

EMERGING TRENDS IN AGRICULTURE AND VETERINARY SCIENCES

Dr. Feyza DÖNDÜ BİLGİN



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PREFACE

Emerging consumer expectations, governmental regulations, technical advancements, innovation, and environmental concerns are some of the major forces influencing agricultural trends. The sector's trade exerts significant pressure on farmers to adapt and change. Additionally, the sector has had to adapt to the ongoing problem of changing seasonal conditions. The worldwide patterns of production and consumption are altering as a result of the Internet-driven digital economy. One answer to the difficulties facing agriculture due to climate change is climate-smart agriculture. Similar to earlier sustainability transitions, technological innovation is emphasised as being crucial, although adoption and spread of these advances are gradual in nations. Because these technologies can be utilised to address some of the crucial questions required to alter sustainable food systems and help us better understand global food security and nutrition, advanced technology and innovation are crucial for advancing sustainable food systems. In addition to being essential for preserving the quality and safety of fresh agricultural products and minimising losses, cold chain logistics also plays a significant role in boosting farmer income and fostering the revival of rural business. Globalisation, agroindustrialization, and international development provide a conceptual framework for comprehending the connections between new problems in developing nations and their implications for employment, poverty, and the environment.

Dr. Feyza DÖNDÜ BİLGİN

CHAPTER 1

OVERVIEW OF GENES, NUTRITIONAL GENOMICS, AND SOME GENOMIC DISEASES CONDUCTING PHENOTYPICAL DIVERSITY IN SHEEP

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INTRODUCTION

Adaptation of animal breeds to local climatic conditions is known to reduce environmental stress on animals, but it is known that it leads to an environmentally friendly production and is important for modern agriculture (Berman, 2011). Because sheep are vulnerable to climate change, they have spread and adapted to a wide variety of agro-ecological environments, especially those distributed in plateau, desert or humid regions (Easterling et al, 2000; Lv et al, 2015). Despite extensive study being done in the previous ten years, many elements of the sheep genome are still unknown (Abied et al, 2020). To properly utilize the information on the sheep genome that is currently accessible, extensive and useful application is required to boost sheep productivity and help develop better breeds of sheep. Various genotypes have been produced during the domestication of sheep (Chessa et al). Sheep's horns, tails, and fur colors are three significant phenotypic features that differ considerably. For instance, huge fat tails in sheep have been shown to develop as an adaptation to harsh environmental conditions and a lack of food. (Moradi et al, 2012; Deng et al, 2020). Various features including fur color, horns, tail, wool, ears, udder contribute to variation in sheep phenotypic characteristics (Deng et al, 2020).

Horns from sheep are typically utilized as defense against predators (Johnston et al, 2012). Significant correlations exist between a number of phenotypic features and production and animal welfare metrics. For quick and direct selection breeding of sheep breeds, it is crucial to

comprehend the genetic basis of these phenotypic features (Kalds et al, 2022).

GENETIC ASPECTS OF PHENOTYPIC TRAITS

SHEEP COAT COLOR

The coat and color of sheep has long been the subject of research to understand its biological and genetic basis (Kalds et al, 2022). Sheep's fur colors vary significantly between and between breeds. (Koseniuk et al, 2018). Parsons et al. (1999) examined the ovine homologous location of the Agouti gene using comparative mapping data to identify pigmentation-related genes in the sheep genome. According to the study, the Australian Merino sheep's recessive pigmentation locus is situated in OAR13 in a location that is similar to the Agouti gene locus regions on murine chromosome 2 and human chromosome 20. (Beraldi et al, 2006). The Extension locus, which contains the MC1R gene in addition to the Agouti locus, which encodes the Agouti gene, has also been demonstrated to play a role in the development of coat color in sheep. (Klunglad and Vage, 2003). The reverse of Agouti is involved; the dark coat phenotype is brought on by its high functional expression (Vage et al, 1999).

EAR SIZE

The length, drooping, and steepness of sheep ears vary widely and are thought to be breed-specific. It is thought that MSRB3 has a role in determining ear phenotype in sheep (Wei et al, 2015).

CURLY FLEECE

Kıvrık sheep, which is one of the medium-sized sheep breeds in the world, has both meat and dairy types, and has attracted attention in terms of combined productivity in recent years. The phenotype in the beef breed is broad, rounded, muscular body and chest. The rump is slightly inclined, the legs are short (Zhao et al, 2021).

Although the dairy breed has a narrow chest and body structure, the legs are long. The dorsal line is slightly concave. They usually have a white body, but are available in black and tawny colors (Liu et al, 2018). The head, underbelly, legs, and in some cases, the neck region are devoid of fleece. It has a coarse mixed fleece type (Liu et al., 2022). Females are hornless, males have spiral horns. It has a long, thin, lean tail structure and is covered with long hairs. While the tail extends down from the tarsus joint in some animals, it extends up to the tarsal joint in others (Wu et al, 2021).

TAIL

Sheep have developed the ability to store fat in their tails to survive in a harsh environment (Pan et al, 2019). Large-scale genetic investigations have been carried out recently to clarify phenotypic variations in sheep tail formation (Li et al, 2020). Multiple comparisons consistently chose the PDGFFD gene. Its manifestation differed across breeds of fat- and thin-tailed sheep. Investigations into allele gene-specific expression (PEE) in thick-rumped Kazakh sheep and thin-tailed Texel sheep (Wang et al, 2022). The study found 2405 PEE genes

in adipose tissue; it has been noted that PDGFFD and LYPLA1 are likely connected to biological processes involving lipids. Given the wide variation in tail phenotype and associated fat deposition levels among sheep breeds and geographical regions, it is likely that several genes affect how sheep tails look. (Kalds et al, 2022).

BODY HEIGHT AND STATURE

Proportional or disproportionate dwarfism is among the conditions that affect the sheep phenotype (Thompson et al, 2008; Boegheim et al, 2017). Dwarfism and angular deformations of the forelimbs have been reported as the result of a 1-bp deletion at position 107 in exon 3 of the SLC13A1 gene in Texel sheep.

NUMBER OF NIPPLES

The extra teat/nipple phenotype was observed and documented in sheep a century ago (Bell, 1899; Phillips et al, 1946). However, it has been argued that this feature has no practical value in sheep production (Phillips et al., 1945). It is also recognized as a morphological defect with adverse effects on overall udder health (Hardwick et al, 2020). PHGDH, KDM3A, GLIS3, FSHR, CSNV2, CSN1S1 and ROBO2 have been reported to be associated with udder and nipple development in sheep (Li et al, 2020).

GENOMIC DISEASES

GASTROINTESTINAL INTERFERENCE RESISTANCE

Infections with gastrointestinal parasites (GIPs) rank among the most significant health issues in the pasture livestock system (Coles et al, 2005). The three parasites that are most frequently seen in sheep in Turkey are nematodes (*Trichostrongylus* spp.), coccidians (*Eimeria* spp.), and tapeworms (*Moniezia* spp.) Studies on the genetic parameter estimate of phenotypes of gastrointestinal parasite infection resistance have demonstrated that gastrointestinal nematode resistance in several sheep breeds has low to moderate heritability (Mpetile et al, 2015; Berton et al, 2019; Gowane et al, 2019).

SCRAPIE

Sheep scrapie is one of a group of diseases known as transmissible spongiform encephalopathies (BSE) or prion diseases that affect various mammalian species (Balton et al, 1982). Infectious substance is called prion (Prusiner, 1998). The hallmark of BSEs is the detection of an abnormal form of the prion protein in the brain and sometimes in lymphoreticular tissues. The hallmark of the infection is the conversion of the normal cellular form of the prion protein to a misfolded, disease-associated form (PrP^{Sc}) with a proteinase K resistant nucleus (PrP^{Pres}) (Prusiner, 1998).

To reduce scrapie in sheep and goat herds, breeding efforts examining host prion protein-encoding gene (PRNP) variations that confer resistance to scrapie are helpful. (Belt et al, 1995). Scrapie resistance is

connected with the sheep genotype A136R154R171 (Hunter et al, 1997).

NUTRITIONAL GENOMICS

NUTRIGENOMIC

Feeding is the most important factor affecting the health status and yield characteristics of animals among environmental factors. Nutrigenomics is a relatively new topic that deals with animal nutrition at the molecular level (Pal, 2022). It meticulously selects foods for better expression of genes that match animal genotypes for health, productivity, and the environment (Wu, 2022). Nutrigenomics integrates nutrition, molecular biology, genomics, bioinformatics, molecular medicine and epidemiology to better understand the role of food as an epigenetic factor in the emergence of these diseases (Malgwi et al, 2022). In recent years, depending on the developments in the field of molecular genetics, increasing knowledge about the compositions and functions of genomes has begun to be transferred to practice. These developments have made it possible to understand how nutrients change gene and protein expression and how they affect cell and organism metabolism (Hag et al, 2022).

CONCLUSION

As a result of the research, several genes known to play a role in determining the sheep phenotype variation, diseases and nutritional genomics are discussed. Understanding the genetic basis of phenotypic trait variation in sheep is of great importance. It is highly correlated

with the characteristics of production and welfare. Because they are the determinants of race. The maintenance of health and the prevention or postponement of disease are caused by nutrient-gene interactions. For a particular genotype, unbalanced diet increases the risk of developing or exacerbating a number of age-related disorders, including obesity, diabetes, and cardiovascular disease. Many of the genes investigated here have unclear genetic ties and expression patterns.. More comprehensive studies are needed to understand them. It is thought that it will contribute to the development of sheep breeding by defining the expression characteristics of genes, genomic-based selection and modern molecular genetic tools.

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CHAPTER 2

**USE OF PHYTOBIOTICS AS NATURAL FEED ADDITIVES IN
LAYING HENS**

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INTRODUCTION

It is known that animal products should be consumed in addition to plant products for an adequate and balanced diet (Ndlovu, 2010). This is based on the fact that the daily protein requirement is met from plant and animal products about half-half (40-50%) (Sarı et al., 2022). Animal products are an important source of quality protein as well as minerals and vitamins (Ndlovu, 2010).

Eggs are very valuable in terms of many nutrients, especially protein (Mine, 2014; Puglisi and Fernandez, 2022). Almost all of the proteins in eggs can be converted into body proteins (Yiğit et al., 2000). Eggs are also rich in many minerals, especially iron and phosphorus, and fat-soluble vitamins (A, D, E, K) and B-complex vitamins (Açıkğöz and Önenç, 2006).

Food additives are used for various purposes such as stimulating growth, enhance product quality and protect animal health in poultry nutrition. The interest in plant-based ones has increased in recent years because they are natural.

This chapter aims to provide detailed information about phytobiotics, their importance and potential uses in laying hens.

FEED ADDITIVES

Feed additives are substances that increase the performance of livestock, improve feed efficiency, increase product quality and protect the health of the population (Pandey et al., 2019). There are also additives used to give form to feed, that is, to improve pellet quality (Ergün et al., 2013).

Furthermore, some additives protect the nutrients in the feed against oxidation (Eray et al., 2017) and molds (Melin et al., 2007; Nabawy et al., 2014) during transportation and storage. Feed additives have an important place in poultry nutrition due to these positive effects.

ANTIBIOTICS AND SYNTHETIC ANTIOXIDANTS

Antibiotics are defined as substances that stop the growth of some microorganisms or destroy them (Darwish et al., 2013).

The use of antibiotics at sub-therapeutic levels as additives in animal nutrition has provided very important contributions such as increasing productivity and protecting animal health (Lagha et al., 2017). However, the use of antibiotics as growth promoters has been banned since 2006 because of the development of bacterial resistance and residues in animal products in the long-term use of antibiotics in animal nutrition (Apatha, 2009). Following this ban, researchers have turned to safe additives that can be an alternative to antibiotics (Murugesan et al., 2015). There are studies investigating the possibilities of using probiotics (Mazanko et al., 2018; Mohammed et al., 2021), prebiotics (Froebel et al., 2019; Kimminau et al., 2021), organic acids (Akaichi et al., 2022; Rezaeipour et al., 2022), enzymes (Shehata et al., 2018; White et al., 2021) and phytobiotics (Alagawany and Abd El-Hack, 2015; Malekzadeh et al., 2018) as an alternative to antibiotics in poultry.

Antioxidants are compounds that slow down or prevent oxidation (Dai and Mumper, 2010). The classification of antioxidants is given in Figure 1 (Basiouni et al., 2023).

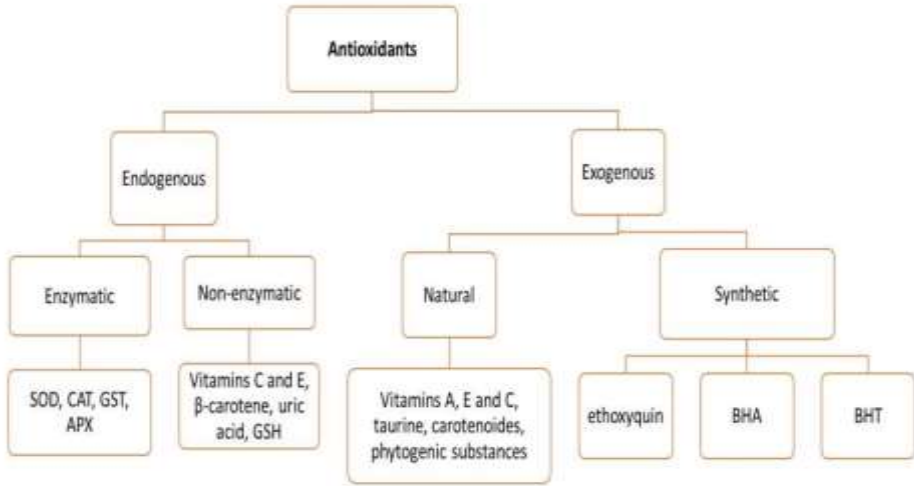


Figure 1. Classification of antioxidants (Basiouni et al., 2023).

(BHT: Butylated hydroxytoluene, BHA: Butylated hydroxyanisole, SOD: Superoxide dismutase, CAT: Catalase, GST: Glutathione S-transferase, APX, Ascorbate peroxidase; GSH, Glutathione)

Butylated hydroxytoluene, butylated hydroxyanisole, tertiary butyl hydroquinone (TBHQ) and propyl gallate (PG) are synthetic antioxidants (Al-Harathi, 2014). The use of synthetic antioxidants as additives is controversial (Al-Harathi, 2014) due to their possible toxic effects (Karre et al., 2013). They have been reported to cause diseases such as cancer (Fahmy and Abdel-Tawab, 2021). These negative properties have increased the interest in alternative antioxidants that can replace them (Rietjens et al., 2002). One of the most important natural antioxidants studied as an alternative to synthetic antioxidants is plants (Candan and Bağdatlı, 2017).

PHYTHOBIOTICS

Phytobiotics or phytochemicals are commonly known as herbal products (Bagno et al., 2018). These include various herbs and spices, as well as extracts and essential oils derived from them (Windisch et al., 2008).

Plants contain various secondary metabolites (Acamovic and Brooker, 2005). Secondary metabolites are also known as phytochemicals (Shakya, 2016). They can be found in parts of plants such as leaves, roots, stems and bark (Anulika et al., 2016).

The steps used in the extraction of phytochemicals from plant sources are given in Figure 2 (Bitwell et al., 2023).

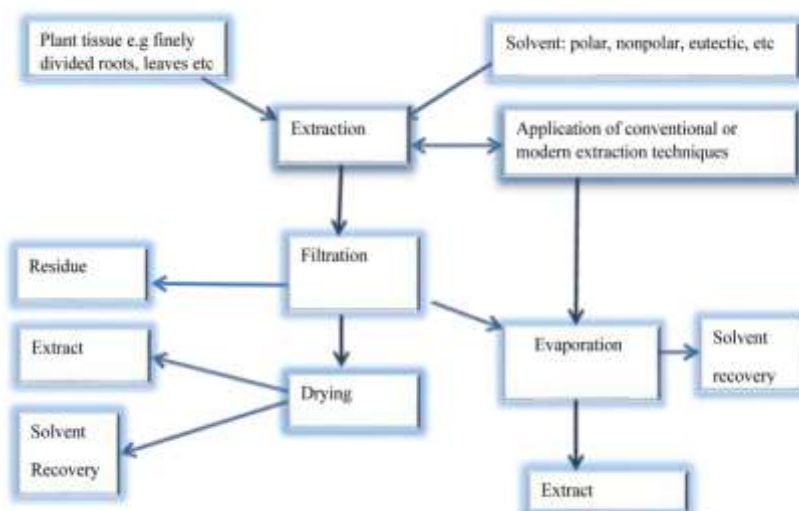


Figure 2. Steps in the extraction of phytochemicals from plants (Bitwell et al., 2023).

It has been reported that alkaloids, flavonoids, tannins, glycosides and saponins are among the most important secondary metabolites in plants (Geetha and Geetha, 2014; Shakya, 2016). These metabolites have

various important activities such as antioxidant, antibacterial, antiviral, antifungal, antiallergic and anti-inflammatory (Ram et al., 2015).

The activity of some secondary metabolites in plants is given in Table 1.

Table 1. Some secondary metabolites and their activities

Secondary Metabolites	Effect/Activity	References
Phenols ve Flavonoids	Antioxidant	Murugan and Mohan (2014) Ram et al. (2015) Shakya (2016)
	Antibacterial	Murugan and Mohan (2014) Ram et al. (2015) Shakya (2016)
	Antiallergic	Murugan and Mohan (2014) Ram et al. (2015) Shakya (2016)
Alkaloids	Antispasmodic	Shakya (2016)
	Analgesic	Ram et al. (2015) Anulika et al., 2016 Shakya (2016)
	Antimalarial	Ram et al. (2015) Anulika et al., 2016 Shakya (2016)
Saponins	Anti-inflammatory	Ram et al. (2015) Shakya (2016)
	Antiviral	Ram et al. (2015) Shakya (2016)
Terpenoids	Antiviral	Ram et al. (2015) Shakya (2016)
	Antihelmintic	Ram et al. (2015) Shakya (2016)
	Anticancer	Ram et al. (2015) Shakya (2016)
	Anti-inflammatory	Ram et al. (2015) Shakya (2016)
Glycosides	Antifungal	Ram et al. (2015) Shakya (2016)
	Antibacterial	Ram et al. (2015) Shakya (2016)

The active substance content of phytobiotics varies widely depending on which part of the plant (seed, leaf, root and bark) is used, harvest time and the geographical region where the plant is obtained (Muthusamy and Sankar, 2015).

The increase in demand for phytobiotics is because they are natural, environmentally friendly and generally safe products (Christaki et al., 2012).

Phytobiotics can be used alone or in mixtures in animal nutrition (Grashorn, 2010). Phytogetic feed additives have been reported to increase livestock performance (Windisch and Kroismayr, 2006) and improve product quality (Windisch et al., 2008).

The general properties/effects of phytobiotics used in animal nutrition are given in Figure 3 (Hashemi and Davoodi, 2011).

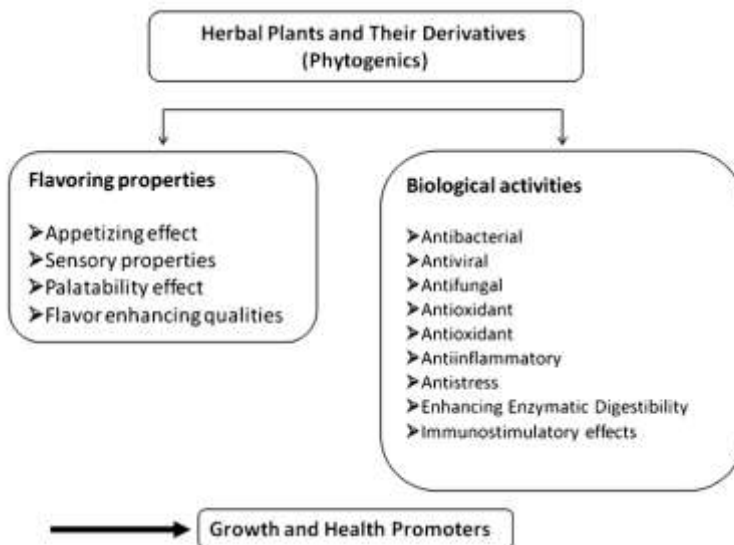


Figure 3. Effects of phytogenics in animal nutrition (Hashemi and Davoodi, 2011).

USE OF PHYTOBIOTICS IN LAYING HENS

There are different studies conducted on the use of some phytochemicals such as oregano (He et al., 2017; Migliorini et al., 2019; Feng et al., 2021), thyme (Kaya and Turgut, 2012; Cimrin, 2019; Yalçın et al., 2020), sage (Kaya and Turgut, 2012; Galamatis et al., 2021), mentha (Kaya and Turgut, 2012; Torki et al., 2021), anise (Yu et al., 2018), black cumin (Aydın et al., 2008; Ghosh et al., 2020), coriander (Çiftçi and Macit, 2018), rosemary (Alagawany and El-Hack, 2015; Cimrin, 2019; Abo-Ghanima et al., 2020), lavender (Torki et al., 2021), garlic (Omer et al., 2019; Ghosh et al., 2020), cinnamon (Abo-Ghanima et al., 2020), wild leek (Kılınç et al., 2023), fennel (Gharaghani et al., 2015), maca (Korkmaz et al., 2016) and jujube (Kılınç et al., 2020) in laying hens.

Some studies on the use of phytochemicals in laying hens are summarized in Table 2.

Table 2. Some studies on the use of phytobiotics in laying hens

Phytobiotics	Form	Dose	Treatment Effects	References
Oregano	Essential oil (EO)	0, 50, 100 and 150 mg/kg	<ul style="list-style-type: none"> - Improved laying rate, average egg weight, feed conversion ratio (100 mg/kg EO) - Increased number of intestinal <i>Bifidobacterium</i> and <i>Lactobacillus</i> - Decreased intestinal <i>Escherichia coli</i> and <i>Salmonella</i> - Increased duodenum villus height - No effects in egg weight, relative eggshell weight, yolk index and Haugh unit value 	He et al. (2017)
Oregano	Essential oil (EO)	0, 50, 100, 150 and 200 mg/kg	<ul style="list-style-type: none"> - Increased serum levels of total proteins and globulins (after 28 days) (150 and 200 mg/kg levels EO) 	Migliorini et al. (2019)
Oregano	Essential oil (EO)	0, 100, 200 and 400 mg/kg	<ul style="list-style-type: none"> - Improved feed conversion ratio (200 mg/kg EO) - Higher egg shell thickness - Lower yolk cholesterol and total saturated fatty acids (%2 thyme) 	Feng et al. (2021)

Thyme	Leaves (dried)	0, 1 and 2%	<ul style="list-style-type: none"> - Increased omega-3 fatty acids (% 2 thyme) - Reduced the yolk malondialdehyde (MDA), blood serum cholesterol and triglycerides levels 	Yalçın et al. (2020)
Thyme Sage Mentha	Extract	0, 150 and 300 ppm	<ul style="list-style-type: none"> - No effects in egg weight, feed conversion rate, shell thickness, shape index, albumen index, Haugh unit and yolk colour - Increased egg production, egg shell strenght and shell weight - Decreased cracked egg rate 	Kaya and Turgut (2012)
Thyme Rosemary	Extract	0 and 1000 mg/kg	<ul style="list-style-type: none"> - Increased egg weight and reduced egg production (thyme) - Reduced the feed intake (rosemary) - Decreased blood serum cholesterol (thyme) - Decreased blood serum triglyceride (both thyme and rosemary) - No effects on serum gamma glutamyl transferase (GGT), alanine 	Cimrin (2019)

Commercial product	Essential oil (encapsulated mix) (EO)	0, 50, 100 and 200 mg/kg	<p>aminotransferase (ALT) and P levels</p> <ul style="list-style-type: none"> - Increased serum aspartate aminotransferase (AST) concentration - Increased serum creatinine (200 mg/kg EO) and decreased Ca concentration (100 mg/kg EO) - Improved alpha-naphthyl acetate esterase and acid phosphatase positive peripheral blood lymphocyte ratios (100 and 200 mg/kg EO) - Improved intestinal morphology (100 and 200 mg/kg EO) 	Arslan et al. (2022)
Commercial product	Essential oil (encapsulated) + Organic acids (EOA)	0, 150, 300 and 450 mg/kg	<ul style="list-style-type: none"> - Increased laying rate (150 mg/kg EOA) (week 21 to 25) - Increased ileal villus height (150 and 300 mg/kg EOA) (week 30) - Decreased secretory immunoglobulin in ileum - Increased the counts of 	Wang et al. (2019)

			<i>Bifidobacterium</i> in cecal (week 25 to 30).	
Coriander	Oil	0, 0.1, 0.3 and 0.5%	<ul style="list-style-type: none"> - No effects in feed conversion rate, feed intake, and cracked egg rate - Increased egg production - Decreased egg weight - Decreased serum cholesterol level - No effect on yolk TBARS (thiobarbituric acid reactive substances) value - Increased lactobacilli counts in the jejunum (90 and 135 mg/kg) - No effects on total coliform bacteria counts (jejunum) - No effects in blood serum albumin, glucose, phosphorus levels and GGT and AST enzyme activities - Lower egg yolk MDA levels (both 0.5% and 1.0% powder levels) - Lower the total number of <i>Enterobacteriaceae</i> in eggshells (1.0% powder levels) 	Çiftçi and Macit (2018)
Jujube	Extract (leaf)	0, 45, 90 and 135 mg/kg		Kılınç et al. (2020)
Sage	Powder	0, 0.5 and 1%		Galamatis et al. (2021)

Maca	Powder	0, 5 and 10 g/kg	<ul style="list-style-type: none"> - No effects in yolk cholesterol content - No effects on some serum parameters (total triglyceride, total cholesterol, glucose, calcium, phosphorus, progesterone, oestradiol - Decreased serum magnesium levels - Increased serum glutathione peroxidase (10 g/kg Maca powder) 	Korkmaz et al. (2016)
St John's wort (SJW) Grape (G)	Extract (SJW) Seed oil (G)	0, 100, 200 and 300 mg/kg	<ul style="list-style-type: none"> - No effects on performance parameters. - Increased egg shell weight (300 mg/kg G and 100 mg/kg SJW levels) - Increased egg albumen index and Haugh unit (100 mg/kg G) - No effects on some blood serum parameters (total cholesterol, triglyceride, LDL, AST, Ca and P) - No effects on body weight changes, egg weight, feed intake, shape index, yolk index, Haugh unit, egg 	Kilinc and Karaoglu (2020)

Mentha Lavender	Essential oil (EO)	0 and 250 mg/kg	shell thickness and shell weight - Increased egg production (lavander-supplementation) - No effects in egg mass, egg weight, egg product and feed conversion (day 29 to day 56) - Improved egg weight and mass, feed intake (day 1 to day 28) - Increased T-SOD (total superoxide dismutase) in serum (day 28 and day 56) - Increased glutathione peroxidase (GSH-PX) in serum (day 56) - Increased GSH-PX (day 28 and day 56) in liver - Increased total antioxidant capacity (T-AOC) in serum and liver (day 56) - Reduced MDA levels in liver (day 28 and day 56) - Decreased the yolk TBARS value (Day 0) - The highest egg weight in 400 mg/kg root powder group	Torki et al. (2021)
Star anise	Essential oil (EO)	0, 200, 400 and 600 mg/kg		Yu et al. (2018)
Ashwagandha	Root powder	0, 200, 400 and 600 mg/kg		Kılınç (2023)

				<ul style="list-style-type: none"> - The highest albumen index, shell weight, Haugh unit in 200 mg/kg root powder group - Improved feed intake (0.75% and 1% powder) - Increased egg mass egg production - No effects on feed conversion ratio (FCR) and egg weight 	Sandeep et al. (2020)
Ashwagandha	Root powder	0, 0.25, 0.5, 0.75 and 1%		<ul style="list-style-type: none"> - Improved the egg production and weight, feed intake and conversion, Haugh unit (both rosemary and cinnamon EO) - Improved some blood serum parameters [cholesterol, urea, alanine aminotransferase (ALT), aspartate aminotransferase (AST), P, Ca], immunity and antioxidant parameters (both rosemary and cinnamon EO) - No effects on live weight, feed conversion ratio, feed consumption, organ 	Abo-Ghanima et al. (2020)
Rosemary Cinnamon	Essential Oil (EO)	0 and 300 mg/kg			

Black cumin	Seed	0, 1, 2 and 3%	weights, and abdominal adipose tissue - Increased egg production (3% seed) - Increased egg weight (2 and 3% seed) - Greater egg shell strength (3% seed) - Decreased yolk cholesterol (2 and 3% seed)	Aydın et al. (2008)
Garlic Black cumin Turmeric	Powder	0 and 1% powder (combined) + 1% linseed oil 2% linseed oil 2.5% linseed oil	- No effects on body weight gain and feed conversion ratio - Increased egg production, egg weight and feed intake	Ghosh et al. (2020)
Garlic Onion	Powder	0, 0.5 and 1%	- No effects in egg weight and consumption/hen/day (both single and combined powder) - Improved egg production, feed conversion, egg mass/hen - No effects on egg shape index, albumin, shell percentages, shell thickness and Haugh unit	Omer et al. (2019)

			<ul style="list-style-type: none"> - Increased egg weight - Decreased serum total cholesterol concentration (single and mix powder) - No effects on nutritional properties of eggs were not affected - Increased egg weight (improved productivity) - Modulator effects on intestinal microbiota (increased <i>Lactobacillus</i> spp. and <i>Bifidobacterium</i>, reduced Enterobacteriaceae populations) 		<p style="text-align: center;">Abad et al. (2020)</p>
<p style="text-align: center;">Allium extract (Commercial Product)</p>	<p style="text-align: center;">Extract (rich in propyl propane thiosulfonate)</p>	<p style="text-align: center;">0 and 1000 mg/kg</p>	<ul style="list-style-type: none"> - Lower serum cholesterol and triglycerides in R, D and C-supplemented groups (under both thermoneutral and heat stress condition) - Increased plasma glutathione peroxidase activity [R+D+C (combination)] - No effects on live body weight, feed conversion ratio, feed consumption and egg weight 		<p style="text-align: center;">Torki et al. (2018)</p>
<p style="text-align: center;">Rosemary (R) Dill (D) Chicory (C)</p>	<p style="text-align: center;">Essential oil (R, D) and Extract (C)</p>	<p style="text-align: center;">0 and 20 ml/100 kg (R) 0 and 15 ml/100 kg (D) 0 and 250 ml/100 kg (C)</p>			

Rosemary	Powder	0, 3, 6 and 9 g/kg	<ul style="list-style-type: none"> - Increased egg numbers and egg mass - Affected serum total cholesterol, IgA and IgM (immunoglobulin A and M) concentrations 	Alagawany and El-Hack (2015)
Wild leek	Powder	0, 1, 2 and 3 g/kg	<ul style="list-style-type: none"> - No effects on egg production, feed intake, internal and external egg quality trait, and egg yolk cholesterol concentration - No effects on MDA and total antioxidant-oxidant status of egg yolk - Increased 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity in yolk - Decreased oxidative stress index in egg yolk - Reduced serum cholesterol concentration - Increased superoxide dismutase enzyme activity level (3 g/kg powder) and total antioxidant status level (2 g/kg and 3 g/kg powders) 	Kılınç et al. (2023)

Fennel	Fruit	0, 10 and 20 g/kg	<ul style="list-style-type: none"> - Increased MDA and carbonyl egg contents under heat stress - Reduced the values of both parameters (MDA and carbonyl egg contents) in hens fed fennel-supplemented (under heat stress) - No effects on yolk cholesterol level and increased yolk triglyceride levels under heat stress - Lower yolk cholesterol and triglyceride levels in hens fed fennel-supplemented (under heat stress) 	Charaghani et al. (2015)
Turmeric (T) Fennel (F) Black pepper (BP)	Essential oil	0 and 1% (single) 0 and 0.5% (combined) (T+F, BP+T, BP+T)	<ul style="list-style-type: none"> - Improved egg weight, hen-day-production and Haugh unit (combined supplementation of phytobiotics) - No significant differences chemical composition of eggs - Lower Cl and K in eggs - Increased Ca, Zn, S, and Cu contents in eggs 	Samantaray and Nayak (2023)

			(combined supplementation of phytobiotics)	
Mrytus Vitex	Essential oil (EO)	0 and 2 ml/kg (single) 0 and 1 ml/kg (combined)	<ul style="list-style-type: none"> - No effects on body weight, egg production, feed intake and feed efficiency (alone and mix EO) - Decreased egg specific gravity (vitex-supplemented) - No effects on some egg quality parameters (Haugh unit, albumen index, egg shell thickness, yolk index) - Decreased egg yolk color (EO mix) 	Karakullukçu et al. (2016)
Nettle	Powder	0 and 15%	<ul style="list-style-type: none"> - Increased feed intake, shell thickness and yolk color - Lower cholesterol contents of the yolk - Increased the total n-3 PUFA concentration in the yolk and reduced the ratio of n-6/n-3 - Improved performance and egg quality - Alleviated the effect of challenge (morphology, cell 	Zhang et al. (2020)

<i>Yucca schidigera</i>	Extract (E)	0 and 500 mg/kg (E) (Note: Half of the hens in each group were orally administrated with <i>Clostridium perfringens</i> type A and coccidia)	apoptosis, antioxidant capacity in the jejunum	Mao et al. (2023)
Ginger Fennel	Essential oil (EO)	0 and 300 mg/kg	<ul style="list-style-type: none"> - No effects on productive performance - No effects in egg shape index and yolk index - Decreased Haugh unit (fennel-supplemented) - Improved egg shell weight and thickness (EO) - No effects in white blood cells (except monocyte count) - Higher the monocyte level (fennel EO) - No effects on some blood biochemistry parameters (Cholesterol, triglyceride, HDL and LDL) - Increased feed consumption (1%) 	Nasiroleslami and Torki (2010)

Ginger	Dried fermented	1 and 5%	<ul style="list-style-type: none"> - Better hen-day egg production and egg mass - No effects on shell thickness, egg shell breaking strength, shell ratio, yolk color, albumen ratio, yolk ratio, and Haugh unit - Tended to be higher villus area, villus height, cell area and cell mitosis in all (duodenum, jejunum and ileum) intestinal segments 	Incharoen and Yamauchi (2009)
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CONCLUSION

It is necessary to add some substances to the diets to reduce production costs by increasing feed efficiency, improve product quality and protect the health of the population in poultry nutrition, as in other livestock animals. Since some of these substances, which are called feed additives, are controversial and limited in use due to their synthetic properties and some of them are completely banned, the interest of researchers and producers in natural additives has increased. One of these natural additives is phytobiotics.

