 6 log CFU/g at the end of the storage period. The initial numbers of *Enterobacteriaceae* were 2.11 log CFU/g and continued to increase rapidly in all sucuk samples throughout the storage period. The initial number of *Enterobacteriaceae* were 2.11 log CFU/g and not fast much more still continued to grow in all sucuk samples during the storage time. Counts of microorganisms did not sufficiently reduce during the storage period with heat treatment (Table 1). High microorganism counts may affect the safety and shelf life of the sausage as they may contain spoilage and pathogenic microorganisms. It was known that the growth of the microorganisms, particularly *Enterobacteriaceae* was affected by pH to reduce, but in this study microorganisms were not affected by pH level. Because, the pH values of sucuks were not sufficiently decreased during the storage time. Also, starter culture was not used in sucuk manufacture and fermentation was not apply in this study. As a result, the pH value and water activity was not reduced and the microorganisms were not affected by these conditions of the sucuk. Effects of these microorganisms may be prevented at certain counts by heat treatment, pH and aw. In order to produce safer sucuk, starter culture must be used and heat treatment should be applied after fermentation. Our results are in deal with many other studies (Gençcelep et al., 2007; Ercoşkun et al., 2010).

3.3. Color Analysis

The effect of adding beetroot powder instead of sodium nitrite on the color of sausages at the beginning and end of storage the evolution of outside and cut surface color parameters is given in Table 2.

Table 2. Overall effect of treatment and storage period on color properties of sucuk (means± SD)

	Color of surface			Color of cut surface		
Treatment	L*	a*	b*	L*	a*	b*
C	30.59±4.25^{b}	12.15±3.44^{ab}	11.28±3.86^{c}	37.26±3.09^{a}	20.85±1.64^{a}	23.97±3.15^{a}
N	37.20±6.29^{a}	11.92±4.90^{ab}	15.34±1.93^{a}	36.53±3.73^{a}	19.45±3.17^{b}	21.54±3.52^{b}
P	29.08±3.53^{b}	11.49±2.95^{b}	10.27±2.27^{d}	33.25±3.13^{c}	17.35±2.79^{d}	18.02±3.23^{c}
NP1	30.36±3.03^{b}	12.65±2.88^{ab}	10.46±2.86^{cd}	33.48±2.90^{c}	17.77±2.91^{cd}	16.43±3.71^{d}
NP2	30.14±3.40^{b}	12.76±3.38^{a}	12.57±1.20^{b}	34.93±1.45^{b}	18.65±1.38^{bc}	17.98±1.07^{c}
Significance	**	**	**	**	**	**
Storage period						
0d	31.05±4.53^{a}	15.95±2.24^{a}	23.97±3.15^{a}	36.81±1.67^{a}	21.04±1.64^{a}	21.66±3.06^{a}
15d	31.65±6.73^{a}	11.93±1.84^{b}	21.54±3.52^{b}	36.30±3.34^{a}	19.14±1.82^{b}	20.92±3.66^{b}
30d	31.74±3.64^{a}	8.70±1.51^{c}	18.02±3.23^{c}	32.17±2.48^{b}	16.26±2.20^{c}	16.18±3.20^{c}
Significance	NS	**	**	**	**	**
TxS	**	**	**	**	**	**

C= control; N 100 ppm nitrite; P= % 1 red beet powder; NP= 150 ppm nitrite + %1 red beet powder; NP_2= 100 ppm nitrite + %1 red beet powder. $^{a–d}$ Any two means in the same column having the same letters in the same section are not significantly different at P>0.05, **P<0.01, NS= not significant; SD= standard deviation

All color measurements (outside and cut surface) of L*, a* and b* levels of the sucuk samples were importantly affected by treatment and storage period and its interaction (P<0.01; Tables 2 and Figures 3 and 4). Addition of red beet powder has an effect on the value of redness and the maximum levels have been monitored from the sample in group NP2 outside color of sucuks. Only nitrite added sucuk had the highest L* values in the samples and multiple comparisons of treatments in the processing time revealed that the lightness of red beet powder added groups were significantly different from the only nitrite samples. The lightness was not changed with the adding RBP (%1) and RBP (%1) + nitrite levels (50 and 100 ppm) in sucuk (Table 2). These can be attributed to the color effect on the L* values of the RBP causing the darker color. Likewise, Sucu and Turp (2018) reported that the addition of beetroot powder as a nitrate source into fermented sausages led to a decrease in the L* and b* values of sausages at the beginning of storage (P<0.05).

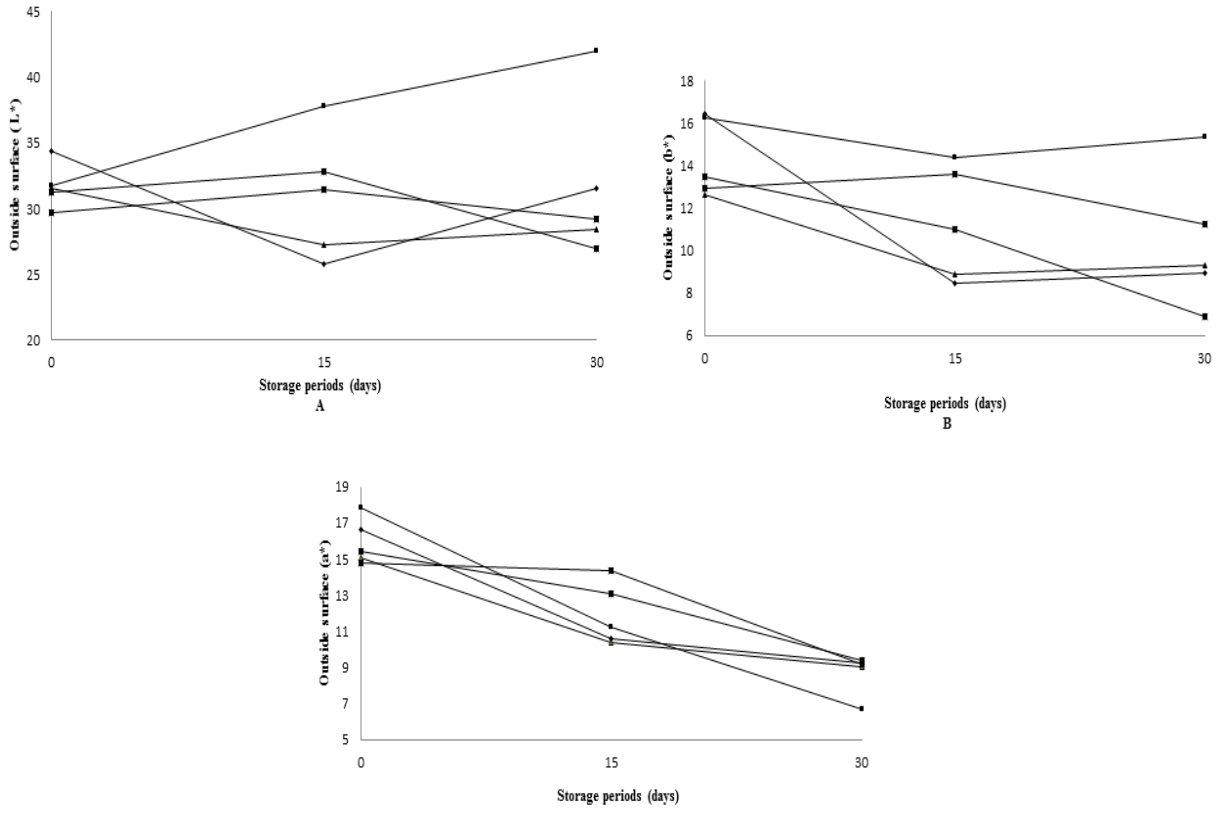

Figure 3. The effect of the interaction between treatments x storage period on colors (outside surface) values. Control (–♦–); 100ppm nitrite (–■–); 1% RBP (–▲–); 50ppmNitrite+1% RBP (–☒–); 100ppm Nitrite+1% RBP (-x-).

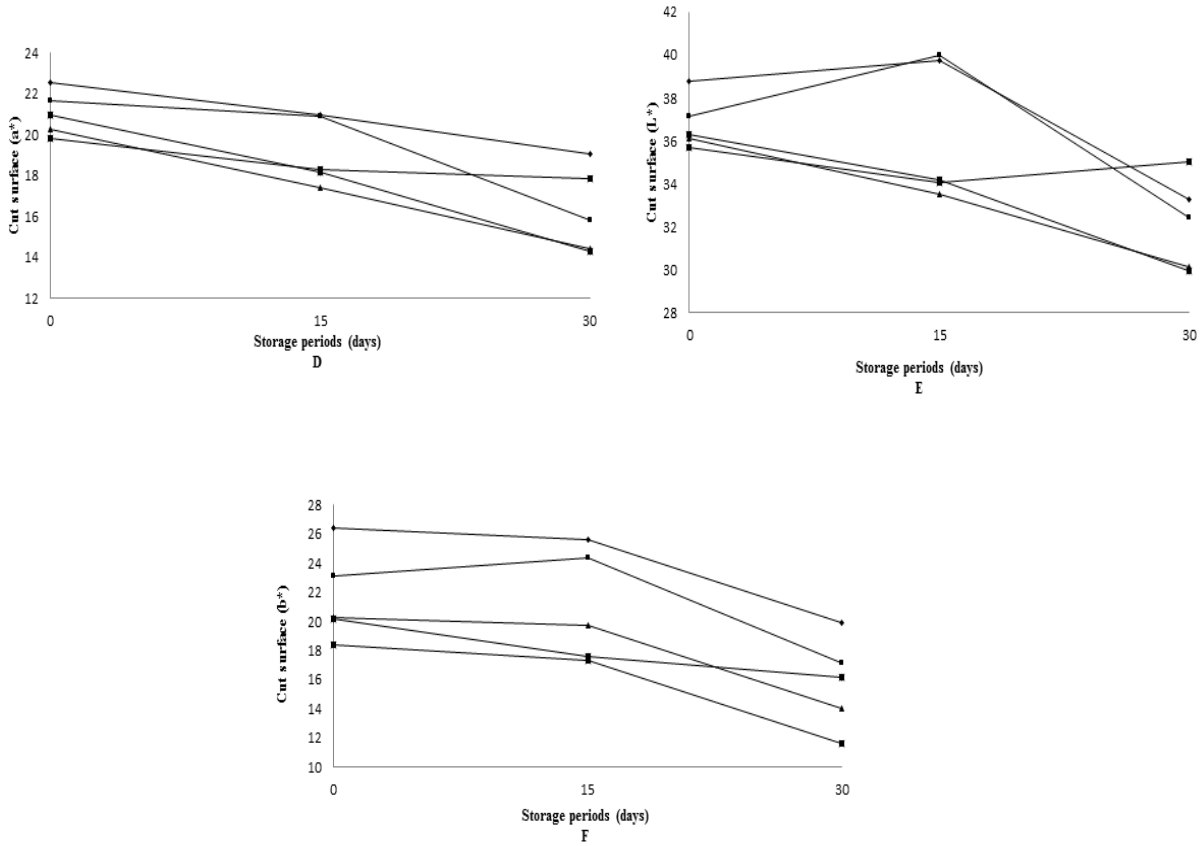

Figure 4. The effect of the interaction between treatments x storage period on colors (cut surface) values. Control(–♦–); 100ppm nitrite (–■–); 1% RBP(–▲–); 50ppmNitrite+1% RBP (–☒–). 100ppm Nitrite+1% RBP (-x-).

The reducing in L* value are represented by the formation of dark color in the sucuk because of the browning reaction. Üren and Babayiğit (1996) determined that the lightness values of sucuk samples were between 35.87 and 45.92, whereas redness and yellowness values found between 6.87 and 14.14, and 10.04 and 17.62, respectively. Similarly, Kayaardı and Gök (2003) reported that L* values of sucuk usually reduced during the 15 days of ripening period. The lightness (L*) values of inside color of heat treated sucuk specimens decreased during storage time (Table 2). The decrease in L* levels of traditional sucuks were also determined by Bozkurt and Bayram (2006), Kayaardı and Gök (2003). The decrease in L* value indicate darkening due to drying (Üren and Babayiğit, 1996). However, heat treatment caused an increase in L* values of sausages ($P<0.01$). Denaturation of myoglobin can cause a light color in sausages (Chasco et al., 1996). Compared to the L* values of the heat-treated sample at different periods of fermentation, traditional sucuk had a lower L* value ($P<0.01$). During the initial days of storage time, nitrogenous compounds (such as nitrite) present in meat colour combined with myoglobin to perform the desired color pigment. This pigment has a red color, therefore, a* values would be different. The pigment formed during this period is denatured and so, it caused some decreases in a* values. Similar results were observed as stated by Kayaardı and Gök (2003) that a* values of sucuks increased during the first 5 days of ripening, but decreased during the later ripening period. Perez-Alvarez et al. (1999) reported that nitrosomyoglobin formation and moisture loss may be related to the reasons for the increase in a* values. A possible reason for the decrease in a* values may be the partial or complete breakdown of nitrosomyoglobin due to lactic acid production (Muguerza et al., 2002). Martínez et al. (2006a) indicated that betalains, natural colorant found in red beetroot, can be easily affected by effects such as light, oxygen, pH, temperature. These results indicate that red beet is effective in increasing the redness of heat treatment sucuk because of the red beet contained betalains (Ravichandran et al., 2013).

This result shows that beetroot powder is effective in providing the desired red color in sausages due to their betalain and also nitrate content. A similar result was reported for mortadella sausage by Baldin et al. (2018). Moreover, the use of beetroot powder caused an increase in the a* values of sausages compared to the control. Also, similar results have been reported by different authors for emulsified pork sausage (Jin et al., 2014), emulsified beef sausage (Turp et al., 2016) and Turkish fermented beef sausage (sucuk) (Sucu and Turp, 2018).

Although redness decreased over storage time, this is probably caused by pigment degradation as noted by Fernandez-Gines et al. (2003). Moreover, high water activity, high storage temperature, high luminosity and presence of oxygen and metal ions have been reported to have negative effects on the stability of betalains, natural colorant found in beetroot (Aykın-Dinçer et al., 2020); therefore, beetroot powder may not protect sausages

from discoloration throughout the storage in spite of increasing redness. The decrease in b* value during storage indicates that the color of the sucuk changes from yellow to blue (nearing a negative b* value) over time. It can be said that the reason for this may be browning reactions because melanoidins have a brown color.

Betalains are considered substrates of peroxidase due to their chemical structure. In addition to oxygen, hydrogen peroxide was also reported to accelerate betanin degradation. As a result of this study, it is thought that the decrease in a* and b* values in sucuk during the storage period is due to the degradation of betalins by peroxidase enzymes produced by microorganisms (Pedreno and Escribano 2000; Herbach et al., 2006).

The color results determined in the study are similar to the results shown by Kayaardı and Gök (2003). Perez-Alvarez et al. (1999) determined that the b* values of Spanish sausages also decreased during fermentation and maturation periods. They explained the decrease in b* values as the decrease in oxymyoglobin, which contributes to the use of oxygen by microorganisms and therefore the yellow color. Jeong et al. (2010) also reported a decrease in redness of low-fat sausage, which added red powder homogenate during storage, but the smoked low-fat sausage with 75 ppm sodium nitrite and 0.5% red powder homogenate showed stable redness during storage. These results disagreed with this study due to differences between the type of red powder (powder and homogenate) and type of sausage (emulsified sausage and smoked low-fat sausage). The addition of red beet decreases lightness and yellowness of heat treatment sucuk; thus, the added level of red beet needs to be adjusted to control desirable properties.

4. Conclusion

The use of beetroot powder instead of nitrite in heat treated sucuk which is a popular traditional meat product affected some quality characteristics of this reformulated product depending on the used amount.

While there was statistically significant difference between the residual nitrite contents of the samples at the end of the storage period, containing only beetroot powder had a significantly higher residual nitrite in comparison with those of the control sample. Color change and lipid hydrolysis in heat treatment sucuk is a big problem during cold storage and the same problem continues in the marketplace. Redness value (a*) of the samples in surface and cut surface decreased and was no well protected during the storage when an increased amount of beetroot powder was used.

Instead of red beet, after pure betalain extraction from red beet, can be a suitable colorant for meat products as well as an additive substitute. In addition, beetroot powder can be considered as an alternative to synthetic colorants and nitrite in cooked, smoked, semi-dried and fermented sausages or less nitrite in all cured meats.