According to the literature review, there are many studies reporting the effects of phytobiotics on performance, product (meat, egg) quality, sensory properties, oxidation parameters, oxidative stress, intestinal microbiology and histomorphology, and some immune and serum biochemistry parameters in poultry. There are also studies that examined the effects of these substances in poultry under heat stress. Also, recently, especially encapsulation studies have come to the forefront. There are also studies investigating the use of herbal extracts, essential oils and various active substances in poultry by encapsulating them.

It was seen in some of the studies summarized in this chapter that different forms and doses of various plants have statistically significant effects on a number of parameters in poultry, and some do not have statistically significant effects.

In conclusion, the use of plants as an alternative to antibiotics and synthetic antioxidants is promising. However, being natural does not mean that plants are always safe. It must be taken into account that they

may also have negative impacts above certain doses. For this reason, dose studies of phytobiotics must be conducted more extensively and their interactions with other substances in the feed must be supported with detailed studies. It is also considered that it would be useful to investigate more comprehensive methods such as encapsulation, which is used to protect the bioactive components of plants from some factors, such as oxygen, heat and light.

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CHAPTER 3

DETERMINATION of LEAD in the BLOOD of WILD BIRDS

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1. INTRODUCTION

The rapidly increasing industrial developments and globalization in the world cause environmental pollution [1]. Lead from heavy metals has an important place in environmental pollution [2,3]. Lead (Pb) is not required for the maintenance of physiological functions in living organisms and they are classified as toxic for many organisms [4]. Wild birds are very vulnerable to environmental pollutants such as heavy metals that enter the food chain easily [5]. While a high level of heavy metal exposure causes death in birds, a low level of heavy metal exposure may lead to teratogenicity, mutagenicity, and carcinogenicity, suppress the immune system, impair body coordination and cause reproductive complications [6,7]. Birds are commonly used as bioindicator organisms in monitoring environmental pollution [3,8]. The most important route that affects the intake of heavy metals by living organisms is their feeding habits. The feeding habits of many bird species are employed in monitoring ecological changes. The food types preferred in feeding habits (seed, fruit, mice, insect, worm, mole, egg, frog, lizard, fish and birds, etc.) and stones such as sands and pebbles they prefer for their gizzards are the means of heavy metal exposure [2,9–11]. The other ways of heavy metal intake to the living organism body are habitat (locations near human activities) and gunshot pellets found in the bodies of the prey of the organisms with carnivore feeding habits. The gunshot pellets can stay in the gizzard of the waterbirds for more than 20 days after once ingested [12,13]. In birds, a blood Pb concentration of ≤ 150 ppb is defined as the absence of Pb exposure [14], whereas blood Pb concentrations of 200-500 ppb, 500-1000 ppb, and >1000 ppb are defined

as the subclinical poisoning range, clinical poisoning range, and severe lead poisoning dose, respectively [12]. Also, the threshold value for Pb concentration has been determined as 500-1000 ppb for waterbirds [15]. In the world and Türkiye, numerous studies have been conducted using birds as the biological materials (egg shell, tissue, feather, faecal pellets, and blood) in the determination of environmental pollution [8,16–21]. Blood is preferred as the biological material to identify the inorganic pollutants since blood analysis is fast, riskless, and reproducible [8]. Various devices (inductively coupled plasma mass spectrometry, ICP-MS; Atomic absorption spectrometer, AAS; Inductively coupled optic emission spectrometry, ICP-OES; Graphite Furnace Atomic Absorption Spectrometry, GFAAS; Flame atomic absorption spectrometry, F-AAS; X-ray fluorescence spectrometry, XRF) are employed for the analysis of heavy metal concentration in biological materials. ICP-MS device is more commonly preferred for allowing low-cost, rapid, precise, and accurate measurement of the trace elements as well as its capability of analyzing various trace elements with high sensitivity and reproducibility [22].

A limited number of studies have been conducted to determine the Pb concentrations in the blood of wild birds in the world and Türkiye. The present study was conducted to monitor the pollution of Pb using an ICP-MS device in the blood of seventeen wild bird species in Türkiye.

2. MATERIALS and METHODS

2.1. COLLECTION of SAMPLES

The approval was obtained from the Animal Experiments Local Ethics Committee of Harran University (Dated 27.11.2019 and numbered 28328) to conduct the study. In the study, blood samples were collected from forty wild birds of 17 species, ages, and sexes that came to “Gölpınar Wilderness Rescue and Rehabilitation Centre” within the body of the Şanlıurfa Branch of the Directorate of Nature Conservation and National Parks because of various reasons (sick, injured, travel weary and need of care) between the years of 2020-2021. Since there is no general method for sex determination in birds, sex determination could not be examined in animals coming to Gölpınar Wilderness Rescue and Rehabilitation Centre [23]. It is challenging to determine chronological age at the individual level in many animal species. Therefore, DNA methylation, as a novel method, is used for age determination of wild birds. However, DNA methylation levels may vary in wild birds as a response to anthropogenic pollution. Therefore, age determination could not be performed in the wild birds used in the study [24].

2.2. PREPARATION and MEASUREMENT of SAMPLES in an ICP-MS DEVICE

Blood samples of 1 ml were taken from the brachial and tibial veins of the wild birds using an injector (25G) into tubes prefilled without anticoagulants. The digestion procedure of the blood samples was carried out as described by Ütme and Temamoğulları (2021). Following the

digestion procedure, Pb concentrations in the blood samples were measured using ICP-MS (Agilent Technologies /7700X) device [21]. The operating conditions of the ICP-MS device are given. (Table 1)

2.3. PLOTTING the CALIBRATION CURVE and CALCULATING LOD-LOQ VALUES

Standard solutions were supplied by Agilent company (27 mixed elements: 8500-6940 2A and Mercury) and used in plotting the calibration curves. All the solutions were prepared with ultra-purified water. Samples were analyzed by 1 ppm internal standards (Agilent 5188-6525) using the EPA 6020 method (Multi-elemental determination of analyses by Inductively Coupled Plasma-Mass Spectrometry). Limit of detection (LOD) and limit of quantification (LOQ) values were separately calculated for element. R, LOD, and LOQ values of the metal were determined as 1.0000, 0.0607, and 0.20031 for Pb. All analyses were repeated three times and mean values were calculated [25].

3. RESULTS

The concentrations of Pb (ppb, $\mu\text{l/L}$) were assessed in the blood samples taken from the wild birds and presented in Table 2. In the study in which heavy metal exposure was determined in the blood samples of a totally of 40 wild birds belonging to 17 species of different ages and sexes in Türkiye; Pb concentration (509.58 ± 0.29 ppb) was detected to be over 150 ppb, which has been determined as the threshold value for lead poisoning in birds [14] and within the clinical poisoning range (500-1000 ppb) [12] in only one blood sample belonging to Golden Eagle. The radiographic image of Golden Eagle detected with high Pb concentration

(Fig 1) revealed numerous lead bullets that hit the body due to firearm injury. It was thought that shots in the body of the wild birds played a role in the detection of high Pb concentration in the blood sample of Golden Eagle. In the study, recovery rates for lead were 100%.

4. DISCUSSION

Non-essential Pb concentrations were assessed in the blood of wild birds in a limited number of studies. The present study is the first research that investigated Pb levels in the blood of seventeen different bird species in Türkiye. In the present study that determined heavy metal exposure in the blood samples of a totally of 40 wild birds belonging to 17 species of different ages and sexes; Pb concentration (509.58 ± 0.29 ppb) was detected to be over 150 ppb, which has been determined as the threshold value for lead poisoning in birds, and within the clinical poisoning range (500-1000ppb) [12,14] in only one blood sample belonging to Golden Eagle. The results obtained in the present study that investigated Pb concentrations in the blood samples of wild birds were compared with the results of international similar studies carried out in the world (Table 3).

Feeding habits and habitats are important in determining the degree of lead exposure in blood. Therefore, in the study, blood lead concentrations were evaluated according to the value of 500-1000 ppb [15] for the wild birds that inhabit and feed in the water and wetlands (Common Moorhen, White stork, Black-crowned Night Heron, and Grey Heron) and based on the value of >150 ppb for the other wild bird species (Golden Eagle, Black Kite, Griffon Vulture, Common kestrel, Sparrowhawk, Barn Owl, Little Owl, Long-eared Owl, Eurasian Eagle-

owl, Western Marsh Harrier, Common Buzzard, Common Crane, and Booted Eagle) [14].

Common moorhen (*Gallinula chloropus*) is a native bird species usually that eats aquatic and marsh plant seeds, fruit, insects, worms, and occasionally egg in the reeds [9,10]. In this study, a lead concentration of 133.50 ± 2.78 ppb was detected in the blood sample of Common moorhen (n=1). The lead concentration in the blood of Common moorhen was lower than the limit value determined for lead [15]. Lead concentrations of 6.75 ± 1.44 and 6.26 ± 0.85 ppb were detected in the blood samples of Common moorhens in respectively Navaseca Lagoon (n=81) and Tablas de Daimie (n=33) of Spain in a study carried out using ICP-MS device [17]. Likewise, lead concentration was determined to be 106 ppb by GF-AAS in another study analyzing the blood samples of Common moorhen (n=12) in Spain [13].

White stork (*Ciconia ciconia*) is a migratory bird that inhabits wetlands and the land and eats the animals such as mice, frogs, lizards, worms, insects, and fish [9, 26]. Since no information concerning the threshold value of blood lead for white storks has been encountered, the lead concentrations in the blood samples of the white storks were evaluated based on the threshold value of lead poisoning determined (500-1000 ppb) for the wild birds that inhabit and feed in the water and wetlands. In the present study, Pb concentrations of 37.98 ± 0.80 , 8.04 ± 4.67 , $<0.000 \pm \text{N/D}$, 56.88 ± 4.77 ppb were detected in the blood samples of white storks (n=4), respectively. Pb concentration was found to be 36.92 ± 5.5 ppb using ICP-MS device in the blood samples of white storks (n=44) in a study carried out in Spain [27]. The Pb concentration

was assessed to be 102.70 ± 90.90 ppb in a study using the same device (ICP-MS), in the blood samples of white storks ($n=70$) in Spain (Los Hornos) [8]. In the blood samples of white stork collected from Caceres ($n=23$) and La Puebla del Río ($n=35$) cities of Spain, blood Pb concentrations were determined as 90.7 ± 51.0 ppb and 44.4 ± 33.6 ppb, respectively using AAS device [28]. Blood Pb concentration obtained in the present study was lower than the value determined from Caceres city and similar to those determined La Puebla del Río city. Likewise, in another study conducted using an AAS device in Spain (La Puebla del Rio, Sevilla), Pb concentration values were detected to be 79.29 ± 37.39 , 76.45 ± 37.96 , 72.23 ± 44.45 , and 52.28 ± 34.94 ppb, respectively in the 1999 ($n=68$), 2001 ($n=62$) and 2003 ($n=93$) blood samples of white storks [29]. From the results of the present study; the Pb concentration of only one blood sample was lower whereas the other results were compatible with the results of that study. In another study conducted using the same device (AAS) in Spain, it was found that Pb concentration was 17 ppb in the blood of 30 white storks [5]. Likewise, in another study carried out in the Southwestern Poland, Pb concentration values of 1336 ± 691 ppb ($n=48$), 2005 ± 1205 ppb ($n=45$), 2276 ± 1131 ppb ($n=56$), and 4554 ± 3156 ppb ($n=37$), respectively, were detected in white stork blood samples using AAS device [30]. The results of both studies were detected to be higher than the Pb values reported in the present study.

Grey heron (*Ardea cinerea*) is a non-migratory native bird that feeds with a diet mostly composed of fish, mammals, birds, and invertebrates in shallow waters [9,10,31]. In the present study, lead concentration was detected as 1.65 ± 2.56 ppb and 32.80 ± 13.22 ppb in the blood samples of

Grey Heron (n=2), respectively. The lead concentration level detected in the blood of Grey Heron was found to be lower than the identified Pb limit level [15]. A Pb concentration of 160 ± 66.7 ppb was detected in a study conducted in Spain with the blood samples of Grey Heron (n=6) using the AAS device [2]. Likewise, a Pb concentration of 15 ppb was identified in the blood samples of Grey Heron (n=20) in a study carried out in Spain using the same device [5]. The results of that study conducted in Spain were found to be higher than the lead concentration values detected in the present study.

Black-crowned night heron (*Nycticorax nycticorax*), as an aquatic ecosystem bird, is a native bird species that usually eat fish, small rodents, and amphibians [9,32]. Pb concentrations of 16.66 ± 3.13 and 349.30 ± 6.61 ppb were measured in the blood samples of black-crowned night herons (n=2), respectively. The Pb concentration in the blood of the black-crowned night herons were detected to be low according to the threshold value of lead poisoning (500-1000 ppb) determined for the wild birds that inhabit and feed around the water and wetlands [15]. It is thought that the low Pb concentration in the blood samples of black-crowned night herons as a native birds may be due to the low industrial structuring in the region.

Common crane (*Grus grus*) that inhabit open meadow, forest, farming fields, and water puddles and has an omnivorous feeding habit migrates partially through Türkiye [9,10,33]. In the present study, a Pb concentration of 160.42 ± 1.92 ppb was determined in the blood sample of Common crane (n=1). Literature reviews revealed no threshold value of blood lead poisoning specific for Common crane; therefore, when the

present study results were evaluated based on the threshold value of Pb poisoning (150 ppb) determined for the birds it was determined that the blood Pb concentration of Common crane was below the identified limits.

Barn owl (*Tyto alba*) is a non-migratory and native bird species and is one of the nocturnal predators and known to eat mostly rodents, insects, and birds [9,34]. The blood samples of the barn owl (n=3) had lead concentrations of $<0.000 \pm \text{N/D}$, 17.43 ± 2.57 , and 17.52 ± 57.48 ppb, respectively. No threshold value of blood lead poisoning has been determined specifically for Barn Owl in the literature reviews, therefore the results were found to be lower compared with the threshold value of Pb poisoning determined for birds in general (150 ppb).

Western marsh harrier (*Circus aeruginosus*), a native and migratory bird, eats primarily rodents, birds, less frequent insects, and occasionally carrions [9,35]. In the present study, lead concentrations of 195.53 ± 1.62 and 36.09 ± 21 ppb were identified in the blood samples of Western marsh harrier (n=2), respectively. The levels of lead measured in the blood sample of Western marsh harrier were lower than the identified limit lead value. The blood Pb concentration values were found to be 58-108 ppb [36]; in a study conducted in France (n=8) with the blood samples of Western marsh harrier using the GF-AAS device and 29-2840 ppb in another study carried out in the blood samples of 94 Western marsh harriers using AAS device also in France between 1990-1991 [37]. A Pb concentration of 4.8 ± 0.2 ppb was detected in the blood samples of Western marsh harrier (n=2) caught in the studies conducted in Spain between 2016-2017 while a Pb concentration of 1 ppb was detected in

the Western marsh harrier (n=1) in the wildlife rehabilitation center between 2004-2020 [38].

The diet of Golden Eagle (*Aquila chrysaetos*), a native bird species, includes mostly big prey animals and occasionally carrions [10, 39]. In the present study, the blood sample of Golden Eagle (n=1) had a lead concentration of 509.58 ± 0.29 ppb. In the study, the level of lead concentration found in the blood sample of Golden Eagle was higher than the determined lead limit value. It is thought that this may be associated with the numerous lead pellets that hit its body as a result of the firearm injury as seen from the radiographic image (Figure 1). Pb concentrations of 1360 ± 260 ppb and $40-80 \pm 190$ ppb were detected in the 70 and 48 blood samples of Golden Eagle in a study carried out in Southeast Montana (USA) between 2008-2010 using an ICP-MS device, respectively. Pb concentrations of 1300 ± 280 ppb were measured in the blood samples of 87 Golden Eagles using the GFAAS device between 1985-1993 in the Montana Region [40]; while Pb concentrations of 20.3 ± 5.6 ppb and 1420 ± 738 ppb were encountered in the blood samples of Golden Eagles (n=5) caught in the studies using GFAAS device between 2016-2017 and of Golden Eagles in the wildlife rehabilitation center between 2004-2020 (n=4) in Spain, respectively [38].

Black kite (*Milvus migrans*) is a migratory raptor that eats reptiles, mammals, birds, insects, and carrions [9,10,41]. Concentrations of 5.04 ± 30.62 , $<0.000 \pm N/D$, 12.49 ± 26.21 , and $<0.000 \pm N/D$ were identified in the blood samples of the Black kite (n=4), respectively. The Pb level in the blood sample of Black Kite was lower than the determined limit value. In Spain, a Pb concentration of 54 ppb was identified by the

AAS device in the blood samples of Black Kite (n=25) [5]. The lead values detected in the present study were lower than those reported in the study conducted in Spain.

Common buzzard (*Buteo buteo*), which is usually a resident species, prefers the fields such as woodland, grasslands, and meadows. It eats small and middle-sized mammals (rabbit and mouse species) [9,41]. In the present study, Pb concentrations of 9.56 ± 3.82 , 24.27 ± 6.13 , 55.05 ± 7.73 , and 13.03 ± 35.07 ppb were found in the blood samples of the common buzzard (n=4), respectively. The level of Pb identified in the blood sample of the common buzzard was lower than the determined lead limit value. A study carried out in Spain using an AAS device in the blood samples of common buzzard reported a Pb concentration of 118 ppb [2]. This value was higher than the results detected in the present study. However, Pb concentrations in the 3 blood samples used in the present study were consistent with the Pb concentration of 35.7 ± 4.6 ppb detected by the GFAAS device in the blood samples of the common buzzards (n=18) caught in Spain between 2016-2017 [38].

The diet of Common kestrel (*Falco tinnunculus*) which is a non-migratory native bird species usually includes insects and small mammals [9,42]. In the present study, Pb concentrations of $<0.000\pm N/D$, $<0.000\pm N/D$, 33.04 ± 10.07 , and $<0.000\pm N/D$ ppb were detected in the blood samples of common kestrel (n=4), respectively. The level of lead identified in the blood samples of common kestrel was lower than the determined lead limit value [14]. In Spain, a Pb concentration of 115.2 ± 52 ppb was identified in the blood sample of Common Kestrel

(n=8) through the ASS device [2]. When compared with the present study, lead levels in the blood samples in Spain were higher.

Booted Eagles (*Hieratus pennatus*) are the summer migratory birds that eat small mammals, reptiles, and birds [9,43]. In the present study, Pb concentrations of 38.60 ± 11.13 and 12.06 ± 36.82 ppb were detected in the blood samples of Booted Eagles (n=2), respectively. The level of the lead identified in the blood sample of Booted Eagle was lower than the determined lead limit level. In Spain, a Pb concentration of 87.5 ppb was determined by using the AAS device in the blood samples of Booted Eagle (n=2) [2]. Also, a Pb concentration of 1.4 ppb was determined in the blood sample of the Booted Eagle (n=1) that was brought to the rehabilitation center for treatment, using a GFAAS device in a study conducted in 2017 in Spain [38].

Sparrowhawk (*Accipiter nisus*) is a native and vagrant bird species eating birds and small mammals [9,44]. In the present study, Pb concentrations of 71.50 ± 2.03 ppb and 34.63 ± 8.24 ppb were analyzed in the blood samples of Sparrowhawk (n=2), respectively. Since literature reviews indicated no threshold value for lead poisoning identified specifically for Sparrowhawk, Pb concentration in the blood samples of Sparrowhawk was found to be low according to the threshold level for lead poisoning (150 ppb) determined for birds in general.

Little owl (*Athena noctua*), which is a non-migratory and resident bird species, is known to usually eat insects, worms, small mammals, and small birds [9,10]. In the present study, it was determined that the blood samples of Little Owl (n=2) had the Pb concentrations of 10.04 ± 17.86 and 32.15 ± 5.59 ppb, respectively. The Pb level detected in the blood

sample of the Little owl was lower than the determined lead limit value. A Pb concentration of 79.3 ppb was identified in the blood sample of a Little owl (n=3) using an AAS device in a study carried out in Spain [2]. It was monitored that both blood samples of Little owl analyzed in the present study indicated a lower level of lead than the results obtained in the study conducted in Spain.

Eurasian Eagle-Owl (*Bubo bubo*) which has a very wide and divergent food network containing usually fish, reptiles, birds, and mammals is a resident and non-migratory largest owl species seen in many habitats [10,45]. In the present study, Pb concentrations of 5.34 ± 75.34 , 27.92 ± 7.12 , and 72.01 ± 1.18 ppb were detected using an ICP-MS device in the blood samples of Eurasian Eagle-Owl (n=3), respectively. Pb level detected in the blood of Eurasian Eagle-Owl was lower than the determined lead limit value. In a study performed in Spain, a Pb concentration of 83.2 ± 66.8 ppb was found by using the AAS device in the blood samples of Eurasian Eagle-Owl (n=5) [2]. Pb concentration detected in only one blood sample in the present study was lower than the results of the study carried out in Spain. However, the results of the other samples were consistent with the results of that study.

Reproducing Griffon vultures (*Gyps fulvus*) eat animal carrions by soaring over wide areas. It has been reported that juvenile and immature individuals migrate [41,46]. In the present study, Pb concentration was found to be 11.90 ± 12.90 ppb in the blood sample of griffon vulture (n=1) by using an ICP-MS device. The Pb level detected in the blood sample of the griffon vulture was lower than the determined lead limit value. Two studies have been encountered in the literature reviews. Pb

concentrations of 379 ± 121 ppb, 384 ± 36.4 ppb, and 316 ± 67.8 ppb Pb were detected by using an AAS device in the blood samples of griffon vulture in Spain ($n=6$) [2] and by using a GFASS device in the blood samples of griffon vulture caught in Spain ($n=118$) between 2016-2017 and in the blood samples of griffon vultures ($n=6$) in the wildlife rehabilitation center between 2004-2020 [38], respectively. The results of both studies showed higher than Pb concentrations detected in the present study.

Long-eared owl (*Asio otus*) is a resident bird species that eats usually rodents and occasionally birds [9,47]. In the present study, Pb concentrations of 19.60 ± 4 ppb and 21.53 ± 2.14 ppb were analyzed in the blood samples of a Long-eared owl ($n=2$), respectively. Since literature reviews indicated no threshold value for lead poisoning identified specifically for the Long-eared owl, the Pb level in the blood samples of the Long-eared owl was very low according to the threshold level for lead poisoning (150 ppb) defined for birds in general.

It was thought that the difference between the Pb concentrations in the blood samples belonging to different wild birds obtained in the present study and Pb concentrations in the blood samples of similar wild birds in the world was associated with the ingestion of the sand and pebble fragments with different geological characteristics for their gizzards, ingestion of animals hit by firearm shots or ingestion of seeds, fruits, insects, worms and eggs containing heavy metals, gunshot pellets that hit their bodies due to firearm injuries, the difference between sensitivity levels of the devices used in the analyses and the distances between their habitats and human activation.

In conclusion, the role of the wild birds used as bioindicators could not be neglected in the determination of environmental pollution. Heavy metals are pollutants that may lead to severe damage in ecosystems over time, particularly due to their accumulation in the environment. In the present study, it was found that Pb concentration in the blood sample of Golden Eagle (*Aquila chrysaetos*) was higher than the threshold value for poisoning whereas Pb concentration in the blood samples of the other wild birds were lower than the threshold value for poisoning. The results of this first study conducted on seventeen wild bird species in Türkiye revealed that heavy metal pollution (Pb) was not determined in wild birds except for one sample. Heavy metal exposure may pose a risk for the continuation of generations of wild birds. Therefore, it is recommended to conduct studies on heavy metals for evaluation of their effects on the environment and human health.

Table 1. ICP-MS working conditions

Instrument	Agilent 7700×ICP-MS
RF power	1550 W
RF matching	2.1 V
Sample depth	8.0 mm
Sample uptake rate	0.1 mL min ⁻¹
Plasma gas flow rate	15 L min ⁻¹
Auxiliary gas flow rate	1.0 L min ⁻¹
Carrier gas flow rate	0.95 L min ⁻¹
He gas flow fate	4.3 mL min ⁻¹
Spray chamber temp.	2°C
Spray chamber	Soft double pass-type
Torch	Quartz glass torch
Number of replicates	3

Table 2. Pb values in blood samples from wild birds (ppb, μL /L, mean \pm Standard deviation)

Wild Birds	Pb
Common buzzard (<i>Buteo buteo</i>)	9.56 \pm 3.82
	24.27 \pm 6.13
	55.05 \pm 7.73
	13.03 \pm 35.07
Eagle-owl (<i>Bubo bubo</i>)	5.34 \pm 75.34
	27.92 \pm 7.12
	72.01 \pm 1.18
Barn owl (<i>Tyto alba</i>)	<0.000 \pm N/D
	17.43 \pm 2.57
	17.52 \pm 57.48
Long-eared owl (<i>Asio otus</i>)	19.60 \pm 4.00
	21.53 \pm 2.14
Little owl (<i>Athene noctua</i>)	10.04 \pm 17.86
	32.15 \pm 5.59
Marsh harrier (<i>Circus aeruginosus</i>)	195.53 \pm 1.62
	36.09 \pm 21.00
Black-crowned night heron (<i>Nycticorax nycticorax</i>)	16.66 \pm 3.13
	349.30 \pm 6.61
Sparrowhawk (<i>Accipiter nisus</i>)	71.50 \pm 2.03
	34.63 \pm 8.24
Black kite (<i>Milvus migrans</i>)	5.04 \pm 30.62
	ND
	12.49 \pm 26.21
White stork (<i>Ciconia ciconia</i>)	ND
	37.98 \pm 0.80
	8.04 \pm 4.67
	56.88 \pm 4.77
Common kestrel (<i>Falco tinnunculus</i>)	ND
	ND
	33.04 \pm 10.07
	ND
Booted eagle (<i>Hieraaetus pennatus</i>)	38.60 \pm 11.13
	12.06 \pm 36.82
Grey Heron (<i>Ardea cinerea</i>)	1.65 \pm 2.56
	32.80 \pm 13.22
Common moorhen (<i>Gallinula chloropus</i>)	133.50 \pm 2.78
Golden eagle (<i>Aquila chrysaetos</i>)	509.58 \pm 0.29
Common crane (<i>Grus grus</i>)	160.42 \pm 1.92
Griffon vulture (<i>Gyps fulvus</i>)	11.90 \pm 12.90

Table 3. Pb mean±standard deviation and (min-max) range in studies conducted with wild poultry blood samples around the World

Wild Birds	Country	Device	Pb (ppb, µl /L)	Reference
Common moorhen (<i>Gallinula chloropus</i>)	Spain	ICP-MS	6.75±1.44 (0.01-112.2)	[17]
			6.26±0.85 (ND-24.43)	
		GF-AAS	106 (21-267)	[13]
Marsh harrier (<i>Circus aeruginosus</i>)	France	GF-AAS	53-108	[36]
	Spain	GF-AAS	4.8±0.2 (4.5-5)	[38]
			1	
	France	AAS	29-2840	[46]
White stork (<i>Ciconia ciconia</i>)	Spain	AAS	71 (2-320)	[5]
			90.7±51.0 (6-214)	[28]
			44.4±33.6 (ND-126)	[28]
			79.29±37.39 (24-157.54)	[29]
			76.45±37.96 (21.67-195.58)	
			72.23±44.45	
			52.28±34.94 (4.52-195.7)	
	Poland	AAS	1336±691	[30]
			2005±1205	
			2276±1131	
			4554±3156	
	Spain	ICP-MS	36.92±5.05 (8.35-186.4)	[27]
102.70±90.90 (ND-497)			[8]	
Golden eagle (<i>Aquila chrysaetos</i>)	USA	ICP-MS	1360±260	[40]
		GFAAS	40-80 ±190	
	Spain	GFAAS	20.3±5.6 (5.9-35.2)	[38]
			1420±738 (53-3250)	
Black kite (<i>Milvus migrans</i>)	Spain	AAS	54 (2-179)	[5]

Grey Heron (<i>Ardea cinerea</i>)	Spain	AAS	160±60.67 (65-250)	[2]
			15 (2-89)	[5]
Common buzzard (<i>Buteo buteo</i>)	Spain	AAS	118 (50-260)	[2]
		GFAAS	35.7±4.6 (5-76.2)	[38]
Common kestrel (<i>Falco tinnunculus</i>)	Spain	AAS	115.2±52 (50-170)	[2]
Booted Eagles (<i>Hieratus pennatus</i>)	Spain	AAS	87.5 (40-135)	[2]
		GFAAS	1.4	[38]
Little owl (<i>Athena noctua</i>)	Spain	AAS	79.3 (48-130)	[2]
Eagle-owl (<i>Bubo bubo</i>)	Spain	AAS	83.2±66.8 (30-200)	[2]
Griffon vulture (<i>Gyps fulvus</i>)	Spain	AAS	379±121 (200-500)	[2]
		GFAAS	384±36.4 (26.1-3360)	[38]
		GFAAS	316±67.8 (108-497)	

ND: Non-determination

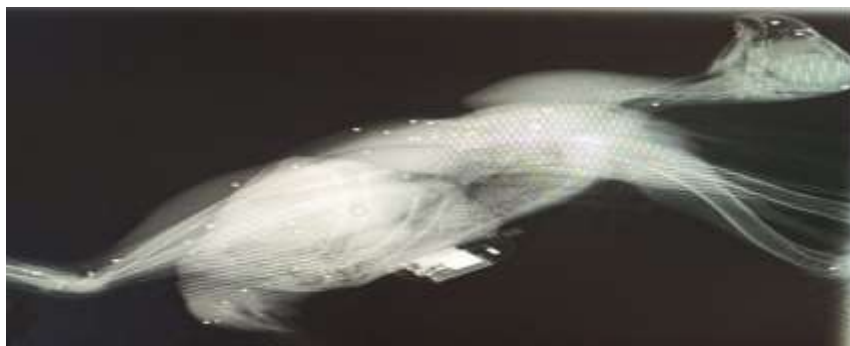


Figure 1. Radiographic image of Golden Eagle (The photo was taken by Resat EKTIREN)

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CHAPTER 4

GLANDULAR & NON-GLANDULAR TRICHOMES

IN CROPS

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1. Introduction

Most plant species have hair-like epidermal structures below and/or above ground. Trichomes, a term derived from the Greek word *trichos*, which means hair, are commonly used to describe them when they appear on aerial plant parts (Schuurink & Tissier, 2020). Trichomes, which are an extension of the above-ground epidermal cells of plants, can be described as unicellular or multicellular appendages. These appendages are found in a wide range of species and are essential to the growth of plants (Xiao et al., 2017). Trichomes act as a barrier to protect plants from threats from the environment such herbivores, ultraviolet (UV) radiation, disease attacks, excessive transpiration, seed dispersal, and seed protection (Wang et al., 2021), as well as retaining heat (Peter & Shanower, 1998).

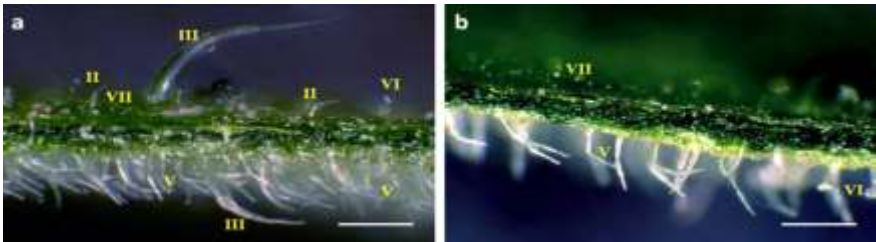


Fig. 1. Trichomes in section of tomato (*Solanum lycopersicum*). Tolerant genotype (a) and susceptible genotype (b) show the trichome density on leaf abaxial and adaxial sides of leaves. The different types of trichomes are marked in the picture. White bars represent 500 μm (D'Esposito et al., 2021).

Trichomes, originating from epidermal cells, are present on nearly all terrestrial plants. They exist in diverse forms (Yang and Ye, 2013).

2. Trichome Types

Trichomes can be classified as "glandular" or "non-glandular" types (Karabourniotis et al., 2020). The fundamental morphological distinction between glandular and non-glandular trichomes is the absence of a glandular head in the latter category (Werker, 2000).

2.1. Glandular trichomes

Glandular trichomes are specialized hairs found on the surface of about 30% of all vascular plants and are responsible for a significant portion of a plant's secondary chemistry (Glas et al., 2012). Large amounts of specialised metabolites are biosynthesized and stored in glandular trichomes, which are epidermal outgrowths. In addition to their function in protecting plants from biotic and abiotic challenges, they have drawn attention because of the significance of the molecules they produce for human use, such as pharmaceuticals, flavour and fragrance components, or insecticides (Schuurink & Tissier, 2020). The storage compartment of glandular trichomes usually is located on the tip of the hair and is part of the glandular cell, or cells, which are metabolically active. This knowledge now assists classical breeding programs, as well as targeted genetic engineering, aimed to optimize trichome density and physiology to facilitate customization of essential oil production or to tune biocide activity to enhance crop protection (Glas et al., 2012).

According to Martnez-Nataren et al. (2011), a glandular trichome is anatomically separated into three regions: the basal, stalk, and head, which can be unicellular or multicellular. Additionally, there are two

categories of glandular trichomes: peltate and capitate. According to Turner et al. (2000), peltate trichomes are considered to be primarily engaged in the generation of essential oils and contain a sizable subcuticular region where the oil is stored. According to reports, capitate trichomes also release a complex blend of polysaccharides, proteins, and mucins in addition to trace amounts of flavonoids and essential oils. Capitate trichomes have a small storage capacity, nevertheless, and the released substances collect up in the cell lumen (Bottega and Corsi, 2000). It is clear that the majority of the essential oil is produced by and stored in peltate trichomes, regardless of its exact composition of the capitate trichome secretory products (Sharma et al., 2003). Thus, it has been discovered that there is a positive correlation between the density of these epidermal structures on the leaves of aromatic plants and the amount of essential oil that can be extracted from them through distillation (Martínez-Nataren et al., 2011).

Particularly in terms of volatility, their morphology appears to be tailored to the kinds of molecules they produce. Capitate trichomes typically produce nonvolatile and frequently sticky resinous compounds such acylsugars or specific diterpenoids (Wagner et al., 2004). They normally have one basal cell, one to several stalk cells, and one or a small number of secretory cells near the stalk's tip. The cells at the apex of the trichome discharge the substances straight onto its surface (Tissier, 2012). Typically, volatile compound-producing trichomes have a storage area that allows the volatiles to be kept in a liquid condition and only release them when trichomes are destroyed

(such as during herbivory). This space can be subcuticular, that is, located between the secretory cells and the cuticle. This is the case in the peltate trichomes of the *Lamiaceae* (mint family) or the biseriate trichomes of the *Asteraceae* (daisy family) (Turner et al., 2000).



Fig. 2. Glandular trichomes on *Petunia*×*hybrida*. a) Long-stalked and short-stalked trichomes on stem, b) Dead thrips on inflorescence trichomes, c) Dead *Diptera* larvae between inflorescence trichomes (Mofikoya et al., 2019).

Numerous plant species have surfaces covered in glandular trichomes. They are incredibly diverse, both in terms of structure and the substances they release. In addition to within species or even individual plants, this diversity is displayed between species. Research into the biosynthetic processes that result in these specialised metabolites has been sparked by the industrial applications of various trichome secretions as well as their potential as a defence barrier, for example against arthropod pests. The secretory cells possess full biosynthetic pathways. Glandular trichomes can be thought of as actual cell factories because they have a significant metabolic capability. To fully exploit the potential of glandular trichomes as breeding or engineering objects, several research areas will have to be further investigated, such as

development, patterning, metabolic fluxes and transcription regulation (Tissier, 2012).

Mint, basil, lavender, oregano, and thyme are grown for the essential oils that are produced in their glandular trichomes (Maleci Bini & Giuliani, 2006). The sesquiterpene lactone artemisinin, which is used to treat malaria, is produced by the annual wormwood *Artemisia annua* in its glandular trichomes (Lommen et al., 2006). Sesquiterpenes produced by the glandular trichomes of the wild tomato (*Solanum habrochaites*) have substantial anti-herbivore properties and could be used as natural insecticides (Glas et al., 2012). Tea trichomes are critical for tea breeding and tea quality as they are rich in nutrients (Li et al., 2020).

Glandular trichomes are a major site of plant natural product synthesis and accumulation for protection against insect predation. Aziz et al., (2005) revealed over 1.000 genes with strong preferential expression in the trichome fraction of the stem, 70% of which are of unknown function. These define a class of genes that are not trichome-specific.

Trichomes and root hairs differentiate from epidermal cells in the aerial tissues and roots, respectively. Because trichomes and root hairs are easily accessible, in the model plant *Arabidopsis*, their development has become a well-studied model of cell differentiation and growth. Molecular genetic analyses using *Arabidopsis* mutants have demonstrated that the differentiation of trichomes and root hair/hairless cells is regulated by similar molecular mechanisms (Ishida et al., 2008). The single-cell trichomes in wild-type *Arabidopsis* are either

unbranched or have two to five branches. Using transgenic *Arabidopsis* plants expressing a green fluorescent protein–microtubule-associated protein fusion protein, which decorates the microtubular cytoskeleton, we observed that during trichome branching, microtubules reorient with respect to the longitudinal growth axis (Mathur & Chua, 2000). *Arabidopsis* trichomes are an excellent model system to study all aspects of cell differentiation including cell fate determination, cell cycle regulation, cell polarity and cell expansion (Schellmann & Hulskamp, 2014).

Both root hairs and trichomes differentiate from epidermal cells and molecular genetic analyses using *Arabidopsis* mutants have demonstrated that the differentiation of root hairs and trichomes is regulated by similar molecular mechanisms. Molecular-genetic approaches have led to the identification of many genes that are involved in epidermal cell differentiation, most of which encode transcription factors that induce the expression of genes active in both root hair and trichome development (Tominaga et al., 2011).

In *Phillyrea latifolia*, the concentration but not the composition of leaf tissue phenylpropanoids varied significantly between sun and shade leaves, with a marked increase in flavonoid glycosides in sun leaves of plant. The light-induced synthesis of flavonoids in glandular trichomes of *P. latifolia* probably occurs in situ and concomitantly inactivates other branch pathways of the general phenylpropanoid metabolism. This is the first report of the key role of glandular trichomes and of

flavonoid glycosides in the integrated mechanisms of acclimation of *P. latifolia* to excess light (Tattini et al., 2000).

Leaf trichomes protect plants from attack by insect herbivores and are often induced following damage. Traw & Bergelson, (2003) addressed the effects of artificial damage, jasmonic acid, salicylic acid, and gibberellin on induction of trichomes in *Arabidopsis*. Artificial damage and jasmonic acid caused significant increases in trichome production of leaves. Salicylic acid had a negative effect on trichome production and consistently reduced the effect of jasmonic acid, suggesting negative cross-talk between the jasmonate and salicylate-dependent defense pathways. They found that gibberellin and jasmonic acid had a synergistic effect on the induction of trichomes, suggesting important interactions between these two compounds.

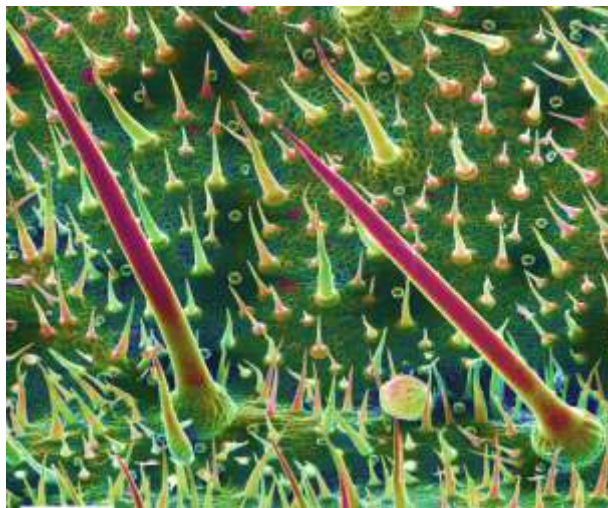


Fig. 3. Trichomes of particularly toxic representatives of *Urticaceae* species *Dendrocnide moroides* stinging hairs, shorter stiff trichomes (both mineralized), and minute, spherical glandular trichomes (Ensikat et al., 2021).

Peiffer et al., (2009) demonstrated that the rupture of foliar glandular trichomes by caterpillar or moth contact induced the expression of defense transcripts (e.g. proteinase inhibitor 2, or PIN2) regulated by jasmonic acid. This generated response did not need chewing or the production of salivary substances.

2.2. Non-glandular trichomes

Non-glandular trichomes serve as surface structures that shield plant organs from a variety of biotic and abiotic stressors. These epidermal appendages play important protective and defensive roles for growing organs, which are due to an ideal combination of structurally advantageous characteristics and chemical reinforcement in the form of phenolic chemicals, particularly flavonoids (Karabourniotis et al., 2020).

According to Quideau et al. (2011), the term "phenolic" refers to carbon-based compounds with one (simple phenols) or more (polyphenols) phenolic groups (phenyl-groups), or at least one hydroxyl-group attached to a benzyl ring. Phenolics are multifunctional secondary metabolites that have a variety of regulatory, defensive, and protective functions in response to biotic or abiotic stressors (Karabourniotis et al. 2014)

At the molecular level, the development of trichomes and the buildup of phenolics are linked processes. Non-glandular trichomes exhibit striking physical similarities to glandular ones in the early stages of development, such as the balloon-shaped apical cells with many

phenolics. Phenolics are transported to the cell walls of the trichomes during secondary wall thickening and subsequent developmental phases. Trichomes act as optical filters to block out wavelengths that can harm sensitive tissues and guard against UV-B radiation as a result of the diffuse deposition of phenolics in the cell walls. Increased surface light reflection also provides protection from powerful visual radiation. Additionally, the trichome phenolic combinations serve as a thin chemical barrier that guards against biotic stressors like herbivores and viruses. Although some trichome cells actually die at maturity, they are still capable of changing their quantitative and qualitative properties as they develop in response to the external biotic or abiotic environment. In fact, the specific light regime, herbivore damage, wounding, water stress, salinity, and the presence of heavy metals may alter the structure and chemical components of trichomes. Trichomes thus serve as dynamic protective structures that could significantly influence the results of many interactions between plants and their environment (Karabourniotis et al., 2020).

There are no analytical investigations mentioning the occurrence of high terpenoids concentrations in non-glandular trichomes, which lack a secretory mechanism. Non-glandular trichomes, on the other hand, appear to be incapable of secretion yet accumulate significant amounts of phenolics, primarily in the early stages of their ontogeny. But at these stages, immature trichomes of holm oak (*Quercus ilex* L.) and olive (*Olea europaea* L.) leaves have striking morphological similarities to

glandular trichomes, including balloon-shaped apical cells with thin cell walls (Karabourniotis et al., 2020).

Non-glandular trichomes are distinguished by their morphological characteristics and display a tremendous variability in their properties such as morphology, size and density often related to their functional purposes. For example, the presence of dense trichome layers is often considered a xeromorphic character (Amada et al., 2017).

Non-glandular trichomes can form a dense layer on the surfaces of plant organs and can be unicellular or multicellular with a narrow apex (Werker, 2000), branching or not (Evert, 2006), or both (Dmitruk & Weryszko-Chmielewska, 2010). The cells of non-glandular trichomes can either remain alive and mature or die and become dry in the first phases of development (Mayekiso et al., 2008). Contrary to what is commonly believed, non-glandular trichomes do not take part in the synthesis, storage, or release of chemical substances that are physiologically active. They are known by their action exclusively on the physical protection of plants against biotic and abiotic stresses, forming a protective barrier against low humidity, high temperatures and sun radiation (Werker, 2000), retaining air film on aquatic plant surfaces and influencing its fluctuation (Barthlott et al., 2009), and deterring the activities of feeding and ovipositing insects. This is because numerous plant species have shown a correlation between the abundance of non-glandular trichomes in leaves and their resistance to herbivore assaults (Santos Tozin et al., 2016).

According to research by Li et al. (2019), non-glandular trichomes of the sunflower (*Helianthus annuus*) can absorb significant amounts of foliar-applied zinc (Zn) in just 15 minutes. This finding shows that trichomes may be crucial to this plant species' ability to absorb foliar fertilisers (Li et al., 2021).

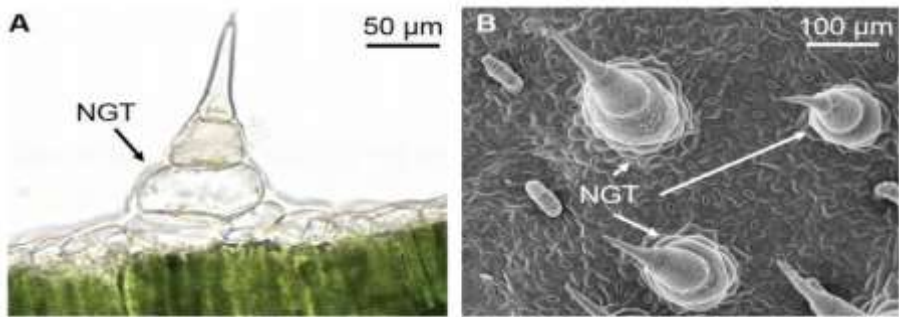


Fig. 4. A) Light micrograph of a cross-section of a sunflower leaf. B) Scanning electron micrograph of the adaxial surface of the leaf showing the non-glandular trichomes (NGTs) (Li et al., 2021).

According to reports from at least 11 species belonging to six different plant families, leaf trichomes are crucial for foliar water uptake (Fernández et al., 2014; Vitarelli et al., 2016; Schreel et al., 2020; Li et al., 2021). According to Guan et al. (2014), *Populus* and *Platanus* leaf trichomes can easily infect humans with respiratory tract infections, lung infections, fever, influenza, and in severe cases, cancer. Cotton seed trichomes are crucial raw materials for the textile industry (Tang et al., 2014).

3. Trichomes in forage crops

New cultivars of alfalfa (*Medicago sativa* L.), have been released with glandular trichomes for resistance to potato leafhopper, *Empoasca fabae* (Harris). The impact of the glandular trichomes on the primary natural enemy of the leafhopper, *Anagrus nigriventris* Girault, is searched. Although the number of leafhopper eggs per stem exposed to wasps did not significantly differ among the four clones, the frequency of foraging and total foraging time were less on the two clones with glandular trichomes than on the two clones with nonglandular trichomes. Wasps tended to fly off of clones with glandular trichomes more often than off of clones with nonglandular trichomes. This study suggests that cultivars with glandular trichomes may interfere with host searching by *A. Nigriventris* (Lovinger et al., 2000).

Experiments were performed to elucidate resistance of glandular-haired alfalfa (*Medicago sativa* L.) to the potato leafhopper (*Empoasca fabae* Harris). The glandular trichomes on FGplh13 alfalfa appeared to provide the major host resistance factor, with resistance to adults being chemically based and resistance to nymphs being chemically and mechanically based. To maintain levels of potato leafhopper resistance, breeders would appear to benefit by continuing to select for the expression of the glandular trichome phenotype (Ranger & Hower, 2001).

C14, C15, C16, C17 and C18 fatty acids were identified as major components of glandular trichome extracts from *Medicago sativa* G98A, an alfalfa genotype resistant to the potato leafhopper (*Empoasca*

fabae). Also C14 through C18 normal fatty acids were minor components. Saturated free fatty acids C12, C13, C14, C15, C16, C17 and C18 were present in trace amounts. They found that fatty acid amides localized in alfalfa glandular trichomes likely contribute to leafhopper resistance (Ranger et al., 2005).

Leafhopper-resistant varieties of glandular-haired alfalfa were made available commercially. Ranger et al., (2001) used four leafhopper-resistant genotypes provided by Cal/West Seed Co., G98A, G98C, and G98D, and the susceptible nonglandular clone Ranger. Studies support the hypothesis that nonvolatile compounds associated with the glandular trichomes on G98A alfalfa are deterrent to the potato leafhopper and are related to resistance.

Seed companies are currently marketing varieties of glandular-haired alfalfa (*Medicago sativa* L.) with resistance to the potato leafhopper (*Empoasca fabae* Harris). Although it took 30 years to develop and release the varieties, little is known of alfalfa glandular trichome chemistry and its role in resistance. To identify these active natural products, Ranger et al., (2002) used the resistant glandular genotype 'G98A' (Cal/West Seeds) and the susceptible nonglandular variety 'Ranger'. Results indicate that alfalfa glandular trichomes do not contain volatile repellent compounds, but do contain nonvolatile, fatty acid derivatives that deter potato leafhopper settling. Because these compounds are nonvolatile, the trichome resistance mechanism probably requires direct contact with the trichome exudate.

4. Conclusions

Trichomes exhibit high diversity between species, within species or even individual plants in shape or the compounds they secrete. Grandular trichomes are an important source of essential oils can be used by the pharmaceutical industry, for protection against herbivores and pathogens. Breeding programs aims to optimize trichome density and physiology to facilitate essential oil production or to enhance crop protection.

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CHAPTER 5

SOIL CONSERVATION and IMPROVEMENT KUDZU (*Pueraria DC.*)

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INTRODUCTION

In proportion to the rapidly increasing population in the world, agricultural lands are getting smaller or less. As the usability of agricultural lands decreases, the use of chemical fertilizers and pesticides has been increased in order to maintain or increase the production level, and chemical pollution has emerged as a natural result of these practices. Under the threat of degeneration or extinction by erosion, "soil" must be protected for sustainable food production and security. Therefore, the soil; It is possible to protect and preserve it with many applications such as reducing soil tillage or direct sowing, reduced tillage practices such as direct sowing, mulch cultivation, using mulch farming techniques, using cover crops, adding organic matter, applying the rotation correctly and duly, and taking more place in rotations of legumes. Many leguminous plants can be used in controlled production as green manure or cover crops. In this context, 'Kudzu' roots, which can be used in many areas and are a good soil protector and cover, tightly wrap the soil and reduce erosion. Also, being a legume plant, it provides a healthier soil accumulation with the nitrogen it fixes.

Kudzu, which has many different uses and many useful functions, is a legume native to Asia and the Pacific. It originates from East Asia and the Pacific islands. Kudzu, which was used to make clothes or paper with the peels peeled from its body in ancient times, is still used as a raw material in weaving, paper and basket making today. It is also used as a medicinal plant in human and animal nutrition and human health. The word 'Kudzu' comes from the Japanese word for plant or kuzu. From the East Asian Arrowroot (*Pueraria montana* var. *lobata*) derived. This plant can also be considered as a noxious

weeds or an invasive plant due to its naturally climbing, winding and rapidly growing structure. It propagation in humid regions of the world, in monsoon forests and on coastal roads. Kudzu, which was brought to North America from its homeland of China and Japan in the 1870s to prevent erosion and to green bare coasts, has become a common weed in the southeastern United States and has propagated rapidly in these regions. Kudzu, which is considered an invasive species with its rapid branch feature, is resistant to cold winters. With its large rapidly developing leaves and late blooming reddish-purple flowers, Kudzu is cultivated for its starch-rich edible roots and fiber. It is also used as a delicious forage legume, a wrapping ornamental plant and a soil protective cover plant.

1. KUDZU ORIGIN AND GEOGRAPHICAL DISTRIBUTION

Kudzu is a perennial vine from the Fabaceae family. Kudzu, a plant native to Asia, is believed to have originated in Japan and later spread to China and Korea. In Japan, Kudzu is found in Hokkaido island, Kuchinoshima island and mountainous regions ranging from many plains and islands, and in Henan, Hunan, Zhejiang and Sichuan provinces in China (Miller and Edwards, 1983). *Pueraria*, which includes 26 different species, is distributed in Asia, North America and South America (Wang et al., 2020). Generally, Kudzu (*Pueraria montana* (Lour.) Merr.) and Tropical kudzu (*P. phaseoloides* (Roxb.) Benth.) are species. Tropical kudzu (*P. phaseoloides*) is a native legume plant of India, cultivated and spread in many tropical countries. It is grown only as a ground cover and green manure in Southeast Asia, India, Indonesia, Belgian Congo, Liberia, and Equatorial Africa. In

Asia and Indonesia, Tropical Kudzu is used as a cover plant, especially to cool and shade the plants. It is stated that kudzu is an invasive legume that was brought to the United States from Asia in 1876 and has adverse environmental problems as it quickly covers everything. Kudzu has been nicknamed "the vine that ate the South". It is a plant of subtropical and temperate regions. Five species in the genus *Pueraria* "*P. montana*, *P. lobata*, *P. edulis*, *P. phaseoloides*, and *P. thomsonii*" are closely related to Kudzu populations in the United States and appear to have multiple ancestry (Mitich et al., 2000).

Kudzu was not recognized as a cover crop in 1953 after the dissemination activities started in 1933 with the aim of controlling soil erosion and it was declared as an invasive weed of the south in 1970. It is estimated that Kudzu currently covers an area of seven million acres in the southeastern United States. It is reported to have spread as far north as Pennsylvania, Illinois, and Connecticut, and from east Texas to central Oklahoma in the west, with the largest invasions being in Mississippi, Alabama, and Georgia (Love et al., 2000). Kudzu (*Pueraria lobata*) is the plant that spreads to the north with increasing temperatures with climatic changes (Sasek and Strain, 1990).

2. KUDZU MORPHOLOGY

Herbaceous or woody, curly, winding, climbing vines like vines. Numerous shoots emerge from the root crown. The leaves are broad and large, pinnately 3-leaf, leaflets whole or with curved lobes, stipules ovate or linear. Each of the leaves, consisting of three leaflets, is in the form of formations attached to a node (Fig. 1). The middle leaf has an

egg-shaped and unclear appearance, expanding from top to bottom. The leaflets are 8-10 cm long. The upper part of the leaflets is smooth, the lower part is covered with dense silvery hairs. Leaf stalks are hairy.

The flowers are medium-sized, clustered along the knuckles of axillary or terminal pseudo panicles. The flowers can reach up to 20 cm in length. Kudzu blooms from July to September and the flowers are purple, reddish purple and have yellow streaks at the base, smelling like grapes. It forms long vines covered with small, brownish hairs that run along the ground, climbing any vertical surface and forming dense leaf bundles. The vexillary filament is usually attached to the tube, but is sometimes free; anthers are uniform.

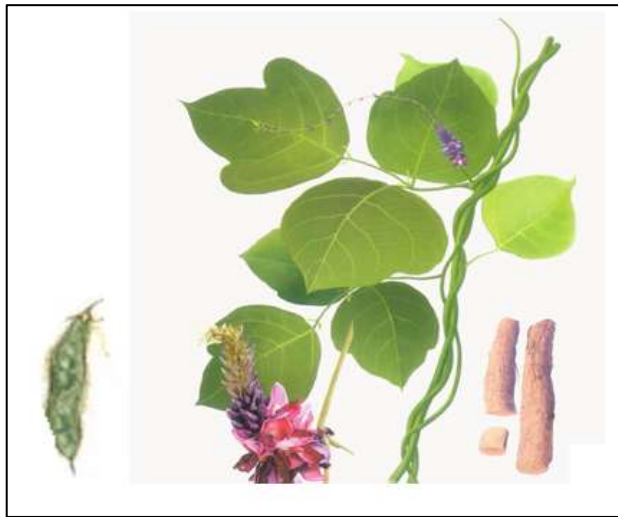


Figure 1. Leaf, flower, pod and root image of Kudzu (Anonymus 2023a)

Kudzu produces small pods about 5 cm long. Pods are long and linear, compressed, with brown hairs (Fig. 1). Seeds are small, oblong, hemispherical or almost cylindrical, kidney-shaped and usually slightly

hairy. Kudzu propagation is usually by clones or seeds. Kudzu's primary propagation method is asexual vegetative propagation (cloning) by rhizomes supported by its rooting ability wherever the stem is exposed to soil. Vegetative propagation is quite rapid (Fig. 2). In propagation by seeds, Kudzu can reproduce completely dependent on pollinators. Propagated by seeds contained in pods. There may be only one or two viable seeds in the pod and the pods are hard, so the seeds may not germinate for several years.



Figure 2. The propagation of Kudzu in nature (Anonymous, 2023b)

3. KUDZU CULTIVATION

“*Pueraria*” genus consists of 26 species native to South Asia, distributed in subtropical and temperate regions of Asia, with the more common species *Pueraria lobata* and *Pueraria thomsonii*. *Pueraria lobata* is a highly adaptable species. It grows on damp slopes, valleys and short shrubs. It grows well in deep, loose, sandy loam soils rich in humus. It grows better in areas with at least 1000 mm of precipitation

where the air temperature is at least 4°C in winter and above 27°C in summer. In short, it prefers high temperature and humidity. Kudzu is very resistant to drought due to the carbohydrates it accumulates in its roots and its growth is quite fast at 30 cm per day. In Korea, kudzu can be grown in regions where the temperature can drop to -30°C (Mitch, 2000).

Kudzu's thriving roots enable plants to survive in extremely dry climates and arid conditions. Ideal living conditions are moist, well-drained, acid or neutral soils (4.5-7.0 pH). It prefers degraded areas such as forest edges or abandoned fields and roadsides as habitat. Kudzu can thrive well on the ground of a closed canopy forest. Although Kudzu can grow in a variety of climates and terrains, certain conditions may produce the best Kudzu. Roots are richer in starch in winter. The colder the winter, the more starch each tuber can produce. In the wild Kudzu harvest, Kudzu can sometimes be confused with poison ivy. Poison ivy stems are hairless while kudzu stems are hairy. Kudzu vines also curl up, and poison ivy does not have this feature. These two features are the best way to tell the two separate. Seeds can be sown directly into the field, or by first growing seedlings from the seeds, and then planting the obtained seedlings in the field. In order to create seedlings, the seeds are sown in the furrows opened at 90 cm intervals in June-July with 2 cm intervals and 1-2 cm soil depth. 7-11 kg of seeds are used for one hectare. When the seedlings have 4-6 leaves, they are planted in the field at 2 m intervals. In vegetative production of kudzu, cuttings taken from root crowns are used, 1250 cuttings are sufficient for one hectare

(Elçi, 1988). Kudzu plant cannot be used in the first year, but two forms of yield can be obtained in the second year. The yield of seeds capable of germinating from kudzu is low. Seeds can be harvested from fruits on branches that hug hedges or trees. Seeds show 50-60% hard-shelled property.

4. KUDZU AS FOOD

Its flowers, leaves, shoots and roots are used in human nutrition, and the flowers are also used by brining and producing starch from the roots. The root and flower buds of *Pueraria lobata* are widely used as food. Kudzu roots used are also known as Ge Gen (Chinese name), Kudzu root (*Radix puerarie*), Yegen, Ye ge or Kudzu vine, Japanese vine. The most common use is the use of starch obtained from Kudzu. The roots contain starch, which is traditionally used as a food ingredient in East and Southeast Asia. The use of kudzu roots of Chinese origin dates back to ancient times (Zhao et al., 2021). In China and Vietnam, Kudzu starch is used as a kind of natural medicinal beverage after being suspended with water and sugar without cooking (Van Hung et al., 2007). Kudzu starch is produced in China, Japan, and other East Asian countries (Liu et al., 2021) and is sold worldwide. Kudzu powder, known as lamb-ko in Japan and often shortened to "kuzu" in England, has been produced commercially in Japan since at least the early seventeenth century. It is used as a summer beverage in Vietnam, arrowroot tea, starch in Korea, and in dishes such as Kuzuko, lambmochi, mizu manjū and lamb in Japan (Fig. 3).



Figure 3. Use of root and starch of Kudzu (Anonymous, 2023c)

It also acts as a thickener for sauces. Its flowers are used to make a jelly that tastes like grape jelly (Shurtleff and Aoyagi, 1977). The leaves, flowers and roots of the plant are used raw, sautéed, deep-fried or as a herbal supplement or root tea. It is also used as a gelling agent for salads, such as agar or gelatin, as well as making noodles. Roots (*Pueraria montana* (Lour.) Merr. roots) are a staple food in Japan. The peeled roots contain about 2.1% protein, 0.1% fat, 27.1% carbohydrates, 1.4% ash. 100 g of root starch; 340 calories, 16.5% moisture, 0.2 g protein, 0.1 g fat, 83.1 g total carbohydrates, 0.1 g ash, 35 mg Ca, 18 mg P, 2.0 mg Fe, and 2 mg contains Na. The nutritional content of 100 g of cooked leaves is; 36 calories, 89.0% moisture, 0.4 g protein, 0.1 g fat, 9.7 g total carbohydrates, 7.7 g fiber, 0.8 fat, 34 mg Ca, 20 mg P, 4.9 mg Fe, 0.03 mg thiamine, 0.91 mg riboflavin, 0.8 mg niacin (Anonymous, 2023d).