Author Contributions

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

	Ö.S	H.G
C	30	70
D	50	50
S	10	90
DCP	20	80
DAI	40	60
L	80	20
W	10	90
CR	30	70
SR	20	80
PM	20	80
FA	20	80

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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UTILIZATION OF GENOME EDITING FOR LIVESTOCK RESILIENCE IN CHANGING ENVIRONMENT

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Abstract: Climate change poses a significant threat to livestock production systems, including changes in temperature and rainfall patterns, increased frequency of extreme weather events, and the spread of diseases. The use of genome editing technologies presents a potential solution to mitigate the impacts of climate change on livestock. This paper reviewed the prospects of utilizing genome editing in mitigating the impact of climate change in livestock. Applications of genome editing in development of heat-tolerant, and disease-resistant as well as animals with improved feed and water use efficiency and reduced methane emissions are explored. Additionally, a potential breeding program for gene edited animals is proposed. There are several different genome editing techniques that can be used in livestock breeding, including CRISPR/Cas9, TALENs, and zinc-finger nucleases. These techniques involve introducing specific changes to the animal's genome, such as deleting or replacing genes, or introducing new ones. The technology has enormous potential for improving livestock breeding, as it allows for the creation of animals with desirable traits in a much shorter time frame than traditional breeding methods. Generally, it may take years or even decades to breed an animal with a specific trait using traditional breeding methods, whereas genome editing can achieve the same result in just a few generations. Genome editing can be used to mitigate the impact of climate change on livestock production by reducing the methane emissions by improving the efficiency of feed conversion and modifying the genes responsible for methane production. Technology can be utilized to improve livestock feeds by modifying genes involved in plant growth, development, and nutrient use. This lead to the creation of forages that are high yielding, more nutritious and better adapted to diverse production environments. Genome editing allows development of animals that are more resistant to diseases, which can help reduce the need for antibiotics and other treatments. This is particularly important given the growing problem of antibiotic resistance, which is a major concern in both human and animal health. Genome editing has the potential of developing animals that are thermo-tolerant, as well as animals with improved feed and water use efficiency. The proposed breeding program for gene-edited animals will ensure that the animals produced are healthy, genetically diverse, and meet the desired traits. In terms of ethical concerns, policies for genome editing ought to consider the potential for unintended consequences or the creation of animals with characteristics that are viewed as undesirable or unethical. Overall, genome editing technology has the potential to revolutionize livestock production and contribute to the global effort to mitigate the impact of climate change.

Keywords: Climate change, Mitigation, Genome editing, Livestock

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1. Introduction

Climate change can have significant impacts on livestock production, including heat stress, changes in forage quality and quantity, changes in water availability and quality, increased disease risk and changes in animal behaviour (Ngeno et al., 2013; Jasrotia et al., 2023). These effects of climate change on livestock pose significant challenges for farmers and the agricultural industry as a whole. Adaptation strategies are necessary to mitigate the impacts of climate change on livestock production. This includes the exploration of genome editing in development forage and livestock breeds that are more resilient to the impacts of climate change. Genome editing is a powerful tool that allows researchers to make precise changes to an animal's DNA (Panda and McGrew, 2022), potentially improving its ability to cope with environmental stressors such as heat, drought, and diseases (Ricroch, 2019; Pramod and Mitra, 2023). By harnessing the power of genome editing, scientists can develop livestock that are better able to withstand the challenges of a changing climate, improving animal health, productivity, and sustainability. Breeding programs have long been used to improve the performance and traits of livestock (Ngeno et al., 2013). With the advent of genome editing technology, breeding programs have the potential to become even more powerful tools for developing animals that are better adapted to the challenges of a changing climate. By incorporating gene-edited animals into breeding programs, researchers can select for traits that improve resilience and productivity, creating more robust and sustainable livestock populations. In this paper, mitigation of impacts of climate change including improving feeds and enhancing resistance to disease,

heat and water stresses in livestock through speed breeding utilizing genome editing techniques are reviewed. Finally, a potential breeding program for gene edited animals is proposed.

2. Genome Editing Techniques

Genome editing techniques are a set of tools used to modify DNA sequences in living organisms. There are several genome editing techniques available, but some of the most popular ones are: (a) CRISPR-Cas9: CRISPR-Cas9 is a revolutionary genome editing technique that allows scientists to cut DNA at specific locations, thereby adding, removing or modifying genes. The technique relies on a protein called Cas9, which acts like a pair of molecular scissors that can cut DNA at a specific location. Scientists can then insert, delete, or replace the cut DNA sequence with a desired sequence (Mehra and Kumar, 2022; Rasheed et al., 2022; Khiabani et al., 2023; Li et al., 2023), (b) Zinc Finger Nucleases (ZFNs): Zinc Finger Nucleases are engineered proteins that can bind to specific DNA sequences and cut the DNA at that location. Like CRISPR-Cas9, ZFNs can be used to add, delete or modify genes (Mehra and Kumar, 2022; Wani et al., 2023a), (c) TALENs: Transcription Activator-Like Effector Nucleases (TALENs) are another type of engineered proteins that can bind to specific DNA sequences and cut the DNA at that location, and TALENs work by using a DNA-binding domain that can be programmed to recognize a specific DNA sequence (Mehra and Kumar, 2022; Kumar and Kues, 2023; Wani et al., 2023a), (d) Homologous Recombination: Homologous Recombination is a technique that uses a DNA template to repair a broken DNA strand. This technique is often used to introduce specific mutations or changes to a DNA sequence (Ranjitha et al., 2022; Park, 2023). These genome editing techniques have many potential applications in livestock breeding. They can be used to mitigate impact of climate change, improve quality and quantity of livestock feeds, address genetic disorders, develop new breeds, create disease, water and heat tolerant animals, and more.

3. Application of Genome Editing

3.1. Mitigating Greenhouse Gas Emissions

Genome editing has emerged as a promising tool for mitigating the impact of climate change on livestock. By making precise changes to the genetic code of animals, scientists can develop animals that are better adapted to the changing climate and can help reduce greenhouse gas emissions. Livestock production is a significant contributor to greenhouse gas emissions, with livestock accounting for around 14.5% of global greenhouse gas emissions (Sakadevan and Nguyen, 2017). Genome editing technology can be used to mitigate the impact of climate change on livestock production by reducing the methane emissions from livestock and improving the efficiency of feed conversion (Anderson et al., 2020; Wray-Cahen et al., 2022). One approach is to use genome editing to modify the genes responsible for methane production in the rumen of cattle and other ruminant animals. Researchers can introduce genes from other organisms or modify the animal's own genes to reduce the production of methane during digestion. This could lead to a significant reduction in greenhouse gas emissions from livestock production (Raza et al., 2022; Wray-Cahen et al., 2022). For examples of genome editing has been used to create cattle with a mutation in *MSTN* gene, which is associated with reduced methane emissions (Dunne et al., 2019). Likewise, Hill et al. (2021) has edited the microbiome of the animal's rumen to reduce methane production. Nitrous oxide emissions in pigs has been reduced by developing a mutation in *GDF8* gene, which is associated with reduced nitrous oxide emissions (Liu et al., 2019).

Another approach is to use genome editing to improve the efficiency of feed conversion in livestock. By modifying the genes responsible for nutrient absorption and metabolism, researchers can develop animals that require less feed to produce the same amount of meat or milk, thereby reducing the overall environmental impact of livestock production (Leisner, 2020; Moloney and McGee, 2023). For example, researchers have used genome editing to develop cows with a mutation in a gene called *DGAT1*, which is associated with improved feed efficiency (Cole et al., 2019).

Mitigating greenhouse gas emissions in both the industry and livestock sectors is crucial to address climate change and reduce the impact of human activities on the environment. While the sources of emissions and mitigation strategies differ between the two sectors, both sectors have significant potential to reduce their greenhouse gas emissions and contribute to global efforts to address climate change. In the industry sector, strategies such as improving energy efficiency, using renewable energy sources, and implementing carbon capture and storage technologies can reduce greenhouse gas emissions (IEA, 2020). Additionally, adopting circular economy principles, such as reducing waste and increasing resource efficiency, can also mitigate emissions in the industry sector (Ellen MacArthur Foundation, 2021). In contrast, mitigation strategies in the livestock sector focus on reducing emissions from enteric fermentation, manure management, and herd management practices (FAO, 2014). Additionally, reducing meat consumption and shifting towards plant-based diets can also help mitigate emissions from the livestock sector (Poore and Nemecek, 2018). Despite the differences in mitigation strategies, both sectors can benefit from implementing circular economy principles. For example, reducing waste in the livestock sector by implementing manure management practices can generate renewable energy sources such as biogas, which can be used to power the industry sector (FAO, 2014). Additionally, the industry sector can implement circular economy principles by using waste materials from the

livestock sector as inputs for their production processes (Ellen MacArthur Foundation, 2021).

3.2. Improving Livestock Feeds

Genome editing can be used to improve livestock feeds by modifying genes involved in plant growth, development, and nutrient use. This approach could lead to the creation of crops that are more nutritious, have higher yields, and are better adapted to different environmental conditions (Moloney and McGee, 2023). One application of genome editing in improving livestock feeds is to modify the genes involved in plant cell walls. For example, researchers have used genome editing to modify lignin, a component of plant cell walls that makes them tough and difficult to digest (Pazhany and Henry, 2019; Yu et al., 2021). By reducing lignin content, it may be possible to create crops that are more easily digested by livestock, leading to better feed efficiency and improved animal health. Another application is to modify genes involved in nutrient uptake and utilization. For example, researchers have identified genes involved in the uptake and storage of nutrients such as nitrogen, phosphorus, and iron (Roell and Zurbriggen, 2020; Matres et al., 2021). By modifying these genes, it may be possible to create crops that are more efficient at using these nutrients, leading to higher yields and improved feed quality. Genome editing can also be used to introduce beneficial traits into crops, such as resistance to pests and diseases (Yin and Qiu, 2019; Matres et al., 2021).This can help to reduce the use of pesticides and other chemicals in crop production, leading to more sustainable and environmentally-friendly livestock feeds. While genome editing for improving livestock feeds is a promising approach, there are also some challenges to consider. One challenge is the potential for unintended consequences, such as off-target effects or unintended changes to other traits. It will be important to carefully evaluate the safety and efficacy of any gene-edited forage crops before they are introduced into the feed supply. Another challenge is the regulatory framework surrounding genome-edited crops (Menz et al., 2020). In many countries, genome-edited crops are subject to the same regulations as genetically modified crops, which can be time-consuming and expensive to navigate. Despite these challenges, genome editing for improving livestock feeds has the potential to improve animal welfare, productivity, and sustainability of livestock production. It will be important to continue research in this area to identify the most effective strategies for improving feed quality and to ensure that any gene-edited crops are safe and beneficial for animals.

3.3. Enhancing Disease Resistance

Genome editing, is a powerful tool for modifying DNA sequences in order to introduce specific changes or traits. It has the potential to revolutionize livestock breeding by creating animals with enhanced characteristics, such as disease resistance, improved growth rates, and better nutritional profiles (Rexroad et al., 2019). In particular, genome editing for disease resistance in livestock has received a lot of attention in recent years. Disease is a major issue in livestock production, as it can lead to significant economic losses and impact animal welfare. Traditional breeding methods have been used for centuries to select for desirable traits, but these methods can be slow and imprecise. Genome editing, on the other hand, allows for precise modifications to be made to an animal's genome in a relatively short amount of time. One potential use of genome editing in livestock is to create animals with increased disease resistance (Ricroch, 2019; Zhao et al., 2019). For example, Pramod and Mitra (2023) and Guo et al. (2019) have used genome editing to create pigs that are resistant to porcine reproductive and respiratory syndrome virus (PRRSV), which causes significant losses in the swine industry. Genome editing has been applied in creating pigs that are resistant to African swine fever, a highly contagious and deadly disease that has devastated pig populations around the world (Gaudry et al., 2021). By introducing specific genetic changes, the pigs are able to produce a protein that helps protect them against the virus. Another example is the use of genome editing to create dairy cows that are resistant to bovine tuberculosis (TB) reported by Pramod and Mitra (2023). Bovine TB is a major issue in some countries and can lead to significant losses in milk production. Researchers have used genome editing to introduce a specific genetic mutation that makes the cows less susceptible to the disease. While the potential benefits of genome editing for disease resistance in livestock are significant, there are also concerns about the technology. One concern is the potential for unintended consequences, such as off-target effects or the introduction of new diseases (Gori et al., 2015). Another concern is the ethical implications of modifying animals in this way, particularly if it involves creating animals that are unable to feel pain or experience other emotions. Despite these concerns, the use of genome editing in livestock is likely to continue to be an area of active research and development.

3.4. Mitigating Heat Stress

Heat stress is a significant issue in livestock production, particularly in areas with high temperatures and humidity. It can lead to reduced productivity, decreased fertility, and even death in some cases (Ngeno et al., 2013). Genome editing has the potential to address this issue by producing animals with improved heat tolerance. One approach to genome editing for heat stress in livestock is to introduce genetic variations that are associated with improved heat tolerance in other species. For example, researchers have identified specific genetic variations in camels that help them to survive in hot, arid environments (Bahbahani et al., 2019). By introducing these variations into the genomes of livestock such as cattle or sheep, it may be possible to create animals with improved heat tolerance. Another approach is to use genome editing to modify genes that are known to be involved in heat stress response pathways. For example, researchers have identified

genes involved in heat shock response, which is a protective mechanism that helps cells survive under conditions of high heat (Haire et al., 2022). By modifying these genes using genome editing, it may be possible to enhance the heat shock response in livestock and improve their heat tolerance.

Examples of applications of genome editing in mitigating heat stress in livestock include editing of genes involved in thermoregulation, such as those related to sweating or panting. For example, knockout of the *TRPM8* gene, which plays a role in thermoregulation, has led to improved heat tolerance in dairy cows (Tian et al., 2019). Another approach has been enhancement of the heat shock response, which is a cellular mechanism that helps protect cells from heat stress. This has been achieved by editing genes involved in the heat shock response pathway, such as *HSF1*. A study published by Liu et al. (2020) demonstrated that overexpression of *HSF1* improved thermotolerance in porcine cells. Heat stress can impair immune function in livestock, making them more susceptible to diseases. Editing genes involved in immune function, such as those related to cytokine production, has been edited to help mitigate this effect. A study published Zhang et al. (2018) demonstrated that knockout of the *IL1B* gene, which encodes a cytokine involved in the immune response, improved heat tolerance in broiler chickens. It has been demonstrated that knockout of the *MC4R* gene, which plays a role in appetite regulation, improved heat tolerance in broiler chickens (Tang et al., 2021). In 2020, scientists in Brazil used CRISPR-Cas9 to modify a gene called *UCP1* in cattle embryos. *UCP1* is involved in heat production and energy metabolism, and the modification was intended to increase the animals' heat tolerance. The resulting calves showed improved growth rates and were able to maintain body temperature under hot conditions, suggesting that the modification had a positive effect on their heat tolerance (Rossi, 2020).

While genome editing for heat stress in livestock is a promising approach, there are also some challenges to consider. One challenge is the complexity of the genetic basis of heat tolerance, which involves multiple genes and pathways (Jahan et al., 2022). It may be difficult to identify all of the relevant genes and modify them in a coordinated way. Despite the challenges, genome editing for heat stress in livestock has the potential to improve animal welfare and productivity in hot environments. It will be important to continue research in this area to identify the most effective strategies for improving heat tolerance and to ensure that any gene-edited animals are safe and beneficial for both animals and consumers.

3.5. Mitigating Water Stress

Water stress is a significant issue in many parts of the world, particularly in tropics with limited access to water. Livestock are often affected by water stress, which can lead to reduced productivity, decreased fertility, and even death in severe cases (Ngeno et al., 2013). Genome editing has the potential to address this issue by creating animals with improved water use efficiency. One approach to genome editing for water stress in livestock is to modify genes that are involved in water use and conservation (Karavolias et al., 2021). For example, researchers have identified genes involved in animal water use efficiency (Lea et al., 2023), which could be introduced into the genomes of livestock such as cattle or sheep. By modifying these genes, it may be possible to develop animals that are more efficient at using water and can better tolerate water stress. Another approach is to use genome editing to modify genes involved in the regulation of water balance in the body. For example, researchers have identified genes involved in the production and regulation of water balance, which are channels that allow water to move in and out of cells (Alamer, 2011; Karavolias et al., 2021). By modifying these genes, it may be possible to create animals with improved water balance and better tolerance of water stress. Water stress is often associated with high temperatures, and animals that are better able to tolerate heat stress may be more resilient to water scarcity. Genome editing can be used to modify genes involved in heat shock response, such as *HSP70*, which help protect cells from damage caused by high temperatures (Durosaro et al., 2023). While genome editing for water stress in livestock is a promising approach, there are also some challenges to consider. One challenge is complexity of the genetic basis of water use efficiency, which involves multiple genes and pathways. It may be challenging to ascertain all of the significant genes and alter them in a synchronized manner. Notwithstanding the challenges, genome editing for water stress in livestock has the latent to mend animal welfare and productivity in regions with water scarcity.

4. Breeding Program for Gene Edited Animals

Designing a breeding program for gene-edited animals would involve several steps, including identifying the desired traits, selecting the appropriate gene-editing techniques, and developing a breeding strategy to propagate the edited traits (Mueller et al., 2015; Ngeno, 2015). The following is an outline of the essential steps in such a program:

- Identification of the desired traits: The first step in designing a breeding program for gene-edited animals is to identify the desired traits. This could be anything from disease resistance to increased growth rates, improved nutrition, or enhanced fertility (Ngeno, 2015).
- Choosing the appropriate gene-editing technique: Depending on the desired traits, different gene-editing techniques may be used. Some common techniques include CRISPR/Cas9, TALENs, and Zinc Finger Nucleases. Each technique has its strengths and weaknesses, and the choice of technique will depend on the specific traits being targeted (Khalil, 2020).

- Editing the genes in the desired animals: Once the appropriate gene-editing technique has been chosen, the next step is to edit the genes in the desired animals. This could involve introducing new genes, deleting or disabling existing genes, or modifying the expression of genes (Mushtaq and Molla, 2023; Wani et al., 2023b).
- Screening and selection of edited animals: After editing the genes, the next step is to screen and select the edited animals. This involve genetic testing to confirm the desired traits have been successfully edited, as well as assessing other factors such as health, temperament, and breeding potential (Bunton-Stasyshyn et al., 2022).
- Developing a breeding strategy: The final step in the breeding program is to develop a breeding strategy to propagate the edited traits. This could involve selecting edited animals with the desired traits and breeding them with other edited animals, or with non-edited animals to introduce the edited traits into a broader population. It's important to carefully monitor the breeding program to ensure the edited traits are being passed down successfully and that there are no unintended consequences (Wang et al., 2022; Whitworth et al., 2022).
- Evaluation of the results: The animals produced should be evaluated to determine if they meet the desired traits and are healthy.
- Establish regulations: There may be regulatory requirements for gene-edited animals, and the breeding program should be designed to comply with these regulations.