5. KUDZU AS FORAGE

Kudzu is a very good pasture and hay crop. The plant is a perennial, although it is difficult to get a good outlet and plant. During the winter,

it is dry and leaves a lot of organic matter in the soil. It begins to develop again towards spring and remains green in the high temperatures of summer and during periods of precipitation. In places with cool climate, *P. thunbergiana* species is more productive. In the Kudzu plant, no cutting or grazing is done in the first year, but mowed from the second year. It gives 2 forms a year. The first is done in the middle of summer and the second is done in the fall (Elçi, 1988). It has a high quality and delicious feed yield during the growing season. The hay yield is in the range of 5 or 10 tons per hectare. Crude protein content is 19.6% in leaves, 5.6% in stems, 13.3% in hay and silage. A small herd can graze 0.40 hectares of Kudzu every day. Sheep, goats and pigs can also graze easily and with pleasure (Fig. 4).



Figure 4. Kudzu grazing by animals (Anonymous 2023e,f)

6. KUDZU AS MEDICINAL PLANT

Especially recently, herbal treatment methods have started to attract attention with the tendency from chemical drugs to natural. Medicinal plants, which come to the fore in the reduction or struggle of some

addictions (cigarettes and alcohol) that negatively affect life in healthy life, and therefore herbal treatment have gained importance. Kudzu, which is one of the remarkable plants in this context, has become one of the well-known plants in our country. It is the root of Ge Gen or Yege *Pueraria lobata*, widely used in human health and known as Kudzu Root. This root is a Chinese herbal medicine (*radix puerariae lobatae*) that was first used in the late Western Han Dynasty and later became widespread. Listed as a distinctive Chinese herbal medicine in the Chinese pharmacopoeia is *Pueraria thomsonii* (Fenge). In ethnic medicine, the root of *P. thomsonii* is also called Ge Gen (Wong et al., 2011). Kudzu root is used as a herbal medicine in the treatment of fever, acute dysentery, diarrhea, diabetes, neck stiffness, sweating deficiency, alcoholism, hepatoprotection, neuroprotection, antioxidant, estrogenic activity, and cardiovascular diseases (Zhao et al., 2021).

Cellular, animal, and human studies have provided support for the use of rabies in cardiovascular, cerebrovascular, endocrine, and metabolic conditions and their associated complications. It has also been evaluated in traditional Chinese medicine as a general health preservative in the improvement of menopausal symptoms and accompanying osteoporosis and heart failure and hangover (alcohol dependence) (Zhang et al., 2021). The compounds called “daidzin” and “daidzein” found in kudzu roots and flowers have shown that it is a safe and effective method in the treatment of alcohol use in order to eliminate the negative effects of alcohol. Although other treatments

interfere with the way alcohol is metabolized, the use of Kudzu helps to suppress or reduce the desire to drink alcohol (Bown, 1995).

Kudzu, which is used in Chinese medicine, has also been investigated in our country for alcohol use disorder. Although the exact mechanism of action is not known, there are studies showing that it acts via aldehyde dehydrogenase or monoamineoxidazacetaldehyde pathways, causing a rapid decrease in blood alcohol level after alcohol use and reducing the amount of alcohol used in heavy drinkers (Evren and Bozkurt, 2015). Some drugs containing Kudzu, which are currently used in the treatment of alcohol or hangover, are sold in our pharmacies.

Kudzu vine, known as Ge Gen in traditional medicine in China, is a plant that is considered one of the 50 basic medicinal plants and is widely used in Chinese herbalism. Isoflavones are colorless and crystalline phenolic ketone structures isolated from plants. They are among the most common flavonoid class of phenolic substances. They show weak estrogenic activity. Soy and its products are the most common (1.0-4.0 mg/g km) sources, and there are other plant sources such as red clover, alfalfa, Kudzu root, flaxseed and chickpea (Liu, 2004). Medicinal compounds are in the root of *P. lobata*. They are flavonoids and isoflavones, including daidzein, genistein and puerarin (8-C-glycoside of daidzein), which have various biological activities such as anti-alcohol, antioxidant, antipyretic and anticancer (Wong et al., 2011).

Kudzu (Ge Gen); puerarin, daidzein, daidzin, genistein, biochanin A, genistin, formononetin, ononin, mirificin, isoliquiritigenin, kudzusapogenol A, kudzusapogenol B, kudzusapogenol C, sophoradiol, soyasapogenol, rhamnosecinglucosine, arachnids, galactosine It contains anthraquinone, starch, dietary fiber, amino acids, fatty acids and alkaloids (Prasain 2003). Amino acids and sugars that increase the nutritional value and flavonoids, isoflavones and phenolic acids that contribute to the medicinal value, It is significantly higher in *P. thomsonii* than in *P. montana* (Shang et al., 2021).

7. KUDZU IN THE GREEN FERTILIZATION AND SOIL CONSERVATION

Kudzu is a plant that can grow not only in soils that have lost some of their characteristics, but also in all kinds of soils. It is easily used for erosion control and reconstruction of depleted soils. Kudzu, a legume member, also adds nitrogen to the soil through the activities of root bacteria. If the Kudzu planted in a harvested field is plowed thoroughly in the spring and replanted on it, the nitrogen requirement of the planted plant is met. Bacteria in the roots of Kudzu, which has a symbiotic relationship with some soil bacteria, form nodules and fix atmospheric nitrogen. Some of this nitrogen is used by the growing plant, while some can be used by other plants growing nearby. Roots can make up 40% of the total plant biomass.

The leaves are capable of fixing atmospheric nitrogen, which can provide the plant with up to 95% of leaf nitrogen in poor soils. It also acts as a 'green manure' by increasing many minerals that should be in

the soil. Kudzu, which dries in heavy frosts in severe winter conditions, also prevents the soil from freezing. Along the vines are nodes (knots), which are points where stems or shoots can breeding increase supporting and attach to structures. As an ivy vine, Kudzu uses stems or branches that can extend from any node in the vine to attach to and climb most surfaces, and can be rooted by further anchoring these nodes to the soil, making it easy to cover bare areas and cover the soil. Twisting the plant provides a lower carbon concentration in the construction of woody stems and a higher concentration of carbon in the roots that aid root growth.

8. KUDZU AS FIBER

Due to its large leaves and fragrant flowers, *Pueraria thunbergiana* is used as an ornamental plant in the decoration of gardens and houses. Kudzu fiber has also been used in fiber art and basketry for many years. The fiber is 2 - 3 mm long and can also be used in paper making. Kudzu vines are an excellent weaving material. The fibers obtained from the stems are also used in the manufacture of weavings, ropes, cables, coarse ropes.

9. CONCLUSION

As a result, Kudzu; The awareness of the use of Kudzu plant, which belongs to China, Korea and Japan and continues to be used in many areas, is very valuable especially in the fields of food, health and soil conservation, needs to be developed. Kudzu, which is harvested at least twice a year in these countries, kept under control, used and evaluated,

uses nitrogen obtained from the air to enrich the soil and help other plant species to grow comfortably. It also helps to cover the soil by moving rapidly in the area with its nodes and to protect the soil by preventing the soil from being exposed to water wind erosion. However, in addition to these advantages, it is a dangerous legume as it spreads rapidly and prevents the lives of other plants and living things by shading the areas it covers. However, this situation can be eliminated by increasing its harvest and use. Kudzu can be a valuable legume plant that can be benefited by grazing and mowing by animals in a controlled way, by using its shoots, leaves, flowers and roots in the field of food and health, weaving and decorative items in both wet or dry ways.

In the words of one researcher, “When Kudzu ate south, why didn't south eat kudzu?” With this approach, thoughts, approaches and researches about “Kudzu eating the south” should be guided.

When Kudzu ate south, why didn't south eat Kudzu? (K. Carter King)

(Anonymous, 2023g)

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CHAPTER 6

HEAVY METALS IN ANIMAL NUTRITION: IMPORTANCE

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INTRODUCCION

Heavy metals are metallic elements that occur naturally in nature and have high atomic weight and density. In general, metals that are 5 times denser or more dense than water are called heavy metals (Fergusson, 1990). Anthropogenic degradation of the biosphere, rapidly developing global industrialization, intensive agriculture, mining, and rapid urbanization have not only destroyed natural resources but also rapidly reduced habitats and caused serious pollution of the basic components of life. Heavy metals play a critical role in various vital functions associated with living biological systems. Some heavy metals, which are ubiquitous in nature, pass from pellets to plants, and secondarily to animals. Their intensive use and prevalence lead to various chronic problems, including carcinogens. A few heavy metals such as lead (Pb), arsenic (As), chromium (Cr), and mercury (Hg) are classified as human carcinogens by the International Agency for Research on Cancer (Koedrith & Seo, 2011).

Heavy metals (silver (Ag), arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), mercury (Hg), nickel (Ni), lead (Pb), and zinc (Zn)) are elements with a specific gravity of 5g/cm^3 and above (Van Roy et al. 2006). The term 'heavy metal', which has various definitions in chemistry and thus is not standardized, is used even more loosely in biology, agriculture, and medicine. For example, "heavy metal poisoning" in medicine as well as real heavy metals also includes health problems caused by excessive amounts of non-heavy metal manganese (Mn), aluminum (Al), or beryllium (Be) according to the above

chemistry definitions. However, labeling a metal as a 'toxic metal' has a meaning on its own. On the other hand, living organisms require trace amounts of some heavy metals such as cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), vanadium (V), strontium (Sr), and zinc (Zn). But excessive amounts of these 'heavy metals' can be harmful to the organism. Other heavy metals such as Hg, Pb, and Cd have no known benefits to organisms. However, their accumulation in the body over time can lead to serious diseases in mammals. Therefore, it is best to use the term 'heavy metal' carefully and not even use it and group some of the metals under a fuzzy term, to deal with the chemical properties of metals in the periodic table separately, to look at which compounds are toxic rather than to talk about the toxicity of a metal.

HEAVY METALS

Iron (Fe)

Iron is one of the most common elements of the biosphere. The importance of iron in nutritional physiology emerged in the 19th century with the treatment of chlorosis, an anemic condition in women, with iron salts. The iron in the animal organism is 60-90 mg per kilogram of body weight. 60-70% of this is in the composition of hemoglobin, 0.10-20% is in the composition of myoglobin, and 21-25% is in the liver and spleen. Iron has taken on important biological functions. Most iron is found in hemoglobin and myoglobin, and iron-protein compounds such as ferritin and transferrin. Absorption in animals is mainly in the duodenum with a simple stomach system and is easily soluble. As iron is mostly found in natural foods, it can be

absorbed after dissolution in gastric hydrochloric acid or organic acids and reduction in the digestive tract. The normal absorption mechanism of iron is through the formation of ferritin in the intestinal mucosal tissue. Iron is absorbed only in this form. While iron deficiency occurs frequently in humans, it may only happen during the lactation period in animals. Iron deficiency never occurs in old animals with regular feeds. The presence of high amounts of iron in feeds is indirectly harmful. Digestion and absorption of other important nutrients are reduced or the appetite for feed consumption is damaged. This negatively affects feed consumption (Kılıç, 1984).

Copper (Cu)

Copper is an element in the structure of enzymes such as cytochrome c oxidase, tyrosinase, p-hydroxyphenyl pyruvate hydrolase, dopamine beta-hydroxylase, lysyl oxidase, Cu-Zn superoxide dismutase, which play a role in many of the biological events in the organism. Copper is necessary for the existence of all kinds of bacteria in the organism. Its density in high-built animals and humans is 1.5-2.5 mg per kg body weight. In newborns, however, the body has significant reserves of copper. The organism has a higher density in the liver, spleen, kidneys, hairs, and brain than in the average copper presence. It has been determined that as the amount of copper in the organism decreases, hemoglobin synthesis also decreases, but copper does not enter into the structure of hemoglobin. Copper affects bone development. It also ensures the regular functioning of the central nervous system. It enters the structures of many enzymes and ensures their activities. Rickets is

observed in young animals grazing on copper-poor soils, and bone diseases similar to osteomalacia are observed in their development. Optimum or above-required copper intake in daily rations has a positive effect on daily weight gain, feed efficiency, and fattening efficiency. After the use of copper, the number of microorganisms in general decreases. This effect is highly dependent on the solubility of the copper compounds used in the stomach and intestinal tract. An increase in copper content in some internal organs such as the liver is not desirable. Feed dosage of 100-250 ppm in ruminants is absolutely toxic for cattle and sheep. The daily copper excretion in sheep is 3 mg or slightly less. Although meadows and pastures contain 3 mg of copper in some regions, copper deficiency symptoms are still observed in sheep. It is known that the daily copper needs of fleece sheep increase up to 10 mg or 10 ppm in ration dry matter. When copper has consumed 10 times the normal dose in ruminant animals and this feeding continues, it shows the toxic effect (Tufan, 2008). Since the absorption of copper is very slow, only very high doses are toxic. Intake of high doses of copper causes a decrease in growth and feed consumption, rapid weight loss, and death in a short time (Tufan, 2008). Copper toxicity in sheep progresses in 2 phases. This phase in the first phase can be seen even with copper intake below 25 mg/kg, animals do not show many clinical signs, and blood copper levels, and liver enzymes are slightly elevated. In the second phase, the hemolytic crisis occurs suddenly and is accompanied by jaundice, loss of appetite, excessive thirst, and hemoglobinuria. The blood hemoglobin and glutathione concentration drops dramatically within a few days and the methemoglobin

concentration increases. While few animals with these symptoms survive, most die within a few days of liver and kidney damage. Because cows have a high tolerance for copper, copper poisoning is rarely seen in these species (Yiğit and Kabakçı, 2018). Poisoning takes place in 2 phases, just like in sheep. In order for the second stage to be seen, the copper level in the liver must exceed 125 mg/kg (Yiğit and Kabakçı, 2018).

Zinc (Zn)

Zinc has great roles in ensuring growth, intracellular functions, protein synthesis, cardiac metabolism, regulation of acid-base balance in the organism, fertility and infertility, activation of insulin, physiology of mucous membrane, and prevention of hyperkeratosis, especially in the stomach (Sevgican 1977). The weight of zinc, an essential element, in the human body is between 20-30 ppm live weight. Zinc is present in all active cells, albeit at different physiological concentrations. In case of excess intake, it is stored in bones, teeth, skin, hair, and wool. About 40% of the zinc retained in the body is found in the liver. 25% of this is removed from the liver within five days. Zinc concentration in the liver is affected by adrenocorticotrophic hormone and parathyroid hormone (Mertz 1986). Poisoning due to excessive zinc intake is not common. It is known that foods and beverages stored in galvanized containers for a long time cause digestive system disorders and diarrhea due to consumption (Saltes and Bailey 1984). Small amounts of zinc are required for egg production, hatchability, growth, and proper feathering in poultry. Due to zinc deficiency, feathering disorders (curly and

tangled feathers) in chicks and chickens, skin deterioration (scaling of the foot skin), and bone disorders (swelling of the knees and deformation of the leg bones) are observed. Since feedstuffs are generally poor in zinc, it is necessary to add zinc to poultry feed in the form of zinc carbonate (57% Zn) or zinc oxide (80.5% Zn). On the other hand, high (1500 ppm and above) zinc contribution to the rations of growing poultry causes growth retardation, exudative diathesis, and muscular dystrophy (Kutlu, 2015). Zinc is one of the various minerals needed for the body to function smoothly in humans. It is a mineral necessary for the strengthening of the immune system, healing of skin wounds, cell production, and the production of approximately 300 essential enzymes. However, excess zinc reduces the amount of other essential minerals such as iron and copper in the body. It also reduces the enzymes in the body, causing the weakening of the immune system (Gerberding, 2005).

Arsenic (As)

Arsenic compounds, which are among the heavy metals that pollute nature, are used in the manufacture of herbicides, insecticides, defoliants, detergents, leather, paper, ceramics, glass, and rubber. The increase in the amount of arsenic in water is the main indicator of environmental arsenic pollution. It has been reported by the World Health Organization that it can be found in waters with a density of up to 10 ppb, and that water containing more than 50 ppb of arsenic should not be used (Yilmaz and Ekici, 2004). The fact that arsenic compounds are mostly odorless, tasteless, and easily miscible with other drugs and

that they have found widespread use until industrial and agricultural warfare, easily explains the prevalence and frequency of poisoning with these compounds. Arsenic, which has a toxic effect on all living things without discrimination, is usually found in elemental trivalent and pentavalent compounds. Their toxicity is closely related to the duration of their presence and elimination in the body. Acute poisoning with arsenic in cattle is rare. There are 3 oxidative forms of arsenic and the forms that cause acute toxicosis in cattle are trivalent (arsenite) and pentavalent (arsenate) forms. In goats, it has been shown that arsenic has a hemolytic and cytotoxic effect on erythrocytes and leukocytes, as well as causes a decrease in T and B lymphocytes, causes a very significant decrease in IgG levels and increases apoptosis in cells due to increased caspase 3 activity (Patra et al., 2013). Herbicides, fly repellants and ectoparasite drugs prepared as bath liquids are among the most dangerous arsenic preparations in terms of posing a poisoning risk in pets.

Cadmium (Cd)

Cadmium is a substance that is not normally found in the body, but accumulates in the kidneys and liver as a result of lifelong exposure and has toxic effects for almost every system in the organism. Along with nickel, it is a heavy metal that pollutes the environment with battery content, phosphate fertilizers, detergents, and petroleum refinery products (Kahvecioğlu et al., 2003). The European Food Safety Agency determined the highest residue level in feeds as 5 mg/kg (Lane et al., 2015). They reported that anemia, kidney disorders, and bone mineral

loss occurred in sheep exposed to 2.5 mg cadmium per live weight experimentally. It has also been shown that toxication and liver damage develop in broilers with 60 mg of Cd per kg of feed, and RBC and MCV are significantly reduced (Çınar et al., 2010; Çınar et al., 2011). Cadmium is an antagonist of more zinc and copper and less iron. Diets containing 5-30 ppm of cadmium generally reduce animal performance by inhibiting the absorption of copper and zinc and often produce symptoms of copper and zinc deficiency. Cadmium binds the intestinal metallothionein very tightly, reducing copper and, to a lesser extent, zinc absorption. The liver and kidneys contain metallothionein proteins that accumulate cadmium throughout the animal's life (Goff, 2018). In ruminants, less than 1% of dietary cadmium is absorbed. Intestinal metallothionein binds cadmium tightly and limits its absorption. Cadmium can be detected in very small amounts in milk, but milk cadmium concentration does not increase with increasing dietary cadmium levels because it limits mammary gland transport (Goff, 2018).

Lead (Pb)

Lead is the first metal to cause the most significant damage to the ecological system through human activities. Lead is the most important heavy metal causing environmental pollution because it is emitted into the atmosphere as a metal or compound and is toxic in any case. Foods grown especially near industrial and urban centers contain lead above normal levels in grains, legumes, garden fruits, and many food and meat products. Lead sources used in water pipes and water installations in

old houses can cause lead to mix with water. They contain lead in many pigments and other main substances found in cosmetic materials. On the other hand, cigarettes and pesticides can be counted among the sources of lead (Kahvecioğlu et al. 2004). Various nutrients contain varying amounts of lead. In ascending order, depending on the soil in which it grows, lead is found in plant-based foods, fish and seafood, meat, and eggs. The use of lead-containing paints, especially in homes, causes toxic effects (Denizli 2008). Lead is taken into the body mainly through digestion, respiration, and skin. Lead uptake through digestion is slow. Lead is chemically similar to calcium, and the body uses lead as if it were calcium. It is distributed to places where calcium plays an important role. It creates problems, especially in places where tooth and bone development is important. Apart from bones, lead concentration in the kidney and liver is important. Lead taken through the blood causes the red blood cells to lose water and potassium by disrupting the water-electrolyte exchange between the red blood cells and the extracellular fluid. The membrane integrity of red blood cells is impaired, and their disintegration becomes easier. As a result, anemia occurs (Denizli 2008). In the food chain, the spread of lead generally occurs through mussel-like calcium-containing crustaceans and depends on calcium. It is known that single-celled organisms and fish can tolerate water containing 0.04 – 0.198 mg/liter inorganic lead, but they show acute poisoning when lower amounts of lead are ingested (Kahvecioğlu et al. 2004).

Nickel (Ni)

Contamination of soils with heavy metals can reduce the quality of agricultural lands, as well as product yield and quality (Khaliq et al., 2016; Nazir et al., 2015; Rehman et al., 2016). On the other hand, heavy metal contamination of agricultural soils has caused serious environmental and health problems (Khan et al., 2016; Zafar et al., 2015). Like most other heavy metals, nickel (Ni) is an essential micronutrient and is necessary for normal growth and plant development. However, Ni toxicity leads to various physiological disorders in plants (Guo et al., 2010; Kamran et al., 2016). Plants grown in nickel-contaminated soils accumulate in the human body through nutrients. (Khan et al., 2016; Zafar et al., 2015). Previous studies have shown higher Ni concentrations in different plant parts of maize (Guo et al., 2010; Marwa et al., 2012), reporting that when rice (Nazir et al., 2016), wheat (Wang et al., 2015), *Eruca sativa* (Kamran et al., 2016), and cotton (Khaliq et al., 2016) are grown with a Ni source, excess Ni accumulation reduces dry weight and grain yield of maize grown in Ni-contaminated soil. Experiments in humans and rodents have shown that nickel exhibits both immunomodulatory and immunotoxic effects, producing allergic dermatitis and immunological urticaria. Nickel exposure may occur as allergic reactions by damaging the immune system (Al-Atar, 2007; Das et al., 2008). Mice lifetime exposure to nickel oxide (42 mg/m^3) produced emphysema and other proliferative and inflammatory changes (Wehner, 1986). Vyskočil et al. (1994) reported that drinking water contains NiSO_4 when exposed to 100 mg/l

in both male and female rats, causing significant increases in kidney weights. Urinary albumin excretion was significantly increased in female rats, but the increase was marginal in male rats. Nickel crosses the placental barrier, directly affecting the developing embryo/fetus in experimental animals. Inhalation exposure to mice and rats is known to cause testicular damage (Benson et al., 1988).

Selenium (Se)

Selenium is an essential element for poultry, but the difference between the required dose of selenium and its toxic dose is very small. While 0.15 ppm selenium in feed is defined as the dose required for the normal course of all physiological functions, 15 ppm selenium causes serious abnormalities such as a decrease in live weight gain, reduction in egg size, and termination of fertility (Kutlu, 2015). Selenium toxicity occurs in two forms, acute and chronic. Acute selenium poisoning causes liver and kidney damage. Hemorrhagic exudate is seen in the lungs and ascites is common. Blindness and stumbling are also common. Gastroenteritis may be present. In chronic selenium poisoning in horses and cattle, lameness, hair loss, and hoof disorders are seen (Goff, 2018). When selenium is taken in large amounts in poultry, it can cause growth retardation, a decrease in output power, embryonal disorders, and anemia. Its optimal level in the ration is 150 ppb. At high levels, it causes toxic effects and growth retardation, and output power decrease. Since selenium passes into meat tissue and eggs, care should be taken when adding it to the feed. In order for it not to pass into these tissues,

at least one month must elapse after the selenium supplement is discontinued (Kutlu, 2015).

Cobalt (Co)

Cobalt is the central building block of vitamin B₁₂. Cobalt deficiency increases the risk of anemia. However, according to the assessment of IARC, high doses of cobalt have a carcinogenic effect on humans. Cobalt sulfide significantly increases the incidence of lung tumors in animals compared to controls. Cobalt also induces all bases, especially Thymine instead of Guanine, Cytosine rather than Adenine in DNA cleavage. Cobalt reacts with H₂O₂ to O₂⁻ and OH⁻ as in the cobalt-oxygen complex. In the presence of chelators, ROS (reactive oxygen species) production is facilitated. Recent studies have found that cobalt causes mitochondrial DNA damage in neuronal cultured cells (Davidson et al., 1993; Plowman et al., 1991; Plowman et al., 1994). Cobalt toxicity causes decreased feed consumption, loss of body weight, and eventually anemia. Toxicity symptoms are similar to deficiency symptoms (Goff, 2018).

Molybdenum (Mo)

Molybdenum is a component of the enzymes xanthine oxidase, sulfide oxidase, and aldehyde oxidase found in milk and most tissues. Milk and plasma molybdenum concentrations increase with increasing dietary molybdenum intake. Dietary molybdenum is important in practice because it is primarily an antagonist of copper and phosphorus and prevents their absorption. Signs of molybdenum poisoning are actually

related to copper deficiency. Molybdenum and sulfate react in the digestive tract to form a thiomolybdate complex with a high affinity for copper. Copper bound to this type of molybdate cannot be absorbed. Molybdenum toxicity can be prevented by supplementation of copper, and copper poisoning can be reduced by supplementation of molybdenum. The ratio of dietary copper to dietary molybdenum required to avoid copper deficiency ranges from 2:1 in Canadian reports to 4:1 in UK reports. The amount of molybdenum in English meadows is high (20-100 mg/kg forage DM) (Goff, 2018).

NUTRITIONAL IMPACT OF HEAVY METALS IN ANIMALS

Some of the metals (eg iron, manganese, zinc, copper, cobalt, molybdenum, etc.) are necessary for the life of organisms. The smallest change in the concentrations of these metals, which have vital functions in the living body, causes destruction in the tissues and thus a second change by preventing the organ and tissue from performing their duties (Merlini, 1980). Heavy metals, which are one of the important pollutants and the amount of which is increasing due to the polluted environment, have become a source of contamination that is a problem in our environment. It is not possible to separate food safety from feed safety for a healthy diet. The health of animal foods offered for human consumption is closely related to the feed that animals eat. Animal products obtained from animals consuming feed raw materials contaminated with heavy metals in environmental transformation reach the human body through the food chain. Thus, consumption of contaminated food can lead to significant health problems (which may

even result in sudden death) depending on the concentration of heavy metals taken into the body and the amount of retention in the body (Kayhan, 2006).

In general, the effects of heavy metals are on the central nervous system, liver, kidneys, spleen and circulatory system, and all tissues in the body. When they start to accumulate, they cause nervous system disorders, dizziness, loss of appetite, cardiovascular diseases, negative effects on blood formation systems, cancer, anemia, sudden death, and many unspecified physiological disorders (Hızel and Şanlı 2006; Kayhan et al. 2006; Yeşilada and Gelegen, 2000).

It has been reported that in ruminants, symptoms such as delayed puberty, increased number of inseminations per pregnancy, ovulation disorder, early embryonic death, fetal development disorders, irregular cycles, subestrus, anestrus, difficult births, excessive bleeding during delivery, and retention of secundarium can be seen (Manickam et al., 1977; Mehta and Gangwar, 1984; Yıldız and Balıkçı, 2004).

In a study conducted on dairy cows, RBC, Hb concentration, PCV, and MCHC values in animals fed with 500 mg/kg zinc methionine feed were found to be significantly higher than the control group (Sobhanirad and Naserian, 2012).

With the increase in pasture lead concentration in dairy cows, a decrease in feed consumption and an increase in digestive problems were observed in animals (Strojan and Phillips, 2002).

In a study conducted in the state of Wisconsin in the USA, heavy metal analyzes were made in TMR (Total Mixed Ration) consumed by dairy cattle, and Cr, Pb, Cd, Zn, Cu, and Ni were determined in the samples. Cadmium and lead levels in dairy cattle were significantly correlated with the levels determined in the ration (Miranda et al., 2003). Based on available information, it has been reported that Cd is not added as a feed additive for animal growth.

In a study conducted in Van, 21 commercial poultry feeds were investigated for some heavy metal levels. In the study, average heavy metal levels in poultry feeds were determined as Cr 1.205 µg/g, Mn 37.147 µg/g, Fe 70.458 µg/g, Co 0.079 µg/g, Zn 18.832 µg/g, Se 0.061 µg/g, and Mo 0.500 µg/g. The level of these heavy metals detected in feed samples does not exceed the maximum amount of heavy metals determined by NRC and some other International organizations. However, as a result of the continuous mixing of these heavy metals in poultry feed, it can cause health problems in people consuming meat and eggs of poultry (Yücel, 2018).

Suleiman et al. (2015) determined the Zn level as 1.43-11.65 and the Mn level as 0.94-3.12 µg/ml in three different brands of feed most commonly used in Sokoto.

Bukar and Sa'id (2014) investigated heavy metal levels in selected poultry feed samples collected from Kano, Nigeria and they found Cd, Co, Cu, Fe, Mn, Ni, Pb, Cr, and Zn levels were 0.53-3.19 mg/kg, 0.13-3.33 mg/kg, 2.03-5.41 mg/kg, 8.79-19.74 mg/kg, 12.50-37.50 mg/kg,

1.03-2.06 mg/kg, 0.27-0.80 mg/kg, 0.47-2.38 mg/kg, and 16.30-38.04 mg/kg, respectively.

Imran et al. (2014), purchased 21 poultry feeds from three feed companies and five poultry farms to determine heavy metal pollution in poultry feed, and they determined the Cr, Fe, Mn, and Zn levels in the feeds as 0.28-7.71 ppm, 76-116.1 ppm, 0.24-0.97 ppm, and 23.6-50.2 ppm, respectively.

In a study by Ukpe and Chokor (2018), the Co level they detected in poultry feeds was 0.39 g/kg, and the Cr level was 0.529 g/kg.

Islam et al. (2007) examined heavy metal concentrations in some poultry feeds sold in Bangladesh and reported that the Se level was 0.0347-0.0069 ppm, the Cr level was 5.7875-0.0926 ppm, the Mn level was 302.2001- 0.0695 ppm, and the Zn level was 422.3023- 0.0232 ppm.

Co level 0.14- 1.73, Zn level 4.57- 34.18, Mn level 9.77- 42.93, Fe level 0.51 - 55.38, and Cr level 3.78 - 4.89 mg/kg were found in some chicken feed samples that were taken from the local market in the Al Qasim region of Saudi Arabia, according to Alkhalaf et al. (2010).

Okoye et al. (2011), in their study to determine the Zn, Fe, Mn, and Co concentrations in chicken feeds obtained in the southeastern part of Nigeria, the detected concentrations were found to be 34.038-49.950 mg/kg for Zn, 50.575-170.075 mg/kg for Fe, 26.913-76.738 mg/kg for Mn, and 0.613-3.200 mg/ kg for Co.

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CHAPTER 7

CURRENT SITUATION IN TURKISH LIVESTOCK AND THE PROBLEM OF ROUGHAGE

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INTRODUCTION

Livestock is the most important and most valuable part of agricultural sectors. Thanks to livestock, animal products such as meat, milk and eggs are obtained, while at the same time sub-sectors such as animal feed, medicine and equipment develop and create employment. Livestock makes a great contribution to a country's economy.

There are many advantages for the development of animal husbandry in our country. Different climatic conditions, rich animal diversity and human resources living in rural areas increase the potential of animal husbandry in our country. However, some problems need to be solved to make use of this potential (Livestock report, 2020).