Generally, designing a breeding program for gene-edited animals requires careful planning and execution. It's essential to identify the desired traits, choose the appropriate gene-editing technique, screen and select edited animals, and develop a breeding strategy to propagate the edited traits successfully. Additionally, it's essential to adhere to ethical and regulatory guidelines to ensure the safety and well-being of the animals involved in the breeding program.

5. Policy for Genome Editing

The use of genome editing in livestock raises important ethical, safety, and regulatory issues, which require appropriate policy frameworks (Kumar and Kues, 2023). Policies for genome editing in livestock should balance the potential benefits of this technology with the potential risks and ethical concerns (Zhang et al., 2020). One key issue to consider is the safety of gene-edited animals for human consumption (Van Eenennaam and Young, 2019). Regulators must ensure that any gene-edited animals intended for food production are safe to consume and do not pose any risks to human health. Another important issue is the welfare of the animals themselves (Ormandy et al., 2011). Genome editing should be used to improve animal welfare (Kramer and Meijboom, 2021), not to create animals that suffer from unintended consequences or health issues. Policies for genome editing in livestock should also consider the potential environmental impacts (Gordon et al., 2021) of gene-edited animals. For example, gene-edited animals may have different interactions with their ecosystems, which could have unintended consequences on the environment. In terms of ethical concerns, policies for genome editing in livestock should consider the potential for unintended consequences or the creation of animals with characteristics that are viewed as undesirable or unethical. This could include concerns related to animal welfare, social acceptance, and cultural values (Eriksson et al., 2018; Gordon et al., 2021). Overall, policies for genome editing in livestock should be based on a careful consideration of the potential risks and benefits of this technology. They should be informed by scientific evidence, ethical considerations, and stakeholder engagement. The policies should also be flexible enough to accommodate new developments and emerging issues, while ensuring the safety of consumers, animals, and the environment.

6. Conclusion

Genome editing has the potential to sustain and revolutionize livestock production by improving animal welfare, productivity, and resilience to various stresses (heat, water and diseases). The technology can be used to develop animals with desirable traits, such as disease resistance, heat tolerance, and water use efficiency, among others. In the future, genome editing in livestock is likely to become more common, as the technology becomes more efficient, precise, and accessible. However, there are still significant challenges and uncertainties associated with the use of genome editing in livestock. These include issues related to safety, regulatory frameworks, ethical concerns, and public acceptance. Thus, further research is needed to fully understand the potential benefits and risks of genome editing in livestock.

Author Contributions

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

	K.N.
C	100
D	100
S	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, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

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Review
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ANTIOXIDANT ADDITIVES IN FISH FEEDS

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Abstract: Aquaculture is a rapidly developing sector in recent years. For humans, one of the most important sources of protein is fish and other products from aquaculture. Antioxidants are used to prevent oxidation problems in the feeds used in the production of these products. The task of antioxidants is to prevent rancidity of fats and to keep feeds stable. Fish fed with oxidized feeds experience many negative effects such as growth retardation, low feed utilization, weak immune system and reduced resistance to diseases. As a result, it can cause great economic losses along with fish losses. Synthetic antioxidants have been used successfully for many years. However, in recent years, some restrictions and regulations have been introduced the use of synthetic antioxidants. Therefore, natural antioxidants have begun to replace synthetic antioxidants. The sources of natural antioxidants are quite abundant, such as fruits, vegetables, plant extracts, marine macro and microalgae. In recent years, research on these natural antioxidants and their use in fish feeds have been increasing.

Keywords: BHA, BHT, Etoxyquin, Natural antioxidants

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1. Introduction

In aquaculture, unbalanced and oxidized feeds and stress conditions make fish more vulnerable to diseases and cause economic losses due to fish deaths (Amer et al., 2018). It is important to use balanced feed formulations, vaccines and immunostimulants to reduce disease risks in cultured fish, thus increasing the resistance levels of fish against infections (Amer et al., 2018; Amer et al., 2019; Al-Khalaifah et al., 2020; El-Araby et al., 2020).

The unconscious use of broad-spectrum chemicals that have toxic or lethal effects on disease-causing bacteria, protozoa, viruses and parasites without harming the host has caused the resistance of microorganisms to these drugs and the increase of pathogens. In other words, it causes the formation of drug resistance (Ai et al., 2011). In this case, the spread of drug-resistant pathogens has caused many other problems, such as environmental hazards and food safety issues.

Prevention of diseases is more important than treatment. Especially when it comes to diseases caused by feed. Decomposed, poorly stored, nutritionally deficient feed can cause disease. Feeds used in aquaculture contain partially or high amounts of fish oil or other vegetable oils. These added oils are based on marine ingredients containing high levels of polyunsaturated ω-3 fatty acids and are therefore susceptible to lipid oxidation. Oxidized feeds adversely affect the resistance of fish against diseases, at the same time they damage both growth and development and fillet quality. (Sutton et al., 2006; Grigorakis et al., 2010). It has been shown that the use of oxidized oils in the feeds increases the plasma glucose, cortisol and osmolarity levels of the fish, therefore the stress factor develops negatively (Van Anholt et al., 2004; Alves Martins et al., 2007).

During the production, transportation and storage of feed, oxidation can be a major process that can reduce its quality. Oxidation can take place through various mechanisms, including autoxidation, photosensitized oxidation, thermal oxidation, and enzymatic oxidation.

Autoxidation occurs when feed comes into contact with atmospheric oxygen, resulting in the formation of radicals. This reaction can lead to the oxidation of feed, which can cause a decrease in its nutritional and physical quality (Cho and Min, 2009). In order to prevent these problems in fish caused by feed, additives with antioxidant properties are added to fish feeds. These additives used both prevent the oxidation of the feed and ultimately help protect the health of the fish. In this article, general information about antioxidants used in fish feeds and information about their effects are given.

2. Antioxidants

Antioxidants are substances that act as shields against the negative reactions of oxygen (oxidation) of complex structures such as proteins, lipids, carbohydrates and DNA in a cell. The organism is constantly under the influence of both internal (digestive, respiratory, disease, injury, etc.) and external (environmental factors) factors.

As a result, oxidant molecules formed during and after these effects damage cells and tissues. In living organisms, there is a system that constantly neutralizes reactive oxygen species (ROS) and other pro-oxidants through low molecular weight free radical scavengers and antioxidant enzymes. Both intracellular and extracellular enzyme and non-enzyme defense mechanisms against reactive oxygen species or oxidant molecules are called antioxidant defense system (Mates et al. 1999; Dündar et al., 1999; Dündar et al., 2000; Ritola et al., 2002; Mclean et al., 2005; Pham-Huy et al., 2008).

If antioxidant defense is mediated by catalase (CAT), superoxide dismutase (SOD), Glutathione peroxidase (GPx) and Glutathione reductase (GR) enzymes, it is called enzymatic antioxidant defense. If the defense is with substances such as tocopherol (Vitamin E), ascorbic acid (Vitamin C), retinol (Vitamin A), it is expressed as a non-enzymatic antioxidant defense system (Valko et al. 2007). There are antioxidants in many different foods that we obtain from nature. As for the classification of antioxidants, many different classifications can be seen in the literature. These; such as where it is obtained from, its mode of action, activities, biochemical properties (Kebede and Admassu, 2019).

It is possible to examine antioxidants in two groups as natural antioxidants and synthetic antioxidants. In general, among natural antioxidants, enzymes, macromolecules and micromolecules can be given as examples (Hilmi, 1994; Sen and Chakraborty, 2011). Some of the plant organisms found in nature (especially vegetables and fruits) are good sources of natural antioxidants. (Grozea, 2012; Akbarirad et al., 2016; Kebede and Admassu, 2019). These foods are very rich of antioxidant compounds such as Vit E, Vit A, Vit C, β-carotene and etc. (Sies et al., 1992; Anbudhasan et al., 2014). The most important antioxidant compounds found in plant extracts are polyphenols (flavonoids) and phenolic acids and carotenoids. (Balasundram et al. 2006; Göktürk et al. 2007; Sicuro et al., 2010).

Some important natural antioxidants and their sources presented in Table 1. (Balasundram et al., 2006; Grozea, 2012; Akbarirad et al., 2016; Kebede and Admassu, 2019). As regards to synthetic antioxidants are chemically synthesized additives to food products as preservatives to help prevent oxidation (Kebede and Admassu, 2019). Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), etoxyquin (EQ) and citric acid are the most well-known (Sicuro et al., 2010; Blaszczyk et al., 2013).

In addition, these synthetic antioxidants have been used for many years to increase stability in foods and animal feeds (Blaszczyk et al., 2013). However, there is conflicting information about their use. In recent years, updates have been made on the usage areas and doses of these additives. Reported due to growing evidence of its adverse effects on aquatic life and humans, the EU commission has suspended the authorization of ethoxyquin as a feed additive for all animal species and categories. According to Gunathilake et al. (2022) due to growing evidence of its adverse effects on aquatic life and humans, the EU commission has suspended the authorization of ethoxyquin as a feed additive for all animal species and categories. On the other hands, new informations have been presented about using of ethoxyquin.

Table 1. Sources of important natural antioxidants

Compounds	Natural Source
Carotenoids	Dark leafy vegetable, carrots, sweet potatoes, yams, tomatoes, apricots, citrus fruits, kale, papaya
Catechins	Green tea, berries, certain oilseeds
Flavonoids (Polyphenols)	Oilseeds, lettuce, berries, eggplants, peppers, citrus fruits, cruciferous vegetables, onions, black tea
Lycopene	Tomatoes, papaya, watermelon, guava
Phenolic acids	Oilseeds and certain oils, cereals, grains
Vitamin C (ascorbic acid)	Fruits and vegetables, berries, citrus fruits, green peppers, potatoes
Vitamin E (tocopherols)	Oilseed, palm oil, nuts, eggs, dairy products, whole grains, vegetables, cereals, margarine and etc.
Extracts	Extract from green tea, rosemary, sage, clove, oregano, thyme, oat, rice bran and etc.

According to the regulation made by the European Commission on 5 August 2022, it is stated as follows: The existing authorisation of the additive ethoxyquin was suspended by Commission Implementing Regulation (EU) 2017/962.

In accordance with Implementing Regulation (EU) 2017/962, the suspension measure is to be reviewed by 31 December 2022 and in any event after the adoption by the Authority of a non-favourable opinion on the safety and efficacy of the additive ethoxyquin. (EU, 2022).

In the regulation made by the European Commission on October 5, 2022, it was stated that BHA can be used in other animal feeds except cats at the rate of 150 mg/kg. This rate should be; 150 mg of active substance / kg of complete feeding stuff with a moisture content of 12% moisture (EU, 2020). Also in the same regulation was stated that, BHA can be used in combination with butylated hydroxytoluene (BHT) up to 150 mg of the mixture/kg of complete feed (EU, 2020).

3. Antioxidants in Aquaculture

Nowadays, antioxidants prevent spoilage in foods and are added to every product consumed to keep them intact for a longer period of time. Among the food compounds, lipids have the highest risk of being oxidized. In general, oxidation of lipids occurs during improper storage, processing, heat treatment of raw materials, and packaging and storage of processed materials. During the oxidation formation, many compounds such as peroxides,

hydrocarbons, aldehydes, ketones, alcohols and acids are formed. These compounds lose their bitterness and sensory properties in foods, these changes reduce food quality and shorten shelf life (Turan et al., 2012).

Fish tissues are more susceptible to lipid peroxidation than other compounds due to the high content of polyunsaturated fatty acids. Among living things, animals have formed a major defense mechanism against in vivo peroxidation caused by antioxidant enzymes, endogenous antioxidants and nutritional antioxidants (Hamre et al., 2004). In order not to deteriorate the food quality and shorten the shelf life of seafood, an effective antioxidant is needed, especially to prevent deterioration of lipids in fish tissues.

Lipids in feeds can be subject to oxidation during production and subsequent under poor storage conditions (Cheng and Hardy, 2003). Oxidized feeds adversely affect the resistance of fish against diseases, at the same time they damage both growth and development and fillet quality (Sutton et al., 2006; Grigorakis et al., 2010). Oxidation of fish oil in feed can be determined by plasma glucose, cortisol and osmolarity levels of fish and impairs the stress response (Van Anholt et al., 2004; Alves Martins et al., 2007).

It has been determined that different effects occur in fish fed with oxidized feeds. For example, in a study conducted in sea bream, it was determined that the activity of some liver enzymes with antioxidant activity such as catalase and superoxide dismutase increased. (Mourente et al., 2002). It has been observed that the immune system is negatively affected in turbot fish fed with oxidized oil feed (Obach and Laurencin, 1992). In sea bass, more fragile erythrocytes and decrease the activity of lysozyme and the complement system (Obach et al., 1993). In addition, the most common adverse effect was low nutritional quality and reduced PUFA ratios in fish fillets (Alves Martins et al., 2007; Zhong et al., 2008).

Synthetic antioxidants such as BHT, BHA and ethoxyquin have been used in fish feeds for many years in order to prevent negative and undesirable effects in fish. (Hamre et al., 2010).

The use of synthetic and natural antioxidants in aquaculture feeds has been using on for many years. However, in recent years, the use of natural antioxidants has tended to increase due to restrictions and regulations in the use of synthetic antioxidants. According to Hernandez et al. (2014), they added BHT and natural plant oil to sea bream feeds and examined the oxidation state of the feeds. According to results of the study, RO and BHT feeds showed the highest protection against induced oxidation from week 8, and at the end of the storage period, RO feed was the least oxidized.

Natural plant antioxidant sources which containing high levels of phenolic compounds have been shown to reduce oxidation as effectively as synthetic antioxidants in fish fillets (Vargas-Sanchez et al., 2019). Different natural antioxidants (ascorbic acid, tocopherol mix, rosemary, ascorbyl palmitate and etc.) were added to the fish feeds obtained entirely from raw marine products and the oxidation status of the feeds were evaluated. At the end of the study, it was concluded that the feed can be protected against oxidation by using natural antioxidants (Hamre et al., 2010).

In general, fat-soluble antioxidants such as Vitamin E (α-tocopherol) in seafood play an important role in preventing the oxidation of unsaturated fatty acids found in high amounts in fish tissues and are the groups that have the greatest antioxidant activity. Therefore, α-tocopherol acetate is a derivative of Vitamin E and is an antioxidant used to reduce oxidation of lipids in foods in general and especially in seafood (Yıldız et al., 2006).

One of the micronutrients, Vitamin C (also known as L-ascorbic acid) is a non-enzymatic antioxidant and plays an important role in reducing oxidative stress (Narra et al., 2015). Most fish cannot synthesize vitamin C and must only obtain it from external sources. Vitamin C is a powerful antioxidant because it can be oxidized and converted to less reactive substances by most of the free radicals in aqueous solution (Kefer et al., 2009; Gombart et al., 2020).

It is a natural antioxidant of vegetable origin, such as vitamin A, ß-carotene, α-tocopherol, and it makes the antioxidant effect more effective in the presence of vitamin E. However, it is known that the retinol form and ß-carotene of vitamin A are not as resistant to oxidation as vitamin E (Bai et al., 1992). Although natural antioxidants originating from vitamins are used in fish feeds, herbal extracts, marine macro and micro plankton have also been included in studies in recent years.

In recent years, it has been understood that in addition to microalgae species, macroalgae also have compounds with high antioxidant effects. The antioxidant effect contains high levels of non-enzymatic antioxidant compounds such as glutathione, ascorbic acid, α-tocopherol, β-carotene, flavonoids, hydroquinones, phycocyanins, proline, mannitol, myoinositol, phenolic compounds and polyamines (Mallick and Mohn, 2000). For example, in a study conducted on a total of 17 macroalgae species, 11 from brown algae, 1 from green algae, and 5 from red algae, it was revealed that *Sargassum spp*. had the highest antioxidant effect (Matsukawa et al., 2000). Also, Catarino et al., (2023) stated that the brown algae *Sargassum spp* is increasing in popularity day by day, especially due to its exceptional antioxidant properties.

Especially seaweed phenolics have become an attractive, sustainable source of antioxidants with a range of biofunctional properties that could potentially replace existing aquatic feed additives (Gunathilake et al., 2022).

Algae polyphenolic compounds show effective activity in delaying fish oil rancidity by acting as a good antioxidant (Mukherjee and Pal, 2021).

In a study conducted on 8 species belonging to the genus Cystoseira distributed in the Mediterranean, it was determined that these species had high antioxidant activity (Ruberto, 2001). It has been reported that algae

species such as Cladophora, Chaetomorpha, Pithophora, Rhizoclonium, Spirulina, Leptolyngbya are used in feeding on goldfish and channel catfish and increase immunity as well as carotenoid content on fish (Promya et al., 2011; Mukherjee et al., 2019).

4. Conclusion

As a result, nowadays, people have started to stay away from foods containing synthetic, processed additives that are harmful to health. Likewise, as a result of the use of synthetic compounds in aquaculture, it has been revealed that there are negative effects on both fish fillet quality and human health. In recent years, it has been determined that natural antioxidants of plant origin give effective results. Therefore, it is thought that natural antioxidants will be preferred more in the future in order to prevent the oxidation of the feed used in aquaculture.