The biggest problem of animal husbandry is animal feed. Feed costs constitute a large part of the expenses of livestock enterprises. For this reason, increasing feed production and improving feed quality are very important for the productivity and profitability of livestock. It is essential to protect and improve pasture areas for feed production. Pasture areas in our country have both decreased in number and become inefficient. Necessary measures should be taken to increase and improve the pasture areas (Alçiçek, 2021).

Another problem of animal husbandry is animal health and genetics. Animal diseases both reduce the presence of animals and reduce the quality of animal products. Veterinary services should be expanded and supported for the prevention and treatment of animal diseases. In addition, studies on animal genetics should also be carried out and

projects should be implemented for the breeding and development of native breeds (Ceyhan et al., 2015).

A following problem of animal husbandry is marketing. Prices of animal products are unstable for both producers and consumers. This situation negatively affects both the producer's income and the consumer's demand. For the marketing of animal products, supply-demand balance should be ensured, price stability should be maintained, and quality standards should be established (Şahinler and Demir, 2016).

The livestock sector is a sector that can be the locomotive of the agricultural sector of our country. For the development of animal husbandry, the above-mentioned problems must be solved. In this way, the welfare of both producers and consumers will increase and added value will be provided to the country's economy.

THE IMPORTANCE OF ROUGHAGE

Roughages are feeds that originate from vegetables and play an important role in the nutrition of animals. Roughage can be given to animals as fresh, dried or in silages. Fresh roughage usually consists of green plants such as grass, clover, and vetch. Dried roughage is obtained by drying plants such as straw, hay, and clover. Silage roughage is prepared by fermentation of plants such as corn, sorghum, and meadow grass. The common feature of roughages is their high crude cellulose content. Crude cellulose is broken down into energy by microorganisms in the digestive system of animals. Roughages are

necessary to maintain the digestive health of animals and increase milk yield.

Roughages have an important place in the livestock sector. Especially in the nutrition of ruminant animals, the role of roughages is great. We can summarize the general characteristics of roughages as follows:

- Roughages are a feed group suitable for the digestive system of ruminant animals. These animals can utilize roughages more effectively. For another group of animals, roughages are not so beneficial.
- Roughages increase the health and productivity of animals. Thanks to the roughages, the productive life of the animals is extended, the fertility of the animals is improved, and the risk of feeding-related diseases is reduced. For example, diseases such as rumen acidosis, acid-base balance disorder, vitamin A deficiency are prevented with roughages.
- Roughages provide mechanical satiety of animals. Roughages, which give the animals a feeling of satiety, ensure that the animals are comfortable and reduce their stress. It is not possible to provide mechanical satiety of animals without using roughages.
- Forages are effective in preventing diarrhoea. Especially in ruminant animals, the diarrhoea problem that develops due to high energy and protein feeds can be solved by using dry roughage. The use of dry roughage is both a safe and inexpensive method.

- Roughages are the basis of pasture livestock. There is no other alternative for roughage obtained from meadow-pasture areas in pasture livestock. These areas can only be used for roughage production.
- Roughages contribute to a country's economy. Roughage production creates employment, makes use of uninhabited lands, and increases animal product production. In addition, roughage production prevents environmental pollution, improves the climate, and the quality of life. It protects the soil by preventing erosion (Gemalmaz and Bilal, 2016).

CLASSIFICATION OF ROUGHAGES

Roughages have a crucial role in animal nutrition. The classification of roughages can be made in accordance with their nutritional values and forms of utilisation. The following criteria can be used in the classification of roughages:

1. Roughages Rich in Water: These are roughages with a water content of 60-90% and meet the water needs of animals. This group includes:

1.1. Green fodders: These are the roughages that are green in colour and given to the animals as fresh. This group includes:

1.1.1. Pasture green fodders: Pasture areas are grasslands that grow naturally or are created by human intervention. Pasture green fodders

are roughages given by grazing or mowing in the natural environment of animals.

1.1.2. Meadow green fodders: Meadows are grasses that grow near water or in swampy areas. Meadow green fodders are roughages given to animals by mowing or grazing.

1.1.3. Produced green fodders: In this group, there are green fodder plants that are specially cultivated and used for animal feeding. These plants can be classified as:

1.1.3.1. Greed fodders produced annually: These plants are green fodder plants that are cultivated and harvested within a year and given to animals fresh or by conservation methods. For example, corn, vetch, sudan grass seed, barley etc.

1.1.3.2. Greed fodders produced perennially: These plants are green fodder plants that are cultivated perennially and harvested for years and given to animals fresh or by conservation methods. For example, sainfoin, clover, black sainfoin, cranberry etc.

1.2. Conservation and fabrication by-products: This group includes roughages obtained as a result of drying, fermentation or processing of green fodder plants. For example, straw, silage, pulp, etc.

1.3. Root and tuber feeds: This group includes roughages used in animal feeding consisting of roots or tubers of plants. For example, beets, potatoes, carrots, etc.

1.4. Fruits and vegetables: This group includes roughages used in animal feeding consisting of plants consumed as fruits or vegetables. For example, apple, pear, cabbage etc.

1.5. Silo feeds (soured feeds): This group includes roughages obtained as a result of anaerobic fermentation of green fodder plants and stored in an acidic environment. For example, corn silage, sainfoin silage etc.

2. Dry roughages: These are the roughages with a water content of 10-15% and do not meet the water needs of animals. This group includes:

2.1. Dry hay: This is the roughage obtained as a result of drying green fodder plants and given to animals as dried. For example, grass straw, sainfoin straw, etc.

2.2 Harvest residues (husks, straw, etc.) These are the roughages used in animal feeding consisting of parts such as stalks or spikes that remain after the harvest of cereal plants. For example, wheat straw, barley straw etc.

The importance of quality roughages in animal nutrition is as follows: In addition to being an inexpensive source for obtaining animal products, quality roughages ensure healthy functioning of the digestive system because they are rich in organic and inorganic nutrients necessary for the development of rumen microflora and fauna of ruminant animals. In addition, high quality roughages are important in terms of improving the performance of animals, preventing many

metabolic diseases related to feeding and providing high quality animal products (Özen and Çakır, 1993; Çolpan, 2016).

QUALITY OF ROUGHAGE SOURCES

Feed quality is a concept that shows how well the feed can be utilized by animals. There are many factors that affect feed quality. Some of these are the physical, chemical, and biological properties of the feed, the production and processing of the feed, the storage conditions of the feed and the compatibility of the feed with the digestive system of animals. Some parameters used in determining feed quality are as follows:

- Determination of quality according to dry matter or water content: Dry matter content of feeds is a value that is taken as a basis for calculating the nutrient content of the feed. Feeds with high dry matter content provide more nutrients. Water content increases the risk of spoilage of the feed.
- Determination of the quality according to crude ash content: crude ash content indicates the mineral content of the feed. Mineral contents are important for the health and productivity of animals. However, feeds with a very high crude ash content reduce digestibility and appetite of animals.
- Determination of quality according to Digestible Crude Protein (DCP) content: DCP refers to the digestible protein amount of the feed. Protein is an essential nutrient required for animal functions such as growth, reproduction, and milk

production. Feeds with higher DCP value are considered better quality (Aydođan and Topçu, 2022).

- Determination of the quality according to crude cellulose content: Crude cellulose is a carbohydrate that forms the fibrous part of the feed. The fibrous part is necessary for the rumen functions of animals. However, feeds with too much crude cellulose content reduce digestibility and lower their energy value (Güney et al., 2016).
- Determining the quality according to energy content: Energy is the most important nutrient required for vital activities of animals. Energy value of feeds is measured by the amount of heat produced by the metabolism of digestible nutrients in the feed. Feeds with high energy values are used more efficiently (Kaya, 2008).
- Determination of quality according to raw nutrients and their degree of digestion: This parameter enables the evaluation of feed in terms of both quantity and quality. The raw nutrients (protein, fat, carbohydrate) of the feed and how much they are digested by the animal (digestion degree) are determined. Feeds with high degrees of digestion are considered better quality.
- Determination of quality according to physical properties and sensory tests: This parameter examines the factors affecting the acceptance of feed by animals. The physical properties of the feed (colour, shape, size, density) and sensory tests (smell,

taste) are performed. Feeds with good physical properties and sensory tests are consumed more.

Feed quality determination forms for these parameters have been developed. Quality is the preservation of the properties of the feed at the time it was obtained. Processes applied from the harvest of the feed to the time it will be used in feeding, for example, drying or storing the feed by siloing, the appropriate processes during the application of these processes, the harvest period, time, drying or souring ability, shredding level, use of additives and, if used, its type and level: all processes such as distribution of feed, contamination rate with foreign matter, and storage have a more or less effective role on feed quality. The main issue here is to prevent the feed given to the animal from being rejected and left behind in the manger and be utilized by the animal in its metabolism. (Çakmakçı and Barut, 1997).

INCREASING THE FEED VALUE (QUALITY) OF ROUGHAGES

Of the available roughage, more than 90% of the roughage sources, excluding harvest residues, are water-rich roughages. Since it is not possible to use all of these water-rich feeds as soon as possible in places and times when their production is abundant, it is imperative that a significant part of them be safely stored in order to be used outside of their proportions. The conservation of roughage is done in two ways as drying and ensiling.

In the natural conditions of our country, no significant problem is encountered in the drying of water-rich feeds. For this, it is sufficient to properly harvest the roughage plant at a suitable vegetation stage, to take precautions to avoid foreign matter contamination, to reduce the excess water it contains to below 14% as soon as possible, and then to make a reliable storage. However, in any way, during drying and canning of water-rich feeds, losses in nutrients occur due to bacterial fermentation during respiration, mechanical crumbling, washing and storage. The level of these losses, in parallel with the care shown in the applied drying technique, has a value between 10-45% in dry matter.

Another conservation method commonly used in practice for safe storage (protection) of water-rich feeds is ensiling. Although the history of ensiling is quite old (about 1000-1500 years), it is a conservation method that has recently been applied in our country. The water-rich feeds preserved by the ensiling method are called silage. Silage is a feed obtained as a result of fermentation (souring) of green and water-rich roughage plants under the influence of lactic acid bacteria in an airless environment. Silage is also known as animal pickles. The place where the silage is stored is called a silo or silage pit. Silage is used intensively as a source of roughage in countries with developed animal husbandry. In our country, importance has been given to silage production in recent years. However, silage is a subject that requires technical knowledge. In order to obtain a quality silage, silo selection, filling, closing, waiting, and emptying during fermentation processes must be carried in accordance with the technique. In addition, scientific principles must be

considered in the selection, harvesting and size of the material to be ensiled. Otherwise, the silage will not reach its purpose and the expected benefit from the obtained silages will not be achieved (Kızılışımşek et al., 2016).

The main purpose of ensiling is to ferment water-rich green plants or plant parts under suitable conditions to obtain a product that preserves its nutritional value and is consumed willingly by animals.

The advantages of silage making are:

- It preserves the nutritional value of green plants rich in water.
- It plays an important role in the nutrition of high yielding animals.
- Provides quality roughage throughout the year.
- It is not affected by the weather conditions at the time of harvest.
- Reduces harvest loss.
- Reduces weed pressure.
- It prevents soil erosion.
- It provides labour and cost savings (Driehuis et al. 2000).

The disadvantages of silage making are:

- If proper technical and hygienic conditions are not observed in silage production, silage quality decreases.
- The plants to be used in silage production should be harvested at the appropriate time.

- If proper compaction and coating is not done in silage production, silage deteriorates as a result of air intake.
- If the appropriate acidity level cannot be reached in silage production, unwanted microorganisms develop.
- If a disease or pest is found in the plants to be used in silage production, it adversely affects the silage quality (Driehuis et al. 2000).

Silo feeds are roughages obtained by fermentation and storage of green and water-rich plants in an airless environment. Silo feeds play an important role in the nutrition of animals. Silo feeds have many advantages over the drying method. Some of these are as follows:

- With the ensiling process, the nutritional value of plants is preserved, and the losses are reduced.
- Silo feeds meet the water needs of animals and regulate the digestive system.
- Silo feeds are loved by animals and increase milk yield.
- Silo feeds enable crops to be harvested in a short time and increase the productivity of agricultural fields.
- Silo feeds are homogeneous and high quality in terms of colour, odour, aroma and structure.
- Silo feeds save storage space and can be stored for a long time.
- Silo feeds contain important nutrients such as carotene and support the health of animals.

- Silo feeds are an easy and inexpensive method of conservation (Konca et al., 2005).

Silo feeds are an ideal option to especially meet the roughage needs of ruminant animals in winter. Among the plants used in silage production are meadow grasses, legumes, corn, wheat, and other roughage crops.

The points to be considered in silage production are as follows:

- Harvesting the plants at the most appropriate time and quickly transporting them to the silos,
- Mowing and compacting plants to the appropriate size,
- Leaving the silos airless, ensuring their tightness,
- Keeping silos in suitable conditions,
- Giving the silage to animals by habituation.

Silo feeds and silage emerge as the most important option in meeting the abundant, cheap, and high-quality roughage needs of cattle and ovine breeding enterprises in an abbreviated time and sufficiently. This option provides a source of feed that can be used throughout the year, preserving the nutrients needed to feed the animals. Factors such as the selection of plants used in silo feed and silage production, harvest time, shape of the crop, silage method and additives affect the quality of the silage. Appropriate application of these factors increases the nutritional value of silage and positively affects animal performance (Acar and Bostan, 2016).

THE PRESENCE OF GRASS AND PASTURE IN TÜRKİYE

In our country, meadow and pasture areas are of great importance for the development and sustainability of the livestock sector. These areas are natural sources of nutrition for animals and reduce feed costs. However, the productivity and quality of meadows and pastures vary depending on many factors. These factors include climatic conditions, soil characteristics, plant diversity, grazing management, improvement studies and support policies. The situation of meadows and pastures in our country has changed over time with the effect of these factors.

According to the data of Turkish Statistical Institute, the meadow and pasture area, which was 38 million hectares in 1950, decreased to 14.6 million hectares in 2022. In the light of this information, although it is calculated that 43,851,000 tons of dry grass was produced from 14,617,000 hectares of meadow and pasture area in our country in 2022, it is thought that the theoretically calculated production value could not be realized due to the poor quality of the meadow and pasture areas in our country. The reasons for this decrease include the expansion of agricultural lands, industrialization, urbanization, erosion, and misuse. The decrease in meadow and pasture areas has led to problems in animal feeding. Especially in the Eastern Anatolia Region and Eastern Black Sea Region, where cattle breeding is common, meadow and pasture areas could not be utilized sufficiently (TÜİK, 2023).

The quality of meadows and pastures is important as well as the quantity. The quality of meadows and pastures is related to the

nutritional value, yield, and durability of plant species. The quality of meadows and pastures in our country is generally low. According to the data of the United Nations Food and Agriculture Organization, the dry grass yield in meadow and pasture lands in our country is 3000 kg/ha, while the world average is 4000 kg/ha (FAO, 2002). In addition, the nutritional value of meadow and pasture plants is low in our country. The reasons for this situation include the lack of plant diversity, low soil fertility, overgrazing, lack of fertilization and inability to control weeds.

Due to the poor quality of meadow and pasture lands, farmers and producers dealing with animal husbandry have turned to search for alternative roughages. For this purpose, cultivated green fodder plants and cultivated roughages are produced. Among the cultivated green fodder plants, there are legumes such as alfalfa, sainfoin, vetch, Hungarian vetch, and species such as triticale from the grasses. Cultivated roughage production is obtained from species such as silage corn and silage sorghum. Cultivated green fodder crops and roughage production play an important role in the nutrition of animals and increase animal performance (Topçu and Özkan, 2017).

As a result, meadow and pasture areas in our country have a strategic importance for the development of the livestock sector. Therefore, these areas need to be protected, developed, and fertilized. For this purpose, appropriate management techniques should be applied, improvement studies should be carried out, support policies should be developed, and the seed problem should be solved. In addition, production of cultivated

green fodder plants and cultivated roughages should be encouraged and expanded (Topçu and Özkan, 2017).

THE PRESENCE OF ROUGHAGE IN TÜRKİYE

Due to the fact that the quality and amount of roughage sources obtained from meadow and pasture areas in our country are not sufficient for animal feeding, breeders have turned to the cultivation of green fodder crops and to roughage production in this way. Considering the 2022 data of the Turkish Statistical Institute (Table 1), alfalfa is planted on 6.435.927 decares, silage corn on 5.298.522 decares, meadow grass on 4.955.951 decares, oat on 3.607.194 decares, and vetch on 3.421 decares. When the amount of green grass produced in the same table is compared, it is seen that 19.064.213 tons of alfalfa, 28.558.923 tons of silage corn, 3.683.405 tons of meadow grass, 4.649.051 tons of oats and 4.020.433 tons of vetch plant were obtained. When compared in terms of hay production, 4.766.053 tons of alfalfa, 7.139.745 tons of silage corn, 920.851 tons of meadow grass, 1.162.262 tons of oat and 1.005.108 tons of vetch were obtained. As can be understood from these production amounts, it is seen that alfalfa, corn and vetch have an important place in the production of roughage in our country in terms of cultivated green fodder crops (TÜİK, 2023).

Table 1. Roughage Crops Planted in Our Country in 2022 (TUIK, 2023)

Roughage Plant	Planted Area (Decare)	Production (Ton)	
		Green Grass (Ton)	Hay (Ton)*
Vetch	3.421.760	4.020.433	1.005.108
Trefoil	72	117	29
Clover	6.435.927	19.064.213	4.766.053
Meadow grass	4.955.951	3.683.405	920.851
Oat	3.607.194	4.649.051	1.162.262
Sorghum	29.205	117.076	29.269
Triticale	619.185	1.072.635	268.158
Chickling	66.994	55.208	13.802
Italian rye	539.944	2.122.105	530.526
Sainfoin	1.618.249	1.786.207	446.551
Bitter vetch	20.432	12.417	3.104
Corn (Silage)	5.298.522	28.558.983	7.139.745
Mangel	11.491	56.360	14.090
Feed turnip	49.459	268.890	67.222
Wheat	168.327	310.966	77.741
Barley	292.728	482.665	120.666
Rye	106.546	150.885	37.721
Manger peas	258.867	475.005	118.751
TOTAL	27.500.853	66.886.621	16.721.655

* ¼ of the green grass production was calculated as dry grass production.

ANIMAL PRESENCE AND ROUGHAGE DEFICIT IN TÜRKİYE

The number of animals in Türkiye is usually shaped depending on the supply-demand relationship, and according to the 2023 data of the

Turkish Statistical Institute, there are approximately 17 million cattle, 171 thousand water buffaloes, 45 million sheep and 12 million goats in the country (TUIK, 2023).

Roughage production and the rate of meeting the needs in our country is one of the prominent issues. As seen in Table 2, the roughage requirement in Türkiye is found by multiplying the number of animals by the Cattle Unit (CU).

Table 2. Animal Presence and Cattle Unit (CU) Presence in our country in 2022.

Animal	Number	Animal Unit	Cattle Unit (CU)
Cow	8.295.825	1	8.295.825
Cow Cross	7.324.866	0.75	5.493.649
Native Cow	1.231.265	0.5	615.632
Water Buffalo	171.835	0.9	154.651
Sheep	44.687.888	0.1	4.468.788
Goat	11.577.862	0.08	926.228
TOTAL	73.289.541		19.954.773

According to Table 1, fodder crops have been planted on an area of approximately 27.5 million decares in our country in 2022, and 67 million tons of green grass has been produced, and in return, a dry grass production exceeding 16.5 million tons has been realized. It has been calculated that approximately 44 million tons of dry grass production has been realized from an area close to 15 million hectares of meadow and pasture areas. As a result, in 2022, a total of 60.572.655 tons of roughage production was realized from the meadow and pasture lands

used in the production of roughage and the lands where forage crops were produced.

Table 3. Existence of Animals in Türkiye in 2022, Need for Roughage, Amount of Dry Grass Produced, Rate of Meeting Needs and Deficiency of Quality Roughage.

Cattle Unit (CU)	Total Roughage Requirement (ton)*	Amount of Dry Grass Produced (Ton)**	Needs Coverage Ratio (%)	Quality Roughage Gap (ton)
19.954.773	78.661.715	60.572.655	77.00	18.089.060

* 10.8 kg of hay was taken as the daily feed requirement of a culture breed cattle (1 CU) with 600 kg live weight. (21), ** Total Amount of Dry Grass Obtained from Roughage Crops and Meadow Pasture Lands.

When the total roughage requirement of one cattle unit is calculated according to AOAC (2005), it is seen that a 600 kg live weight cultured cattle needs 3% of its weight for dry feed, and this need is based on roughage up to 60%. According to this calculation, it is determined that a culture cattle weighing 600 kg needs 18 kg of dry feed and 10.8 kg of roughage. It is calculated that the roughage requirement of a 600 kg culture cattle for a year is 3.942 kg. The amount of dry grass produced from meadow and pasture lands and roughage crops in our country in 2022 was calculated as 60.572.655 tons (Table 3). As a result, based on the fact that the need for roughage in our country is 78.661.715 and the amount of hay produced is 60.572.655, it can be calculated that 77% of the roughage needs is met and there is a roughage deficit of 18.089.060 tons. However, these calculations consist of mathematical and relative values, and when we look at the real situation of our country, it is thought that the roughage deficit is much higher in reality. In order to

fill the quality roughage deficit, breeders tend to use poor quality alternative roughage sources (stalk, straw, husk, etc.). This tendency, on the other hand, leads to the inability to obtain the desired efficiency from animal husbandry, and the decrease in the contribution of agriculture and animal husbandry to the country's economy.

QUALITY ROUGHAGE PRODUCTION IN TÜRKİYE PROBLEMS AND RECOMMENDATIONS

In order for cattle and ovine animals to be healthy and productive, it is essential that they be fed in a balanced way with roughage and concentrated feeds.

Quality roughage production is insufficient in our country. Despite the support given to roughage crops, the desired level has not been reached in the production of plants such as alfalfa, vetch, forage pea, and sainfoin.

Feeding animals with roughage sources such as straw is not a suitable method in terms of productivity and health. Such feeds strain the digestive system of animals, have low nutritional value and low energy efficiency. In addition, the production and storage of these feeds is costly and laborious. For this reason, it is more beneficial to use quality green feeds in animal feeding. Green feeds provide protein, vitamins, minerals, and water needed by animals, facilitate digestion, and increase milk or meat yield. Among the green roughages, the most common are alfalfa, sainfoin, fodder peas, vetch, and meadow grasses. Growing and harvesting these plants are also easier and more

economical than straw. The importance of green feeds in animal nutrition is an issue that should not be overlooked.

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CHAPTER 8

ENVIRONMENT OF SOYBEAN (*Glycine max* L.): AN OVERVIEW

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1. Introduction

The soybean is a significant protein crop in the world. Plants can adapt to a very diverse range of environments. The interface for this adoption is their phenotype. Soybean is a nitrogen fixing legume and diseases and insects are additional levels increasing complexity of the crops interaction with abiotic environment. Major soybean crop acreages for food, feed, and biodiesel are expanding globally into unfavourable conditions relative to their gene-origin conditions. Both soybean crop yield and quality are impacted by high temperatures during the post-flowering stage and cold both during seedling emergence and seed maturation period.

We now understand how soybeans adapt to nutrient deficiencies in the soil, the molecular basis of symbiotic nitrogen fixation, the tolerance to biotic and abiotic stress, and the effects of flowering time on regional adaptation, plant architecture, seed yield, and seed quality. However, there are still multiple challenges.

Soybeans (*Glycine max* L. Merrill) are one of the five crops that dominate world agriculture along with maize, wheat, cotton, and rice (Karges et al., 2022). The most widely grown oilseed crop in the world is soybean, which is a significant source of food, animal feed, and biodiesel (Ziegler et al., 2016a). Worldwide, this crop is a significant source of protein and oil (Wiebbecke et al., 2012). According to Prabakaran et al. (2018), soybeans are high in dietary fibre, low in saturated fat, and contain an abundance of bioactive components such phenolic acids, flavonoids, carotenoids, isoflavones, and tocopherols

(Ziegler et al., 2016b). For monogastric animals in particular, soybean is regarded as an effective feed supplement due to its high protein content and optimal amino acid composition (Montoya et al., 2017).

Soybean is an annual species of *Fabaceae* native to China. For more than 5000 years, China has been consuming it (Modaresi et al., 2011). This crop can grow successfully in both temperate and tropical climates (Bandillo et al., 2017). A key element of its latitudinal adaptation is photoperiod responsiveness. First varieties introduced from temperate regions to lower latitudes in the past developed rapidly and produced little to no grain. However, the introduction of the long-juvenile trait was a successful genetic strategy to lengthen the vegetative phase, producing a larger yield in areas with short days. This increased soybean cultivation in tropical regions (Lu et al., 2017). World production increased from approximately 160 million tonnes on 70 million ha in 1998–350 million tons on 125 million ha in 2018 (Faostat, 2021) (Table 1).

Table 1. Soybean acreages of top 10 soybean producer countries in 2019 (FAOSTAT, 2021)

Rank	Area	Acreage (million ha)
1	Brazil	36,0
2	USA	30,3
3	Argentina	16,7
4	India	11,1
5	China	8,4
6	Paraguay	3,6
7	Russia	2,8
8	Canada	2,3
9	Ukraine	1,6
10	Bolivia	1,4

In Europe, soybean cultivation is still relatively small-scale and concentrated in the South and East. The potential for soybean in higher latitudes with relatively cool climates is mostly unknown (Karges et al., 2022). Over the past century, a significant amount of germplasm has been produced due to the efforts of soybean breeders and the advancement of breeding technologies. Nevertheless, soybean breeding must be accelerated significantly in order to support sustainable agriculture, meet the demands of a growing global population, and adapt to future environmental changes. The advancement heavily depends on discoveries in gene functional investigations. The development of soybean functional genomics has been greatly aided by the release of the reference soybean genome in 2010. In the last ten years, significant advancements in soybean research have been made in the fields of omics (genomics, transcriptomics, epigenomics, and proteomics), germplasm development (germplasm resources and databases), gene discovery (genes governing important soybean traits like yield, flowering and maturity, seed quality, stress resistance, nodulation, and domestication), and transformation technology (Zhang et al., 2022).

According to Cheng-Wen and Li-Zhi (2017), wild soybean (*Glycine soja*) is the wild ancestor of cultivated soybean (*Glycine max*), which has a variety of unique gene resources to use in breeding programmes. In domesticated germplasm, flowering time variation for soybean is well-characterized and essential for current production (Lu et al., 2020). Loss of seed dispersal was a key factor in the ancient human domestication of the soybean and a key step in the domestication of this

species (Sun et al., 2015). Examining wild accessions could assist in identifying valuable genetic resources that were lost during domestication (Qi et al., 2014).

Functional genomics advancements have been beneficial for soybean breeding. Our knowledge of how soybeans adapt to soil nutrient deficiencies, the molecular mechanism of symbiotic nitrogen fixation, biotic and abiotic stress tolerance, and the roles of flowering time in regional adaptation, plant architecture, and seed yield and quality have all improved as a result of the release of reference genomes for soybeans. However, there are still many of challenges in the way of soybean functional genomics and molecular breeding, particularly when it comes to increasing grain yield through high-density planting, maize-soybean intercropping, utilising wild resources, heterosis, genomic prediction and selection breeding, and precise breeding through genome editing (Du et al., 2023).

2. Pathogens

Development of new crop varieties with improved resistance mechanisms is necessary due to the emergence of new virulent strains of plant diseases (Ramalingam et al., 2020). According to Zhang et al. (2002), the pathogen *Phytophthora sojae* causes root and stem rot in soybeans. Globally, it affects soybeans in a significant degree (Zhang et al., 2017a). The major strategy for managing the disease is to cultivate resistant cultivars (Sahoo et al., 2021). Insects may help distribution and dispersal of root rots. Soybean root rot dispersion and

transport by *Cycloneda sanguinea* (Coleoptera: *Coccinellidae*) insect is reported by Salgado-Neto et al., (2018).

Phakopsora pachyrhizi, the agent that causes Asian soybean rust, is a severe foliar disease that affects soybeans all over the world (Ishiga et al., 2015). This fungus can reduce soybean yields and losses by up to 90% (Silva et al., 2020). The pathogen of white mould disease, which affects more than 500 plant species, is the fungus *Sclerotinia sclerotiorum*. Following infection, soybean yield losses may reach up to 70%. Antimicrobial substances generated by symbiotic bacteria of entomopathogenic nematodes *Xenorhabdus* spp. are described as a viable source for bio-fungicide as a biological control option of this severe pathogen. *Sclerotinia sclerotiorum* disease in soybeans could be effectively suppressed by volatile *Xenorhabdus* spp chemicals (Chacon-Orozco et al., 2020).

3. Symbiotic nitrogen fixation

Soybean yield and nutrient uptake are influenced by climate and soil fertility (Zhao et al., 2020). For crops to have access to nutrients and for soil health, microorganisms in the soil are essential (Zhaof et al., 2020). Due to the high protein and oil content of its seeds as well as its ability to "atmospheric nitrogen fix" through symbioses with soil microbes, soybeans are a particularly significant crop (Schmutz et al., 2010). Development of biological nitrogen-fixing nodules has a direct impact on nitrogen fixation efficiency during soybean growth (Yuan et al., 2017). The complex mechanism of nitrogen fixation in soybeans involves interactions between the plant and symbiotic rhizobia (Munoz

et al., 2016). *Rhizobia* and soybean nodulation competition is a significant barrier to nitrogen fixation. Soybean root exudates act as signals influencing ability of *Rhizobium* to colonize on roots and survive in the rhizosphere of plant (Liu et al., 2017). The symbiotic nitrogen fixing nodules are also important sites of sulfur assimilation to supply sulfur to whole soybean plant tissues (Krishnan et al., 2018).

4. Insects

Heterodera glycines Ichinohe, a parasitic cyst nematode, is the cause of extensive damage to soybeans (Kahn et al., 2021). A small roundworm known as a soybean cyst nematode feeds on the roots of soybean plants (Liu et al., 2012). In order to find their host roots, plant parasitic nematodes respond to root exudates (Hu et al., 2017). It is the soybean crop's most harmful pest globally (Zhang et al., 2017b). Although the majority of the resistant soybean cultivars were developed from the gene pool of farmed soybeans, resistance is declining as a result of this nematode's race changes (Kofsky et al., 2021). The topic of resistance to the soybean cyst nematode (SCN) involves a variety of processes and scientific disciplines. It is also a topic of substantial agricultural importance, as SCN is estimated to cause more yield damage than any other pathogen of soybean, one of the world's main food crops. Both soybean and SCN have experienced jumps in experimental tractability in the past decade, and significant advances have been made. Additional understanding of SCN and other cyst nematodes will also grow in importance and lead to novel disease control strategies (Bent, 2022). New solutions are urgently required to control soybean cyst

nematode, due to the slow decline in effectiveness of the widely used native soybean resistance (Kahn et al., 2021).

Two significant soybean pests in the United States are the soybean cyst nematode and the soybean aphid (*Aphis glycines* Matsumura) (Neupane et al., 2019). The management of soybean aphids can also be achieved by increasing host plant resistance. Four resistance genes were located on several soybean chromosomes, and resistant soybean lines were found (Jun et al., 2013). *Nezara viridula* is a significant agricultural pest in both North and South America. This bug's oral secretion causes damage to soybean crops. Serious injury to seeds sources from feeding behavior, morphology of mouth and saliva of this bug (Giacometti et al., 2020).

Neonicotinoids are main insecticides globally and used as seed treatments in soybeans and many crops. But results demonstrate that widespread prophylactic use of neonicotinoids in main soybean production regions in the US should be re-evaluated by producers due to low or zero effect of these pesticides under many conditions (Mourtzinis et al., 2019).