Author Contributions

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

	O.T.	C.E.
C	60	40
D	60	40
S	100	
L	50	50
W	60	40
CR	60	40
SR	60	40

C=Concept, D= design, S= supervision, L= literature search, W= writing, CR= critical review, SR= submission and revision.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

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

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ANALYSIS OF SELECTED MARKER STUDIES ON MINOR POACEAE FORAGES

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Abstract: Pastures are very important for agriculture since the main feeding source of the enormous cattle herd in the world is forage. Pastures comprise plants from several genera of grasses and legumes. In terms of the physical variety, ecology, and economic significance, the Poaceae is among the most significant families of Angiosperms. Regarding stress tolerance, species within this family exhibit a very wide range of variation. In recent years, the importance of using molecular markers in phylogenetic analyses of numerous organisms has increased. The development of genomic technologies and infrastructure has progressed sufficiently for their use in marker- aided selection (MAS) to be studied in several important perennial fodder species. Differences can be directly attributed to minor variations in the genetic code such as phenotype, single sequence repeats (SSRs), and single nucleotide polymorphisms (SNPs). Therefore, breeders can benefit significantly from developing and characterizing new genetic markers. This paper gives a brief analysis of some international studies on some minor Poacea forages.

Keywords: Markers, Forages, Poaceae, Minor

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1. Introduction

The emergence of several genetic markers as a result of molecular biology advancements has transformed our understanding of the structure and evolution of crop genomes. Finding genetic variety in crops provides a chance to comprehend the molecular basis of several biological occurrences (Adhikari et al., 2017). In the last years, the importance of using molecular markers in phylogenetic analyses of numerous organisms has increased. Fast DNA sequencing methods' accessibility and the advancement of reliable statistical analysis techniques gave this discipline a new start. Traditional classification systems of organisms based on morphology have major drawbacks despite still being frequently used. On the other hand, it seems that the classic morphology-based method for phylogenetic investigations can be supplemented via the use of molecular markers, despite recent popularity and the fact that they are not without flaws. Nuclear genome sequencing (NGS) and EST (expressed sequence tag) programs should enhance the number of genes used for phylogenetic analysis of crops, animals and microorganisms. As they exhibit significant commonality across a wide range of organisms, the power of genes involved in an organism's physiology, such as salt tolerance genes, the cell division (cdc) genes, heat shock genes, receptor genes, homeotic genes, etc., should also be investigated (Patwardhan et al., 2014).

Estimation of genetic diversity using a variety of methods is possible by dominant markers (such as DNA Amplification Fingerprinting (DAF), Random Amplified Polymorphic DNA (RAPD), Arbitrarily Primed Polymerase Chain Reaction (APPCR), Amplified Fragment Length Polymorphism (AFLP), Inter-Simple Sequence Repeat (ISSR) and co- dominant markers (such as Restricttion Fragment Length Polymorphism (STSs). New methods are always being developed today. Each method has its own benefits and drawbacks (Idrees and Irshad, 2014).

The development of molecular markers is now moving in new directions owing to public genomic databases, which have also changed the sorts of PCR-based procedures frequently employed in plant science. In addition to the frequently used DNA markers, different approaches have been proposed. Targeted fingerprinting methods include non-identical methodological improvements such as incorporating gene or promoter components into primers using amplified DNA technology applications. Although these semi-random markers have promising qualities, they can cause a collision and heterogeneous issues similar to those discovered with randomly generated fingerprints. Many elements in motion found in plant genomes can also be used to produce fingerprints. By exploiting certain targeted sites, these markers boost genome coverage and generate bands that primarily resemble one another. Another class of recently developed methods takes advantage of the

indicated length polymorphism in different transcript gene families, such as the cytochrome P450 and tubulin genes, to enable interspecies amplification and transferability. In addition, markers can be generated from functional and/or transcribed parts of the genome using different gene targeting methods associated with RNA information (Poczai et al., 2013).

2. Poaceae

Poaceae includes plants such as maize (*Zea mays*), wheat (*Triticum aestivum*), oats (*Avena sativa*), rice (*Oryza sativa*), rye (*Lolium perenne*), sorghum (*Sorghum bicolor*), and barley (*Hordeum vulgare*) that are the main source of nutrition for mankind and grazing animals (Guisinger et al., 2010). In terms of the physical variety, ecology, and economic significance, the Poaceae is among the most significant Angiosperm families. Regarding salinity tolerance, species within this family exhibit a very wide range of variation (Céccoli et al., 2015). Concerning the Poaceae family of cultivated plants, there has been considerable variation in low-temperature tolerance between and within individual species (Tondelli et al., 2011). Poaceae is the most important group of crops susceptible to abiotic stress. Several significant cultivable species exhibit distinct behaviors in response to abiotic stresses: wheat and rice are sensitive, displaying significant production reductions in response to water scarcity and soil salinity, whereas barley has a natural resistance to drought and salt (Landi et al., 2017). The most frequent abiotic stresses that crops can experience in fields are extreme temperatures, droughts, salt, and soil pollution; these changes serve as a general warning to plant productivity and survival, becoming more detrimental when combined. Several biological pathways, including sensing, signaling, transcriptional reprogramming, and protein changes, are activated in plants in response to such conditions. Plant cells typically undergo metabolic and transcriptional reprogramming in response to stress, which results in a global response that ultimately influences plant physiology and development (Chirivì and Betti, 2023).

3. Markers of Poacea Forages

Tropical and subtropical rangeland systems provide core ecosystem services for the welfare of human populations that depend on the availability and quality of fodder resources. Forage species, however, are still largely disregarded in molecular biology studies (Dell'Acqua et al., 2014). Some less- studied minor forage species were extracted from academic databases and summarized here below

3.1. *Themeda triandra*

Themeda triandra is a perennial C4 grass found in many subtropical and tropical regions including Asia, Africa, Australia and Türkiye. It is a source of wild grassy forage in rangeland habitats. As a forage researched primarily for soil remediation, as a competitor for foreign invasive species, and as a vegetation filter, some authors identify it as synonymous with Australian *T. triandra.* It is a member of the *Poaceae* family with a very high protein content. It is an important feed source for cattle and wildlife, particularly in Africa and other dry grasslands (Dell'Acqua et al., 2013) (Figure 1).

Figure 1. Frequently burnt eucalypt forest in Queensland (Avustralia), dominated by kangaroo grass (*Themeda triandra*) (Williams et al., 2022).

Dell'Acqua et al., (2014) presented the first molecular characterization of 71 different genotypes of Themeda. A total 65 of these were from three sites along an old cattle migration route in Kenya. Using AFLP markers gave a basic picture of the genetic variation in Kenya- originated *T. triandra*. To generate the Cluster Analysis unweighted pair group method with arithmetic mean (UPGMA) phylogram,366 polymorphic AFLP loci were employed in total. Based on the Jaccard similarity index, a UPGMA phylogram was generated for the genotypes examined in this study. The distribution of genomic variation was not necessarily consistent with the geographic sample of the population, according to the Principal Component Analysis (PCA) grouping of the genotypes examined in this work. The matrix incompatibility (MI) contribution was determined for each genotype to detect recombination and determine if the individuals used various reproductive techniques. There is statistical correlation between eleven AFLP loci and environmental variation. In Kenyan T. triandra populations, recombination rates, genetic diversity, and population genetic structure were all thoroughly studied.

3.2. *Andropogon* spp.

Andropogon spp. Is an excellent forage for both livestock and wildlife due to its palatability and high biomass which are important characteristics for the selection of monocultures suitable for grassland establishment or extensive grazing practices (USDA- NRSC, 2002) (Figure 2).

Figure 2. Clumps of mature flowering plants of gamba grass (*Andropogon gayanus*) showing a tussocky growth habit (Bebawi et al., 2018).

Akinyemi et al. (2021) studied the genetic diversity of 9 different Andropogon spp. obtained from Ogun State using four microsatellite markers (Xcup63, Phil227562, Xcup14 and CTM59). Using the DNA extraction method of Zymo spinTM technology, genomic DNA was isolated from the succulent leaf portion of Andropogon grass. Each and every locus-population is in Hardy-Weinberg equilibrium. At every locus, there were fewer viable alleles than noted alleles. For all markers of the grass taken into consideration, the determined heterozygosity was higher than expected.

3.3. *Bothriochloa ischaemum*

A bunchgrass with a wide natural distribution spanning Europe, Africa, and Asia is *Bothriochloa ischaemum* (Gabbard and Fowler, 2007). Microsatellite primers for *B. ischaemum* were generated in work by Matakis et al. (2011) to examine the structure of invasive populations in Texas and identify the source of introduction from the native region. They constructed an enhanced genomic library using the biotinylated nucleotide technique, and then used it to isolate and describe ten polymorphic microsatellite markers. Additionally, the primers were examined for amplification in the plants *Dichanthium annulatum*, *Andropogon gerardii*, *Bothriochloa saccharoides*, and *Schizachyrium scoparium var*. scoparium. Researchers oncluded that using microsatellite markers could help understand the path of dissemination, identify the origin of invading, populations, and develop biological control agents for *Bothriochloa ischaemum* invasive populations.