5. High temperature stress

In nature, soybeans are simultaneously subjected to temperature stress (Singh et al., 2018), which stunts plant growth and development and causes significant economic losses (Vital et al., 2019). During its growth, soybean is typically exposed to high daytime and nighttime temperatures. Mean daily temperature threshold for soybean respiration, photosynthesis and reproductive processes is $\geq 26^{\circ}\text{C}$. High

temperature (daytime 20/39°C or nighttime 29/30°C) decreases photosynthetic rate, pollen germination, pod-set and seed weight (Djanaguiraman et al., 2013a). In addition, soybean production continues to expand into both warmer and colder climates. The rate of leaf emergence and nodal primordium development are influenced by temperature. Both of these affect plant growth and yield, potential leaf area, and light absorption (Tenorio et al., 2017). In semi-arid locations, high temperatures are a significant environmental stressor that affects soybean yield. Pollen anatomical alterations induced on by high temperature stress reduce pollen germination, which in turn reduces pod set (Djanaguiraman et al., 2013b). Temperature fluctuations might affect seed production, particularly during the reproductive growth stage (Puteh et al., 2013). Lower seed yields are expected from early-maturing soybean varieties, but yield reduction is expected to be smaller in late-maturing soybean varieties under high temperature. Yield improvements is expected in cool regions by temperature increases for late-maturing varieties due to increased leaf area, leaf photosynthesis, sink size (number of flowers, pods and seeds) and longer flowering period (Kumagai & Sameshima, 2014). Leaf senescence during late reproductive development stages can be triggered early or enhanced as a result of high temperature stress in soybeans (Djanaguiraman & Prasad, 2010).

High temperature affects metabolic activity. Heat shock transcription factors cause heat shock proteins to be activated in hot environments, resulting in heat stress defence (Li et al., 2014). In reaction to heat, proteins involved in photosynthesis, secondary plant metabolism,

amino acid production, and protein biosynthesis are down-regulated. To combat heat stress, soybean seedlings activate three types of defence mechanisms: tissue-specific defence, general defence, and adaptive mechanisms (Ahsan et al., 2010).

According to Djanaguiraman et al. (2011), high temperatures during soybean flowering increase the rate of ethylene synthesis, leaf senescence, flower abscission, and reactive oxygen generation, damage membranes, and reduce pod set. Some soybean yield components (number of flowers and pods) are expected to be impacted by global warming. However, the majority of these late flowers do not successfully reproduce due to high temperatures during the late growth period. Under high temperatures above 29.4°C, the pod set ratio and pod number decline (Kim et al., 2020).

Seed development is much more vulnerable than vegetative tissues to temperature stress. High temperatures have an adverse effect on seeds throughout the growth of soybeans, leading to poor seed germination, increased pathogen infection, and decreased economic value (Chebrolu et al., 2016). High temperatures during the seed development stage have an impact on soybean seed quality and production. During seed filling, under high temperature, soybean prefers to accumulate lipid than protein in seed. Therefore, decreased protein content in soybean seeds was partly related to reduced seed yield (Nakagawa et al., 2020). High temperatures and humidity during the development and maturation of soybean seeds cause pre-harvest seed damage (Wang et al., 2012). High

temperature reduces soybean seed quality at different positions on the plant (Khalil et al., 2010).

6. Chilling temperature stress

Chilling stress (10-18°C) affects growth and yield of soybean (Kim et al., 2012). Soybean is widely distributed beyond its original habitat and chilling temperature is a major abiotic factor limiting its growth and yield. Many molecular mechanisms related to chilling stress and plant responses are discovered. Chilling temperature damage is maximum on soybean seeds when sown in wet soils. During imbibition, it results with low germination rate and vigor, reduced emergence and severe yield loss (Cheng et al., 2010). Flowering time is critical for soybean successful reproduction and yield. Soybean is a short day plant and it was determined that low temperature can delay flowering time of soybean (Lyu et al., 2020).

Pod set is a critical stage and sensitive to low temperature. Especially at higher latitudes, pod set at low temperatures is important for high yielding soybean production (Ohnishi et al., 2010). Chilling tolerance is an important feature of soybeans in cool climates (Toda et al., 2011). In Japan, in Hokkaido, early maturity is an important trait for soybean due to short growth period restricted by short autumn season and early snowfall. Days to maturity and seed yield have positive correlation and development of an early genotype without decreased seed yield is hard to achieve (Yamaguchi et al., 2015a). In Hokkaido, chilling temperatures result in the appearance of cracked soybean seeds. Seed coats severely split on the dorsal side and cotyledons are frequently

separated resulting with seeds have no commodity value (Yamaguchi et al., 2015b).

Temperature affects aggressiveness and fungicide sensitivity of *Pythium* pathogens causing damping off in soybean (Matthiesen et al., 2016). *Fusarium tricinctum* and *Fusarium solani* in soybean are important root rot pathogens. *F. solani* disease development is favoured by warmer temperatures, but in *F. tricinctum* disease development is favoured by cooler temperatures (Yan & Nelson, 2020).

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CHAPTER 9

THE CHARACTERISTICS and IMPACTS of BRACKEN FERN (*Pteridium aquilinum* (L.) Kuhn) on FARM ANIMALS and HUMANS

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1. INTRODUCTION

Ferns (*Pteridophyta*) are the most developed group of cryptogams (sporophyte) and are distributed widely in all climatic regions of the earth's surface, except for the polar regions and deserts, where a complete drought prevails. They are also the earliest vascular plants in geological time (Tuyji, 1994), which are non-flowering plants, albeit frequently distinguished by their fragmented leaves resembling bird feathers. Ferns dating back to ancient times originally arose on land in the Devonian period, 300 million years ago, when no animal was yet to live on earth. Approximately 250 million years ago, in the absence of flowering plants and trees, in the Carboniferous, all earth wetlands were covered with dense fern forests. Hence, this period is also called "The Ferns Era." These fern forests are the primary basis of coal deposits in the world. One of the most typical fern types is the bracken fern (*Pteridium aquilinum* (L.) Kuhn) (Kekil and Mendi, 2016), which can be found nearly everywhere from the equator to the North Pole, from America to New Zealand.

P. aquilinum is frequently referred to as "fern" in Turkey. This species retains no medicinal value or application. Farmers use their fresh leaves as bedding in melon and watermelon displays and feed their animals with dry fern leaves in the Black Sea Region (Baytop, 1984). Genckan (1985) cited that *P. aquilinum* is among the deadliest weed species, and correspondingly, Tokluoglu (1986) reported that fern (*Pteridium sp.*) is one of the most detrimental plants found in our country's pastures, with harmful effects for sheep, goats, and cattle. *P. aquilinum* poisoning

among animals is a significant problem in Turkey, especially along the Black Sea coastline, from Zonguldak to Hopa, as well as in Bolu and Kastamonu surroundings. The plant also poses a toxic effect on both humans and animals (Ozkara et al., 2003). Aged animals do not consume fresh plants. However, young animals suffer severe poisoning and typically pass away with internal bleeding within 3–4 days. Gallbladder, intestinal, and stomach tumors can develop in animals fed with dried plants in the Eastern Black Sea Region. Therefore, this plant appears to retain a carcinogenic substance (Baytop, 1984).

P. aquilinum is an aggressive colonist and potentially appears in many different plant communities. Once ingrained in plant communities, it is almost impossible to eradicate its deep rhizomes (Štefanić et al., 2022). Bracken fern, which tends to colonize open spaces and pastures and lower the qualitative value of forages, is one of the most common intrusive weeds on acidic soils, especially in many regions of abandoned land (Le Duc et al., 2000). After defoliation, the plant leaves decompose extremely slowly, leaving the soil covered by a thick layer of litter and hard humus that hinder the growth of other plant species (Blackford, 2001). The defoliated leaves may also release several allelopathic compounds that impede the development of other plant species (den Ouden, 2000). *P. aquilinum* is one of several species groups that can proliferate under grazing pressure by merging grazing avoidance with the ability to sustain high-intensity dominance (Birch et al., 2000). Asav (2011) reported that *P. aquilinum*, an invasive and toxic

plant, damaged pasturelands and intruded in large plant communities in the research area.

2. SYSTEMATIC and MORPHOLOGY

2.1. Systematic

P. aquilinum is a species that belongs to the genus *Pteridium* of the *Dennstaedtiaceae* family (Table 1). The genus *Pteridium* comprises five species: *P. aquilinum*, *P. esculentum*, *P. arachnoideum*, *P. caudatum*, and *P. semihastatum* (Hojo-Souza et al., 2010; Thomson 2004). In addition to this classification, these ferns can be categorized based on their morphology and genotype associated with their geographical locations. Among these species, the diploid *P. arachnoideum* and *P. esculentum* are predominantly found in the Southern hemisphere, whereas *P. aquilinum* in the northern hemisphere, allotetraploid *P. caudatum* in South and Central America, and *P. semihastatum* commonly in Southeast Asia and Australia (Thomson 2004).

There are 12 varieties and two subspecies of *P. aquilinum* (*P. aquilinum* (L.) Kuhn ssp. *aquilinum* and *P. aquilinum* ssp. *caudatum* (L.)) (Tryon, 1941; Costa et al., 2012), and they all contain 104 chromosomes (Marrs and Watt, 2006). The genus name *P. aquilinum* refers to wing/feather in Greek, and the species name means eagle-like in Latin. In Turkey, however, it is widely denominated as Papra, Eylentü, Eyraltu, Güllük, Ifteri, Red grass, and Bracken fern (Doğan, 2022).

Table 1. Taxonomic classification of *P. aquilinum* (Sarigul, 2018)

Kingdom	: <i>Plantae</i>
Division	: <i>Polypodiophyta</i>
Subdivision	: <i>Polypodiophytina</i>
Class	: <i>Polypodiopsida</i>
Subclass	: <i>Polypodiidae</i>
Order	: <i>Polypodiales</i>
Family	: <i>Dennstaedtiaceae</i>
Genus	: <i>Pteridium</i>
Species	: <i>Pteridium aquilinum</i> (L.) Kuhn

2.2. Morphology

P. aquilinum is a polycarpic geophyte reproducing by spores and widely branching its rhizomes on plowed fields (Štefanić et al., 2022). The plant can grow 40-200 cm tall under optimum conditions (Yılmaz and Coskun, 2022). Leaves emerging from the rhizome are in the form of three-pinnate. The petiole is as long as the leaf blade and is stiff and upright. The leaves are triangular, and the pinnae are 10-20 pairs (Kaynak et al., 2008). The colors of the leaves range from yellowish-green to dark green (FEMTT, 2017). The fiddleheads, young leaves, are acinaciform and covered by silver-gray villus that emerges from the rhizomes in early springtime. In temperate climates, the leaves gradually change color in late summer and die after the first frostiness of autumn. Mature fronds can grow up to 0.6-1.8 m and even elongate up to 3 m, depending on environmental conditions. The leaves can reach their maximum width in a few months (Caçador, 2014). The underground rhizome is stiff and hairy (Akkaya, 2005) and can grow to more than 1 meter in height, extending to 0.5-1 m deep in the soil (FEMTT, 2017). The plant rhizomes serve as a nutrient storage organ

and support colonization by moving through the soil. The plant dies in the fall, whereas it begins to regrow from its rhizomes in the spring. The plant spores potentially spread over hundreds of kilometers (Caçador, 2014). The size and shape of spore-bearing leaves resemble those of sterile leaves (Štefanić et al., 2022). The spore season of the fern is July and August, and the fern spores potentially remain viable for ten years (Štefanić et al., 2022; Yılmaz and Çoskun, 2022). The plant reproduces by spores aligned through the edge of the leaves in the sporangium (FEMTT, 2017). The plant produces 1400 g/m² of dry matter (Caçador, 2014).

3. DISTRIBUTION

Several studies focused on the distribution of ferns in the world and their ecological relations with their habitat (Vyherry, 1920; Pickett, 1931; GAMS, 1938; Tryon, 1957; Hevly, 1963; Bremer, 1980; Edwards and Birks, 1986; Benlioglu et al., 1997; Kaynak, 1989). These studies have revealed that ferns are highly dependent on the chemical composition of the soil. Researchers have also indicated that in addition to physical factors such as climate, soil composition, and moisture, ferns are often a species potentially living in diverse soils with different physical characteristics for distribution while remaining permanently rooted in a particular type of rock. *P. aquilinum* is a cosmopolitan fern found at various altitudes on all continents except for Antarctica (den Ouden, 2000; Štefanić et al., 2022, Fig. 1). In our country, however, it is widespread on the Anatolian coasts, especially in forest clearings in the Aegean, Mediterranean, and Black Sea regions (Tuyji, 1987;

Ozkara et al., 2003; Sarigul, 2018, Fig. 2). According to Davis (1965-1988), *P. aquilinum* is widely distributed in cleared forestland and steppe areas between 0-1,900 m altitudes in Anatolia. GAMS (1938) reported that this fern grows in trimmed grassland and moist pastureland in soils with a pH ranging from 3.2 to 7.6. In a study performed in Kütahya province, Cakmak (1997) stated that this species mostly prefers neutral settings. In another study conducted in the Black Sea region, Benlioglu et al. (1997) discovered that *P. aquilinum* thrives on siliceous and calcareous rocks and neutral soil conditions, albeit poor in lime. Marrs and Watt (2006) also reported that the *latiusculum*, *decompositum*, and *wightianum* varieties of this species can survive at an altitude of 0-2700 m, 300-3000 m, and 700-3300 m, respectively, whereas the *aquilinum* variety perfectly grows in the Alps at an altitude of 0-1800 m and 3100 m in the mountains.



Figure 1. Distribution of *P. aquilinum* in the world

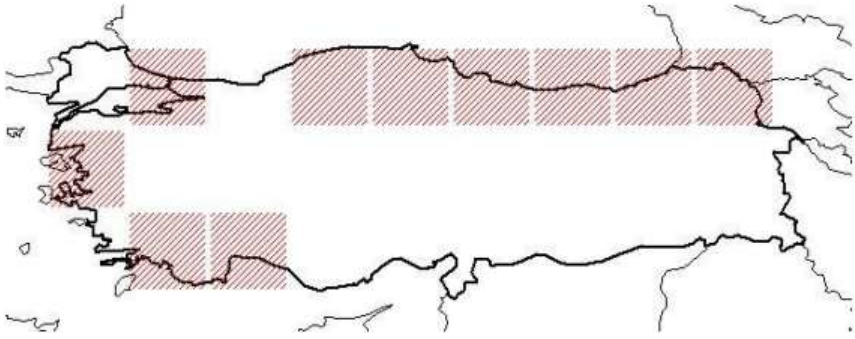


Figure 2. Distribution of *P. aquilinum* in Turkey (Sarigul, 2018).

Numerous studies on alien plant invasion revealed that *P. aquilinum* is present and widespread in Turkey. This plant is one of the invasive species that has been identified in pastures (Fig. 3). For instance, Akkaya (2005) reported that *P. aquilinum* is a wide-tolerant cosmopolitan fern species that can grow in all kinds of environments in his study in which he analyzed the ecological characteristics of fern species thriving in the region, taking soil and rock analysis in the area within the borders of Mula-Denizli-Antalya provinces. Correspondingly, while researching the flora of Katırlı Mountain (Bursa), Erdogan et al. (2011) discovered a wide range of *P. aquilinum* distribution at 400-650 m altitudes on the North-Western slopes of Orhangazi, Karsak village, Uçkaya Hill. Daskın (2012) reportedly located the *P. aquilinum* species in Söğütöinar village of Mudanya district in his study in which he analyzed the vascular plants of the Mudanya (Bursa, South Marmara) coast. Sarigul (2018) also recorded vast distribution of the *P. aquilinum* species in Çat, Zilkale, Ayder, Hazındak, Amlakit, Samistal, Sıraköyler (Palovit-Trovit-Elevit), and

Apivanak in his research on the fern flora of Kackar Mountains National Park (Rize). Zeybek et al. (2020) discovered the extending distribution of this species in the Azdavay/Kastamonu forest flora. Ozel et al. (2021) also observed that the *P. aquilinum* community, distributed from the western slopes of Karadag towards Bandırma, possibly occurred owing to the elimination of forest cover by anthropogenic effects, and the elevation level of the plant community sampled was 354 m. *P. aquilinum* is an invasive plant and widely intrusive in pasturelands. For instance, Ozcan (2010) reported that the incidence rate of *P. aquilinum* was 23.63% in the surveys he carried out on the interior of the forest pastures in the Yuvacık basin of İzmir. Similarly, Asav (2011) informed that weeds have proliferated due to intensive and early grazing practices in Trabzon pasturelands, and *P. aquilinum* is one of the most intrusive weeds in this area based on his study to specify the intrusiveness of the weed species in the pasturelands of Trabzon province. The researcher also documented that *P. aquilinum* has an average incidence rate of 36.24% in the pasture areas of Trabzon province in the survey studies. However, this rate greatly varied among ten different districts of Trabzon province (66.67%, 26.32%, 46.43%, 30.77%, 33.33%, 28.57%, 36.14%, 29.41%, 47.83%, and 55.56% in Akçaabat, Araklı, Çaykara, Dernekpazarı, Hayrat, Köprübaşı, Maçka, Sürmene, Tonya, and Vakfikebir districts, respectively). Despite being a toxic plant, *P. aquilinum* serves several purposes for human usage. For instance, Sargin (2019) noted that local communities used dried and ground *P. aquilinum* leaves as tea in the Mersin-Bozyazı region.



Figure 3. *P. aquilinum* in the pasturelands of Fevziye village in Bursa-Gemlik (Budakli Carpıcı, 2022)

4. CONTROL

Timing is crucial to fight against *P. aquilinum* and its management (Štefanić et al., 2022). It is feasible to avoid the spread of this plant species through cultural, mechanical, chemical, and biological control methods. However, the literature review indicated that weed-mowing (cutting) and chemical control applications were reportedly successful (Lowday, 1987; Marrs et al., 1993; Dolling, 1996; Marrs et al., 1998; Paterson et al., 1997; Stewart et al., 2005; Novák, 2007; Sellers et al., 2017).

4.1. Cultural control: Frequent liming, fertilizing, or planting forage mixtures are recommended to minimize the *P. aquilinum* invasion (Štefanić et al., 2022).

4.2. Mechanical control: Applying the mechanical control method for a long time (over ten years) is necessary for a satisfactory conclusion. The primary objective of the mechanical control method is to

continuously cut the leaves and allow the plant to deplete the rhizome reserves (Marrs et al., 1998; Måren et al., 2008; Milligan et al., 2016; Milligan et al., 2018; Akpınar et al., 2023). Mowing the plant leaves when the carbohydrate reserves are consumed extensively is necessary to attain this result. Besides, removing the rhizomes from the soil using a plow or harrow will be a successful management strategy for mechanical control (Snow and Marrs, 1997; Marrs et al., 1998). Argenti et al. (2012), studying pasture improvement in a preserved area in the Apennine mountains invaded by *P. aquilinum* species, focused on two distinct control methods (a: mowing of *P. aquilinum* + plowing at 30 cm depth + planting of forage crop mixture + mowing of *P. aquilinum* in the following years of planting; and b: mowing of *P. aquilinum* + harrowing and planting of forage crop mixture). As a result, they reportedly identified that the deep plowing and frequent mowing of *P. aquilinum* every year was the most effective application. They also documented that while the rate of *P. aquilinum* was 3.7%, 0.9%, 1.0%, and 0.6% in this application, the other application resulted in 21.8%, 22.5%, 28.7%, and 23.7%, respectively. Correspondingly, Akpınar et al. (2023) noted that mowing twice a year is the most effective method, albeit costly.

4.3. Chemical control: This method focuses on the transportation of herbicides through the phloem. The herbicides sprayed on the leaves are carried to the rhizomes through the phloem to function (Akpınar et al., 2023). Various chemicals, including asulam and non-selective glyphosate, are the most effective herbicides for this purpose, followed

by 2,4 -D, 2,4-D + aminopyralid, Triclopyr ester, Triclopyr + fluroxypyr, Chlorsulfuronglyphosate, dicamba, picloram, and metsulfuron-methyl (Veerasekaran et al., 1978; Pakeman et al., 2005; Sellers et al., 2017; Štefanić et al., 2022). Herbicide application time is crucial, according to Veerasekaran et al. (1978), and for a long-term application, especially for an effective result, 4.4-8.8 kg ha⁻¹ application is recommended in the stage of full frond expansion. Numerous studies reported that leaf density lessened by 99%, 95%, 85%, and 73% within four years after 4.4 kg ha⁻¹ asulam application on July 26. Sellers et al. (2017) also documented that the control rate after one and eight months of metasulfuron and chlorsulfuron application resulted in a 60% and 90-95% success rate in the fight against fern, respectively. Finally, Štefanić et al. (2022) noted that glyphosate, dicamba, picloram, and metsulfuron-methyl are the potential herbicides used in chemical control and that the application time should preferably be the autumn season when the plant is actively growing and at fully frond expansion period.

4.4. Biological control: The use of biocontrol methods developed so far is limited (Akpınar et al., 2023). Several biological control agents severely harm leaves in the spring; however, the rhizome has yet to be damaged by any known biological control agents. Although *Panotina angularis* and *Conservula cinisigna* are promising agents, there are no conclusive research results (Štefanić et al., 2022).

5. BRACKEN FERN-INDUCED TOXICITIES

Poisonous plants potentially cause toxic effects through false contact or accidental feeding. Another source of toxicity is that some plants can be harmful, especially consumed frequently (Norton, 2008). Indeed, most toxic plants are not palatable, and farm animals ordinarily avoid feeding them. However, management issues may lead to plant-based toxicity, usually by poor forage and pasture management, or excessive contamination of grain or other feed raw materials with toxic plants (Bischoff and Smith, 2011). In many parts of the world, *P. aquilinum* is known as a highly-toxic plant (Anjos et al., 2008; Bischoff and Smith, 2011). While fern directly poses a risk for its toxicity when fed by ruminants, it indirectly provides a habitat for parasites such as acarids (Smith, 1977). It is also the sole plant scientifically proven to be naturally carcinogenic in animals when consumed (Yamada et al., 2007; Norton, 2008). In 1965, Evans and Mason reported that mice fed by *P. aquilinum* developed tumors, thus proving its carcinogenic potential. Since then, scientists put the utmost effort to comprehensively investigate the biological and chemical properties of *P. aquilinum* to ascertain the susceptibility of animals. Rasmussen and Pedersen (2017) also detected ptaquiloside and pterosin B only in *P. aquilinum* among the 21 bracken fern species analyzed. *P. aquilinum* contains several toxic compounds, including ptaquiloside and thiaminase. Especially young shoots and fiddleheads retain high levels of toxic compounds. In ferns, thiaminase is the primary toxin responsible for neurological syndrome in horses. It causes a thiamine deficiency by cleaving

thiamine and agonistically inhibiting thiamine activity (Knight 2012). Similarly, ptaquiloside is an illudant-type norsesquiterpene glycoside isolated from fern as a fern carcinogen (Bates, 2023). Illudants are a subclass of sesquiterpene synthesized by ferns and are highly toxic (Potter and Baird, 2000). All parts of the plant retain ptaquiloside (leaves, rhizomes, spores, and roots), and its concentration varies in relation to the annual growth cycle and growing conditions (Rasmussen et al., 2015; Zacccone et al., 2014). Young fiddleheads typically tend to have higher PTQ concentrations than mature fronds (Rasmussen et al., 2003). The leaves are known to have the highest concentration in spring, with 1000 to 50,000 $\mu\text{g g}^{-1}$ (dry weight). The concentration in the rhizomes is negligible for most of the year; however, its concentration may increase in the fall due to nutrient transportation from the leaves to the storage rhizomes since bracken fern is a deciduous perennial species. The ptaquiloside content in spores and roots is considerably low when compared to that found in leaves (Rasmussen et al., 2013, 2015; Kisielius et al., 2020). The ptaquiloside quantity typically varies in bracken fern between ten and several grams per kg (Kalac, 2017). Young fiddleheads of various bracken genotypes may retain ptaquiloside between 0.032 and 3.9 mg per gram of biomass (mg/gbm) (Alonso-Amelot et al., 1995).

Ptaquiloside is an unstable compound and rapidly aromatizes under acidic or alkaline aqueous states by de-glycosylation of d-(+)-glucose to synthesize pterosin B, a non-carcinogenic chemical (Fletcher et al., 2011). In other words, the stable and non-toxic metabolite pterosin B is

released naturally as the end product of acidic and alkaline hydrolysis of ptaquiloside. Yet, the compounds synthesized during the conversion of ptaquiloside to pterosin B are considered 'genotoxic' and are known to have an immunomodulatory effect. However, when ptaquiloside interacts with complex biological systems such as in the gastric mill, it results in several reactions, depending on its hydrolysis under different variables, such as temperature, the amount of water animals drink, and its interactions with enzymes (Arapha et al., 2019). *P. aquilinum* leaves retain comparable levels of pterosin B and ptaquiloside, and pterosin B is permanently synthesized as a reactional by-product in aqueous solutions, indicating the previous presence of ptaquiloside (Yamada et al., 2007). Ptaquiloside may also interact with animal DNA when digested due to its alkylating characteristics (Rasmussen et al., 2005; Ugochukwu, 2019). Ojika et al. (1987) reported that ptaquiloside carcinogenicity originates from DNA damage through the alkylation of adenine mononucleotide via the reactive cyclopropyl ring. The resultant carcinogenic dienone, synthesized from ptaquiloside under poor alkaline conditions, acts as an agent with a significant alkylating effect against amino acids and nucleic acid bases, causing DNA cleavage (Kigoshi et al. 1995). In addition to these biotoxins, *P. aquilinum* contains biotoxins that are undesirable for animal feeding, such as prunasin. This chemical is a cyanogenic glycoside, a toxic chemical in the tissues of the young plant to protect them from defoliation (Gil da Costa et al. 2012). However, cyanogenic glycosides rarely cause cyanide toxicity in animals (Fenwick 1988).

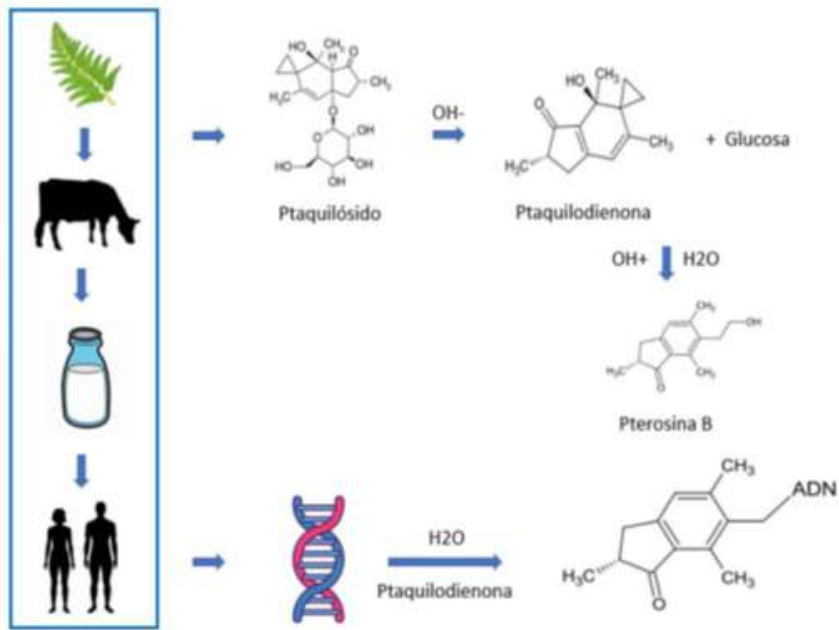


Figure 4. Fern (*Pteridium*) food chain and reactivity under physiological conditions of ptaquiloside. Source: Rodríguez-Salazar and Chacón-Villalobos (2023)

These biotoxins induce DNA damage due to cleavage when feeding animals with a high quantity of *P. aquilinum*, whereas it causes cell cycle arrest in case of its consumption at a low quantity (Aranha et al., 2019; Tourchi-Roudsari 2014; Ugochukwu, 2019). Apart from the development of cancers in humans and animals, studies extensively focused on the Ptaquiloside biogeochemistry and its health effects in relation to several critical acute and chronic diseases among humans and animals associated with *Pteridium* spp. However, Yamada et al. (2007) reported that *P. aquilinum* is likely to retain unknown chemicals, similar to ptaquiloside, and cause toxicities yet to be identified. Correspondingly, O'Connor et al. (2019) emphasized the need for more

research and analysis of the processes since *P. aquilinum* potentially contain other structurally similar illudants at higher concentrations than ptaquiloside since these substances may also contribute to the overall toxicity and carcinogenicity of *P. aquilinum*.

6. SIGNS ASSOCIATED with BRACKEN FERN TOXICITY in FARM ANIMALS

P. aquilinum toxicosis manifests itself as various clinical syndromes in different animals, and its toxic effects vary depending on its consumption quantity(ies) (Gil da Costa et al., 2012). In addition to its carcinogenicity, fern causes severe fever (41-42 °C), high pulse and respiratory rate, salivating, bleeding in various organs such as gums, nostrils, and gastrointestinal tract, reflux of ruminal contents from the nostrils, and also induces acute toxicity symptoms such as hemorrhagic lesions in the mammary gland and blood in the milk (Anjos et al., 2008; Lucena et al., 2011; Plessers et al., 2013). Cattle develop an acute hemorrhagic syndrome that commonly leads to bleeding and death. Acute hemorrhagic syndrome has a generally dismal prognosis, and most livestock (>90%) die within 1-10 days after the onset of symptoms (Plessers et al., 2013). If fern consumption intensively persists, enzootic bovine hematuria, characterized by tumor induction in the upper digestive tract and urinary bladder, develops (Carvalho et al., 2006; Sharma et al., 2013; O'Connor et al., 2019). The hematological changes visualized in fern poisoning are associated with acute toxicity, and these hematological changes result from suppression of the activity of all blood cells and immune system cells in the body. As a result, the low

platelet count in the blood leads to severe bleeding (Gil da Costa et al., 2012). Similarly, Campo et al. (1992) reported that immunosuppression was observed in cattle grazing on fern-invaded pastures. Such immunosuppressive consequence is attributed to its genotoxic effect because it interferes with biochemical processes such as the synthesis of the P53 protein responsible for apoptosis in cells, as well as reducing the activity of natural killer cells, which are involved in the regulation of the immune system response in cells and the elimination of infected or cancerous cells by improving metallothionein expression (Alonso-Amelot and Avendaño 2002; Latorre et al., 2013). Aranha et al. (2019) reported that cattle fed by fern for only seven days developed liver lesions and pathological alterations in the urinary bladder. They additionally identified that most of the ptaquiloside underwent rapid hydrolysis in the rumen via conversion into non-toxic pterosin B; subsequently, it either infiltrated the bloodstream or was partly removed by the urinary spillage, indicating that the transfer of the carcinogenic ptaquiloside metabolites took place through the rumen membrane. Gil da Costa et al. (2012) and Medeiros-Fonseca et al. (2021) also documented that inactivation driven by ptaquiloside stimulates the persistence of viral infections such as bovine papillomavirus and induces chromosomal aberrations. Rocha et al. (2022) discovered that buffaloes are more resistant to *P. aquilinum* toxins than cattle. Prolonged ingestion of ptaquiloside in sheep induces tumors in the bladder. In addition to the bleeding symptoms, it may also compel cancer development, and extended chronic fern intake potentially causes retinal neuropathy and eventually permanent blindness (Potter

and Baird, 2000; Bates, 2023). Goats, however, are the only farm animals resistant to the effects of ptaquiloside toxicity (Plessers et al., 2013). Cystitis is one of the most frequently observed conditions during slaughter in animals fed with fern for a prolonged period (Peixoto et al., 2003; Carvalho et al., 2006). Inflammation and preneoplastic bladder alterations in the bladder mucosa have been attributed to ptaquiloside-like chemicals or other toxic compounds (Ngomuo and Jones, 1996; Takanashi et al., 1983). Additionally, red tissue necrosis and marked bone marrow aplasia were observed in the liver in an autopsy on animals that died from fern toxicity (Anjos et al., 2008). The toxic effects of fern are cumulative; in other words, signs of acute fern toxicity become more visible one to three months after transferring the herd to a fern-contaminated pasture and even up to two weeks after removing the animals from that range (Vetter, 2009).

Bracken fern toxicosis arises from induced vitamin B1 deficiency in monogastric animals such as pigs and horses (Bates, 2023). Yet, the ration fed by horses and pigs is crucial for supplying sufficient vitamin B (thiamine) levels. Since *P. aquilinum* retains thiaminase, an effective vitamin B-degrading enzyme, the resultant toxicity effects are the primary signs of vitamin B deficiency (Fenwick, 1988). Since ruminants potentially synthesize complex B vitamins in their rumen, these symptoms do not appear immediately; however, long-term fern consumption may result in thiamine deficiency among these animals (Marín, 2019). Thus, the primary *P. aquilinum* toxin in monogastric animals is thiaminase. Thiaminase degrades thiamine (vitamin B1), a

required compound for the proper functioning of the nervous system, and thus bracken fern-driven toxicity in monogastric animals can be visualized by the symptoms of vitamin B1 deficiencies. For instance, thiaminase potentially triggers anorexia, weight loss, depression, constipation, sluggishness, paralysis, and a squatting stance with feet open in horses. Animals may also tremble when forced to move. Animals with severe disease develop cardiac arrhythmias and frequently pass away. Pigs are more resilient than horses, so much so that they display disease symptoms only when forced to feed only ferns exclusively. Fatigue, weight loss, and abrupt onset of respiratory distress are among these symptoms observed in pigs. However, these symptoms are not explicit but usually ensue as sudden death (Seva et al., 2010). Animals are affected by the active ingredient toxins found in the fern, which causes significant livestock losses every year (Gil da Costa et al., 2012). The disease may even manifest days or weeks after ending fern feeding. Hence, all animals fed by fern should be treated equally, even if they are asymptomatic. Indeed, animals may be asymptomatic for a long time without displaying any symptoms until the evident blood in the urine, a point of no return for a rapidly progressive decline (Marrero Faz and Calderón Tobar, 2012). Detecting occult blood in the urine with test strips may be beneficial without disregarding clinical and histopathological symptoms (Verde et al., 2017). Rectal inspection to examine hematological analyses and the bladder wall and the use of ultrasonography may be helpful for diagnosis (Calderón Tobar et al., 2011; Ugochukwu, 2019). Plumlee (2004) reported the significance of blood or platelet transfusions from

healthy animals; however, he indicated that the procedure required large volumes of blood—at least 2-4 liters. Antimicrobial agents can be applied to prevent secondary infections, and animals should be removed from fern-invaded pastures immediately (Plessers et al., 2013). Clinical symptoms, exposure history, and typical postmortem findings all contribute to diagnosing *P. aquilinum* toxicosis. Clinical syndromes in cattle and sheep are progressive, and humane euthanasia is frequently necessary for animals with advanced diseases. In monogastric animals with *P. aquilinum* toxicosis, symptoms are non-specific and sudden death is an ordinary case. However, vitamin B supplementation and pasture maintenance may often lead to a full and speedy recovery, provided that detecting the symptoms earlier, halting fern consumption, and changing the feeding habit (Fenwick, 1988; Bates, 2023). Latorre et al. (2013) discovered that selenium protects and reverses ptaquiloside-induced immunosuppression, reporting that selenium supplementation may also help prevent some toxic consequences of ptaquiloside where foods of animal origin, water, and air are likely to contain ptaquiloside and in humans exposed directly or indirectly to *P. aquilinum* infested areas.

7. EFFECTS of BRACKEN FERN TOXICITY in HUMANS

Studies also emphasized that those individuals exposed to bracken fern in childhood are at higher risk of developing gastric cancer twice as much as those who were not exposed (Galpin et al., 1990). However, *P. aquilinum* plant is not only utilized as animal feed in many parts of the world, but it has also been used by humans for many years in the

field of traditional medicine, as a food supplement, or direct consumption (Bryan and Pamukçu 1979; Marlière et al., 1998; Hojo-Souza et al., 2010; Gil da Costa et al., 2012; Rasmussen et al., 2021). Consequently, the IARC has categorized fern as "possibly carcinogenic to humans (group 2B)". Despite this categorization, young fiddleheads (or croziers) are used for human consumption in the vast majority of the world (IARC, 2023). Humans have exploited the fern's rhizomes as food sources in drought seasons (Alonso-Amelot and Avendaño 2002). Based on the extensive use of pickled fern in Japan, Smith (1977) claimed that the incidence of stomach and rectal cancer in the world is very high in this country, emphasizing that there might be a relationship between them. Yet again, this data was also spatially associated with fern cover in basins with acidic soils containing higher than average heavy metal concentrations and a higher incidence of gastric cancer among residents. Nonetheless, it is difficult to assume that fern alone is to blame for higher than regular gastric cancer rates; however, ptaquiloside is reportedly found in the milk of animals grazing on pastures with dense fern cover (Bonadies et al., 2011; Virgilio et al., 2015). Bonadies et al. (2011) and Virgilio et al. (2015) identified the ptaquiloside quantity between 0.53 and 1.03 µg/l in milk samples from cattle, sheep, and goats grazing on fern-invaded pastures. In fern-fed calves, ptaquiloside-like compounds were identified in muscle, liver, kidney, and heart tissue even 15 days after ending fern-based feeding (Fletcher et al., 2011). Correspondingly, Pamukcu et al. (1978) reported that the milk produced by fern-fed cows contained more carcinogenic, toxic, and mutagenic metabolite(s) than the regular milk, proving that

fern-fed cows' milk was mutagenic. They also documented that the fern-feeding process induced intestinal and urothelial carcinomas among mice. Evans et al. (1972) similarly remarked that milk with ptaquiloside induced acute hemorrhagic syndrome in calves. Park et al. (2023) detected a total of 0.1 g/kg ptaquiloside in the bovine beef grazing naturally on the pastureland, whereas the limit of the daily ptaquiloside intake of humans was about 3.0×10^{-5} g/kg body weight/day. Potter and Baird (2000) documented that the milk produced by a fern-fed cow contained approximately 8.5% of the total ingested ptaquiloside quantity. Interestingly, Kalac (2017) found almost the same results, concluding that $8.6 \pm 1.2\%$ of ptaquiloside taken by fern-fed cows transferred into the milk depending on its doses. However, he observed that pasteurization and boiling processes reduced the ptaquiloside level in milk by 50% and 75%, respectively. Alonso-Amelot et al. (2002) stated that farm ranch consumes a significant amount of milk produced in small-scale, low-tech dairy farms in rural areas. As a result, these individuals are highly at risk and open to ptaquiloside contamination since they consume the milk produced by dairy cattle grazing on unfavorable pastures invaded by *P. aquilinum*. They additionally indicated that each glass of milk these people consumed averagely contained 0.7 to 1.2 mg of ptaquiloside. Potter and Baird (2000) stated that ptaquiloside quantity in milk is the primary reason for gastric cancer cases observed in farmer populations living in countries where ferns are abundant in pasture lands. Hence, *P. aquilinum* toxin residues in the tissues of fern-fed cattle and their potential to reach humans via contaminated milk, dairy products, or

meat from cattle in fern-fed pastures raises health concerns for consumers. It is also a significant concern that carcinogens can be transmitted from water supply locations, where ferns are abundant, or by breathing their spores. Indeed, the natural carcinogen ptaquiloside and its transformation product, pteroesin B, have been detected in both groundwater and surface water where bracken fern-invaded lands. Since ptaquiloside is a highly soluble chemical in water, it is prone to leaching from the topsoil into the surface and groundwater. Ptaquiloside is also a pollutant since it potentially contaminates human beings through potable water (Skrbic et al., 2020). Depending on the carcinogenic qualities, the maximum tolerated ptaquiloside concentration in potable water should be between 0.005 and 0.016 g L⁻¹ (Rasmussen et al., 2005; Skrbic et al., 2020; Wu et al., 2021). However, while ptaquiloside concentrations in groundwater were reportedly 0.6 µg L⁻¹ and 0.09 µg L⁻¹ in Ireland and Denmark, respectively, these rates escalated to 5.3 µg L⁻¹ in surface waters during rainstorms (Clauson-Kaas et al., 2014; 2016; O'Driscoll et al., 2016; Skrbic et al., 2020; Kisielius et al., 2022). Furthermore, studies indicated that spore density may be extreme in areas where *P. aquilinum* is abundant. As 1 mg quantity contains 2×10⁶ spores, a single frond may yield almost 150 mg spores (Marrs and Watt, 2006). *P. aquilinum* spores are brown in color, tetrahedral-globose in shape, and very thinly spiky. Despite seasonal variations, bracken fern produces a remarkable quantity of microscopic spores distributed across large distances by wind or animals (Alonso-Amelot et al., 2001). *P. aquilinum* spores were found in a Spanish city center 70 km from fern

fields, indicating how far they can travel (Rodríguez de la Cruz et al., 2009). Furthermore, *P. aquilinum* produces a large number of spores during sporulation, the quantity of spores released per cubic meter of air at particular times is very high, and spores are carcinogenic, putting workers and visitors in sports facilities at risk for health issues (Caulton et al., 2000). Rasmussen et al. (2013) reportedly identified ptaquiloside in all spores analyzed, albeit in low quantities compared to other parts of *P. aquilinum* plants. The ptaquiloside quantity in spores ranged from 4.5 to 29 $\mu\text{g g}^{-1}$. However, this data still implies that considerable amounts of ptaquiloside can be released into the environment via spores in areas where *P. aquilinum* is common. For instance, during harvest, up to 3 g of ptaquiloside may be released into the air from a 1000 m² area of *P. aquilinum* with ten sporulated leaves per m² and 10 g of spores produced per leaf. Therefore, it should not be ruled out that the data related to ptaquiloside concentration in *P. aquilinum* spores could explain the high incidence of some cancer types observed in people living in fern-rich environments.

Several recent studies displayed critical evidence that humans are potentially open to *P. aquilinum* carcinogens via consuming meat or milk produced by animals grazing on fern-invaded pastures, inhaling its spores, or drinking fern-contaminated water (Taylor, 1981; Alonso-Amelot et al., 1993; Aranha et al., 2014; Bonadies et al., 2010; Calderón Tobar et al., 2014; Clauson-Kaas et al., 2014; Tourchi-Roudsari, 2014; Fletcher et al., 2011; Marlière et al., 2002; O' Driscoll et al., 2016; Virgilio et al., 2015). Hence, controlling the spread of ptaquiloside into

the food chain has taken on significant importance in food safety research due to the elevated risk of gastric and esophageal cancer among people living in fern-invaded areas and exposed to foods contaminated with ptaquiloside (Alonso-Amelot et al., 2002). Rasmussen et al. (2021) also reported that the ptaquiloside chemical is present in various food products, whereas its content varies depending on the food process and source. However, considering the food safety issues, they stated that collecting ferns and consuming them as dried may result in serious health problems in humans due to their high ptaquiloside content. As reportedly noted, numerous videos on several internet websites provide instructions to collect ferns and share recipes to prepare various delicious dishes, and unfortunately, a sizable mass of viewers follow and watch these contents. Therefore, the lack of awareness of fern toxicity poses a significant threat to human health (O'Connor et al., 2019; Rasmussen et al., 2021).

The quantification of ptaquiloside in plants was evaluated directly by HPLC or by converting this substance to Pterosine B (Rasmussen et al. 2003). Hao et al. (2021) successfully identified the ptaquiloside quantity in the plant using a solid phase extraction technique via TOF-MS/MS and HPLC-DAD analysis. Jensen et al. (2008) developed a method to measure the ptaquiloside concentration in soil and groundwater. However, Francesco et al. (2011) used gas chromatography and successfully identified the ptaquiloside concentration at 'ppb level' in the milk of poisoned dairy cattle fed on fern-rich soils and in milk samples collected from healthy dairy cattle.

Consequently, they described this method as a strategy that makes the ptaquiloside easy to screen animal foods appropriate for human consumption, significantly improving the traceability of food of animal origin and making them safer for human consumption. However, there is no sufficient data on what commercially accessible products contain. As a result, it is crucial to develop a fast and accurate method for calculating the ptaquiloside quantity in food of animal origin to ensure food safety. Park et al. (2023) developed a quick, sensitive, and simple method to quantitatively analyze the ptaquiloside using the LC-MS/MS in 26 commercial products, concluding that all potential ptaquiloside contamination sources in the food chain of animal origin could be traceable.

8. CONCLUSION

P. aquilinum is one of the most aggressive and invasive weeds in pasturelands. It is also capable of synthesizing several secondary metabolite groups with a variety of biological functions, including prunasin-like cyanogenic glycosides, thiaminase-like antihistaminic chemicals, and ptaquiloside- and dienone-like carcinogenic compounds. Feeding farm animals with *P. aquilinum* potentially result in several ailments. Fern consumption also induces esophagus (oesophageal) and stomach cancers among humans when consumed directly (through consumption) or indirectly (through ingesting food of animal origin, inhaling spores, and using contaminated water supplies). *P. aquilinum* poses a risk not only for agricultural areas but also for public health. Grazing livestock systematically on the pasture

camouflages its existence; thus, the pastureland should be tested thoroughly before taking the herd onto the area. The proper feed should be supplied to the animals, especially in dry periods, before the current feedstocks deplete. Animal breeders need to be aware of *P. aquilinum* toxicity. Parties should put the utmost effort into the risk assessments when feeding livestock directly with *P. aquilinum* and its risk to humans when exposed to fern-based toxins from secondary sources such as residues in milk or beef. Hence, farmers, botanists, toxicologists, oncologists, and public health professionals should collaboratively work on the subject. Society should also receive education to raise awareness about the biological effects of *P. aquilinum* and its carcinogenicity; in other words, the public should acknowledge its dangers in terms of public health and strive to minimize, if not eliminate, its adverse effects on both farm animals and human beings.

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CHAPTER 10

FIELD CROP CULTIVATION IN THE SOUTHEASTERN ANATOLIA REGION IN TÜRKİYE

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INTRODUCTION

Southeastern Anatolia Region, which constitutes a very important part of Türkiye's agricultural areas; It consists of large plains with less roughness, surrounded by the borders of Iraq and Syria in the south of the Southeast Taurus Mountains. The area has approximately 7.5 million hectares of land, of which 3.2 (42.6%) million hectares are arable land. This region constitutes 9.6% of Turkey's land assets. With the implementation of a large part of the Southeastern Anatolia Project (GAP) in recent years, the Southeastern Anatolia Region has taken important steps in agricultural production and has become an important agricultural production center in Turkey. Our region is one of the gene centers of wheat and lentils in Turkey. In addition, with the increase in irrigated areas with the GAP in recent years, significant increases have been observed in corn production in the region. (Kaplan, 2016).

The GAP region includes the Upper Mesopotamian plains of the Fertile Crescent, known as Mesopotamia at the time of the first agricultural culture in the world, and the plains in the Euphrates and Tigris basins. These plains in the south of the arc formed by the Southeast Taurus Mountains; The coastal plains of the Tigris and Euphrates and the plains of Suruç, Harran, Ceylanpınar, Nusaybin and Silopi (Balaban, 1986). GAP Region is implemented in the Southeastern Anatolia Region, where 9 provinces (Adıyaman, Batman, Diyarbakır, Gaziantep, Kilis, Mardin, Siirt, Şanlıurfa and Şırnak) are located. The land and population size of the provinces within the scope of the GAP is around 10 percent of Turkey's average. GAP was initially considered as a

program based on the development of the region's water and soil resources; Irrigation investments for irrigation and hydroelectric power generation in the Euphrates and Tigris Basin are foreseen (Bengisu, 2014). The total surface area (75,410 km²) of the provinces included in the GAP Region constitutes 9.7% of the country's surface area (779,452 km²). The region covers 11.7% of the agricultural lands, 10.3% of the grassland (grassland-pasture) areas, 6.2% of the forest-heathland and 11.4% of the area allocated to other areas in Turkey. forms. Agricultural areas in the region (43.6%) have a more advantageous situation compared to agricultural areas in Turkey (36%) (TSI, 2006).

The main purpose of the GAP is to increase agricultural production and diversity by irrigating dry lands, to develop the resources of the region with the contributions of the people of the region and to ensure their sustainability. It is thought that with the increase in agricultural production, agriculture-based industry will develop, the economy will revive, new employment areas will be opened to the people of the region, and thus migration from the region will be prevented, the income and welfare level of the people of the region will increase and the development gap between them will decrease (Erkan, 2003). Bringing water to dry lands, increasing agricultural production and diversity, developing the resources of the region with the contributions of the people of the region and ensuring their sustainability are the objectives of the Southeastern Anatolia Project (Benek, 2009). In this section, information is given about field crops grown in the Southeastern Anatolia Region of Turkey.

FIELD CROP CULTIVATION IN TÜRKİYE

The territory of Turkey is located between 36-42° North latitude and 26-45° East longitude. There is a time difference of 76 minutes between east and west. Its shape roughly resembles a rectangle and its width is 1,660 kilometers. The actual area it covers, including lakes and islands, is 814,578 km², and its projection area is 783.562 km². Turkey is a country with a very rich production pattern in terms of agricultural production diversity, where four seasons are experienced. Türkiye consists of 7 geographical regions. These regions are the Black Sea, Marmara, Aegean, Central Anatolia, Eastern Anatolia, Mediterranean and Southeastern Anatolia Regions. There is an agricultural area of 23,845,049 ha in Turkey and 2,972,893 ha in the Southeastern Anatolia Region. Agricultural areas in the Southeastern Anatolia Region constitute 8% of Turkey's agricultural areas (TSI, 2022).

Table 1. Field crops cultivated in Türkiye (TSI, 2022)

Field Crops	Production Area (decare)	Production Quantity (ton)	Yield (kg/da)
Durum Wheat	11.946.883	3.750.000	314
Wheat, Excluding Durum Wheat	54.071.460	16.000.000	296
Corn	9.114.988	8.500.000	933
Barley (Beer)	1.109.454	400.000	361
Barley (Other)	30.775.796	8.100.000	263
Rye	978.391	273.000	279
Oat	1.372.503	365.000	266
Sorghum	58	17	293
Millet	12.293	4.168	339
Kaplıca, wheat	17.882	3.447	193
Bird Food	18.627	4.848	260
Triticale	996.306	320.000	321

Buckwheat	8.482	1.359	160
Beans, Dry	970.490	270.000	278
Broad Beans, Dried (For Human Consumption)	17.638	4.234	240
Chickpeas, Dry	4.564.804	580.000	127
Lentils, Dry (Red)	2.997.868	400.000	133
Lentils, Dry (Green)	427.890	45.000	105
Peas, Dry	8.873	2.392	270
Cowpea, Dried	11.521	1.161	101
Burçak (Grain)	14.478	2.307	159
Buy (Fenugreek)	8.903	1.044	117
Grasspea	16.146	2.056	127
Lupine (For Human Consumption)	1.185	114	96
Soybean	380.090	155.000	408
Peanuts in Shell	457.015	186.340	408
Cotton Seed (Rawberry)	5.732.233	1.650.000	288
Flaxseed	95	8	84
Canola Or Rapeseed Seed	410.805	150.000	365
Sesame Seed	242.851	17.366	72
Sunflower Seed (Oil)	8.992.542	2.350.000	261
Sunflower Seed (Snack)	804.355	200.000	249
Cannabis Seed	1.943	159	82
Poppy Seed	265.012	12.240	46
Safflower Seed	262.344	30.000	114
Paddy	1.205.110	950.000	788
Potatoes (Excluding Sweet Potatoes)	1.390.724	5.200.000	3.739
Sweet potato	345	1.269	3.678
Jerusalem Artichoke	640	1.305	2.039
Sahlep	257	117	455
Sugar beet	2.745.237	19.000.000	6.921
Sugar Beet Seeds	10.627	2.281	215
Sugar cane	47	237	5.043
Tobacco, Unprocessed	750.510	82.250	110
Cotton, Unginned	5.732.233	2.750.000	480
Cotton, Ginned (Fibered)	5.732.233	1.017.500	178
Linen, Fiber	87	6	69
Hemp, Fiber	265	31	117
Vetch (Common) (Green forage)	2.078.824	2.501.907	1.204

Vetch (Hungarian) (Green forage)	748.983	995.995	1.330
Vetch (Other) (Green forage)	573.315	522.531	911
Burçak (Green forage)	20.432	12.417	608
Clover (Green forage)	6.411.294	19.064.213	2.974
Sainfoin (Green forage)	1.608.747	1.786.207	1.110
Clover (Green forage)	72	117	1.625
Oat (Green forage)	3.602.676	4.649.051	1.290
Sorghum (Green forage)	29.181	117.076	4.012
Triticale (Green forage)	619.185	1.072.635	1.732
Plum (Green forage)	66.994	55.208	824
Corn (Grow)	50.607	114.672	2.266
Corn (Slage)	5.245.654	28.558.983	5.444
Fodder beat	11.491	56.360	4.905
Feed Turnip	49.459	268.890	5.437
Wheat (Green forage)	168.307	310.966	1.848
Broad Bean, Dry (Forage)	8.165	2.452	300
Sudan Grass (Forage)	2.424	9.169	3.783
Meadow Grass (Green forage)	4.929.032	3.683.405	747
Barley (Green forage)	292.228	482.665	1.652
Rye (Green forage)	106.546	150.885	1.416
Peas (Feeder)	258.767	475.005	1.836
Italian Grass (Forage)	539.819	2.122.105	3.931
Other Forage Crops Not Elsewhere Classified (Excluding Cereal Straw and Shells)	16.932	28.424	1.679
Sainfoin Seed	3.096	171	55
Vetch (Common) Seed	204.045	26.229	129
Vetch (Hungarian) Seed	65.631	8.927	136
Vetch (Other) Seed	18.045	2.214	123
Grass Seed	14.275	2.532	177
Clover Seed	34.224	2.049	60
Hops Cone	1.828	1.051	575
Poppy Capsule (Poppy Head)	265.012	12.240	46
Sage	12.677	2.356	186
Dead nettle	6	1	167
Lavender	46.479	7.722	166
Melissa	697	324	465
Rose, Oil	41.675	19.879	477

Field crops cultivated in Turkey; wheat, barley, corn, rye, oats, sorghum, millet, birdseed, triticale, dried beans, broad beans, chickpeas, lentils, dried peas, dried cowpeas, vetch fenugreek, prunes, lupine, soybean, peanuts, cotton, flax, canola (rapeseed), sesame, sunflower, hemp, poppy, safflower, rice, potato, sweet potato, yam, salep, sugar beet, sugar cane, tobacco, vetch, clover, sainfoin, clover, animal beet, fodder turnip, bluegrass, sudangrass, Italian grass, hops, sage, nettle, rose, lemon balm and lavender. The most cultivated field crop in Turkey in terms of production area is wheat. 20 million tons of wheat was produced in approximately 66 million decares of land. Ranking in the second place, barley was produced in an area of 32 million decares, with 8.5 million tons. Sunflower took the third place. 2,5 million tons of sunflower was produced in an area of approximately 10 million decares. Species that are used extensively in human and animal nutrition have been produced more (Table 1).

FIELD CROPS CULTIVATION IN THE SOUTHEASTERN ANATOLIA REGION

Southeastern Anatolia Region is one of the seven geographical regions of Turkey. It covers the areas from the south of the Southeast Taurus Mountains to the Syrian border. The region is surrounded by the Eastern Anatolia Region in the east and north, the Mediterranean Region in the west, Syria in the south and Iraq with a short border. The weather is dry in the summer months, and rainy and warm in the winters. Compared

to the Mediterranean climate, winters are colder and summers are warmer.

Table 2. Field crops cultivated in the Southeastern Anatolia Region (TSI, 2022)

Field Crops	Production Area (decare)	Production Quantity (ton)	Yield (kg/da)
Durum Wheat	4.101.433	1.460.523	356
Wheat, Excluding Durum Wheat	6.027.433	1.855.836	308
Corn	2.270.428	1.798.952	792
Barley (Beer)	42.930	15.051	351
Barley (Other)	3.199.329	709.126	222
Oat	150	71	473
Millet	1.440	333	231
triticale	7.210	2.968	412
Buckwheat	196	31	158
Beans, Dry	2.909	736	253
Chickpeas, Dry	415.066	53.628	129
Lentils, Dry (Red)	2.874.670	381.512	133
Lentils, Dry (Green)	53	4	75
Peas, Dry	250	125	500
Burçak (Grain)	10.311	1.868	181
Soybean	6.814	2.050	301
Peanuts in Shell	64.370	24.599	382
Cotton Seed (Rawberry)	3.587.978	1.004.777	280
Sesame Seed	6.197	464	75
Sunflower Seed (Oil)	84.785	19.748	233
Safflower Seed	33	3	91
Paddy	14.746	6.363	432
Potatoes (Excluding Sweet Potatoes)	10.031	37.434	3.732
Sugar beet	20.665	160.925	7.787
Tobacco, Unprocessed	128.101	23.144	181
Cotton, Unginned	3.587.978	1.674.630	467
Cotton, Ginned (Fibred)	3.587.978	619.609	173
Vetch (Common) (Green forage)	136.104	137.038	1.007
Vetch (Hungarian) (Green forage)	13.238	12.974	980
Vetch (Other) (Green forage)	4.690	3.491	744

Burçak (Green forage)	8.709	2.446	281
Clover (Green forage)	106.247	145.892	1.373
Sainfoin (Green forage)	10.940	10.551	964
Sorghum (Green forage)	300	78	260
Triticale (Green forage)	3.182	4.218	1.326
Plum (Green forage)	1.948	1.320	678
Corn (Slage)	284.210	1.324.239	4.659
Meadow Grass (Green forage)	345	863	2.501
Barley (Green forage)	200	420	2.100
Rye (Green forage)	762	1.524	2.000
Peas (Feeder)	10.552	7.089	672
Italian Grass (Forage)	3.302	17.642	5.343
Other Forage Crops Not Elsewhere Classified (Excluding Cereal Straw and Shells)	15	7	467
Vetch (Common) Seed	13.705	1.402	102
Vetch (Hungarian) Seed	2.350	299	127
Clover Seed	12.000	396	33
Lavender	501	66	132

Field crops cultivated in the Southeastern Anatolia Region; wheat, barley, maize, oats, millet, triticale, dried beans, chickpeas, lentils, dried peas, vetch, soybean, peanuts, cotton, sesame, sunflower, safflower, paddy, potatoes, sugar beet, tobacco, cotton, common vetch, Hungarian vetch, clover, sainfoin, sorghum, damson, meadow grass, rye, Italian grass and lavender. Wheat is the most cultivated field crop in the Southeastern Anatolia Region, as it is throughout Turkey. The total wheat production area is approximately 10 million decares and the production amount is close to 3.3 million tons. The second most grown field crop is cotton. The seed yield of 2.2 million tons of cotton produced in 3.5 million decares of land is up to 467 kg/da. Red lentils took the third place. In 2022, 381 thousand tons of lentils were produced

in an area of 2.8 million decares in the Southeastern Anatolia Region. The least cultivated field plant is safflower (Table 2).

Table 3. Field crops cultivated in Şanlıurfa province (TSI, 2022)

Field Crops	Production Area (decare)	Production Quantity (ton)	Yield (kg/da)
Durum Wheat	1.538.920	548.482	356
Wheat, Excluding Durum Wheat	1.383.412	459.409	332
Corn	1.211.372	870.822	719
Barley (Other)	1.063.270	167.302	157
Millet	60	17	283
Chickpeas, Dry	55.733	5.950	107
Lentils, Dry (Red)	1.349.231	143.691	106
Burçak (Grain)	931	82	88
Soybean	2.353	523	222
Cotton Seed (Rawberry)	2.424.783	661.716	273
Sesame Seed	343	27	79
Sunflower Seed (Oil)	74.485	17.470	235
Paddy	4.850	1.928	398
Potatoes (Excluding Sweet Potatoes)	588	2.540	4.320
Sugar beet	1.500	8.037	5.358
Cotton, Unginned	2.424.783	1.102.859	455
Cotton, Ginned (Fibered)	2.424.783	408.055	168
Vetch (Common) (Green forage)	87.360	82.150	940
Burçak (Green forage)	55	61	1.109
Clover (Green forage)	32.583	38.220	1.173
Sorghum (Green forage)	300	78	260
Triticale (Green forage)	300	270	900
Corn (Slage)	92.946	460.860	4.958
Barley (Green forage)	200	420	2.100
Peas (Feeder)	8.490	5.509	649
Vetch (Common) Seed	9.307	698	75
Clover Seed	12.000	396	33

In Şanlıurfa province, in 2022, wheat, corn, barley, millet, chickpea, red lentil, vetch, soybean, cotton, sesame, sunflower, paddy, potato, sugar beet, common vetch, clover, sorghum and triticale were grown. The most grown field crop in Şanlıurfa is wheat. Approximately 1 million tons of wheat was produced in 2.8 million decares of land. Wheat yield (332 kg/da) in Şanlıurfa is above the yield average of Turkey and Southeastern Anatolia Region. The second most produced field crop is cotton. 1.5 million tons of cotton was produced in 2.4 million decares of land. Red lentils took the third place. 143 thousand tons of red lentils were produced in 1.3 million hectares. Red lentil yield is 106 kg/da in Şanlıurfa. The yield of red lentils in Şanlıurfa is below the red lentil yield average of Türkiye and Southeastern Anatolia Region (Table 3).

Table 4. Field crops cultivated in Gaziantep (TSI, 2022)

Field Crops	Production Area (decare)	Production Quantity (ton)	Yield (kg/da)
Durum Wheat	171.497	69.360	404
Wheat, Excluding Durum Wheat	454.304	157.864	347
Corn	63.695	44.286	695
Barley (Beer)	11.330	3.550	313
Barley (Other)	202.843	31.951	158
Beans, Dry	81	14	173
Chickpeas, Dry	77.385	9.004	116
Lentils, Dry (Red)	52.530	3.765	72
Lentils, Dry (Green)	53	4	75
Soybean	215	69	321
Peanuts in Shell	10.000	4.284	428
Cotton Seed (Rawberry)	59.074	16.120	273
Sunflower Seed (Oil)	4.653	1.592	342
Potatoes (Excluding Sweet Potatoes)	3.100	10.910	3.519
Sugar beet	17.350	143.071	8.246

Tobacco, Unprocessed	15	4	267
Cotton, Unginned	59.074	26.865	455
Cotton, Ginned (Fibred)	59.074	9.940	168
Vetch (Common) (Green forage)	9.456	13.935	1.474
Clover (Green forage)	1.105	1.637	1.481
Corn (Slage)	94.142	446.477	4.743
Peas (Feeder)	100	165	1.650
Italian Grass (Forage)	3.009	17.177	5.709
Vetch (Common) Seed	100	17	170

Field crops cultivated in Gaziantep province; wheat, barley, corn, dried beans, chickpeas, lentils, soybeans, peanuts, cotton, sunflowers, potatoes, common vetch, tobacco, alfalfa and Italian grass. The most cultivated field crop in terms of production area is wheat. 227 thousand tons of wheat was produced in 625 thousand hectares of land. Wheat yield (404 kg/da) in Gaziantep is above the yield average of Turkey and Southeastern Anatolia Region. In terms of production area, barley took the second place. 35 thousand tons of barley was produced in 224 thousand hectares of land. Both feed and brewed barley were produced in Gaziantep. Corn is in third place. 490 thousand tons of corn was produced in a total area of 159 thousand hectares, including grain and silage. The least grown field crop is tobacco. 4 tons of tobacco was produced in a 15 decare area (Table 4).