3.4. *Arrhenatherum elatius*

A perennial grass known as *Arrhenatherum elatius* has been introduced worldwide and can be seen growing in various of ecological settings. It is believed that this species was introduced after the cultivation of grasslands increased around the end of the Middle Ages rather than being a native of Central Europe (Michalski et al., 2010). Three taxa from the genus Arrhenatherum, including *A. elatius* subsp. elatius, *A. kotschyi*, and *A. palaestinum*, are listed in Flora of Türkiye (Cabi and Dogan, 2012). Michalski et al. (2010) examined 186 AFLP (amplified fragment length polymorphism) loci in 46 European accessions of A. elatius and discovered a significant level of genetic heterogeneity in this species.

Meng et al. (2011) used 100 inter-simple sequence repeat (ISSR) primers to examine the genetic diversity of 19 different *Arrhenatherum elatius* accessions, of which 11 produced unique amplification results. Out of the 152 bands detected, total 107 were polymorphic. The 19 A. elatius accessions were split into three groups with related circumstances based on the results of the PCA and UPGMA cluster analysis. Among the 19 *A. elatius* accessions under study, genetic distance and geographic distance were correlated.

3.5. *Rhodes grass (Chloris gayana)*

In all tropical and subtropical areas of the world, Rhodes grass is a significant tropical C4 grass. It is a high-yielding and high-quality grass that is either annual or perennial forage. It is also a cover crop to increase soil fertility and decrease soil nematodes (Cook et al., 2005). Diploid and tetraploid varieties of rhodes grass exist. They can vary in characteristics including growth pattern, flowering time, dry mass production, seed production, quality, and tolerance to salinity, frosts, and drought (Loch et al., 2004).

There is intra- and inter-cultivar diversity for the salt tolerance of rhodes grass. Taleisnik et al. (2021) cloned and analyzed plants of the Boma for salt tolerance at the seedling and late stages using AFLP and RAPD amplification patterns. For fingerprinting these clones, both methods were equally effective. Despite AFLP producing more bands, both methods had the same ratio of polymorphic bands, and the fraction presented only intolerant clones. These bands could serve as markers for aided selection, coupled with those only present in sensitive clones.

Negawo et al. (2021) used DArtSeq markers to characterize 104 Rhodes grass genotypes for conservation in the ILRI forage genebank to indicate the collection's population structure and genetic diversity and generate representative subset groups. As a result of the characterization, the average polymorphism information content was between 0.18 and 0.26, and a total of 193,988 SNP markers and 142,522 SilicoDArT markers were formed.

Hierarchical clustering using specific informative markers with a cophenetic correction coefficient of 82% resulted in three and two main clusters with SilicoDArT and SNP markers, respectively. The presence of two primary subpopulations employing both marker types, as revealed by a Bayesian population structure analysis, further demonstrated the collection's substantial genetic diversity. A representative subset of 21 inheritances from

different origins was developed using SNP markers.

In order to develop salt-tolerant clones, Ribotta et al. (2013) assessed the survival percentage under salt stress in 46 diploid and tetraploid clones of rhodesgrass (*Chloris gayana* K.). At 600 mM NaCl, fifteen clones were selected hydroponically. By using survival percentage, salt-tolerant rhodesgrass clones were produced. Using the AFLP approach, genetic diversity in a subset of clones was evaluated. Tetraploid and diploid clones could be distinguished using AFLP. Researchers observed genetic diversity at every ploidy level. Researchs chose Clone parents to produce new synthetic varieties.

3.6. *Agrostis* spp.

From a taxonomic standpoint, Agrostis is regarded as one of the most challenging and complex grass genera (Warnke, 2003). Because of the uniparental mode of transmission, chloroplast markers are helpful for identifying species, studying the evolution of hybrids in plant taxa, and dispersing seeds (Ennos et al., 1999). Within the Agrostis complex and the connected genera of Polypogon, Zapiola et al. (2010) generated 12 novel polymorphic chloroplast microsatellite markers to help identify species that received transgenic pollen.

Agrostis species that are utilized for turf have ambiguous genetic relationships. Between 150 and 200 Agrostis species are thought to exist, and interspecific hybridization is a method that has been used to improve one Agrostis species (Amundsen and Warnke, 2011). Recent research on the chromosomal pairing behavior of inter-specific hybrids has either supported or rejected previously postulated genetic links (Honig et al., 2016).

Using recently developed A. stolonifera microsatellite (SSR) markers, Honig et al. (2016) evaluated the genetic linkages among Agrostis cultivars and accessions. 74 Agrostis cultivars and accessions were utilized to genotype 16 individuals using nuclear SSR (nuSSR) and chloroplast SSR (cpSSR) markers. Agrostis species and cultivars can be distinguished by SSR markers, which are helpful in examining the genetic diversity and connections within the genus Agrostis. The species relationships proposed by Jones in the 1950s were most closely mirrored by genetic relationships based on SSR markers. NuSSR marker-based genetic linkages within the Agrostis species closely matched known pedigree links.

3.7. Timoty (*Phleum pratense* L.)

Phleum pratense L. is a perennial grass species that is cultivated in temperate regions of Europe, North America, and Asia. According to Tanhuanpää et al. (2016), the Nordic countries use timothy as their primary forage grass. The cool-season perennial grass species timothy, which can live in a short growing season and is resistant to frost and ice- encasement, is one of Norway's most significant forage grass species (Kovi et al., 2021). High output, feed quality, and winter survival are the key objectives of timothy breeding. Abiotic and biotic factors also contribute to winter injuries. Low-temperature parasitic fungi are one of the main causes of inadequate overwintering. *Typhula ishikariensis* Imai (syn. *T. idahoensis* Remsb.), speckled snow mold, is one of the most important pathogens in the cold climates of the northern hemisphere (Smith et al., 1989).

To break the forage yield plateau in breeding timothy (*Phleum pratense* L.), molecular markers may be useful (Tanaka et al., 2015). Using bulked-segregant analysis, conducted a study to identify DNA markers linked to timothy's resistance to *Typhula ishikariensis*. The cross of the Japanese sensitive cultivar Nosappu with the Finnish resistant cultivar Tammisto II resulted in a progeny of 161 F1 individuals. With a total of 292 primer combinations, resistant and susceptible bulks of eight individuals in each were examined. Together, these six DNA markers and their associations with resistance account for 15% of the phenotypic diversity in Typhula resistance. One linkage group, made up of four markers, contained a QTL that accounted for 7% of the variation in Typhula resistance.

3.8. *Paspalum* spp.

Paspalum is an important genus in the class *Paniceae*, with a complex taxonomic classification as well as diverse forage, ornamental, and weed commercial value. Different species of these plants have been preserved in germplasm banks and dispersed around the world, mostly for cultivar development and cytogenetic research, due to the great interest shown in many species of this genus. Accurate identification of germplasms and measurement of their variability is essential for their use in breeding programs and their appropriate containment (Cidade et al., 2013).

Silveira et al. (2022) conducted a study to measure a range of genetic parameters and predict yield increases in the P. notatum in-species hybrid population. High genetic variability for the production of fodder was evident in the genetic material under study. According to the analysis, new crosses should include 30N male parents and 132,332,336,437 crosses to improve dry matter production of P. notatum. Parents must be chosen from several groups to maximize genetic heterosis and variety. These parents must also be a part of diallel crosses. For a triat of interest, they constructed divergent groups containing genotypes, each distinctly identical, and allowed the selection of superior parents from each group. Selection os superior P. notatum forage hybrids for pastoral systems can be performed using different analyzes and genetic parameters estimated by REML (residual maximum probability). In plant breeding, multivariate analytics are essential tools.

A total of 214 isolates of Paspalum (177 were sampled from 35 species and 37 unclassified) were included in the study by (Cidade et al., 2013). Seventeen novels of SSR polymorphism loci were discovered for the Paspalum species under investigation. Of the 23 microsatellite primer pairs examined for transferability to other species, 12 (52%) amplified their loci in most species.

3. Conclusion

In recent years, the importance of using molecular markers in phylogenetic analyses of numerous organisms has increased. Genetic diversity, population genetic structure and recombination rates in populations of different species were investigated in detail in studies. Genetic distance and geographic distance were studied in many types of research. Some research also chose Clone parents to produce new synthetic varieties. Chloroplast microsatellite markers helped to identify species that received transgenic pollen. Accurate identification of germplasms and determination of their variability rates is essential for the development of more effective conservation and breeding programs. Adopting a molecular genetics method using microsatellite markers in the initial assessment of germplasms helps identify the species and assess the likelihood of successful hybridization.

Author Contributions

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

	B.B.	N.B.
C	50	50
D	50	50
S	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, 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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