Table 5. Field crops cultivated in Adiyaman province (TSI, 2022)

Field Crops	Production Area (decare)	Production Quantity (ton)	Yield (kg/da)
Durum Wheat	36.860	11.514	312
Wheat, Excluding Durum Wheat	521.631	160.378	307
Corn	28.682	23.553	821

Barley (Beer)	31.600	11.501	364
Barley (Other)	536.028	165.789	309
Oat	150	71	473
triticale	7.210	2.968	412
Beans, Dry	924	208	225
Chickpeas, Dry	161.997	22.639	140
Lentils, Dry (Red)	32.965	4.643	141
Peanuts in Shell	1.370	532	388
Cotton Seed (Rawberry)	81.888	22.959	280
Sesame Seed	1.724	124	72
Sunflower Seed (Oil)	3.087	403	131
Potatoes (Excluding Sweet Potatoes)	1.773	6.418	3.620
Sugar beet	975	5.328	5.465
Tobacco, Unprocessed	120.187	19.968	166
Cotton, Unginned	81.888	38.264	467
Cotton, Ginned (Fibered)	81.888	14.158	173
Vetch (Common) (Green forage)	11.523	10.398	902
Clover (Green forage)	6.124	8.214	1.341
Sainfoin (Green forage)	20	30	1.500
Triticale (Green forage)	2.882	3.948	1.370
Plum (Green forage)	586	625	1.067
Corn (Slage)	24.213	80.910	3.342
Rye (Green forage)	762	1.524	2.000
Peas (Feeder)	1.293	1.144	885
Italian Grass (Forage)	205	238	1.161
Vetch (Common) Seed	636	104	164
Lavender	100	0	0

Field crops cultivated in Adıyaman province are wheat, corn, barley, oats, triticale, dried beans, chickpeas, red lentils, peanuts, cotton, sesame, sunflower, potato, sugar beet, tobacco, common vetch, clover, sainfoin, mulberry, rye, peas, Italian grass and lavender. The most cultivated field crop in terms of production area is barley. Wheat is in the second place and chickpea is in the third place. In 2022, on an area

of 567 thousand hectares, 177 thousand tons of barley; 172 thousand tons of wheat on an area of 558 thousand hectares; 22 thousand tons of chickpeas were produced on an area of 162 thousand hectares. The least cultivated field crop is sainfoin, which produces 30 tons in 20 decares (Table 5).

Table 6. Field crops cultivated in Diyarbakır province (TSI, 2022)

Field Crops	Production Area (decare)	Production Quantity (ton)	Yield (kg/da)
Durum Wheat	820.832	253.774	309
Wheat, Excluding Durum Wheat	1.944.340	545.436	281
Corn	179.281	185.139	1.033
Barley (Other)	692.009	185.789	268
Millet	1.310	288	220
Buckwheat	196	31	158
Beans, Dry	1.269	252	199
Chickpeas, Dry	52.776	7.787	148
Lentils, Dry (Red)	786.789	131.701	167
Burçak (Grain)	3.720	626	168
Soybean	4.046	1.416	350
Cotton Seed (Rawberry)	829.151	245.032	296
Sesame Seed	750	30	40
Sunflower Seed (Oil)	2.560	283	111
Safflower Seed	33	3	91
Paddy	9.046	4.138	457
Potatoes (Excluding Sweet Potatoes)	2.500	9.646	3.858
Sugar beet	840	4.489	5.344
Tobacco, Unprocessed	1.970	527	268
Cotton, Unginned	829.151	408.389	493
Cotton, Ginned (Fibered)	829.151	151.104	182
Vetch (Common) (Green forage)	13.705	24.362	1.778
Vetch (Hungarian) (Green forage)	6.858	9.557	1.394
Vetch (Other) (Green forage)	40	16	400
Burçak (Green forage)	1.554	1.043	671

Clover (Green forage)	7.225	14.017	1.940
Sainfoin (Green forage)	120	96	800
Plum (Green forage)	185	326	1.762
Corn (Slage)	36.880	158.758	4.305
Meadow Grass (Green forage)	345	863	2.501
Peas (Feeder)	19	76	4.000
Italian Grass (Forage)	88	227	2.580
Other Forage Crops Not Elsewhere Classified (Excluding Cereal Straw and Shells)	15	7	467
Vetch (Common) Seed	3.662	583	159
Vetch (Hungarian) Seed	2.350	299	127

Field crops grown in Diyarbakir are wheat, barley, corn, millet, dry beans, chickpeas, red lentils, vetch, soybean, cotton, sesame, sunflower, safflower, paddy, potato, sugar beet, tobacco, common vetch, Hungarian vetch, clover, sainfoin, mulberry, meadow grass, pea and Italian grass. The most grown field crop in Diyarbakır is wheat. In 2022, 800 thousand tons of wheat was produced in 2.7 million decares of land. Cotton took the second place. In an area of 829 thousand hectares, 408 thousand unseed cotton was produced. In terms of production area, red lentils are in the third place. 131 thousand tons of red lentils were produced in an area of 786 thousand hectares. The least cultivated field crop is safflower. 3 tons of safflower seeds were produced in a 33 decare area (Table 6).

Table 7. Field crops cultivated in Batman province (TSI, 2022)

Field Crops	Production Area (decare)	Production Quantity (ton)	Yield (kg/da)
Durum Wheat	63.647	23.003	361
Wheat, Excluding Durum Wheat	293.155	97.370	332
Corn	56.396	60.266	1.069
Barley (Other)	73.085	23.327	319
Chickpeas, Dry	2.547	411	161
Lentils, Dry (Red)	189.855	33.490	176
Cotton Seed (Rawberry)	13.085	3.711	284
Tobacco, Unprocessed	5.529	2.571	465
Cotton, Unginned	13.085	6.186	473
Cotton, Ginned (Fibred)	13.085	2.289	175
Clover (Green forage)	510	1.352	2.651
Plum (Green forage)	67	37	552
Corn (Slage)	6.340	28.530	4.500

In 2022, wheat, corn, barley, chickpea, red lentil, cotton, tobacco, clover and mulberry were grown as field crops in Batman. The most grown field crops in terms of production area and amount are wheat, red lentil and barley, respectively. In 2022, 120 thousand tons of wheat on an area of 356 thousand hectares in Batman; On 189 thousand decares, 33 thousand tons of red lentils and 23 thousand tons of barley were produced on 73 thousand decares. The least cultivated field crop is mulberry, produced 37 tons in 67 decares (Table 7).

Table 8. Field crops cultivated in Siirt province (TSI, 2022)

Field Crops	Production Area (decare)	Production Quantity (ton)	Yield (kg/da)
Durum Wheat	126.940	44.479	350
Wheat, Excluding Durum Wheat	178.310	55.227	310
Corn	9.560	11.100	1.161
Barley (Other)	34.330	8.249	240
Beans, Dry	450	228	507
Chickpeas, Dry	1.284	185	144
Lentils, Dry (Red)	135.510	26.520	196
Cotton Seed (Rawberry)	11.700	3.931	336
Sesame Seed	650	56	86
Paddy	250	83	332
Cotton, Unginned	11.700	6.551	560
Cotton, Ginned (Fibered)	11.700	2.424	207
Vetch (Common) (Green forage)	14.060	6.193	440
Clover (Green forage)	8.030	4.292	534
Sainfoin (Green forage)	5.500	2.475	450
Corn (Slage)	1.884	6.046	3.209
Lavender	140	35	250

Field crops grown in Siirt province are wheat, corn, barley, dried beans, chickpeas, red lentils, cotton, sesame, paddy, common vetch, clover, sainfoin and lavender. The most cultivated field crops in terms of wheat, red lentil and barley are respectively. In 2022, 100 thousand tons of wheat on an area of 305 thousand hectares in Siirt; 26 thousand tons of red lentils were produced on 135 thousand hectares and 8 thousand tons of barley was produced on 34 thousand hectares. The least grown field plant is lavender. 35 tons of lavender was produced in an area of 140 decares (Table 8).

Table 9. Field crops cultivated in Mardin province (TSI, 2022)

Field Crops	Production Area (decare)	Production Quantity (ton)	Yield (kg/da)
Durum Wheat	1.034.533	428.340	414
Wheat, Excluding Durum Wheat	857.785	315.842	368
Corn	673.193	568.580	845
Barley (Other)	395.911	96.415	244
Millet	70	28	400
Chickpeas, Dry	48.244	6.247	129
Lentils, Dry (Red)	174.435	24.050	138
Burçak (Grain)	5.660	1.160	205
Soybean	200	42	210
Cotton Seed (Rawberry)	101.977	32.514	319
Paddy	600	214	357
Potatoes (Excluding Sweet Potatoes)	2.070	7.920	3.826
Tobacco, Unprocessed	400	74	185
Cotton, Unginned	101.977	54.193	531
Cotton, Ginned (Fibred)	101.977	20.051	197
Vetch (Other) (Green forage)	1.000	180	180
Burçak (Green forage)	1.000	180	180
Clover (Green forage)	6.200	8.220	1.326
Plum (Green forage)	610	244	400
Corn (Slage)	1.000	5.600	5.600
Peas (Feeder)	650	195	300
Lavender	50	8	160

Wheat, corn, barley, millet, chickpea, red lentil, vetch, soybean, cotton, paddy, potato, tobacco, cotton, vetch, clover, mulberry, pea and lavender were grown as field crops in Mardin. The most cultivated field crops in terms of production area are wheat, corn and barley, respectively. In 2022, 750 thousand tons of wheat on an area of 1.9 million decares; On an area of 674 thousand hectares, 575 thousand tons

of corn; 96 thousand tons of barley was produced on an area of 395 thousand hectares. The least cultivated field plant is lavender, which is produced in 8 tons on an area of 50 decares (Table 9).

Table 10. Field crops cultivated in Şırnak province (TSI, 2022)

Field Crops	Production Area (decare)	Production Quantity (ton)	Yield (kg/da)
Durum Wheat	220.270	53.697	244
Wheat, Excluding Durum Wheat	260.054	36.985	142
Corn	42.718	31.179	730
Barley (Other)	113.818	16.867	148
Beans, Dry	185	34	184
Chickpeas, Dry	1.315	116	88
Lentils, Dry (Red)	118.897	10.318	87
Peanuts in Shell	53.000	19.783	373
Cotton Seed (Rawberry)	62.200	17.635	284
Sesame Seed	2.380	199	84
Cotton, Unginned	62.200	29.392	473
Cotton, Ginned (Fibred)	62.200	10.874	175
Vetch (Hungarian) (Green forage)	6.380	3.417	536
Burçak (Green forage)	6.100	1.162	190
Clover (Green forage)	44.020	68.050	1.546
Sainfoin (Green forage)	5.300	7.950	1.500
Plum (Green forage)	500	88	176

Şırnak province is located on the border of both Syria and Iraq. Field crops cultivated in Şırnak province are wheat, corn, barley, dry beans, chickpeas, red lentils, peanuts, cotton, sesame, Hungarian vetch, vetch, clover, sainfoin and mulberry. In terms of production area, wheat is the most cultivated field crop in Şırnak province. 90 thousand tons of wheat was cultivated in 480 thousand decares of land. Durum wheat constituted 53 thousand tons of this in an area of 220 thousand hectares.

Red lentils took the second place. 10 thousand tons of red lentils were produced in an area of 118 thousand tons. Barley took the third place. 16 thousand tons of barley was produced in an area of 113 thousand decares. The least produced field crop is dry beans. 34 tons of dry beans were produced in a 185 decare area (Table 10).

Table 11. Field crops cultivated in Kilis province (TSI, 2022)

Field Crops	Production Area (decare)	Production Quantity (ton)	Yield (kg/da)
Durum Wheat	87.934	27.874	317
Wheat, Excluding Durum Wheat	134.442	27.325	203
Corn	5.531	4.027	728
Barley (Other)	88.035	13.437	153
Chickpeas, Dry	13.785	1.289	94
Lentils, Dry (Red)	34.458	3.334	97
Peas, Dry	250	125	500
Cotton Seed (Rawberry)	4.120	1.159	281
Sesame Seed	350	28	80
Cotton, Unginned	4.120	1.931	469
Cotton, Ginned (Fibered)	4.120	714	173
Vetch (Other) (Green forage)	3.650	3.295	903
Clover (Green forage)	450	1.890	4.200
Corn (Slage)	26.805	137.058	5.113

Kilis is the smallest area in the Southeastern Anatolia Region. Wheat, corn, barley, chickpeas, red lentils, dry peas, cotton, and vetch were grown here. Wheat is the most cultivated field crop in Kilis province in terms of production area. 55 thousand tons of wheat was cultivated in a total of 88 thousand hectares of land. In Kilis, the average wheat yield is 203 kg/da, and the average durum wheat yield is 317 kg/da. After wheat, the most cultivated field crop is barley. 13 thousand tons of

barley has been cultivated in 88 thousand hectares of land. The third most grown barberry in terms of production area is red lentil. 3 thousand tons of red lentils were grown in 34 thousand hectares of land. Although the production area is less, the field crop with the highest production amount is maize for silage. The average yield of silage maize in Kilis is 5.113 kg/da. The least cultivated field crop is 125 tons of dry peas in an area of 250 decares (Table 11).

CONCLUSION

Field crops are divided into groups as cereals, edible legumes, industrial crops and forage crops. Most of the plants in the four groups are grown in Turkey and the Southeastern Anatolia Region. Turkey is the gene center for field crops (wheat, barley, rye, oats, flax, lentils, chickpeas and peas), pasture crops (clover, clover, sainfoin, vetch and grass forage crops) as it is located at the intersection of the Near East and Mediterranean Region gene centers. . Wheat is the most produced field crop in 9 provinces in the Southeastern Anatolia Region. In addition, red lentils, barley, corn and cotton cultivation are intensively produced. Southeastern Anatolia Region has strategic importance in terms of red lentil cultivation. In the southeast of Turkey, irrigated agriculture was started with the Southeastern Anatolia Project (GAP). With this project, it has also enabled the cultivation of products that were not grown here before. Before this project, cotton was grown mostly in the Mediterranean and Aegean regions, but after this project, its cultivation increased in the Southeastern Anatolia region. Common vetch, Hungarian vetch, alfalfa, sorghum, animal beet, Italian grass and

mulberry are produced as fodder crops. Sunflower, sesame, cotton, tobacco, safflower, potato, sugar beet, Jerusalem artichoke, rose and lavender are produced as industrial plants. Increasing the production of field crops in the Southeastern Anatolia Region is extremely important in terms of meeting the raw material needs of the agriculture-based industry and increasing the economic power of the region.

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CHAPTER 11

USE OF PHYTOBIOTICS IN BROILER CHICKENS

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INTRODUCTION

The use of antibiotics as additives in animal nutrition has provided important contributions such as increasing performance and protecting animal health (Lagha et al., 2017). However, the use of antibiotics as growth promoters has been banned since 2006 because of the development of bacterial resistance and residues in animal products (Gouvêa et al., 2015). Following this ban, researchers and producer have turned to safe additives that can be an alternative to antibiotics (Murugesan et al., 2015). One of the most important studied as an alternative to antibiotics is phytobiotics (Candan and Bağdatlı, 2017).

Phytogetic feed additives or phytobiotics refer to plant-based feed additives (Mohammadi-Gheisar and Kim, 2018; Stevanović et al., 2018). Possible mechanisms of mode of action in effects of phytobiotics is given in Figure 1 (Kikusato, 2021).

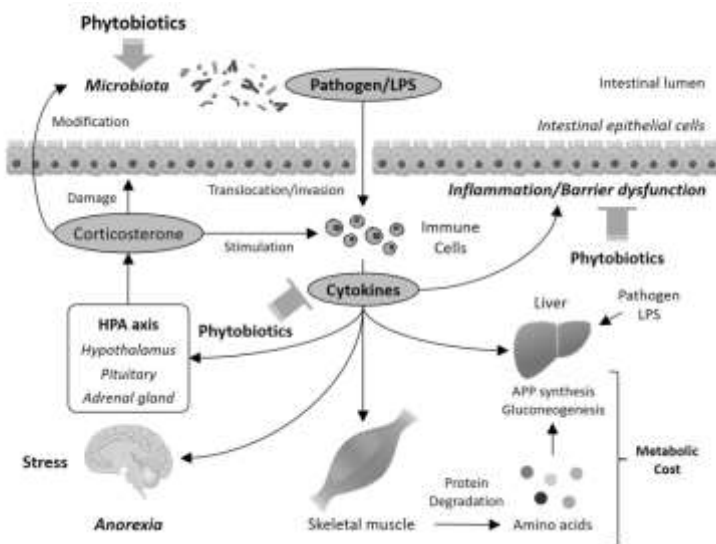


Figure 1. Possible mechanisms of mode of action in effects of phytobiotics (Kikusato, 2021).

Phytobiotics have an important role in promoting growth in animal nutrition (Windisch and Kroismayr, 2006). Phytobiotics, like probiotics, not only balance the microbial flora but also contribute positively to the digestion process of nutrients. Therefore, phytobiotics, which are of great importance in maintaining a balanced microbial flora, stimulate digestive secretions and positively affect the morphological characteristics of the gastrointestinal mucosa (Bagno et al., 2018). They also have positive effects as flavor enhancers in poultry diets (Grashorn, 2010). These multiple effects make them more important than many other additives that can be an alternative to antibiotics.

This chapter aims to provide detailed information about potential uses in broiler chickens of phytobiotics.

USE OF PHYTOBIOTICS IN BROILER CHICKENS

There are different studies conducted on the use of some phytobiotics such as oregano (Karimi et al., 2010; Roofchae et al., 2011; Mathlouthi et al., 2012; Ghasemloo et al., 2017), thyme (Al-Mashhadani et al., 2011), rosemary (Mathlouthi et al., 2012; Sierzant et al., 2021; Liu et al., 2022), clove (Petrovic et al., 2012; Meligy et al., 2023), cinnamon (Kanani et al., 2016; Meligy et al., 2023), sage (Farhadi et al., 2020; Rasouli et al., 2020), mint (Ashayerizadeh et al., 2009; Akter and Asaduzzaman, 2023), peppermint (Toghyani et al., 2010), black cumin (Ashayerizadeh et al., 2009; Toghyani et al., 2010), lemon balm (Petrovic et al., 2012), garlic (Ashayerizadeh et al., 2009; Sözcü, 2019; Kairalla et al., 2022a), anise (Al-Mashhadani et al., 2011; Ding et al.,

2020), ginger (Al-Khalaifah et al., 2022; Kairalla et al., 2022b) and rose (Yıldız et al., 2020) in broiler chickens.

Some studies on the use of phytobiotics in broiler chickens are summarized in Table 1.

Table 1. Some studies on the use of phytochemicals in broiler chickens

Phytochemicals	Form	Dose	Effects	References
Oregano (Mexican and Mediterranean origin)	Leaf	0, 2.5, 5, 10 and 20 g/kg (Positive control: 55 mg/kg penicillin)	<ul style="list-style-type: none"> - No effects on body weight, feed conversion ratio (FCR) and mortality rate (oregano leaf-supplemented) - Higher body weight in penicillin-supplemented group (at 18 day of age) 	Karimi et al. (2010)
Oregano	Essential oil (EO)	0, 300, 600 and 1200 mg/kg	<ul style="list-style-type: none"> - Increased body weight gain (600 mg/kg EO in grower diet) - Improved feed conversion ratio in grower and overall experimental periods (600 and 1200 mg/kg of EO) - No effect on population of cecal lactic acid bacteria - Lower cecal <i>Escherichia coli</i> (300 and 600 mg/kg EO) - No effect on serum antioxidant activity 	Roofchae et al. (2011)
Oregano (O) Rosemary (R) Commercial	Essential oil (EO)	0, 50 and 100 mg/kg (O, R) 0 and 1 mg/kg CB	<ul style="list-style-type: none"> - Improved body weight, body weight gain (avilamycin or EO-supplemented groups) 	Mathlouthi et al. (2012)

blend (CB)		[Positive control: 44 mg/kg avilamycin (A)]	<ul style="list-style-type: none"> - No effects in growth performances among birds fed A and EO diets - Improvement in feed conversion ratio and body weight gain (higher levels of EO) - Increased beneficial bacteria in intestinal content - Increased some bacterial metabolites (butyric acid, valeric acid, propionic acid, butyric acid, acetic acid, and total short-chain fatty acids) - Reduced populations of pathogenic bacteria 	Meligy et al. (2023)
Oregano Cinnamon Clove	Essential oil (EO) (Encapsulated)	0, 200, 300 and 400 mg/kg	<ul style="list-style-type: none"> - No effects on performance at the end of 42 days - The highest lactobacillus colony numbers in encapsulated EO-supplemented group - No effects on length and weight of parts of digestive tract (except ileum weight) 	Ghasemloo et al. (2017)
Oregano	Essential oil (EO) (Encapsulated)	0, 0.2 and 1 g/kg (Non-encapsulated: 0.2 g/kg) (Encapsulated: 1 g/kg)		

Sage	Leaf extract	0, 100, 200, 300 and 400 ppm	<ul style="list-style-type: none"> - No effects on body weight gain, feed conversion ratio, feed intake - Decreased some (blood parameters plasma triglycerides, total cholesterol and low-density lipoprotein (LDL)) - Increased high-density lipoprotein (HDL) - Enhancement of the immunity response of broilers 	Rasouli et al. (2020)
Sage	Powder (P)	0, 0.2, 0.5 and 1.2%	<ul style="list-style-type: none"> - Increased body weight and reduced feed conversion ratio (0.5% P) - Increased eosinophil, monocyte and heterophil (0.2% P) - Reduced some blood parameters (plasma cholesterol, LDL, and triglyceride concentrations) - Increased HDL level (0.2% P) - Increased ileal villus height and intestinal health index 	Farhadi et al. (2020)

				<ul style="list-style-type: none"> - Reduced crypt depth and number of goblet cells (0.2% P) - Increased body weight (4 g/kg P) (28 days of age) - The most efficient feed conversion ratio in 4 g/kg BC-supplemented group (0-42 days) - No effects on internal organ weights and carcass traits - Increased weight of lymphoid organs (BC supplementation) (42 days) - No effects on serum total cholesterol, LDL, HDL, triglyceride, albumin levels - No effects in serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) enzymes concentrations - Increased red blood cell (RBC) count, hemoglobin concentration and hematocrit percentage in 	
Peppermint (P) Black cumin (BC)	Powder (P) Seed (BC)	0, 2 and 4 g/kg (BC) 0, 2, 4 and 8 g/kg (P)		Toghyani et al. (2010)	

<p>Black cummin (BC) Wild mint (WM) Garlic (G)</p>	<p>Powder</p> <p>0 and 1000g/ton</p>	<p>BC-supplemented groups (compared to control birds)</p> <ul style="list-style-type: none"> - No effects on feed consumption (both 0-21 days and 0-42 days) - The highest body weight in BC-supplemented group - The best FCR in BC-supplemented group (All growing periods) - The lowest abdominal fat percent in G and BC-supplemented groups - The highest carcass percent in G and BC groups - No effects on percent of thigh 	<p>Ashayerizadeh et al. (2009)</p>	
<p>Mint</p>	<p>Leaves (L) (dried)</p> <p>0, 1.5, and 2%</p> <p>(Note: positive control: basal diet + antibiotic)</p>	<ul style="list-style-type: none"> - Increased live weight and body weight gain (2.0% mint L) - Improved FCR (2.0% mint L) - Significant effects on wing, thigh, back, neck, liver, heart, gizzard intestine, spleen and bursa (2.0% mint) 	<p>Akter and Asaduzzaman (2023)</p>	

			<p>- Significant effects on some immune parameters [white blood cell (WBC), lymphocyte, granulocyte] in mint L-supplemented groups than in the control (better immunity)</p> <p>- Lower <i>Escherichia coli</i> and <i>Salmonella</i> in caecum in 2% mint L-supplemented groups (compared with the control)</p>	
<p>Anise (A) Thyme (T)</p>	<p>Essential oil</p>	<p>0 mg/kg basal diet 300 mg/kg (A) 300 mg/kg (T) 200 mg/kg A+ 200 mg/kg T (mix)</p>	<p>- Increased final body weight and weight gain (A, T and mix)</p> <p>- Improved in terms of FCR in all treatment groups when compared to control group (0-38 day)</p> <p>- Decreased serum glucose and cholesterol concentrations (A, T and mix groups)</p>	<p>Al-Mashhadani et al. (2011)</p>
<p>Anise</p>	<p>Seed powder (SP)</p>	<p>0, 500, 750 and 1000 mg/L drinking water</p>	<p>- Improved blood RBC, WBC, hemoglobin, hematocrit, total protein, albumin, globulin, glucose, P, and Ca (750 and 1000</p>	<p>Al-Shammari et al. (2017)</p>

				mg/L SP) (days 28 and 56, and on average) - Higher body weight (SA and EO) - No effects on the carcass yield, half chamber rate, eviscerated rate, and percentages of thigh muscle and breast muscle - Higher thymus weight (SA and EO). - Highest villus height in ileum and villus height/crypt depth ratio in ileum and jejunum (EO) - Reduced blood serum total cholesterol, triglyceride and LDL levels - Increased blood serum glucose and HDL concentration - Significant differences on hepatic enzymes and immunoglobulin levels (35 days of age) - Increased (0.1% or 0.2% P) packed cell volume, total white blood cells, monocyte,	Ding et al. (2020)
Star anise	Essential oil (EO) Leaf (L) Star anise (SA)	0 and 0.22 g/kg EO 0 and 5 g/kg L 0 and 5 g/kg (SA)			
Garlic	Extract	0 and 1 ml/L drinking water			Sözcü (2019)

Garlic	Powder (P)	0, 0.1, 0.2 and 0.3%	<p>neutrophil, eosinophil and lymphocyte</p> <ul style="list-style-type: none"> - Decreased triglycerides, total cholesterol, LDL, alanine aminotransferase (ALT) and increased HDL levels - No effects on weight gain and FCR - No effects on carcass characteristics - No effects on oxidative stability of chicken breast muscles - Reduced MDA in frozen thigh muscles. 	Kairalla et al. (2022a)
Rosemary Blackcurrant	Extract	0, 2.5 and 5 g/kg	<ul style="list-style-type: none"> - Reduced FCR (500 and 750 mg/kg E) (0-21 d) - Reduced some serum biochemical parameters (albumin, total cholesterol, LDL and HDL) (500 and 750 mg/kg E) - Reduced some serum biochemical parameters (glucose, total protein and triglyceride) (750 mg/kg E) - Increased T-AOC (total antioxidant capacity), SOD 	Sierżant et al. (2021)
Rosemary	Extract (E)	0, 250, 500 and 1000 mg/kg	<ul style="list-style-type: none"> - Reduced FCR (500 and 750 mg/kg E) (0-21 d) - Reduced some serum biochemical parameters (albumin, total cholesterol, LDL and HDL) (500 and 750 mg/kg E) - Reduced some serum biochemical parameters (glucose, total protein and triglyceride) (750 mg/kg E) - Increased T-AOC (total antioxidant capacity), SOD 	Liu et al. (2022)

			<p>(superoxide dismutase), CAT (catalase), GSH-Px (glutathione peroxidase) and, Immunoglobulins A, G and M (IgA, IgG, IgM) levels</p> <ul style="list-style-type: none"> - Decreased serum MDA level 	
<p>Rose</p>	<p>Rose water (By product of rose oil production)</p>	<p>0, 2 and 4%</p>	<ul style="list-style-type: none"> - No effects on feed intake, live weight, live weight gain (0-35 days) - Increased FCR (0-35 days) FCR (negative effect) - Postive effect on hot carcass weight - No effect on some internal organ (liver, heart, spleen, bursa Fabricius and abdominal fat) weights - No effects on some (villus height, crypt depth and villus height: crypt depth ratio) ileum and jejunum histomorphological parameters (21st and 35th days) - No effects on growth performance (body weight, 	<p>Yıldız et al. (2020)</p>

<p>Clove (C) Lemon balm (LB) Agrimony (A)</p>	<p>Flower buds powder (C) Extract (LB) Extract (A)</p>	<p>0% (Basal diet) 1% C + 0.2% LB 1% C + 0.2% A (C: supplementation to basal diet) (LB, A: supplementation drinking water)</p>	<p>total feed intake, FCR and carcass yield) - No effects on some blood parameters (SOD, and GSH-Px, concentration of vitamin A) - Reduced MDA level in plasma - Some indices of the antioxidant status (sulfhydryl group and vitamin E concentrations)</p>	<p>Petrovic et al. (2012)</p>
<p>Orange peel</p>	<p>Essential oil (EO)</p>	<p>0, 50, 100 and 150 mg/kg</p>	<p>- Significant effect on live weight (except for 1st and 5th weeks) - Improved feed efficiency (150 mg/kg EO) - Increased carcass weight, carcass yield, abdominal fat, things, breast, wing, back, and heart weights</p>	<p>Aydın and Alçiçek (2018)</p>
<p>Cinnamon</p>	<p>Essential oil (EO)</p>	<p>0, 0.025, 0.05 and 0.1%</p>	<p>- Decreased MDA concentration in plasma and duodenal mucosa (0.1% EO) - The higher activities of glutathione peroxidase in plasma (0.1% of EO)</p>	<p>Faix et al. (2009)</p>

			<ul style="list-style-type: none"> - Reduced alanine amino transferase (ALT) activity in plasma (0.05 and 0.025% EO) - Increased feed intake and body weight (C, T and mix) - Decreased blood uric acid concentration and lactate dehydrogenase activities - Decreased blood MDA level (C, T, C + T) - No effects on blood aspartate aminotransferase, creatinine and urea - No effects on blood potassium, sodium, chlorine, hematocrit and rectal temperature levels 	Kanani et al. (2016)
<p>Cinnamon (C) Turmeric (T)</p> <p>(Note: Experiment of broiler chickens under heat stress)</p>	Powder	<p>0 and 0.50 (C, T) 0.25 mix (C + T)</p>	<ul style="list-style-type: none"> - Higher body weight gain - Improved antioxidation indices in broiler liver and serum - Reduced total feed consumption - Increased white blood cell counts and the percentage of heterophils 	Al-Khalaifah et al. (2022)
Ginger	Powder	0, 5, 10 and 15 g/kg		

			<ul style="list-style-type: none"> - Decreased blood cholesterol, triglyceride, and very low-density lipoprotein (VLDL) levels - Increased high-density lipoprotein (HDL) - Lower feed intake and higher feed conversion - Improved some hematological parameters (white blood cells, heterophils, lymphocytes, and the lymphocyte to heterophil ratio) - Decreased serum total cholesterol and triglycerides, VLDL levels - Increased serum HDL concentration 	
Ginger	Powder	0, 0.2, 0.4 and 0.6%	<ul style="list-style-type: none"> - No effects on some in parameters (triglyceride, cholesterol, glucose, LDL, AST, ALP, ALT and MDA concentrations) - Reduced ileal <i>Escherichia coli</i> and <i>Lactobacillus</i> spp. Populations 	Kairalla et al. (2022b)
Fennel	Essential oil	0 and 200 mg/kg		Ghiasvand et al. (2021)

CONCLISION

In some of the studies summarized in this chapter, it is seen that different forms and doses of various plants have positive effects. In conclusion, the use of some plants as an alternative to antibiotics is promising in broiler chickens nutrition.

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