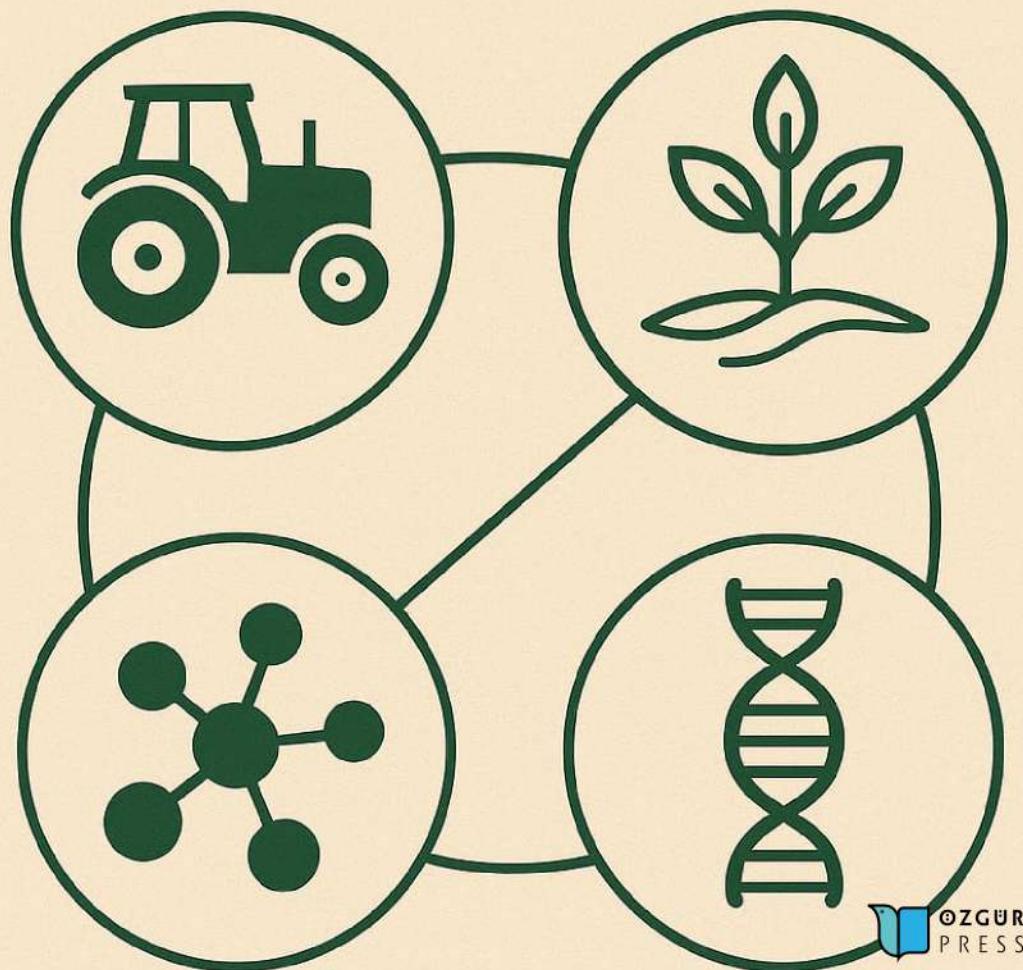


# Integrated Perspectives in Agriculture and Biology: Theory and Practice

Editors:

Assoc. Prof. Dr. Hülya Doğan  
Prof. Dr. Hatice Baş



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Published by  
**Özgür Yayın-Dağıtım Co. Ltd.**  
Certificate Number: 45503

📍 15 Temmuz Mah. 148136. Sk. No: 9 Şehitkamil/Gaziantep  
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Editors: Prof. Dr. Hatice Baş • Assoc. Prof. Dr. Hülya Doğan

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Language: English  
Publication Date: 2025  
Cover design by Mehmet Çakır  
Cover design and image licensed under CC BY-NC 4.0  
Print and digital versions typeset by Çizgi Medya Co. Ltd.

**ISBN (PDF):** 978-625-5757-89-0

**DOI:** <https://doi.org/10.58830/ozgur.pub972>

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Suggested citation:

Bas, H. (ed), Doğan, H. (ed) (2025). *Integrated Perspectives in Agriculture and Biology: Theory and Practice*. Özgür Publications. DOI: <https://doi.org/10.58830/ozgur.pub972>. License: CC-BY-NC 4.0

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# Perface

In an era marked by unprecedented global challenges ranging from climate change and biodiversity loss to food security and sustainable development the intersection of agriculture and biology has never been more critical. The synergy between these two disciplines holds transformative potential, not only for improving agricultural productivity and resilience but also for advancing our fundamental understanding of life systems and their interactions with the environment.

Integrated Perspectives in Agriculture and Biology: Theory and Practice emerges from this dynamic context. This book consists of several chapters written by leading researchers, practitioners, and educators in the field. Each contribution reflects a unique viewpoint, yet all share a common commitment: to explore and apply integrated approaches that bridge theory and practice, tradition and innovation, and the micro and macro scales of biological and agricultural systems.

The book covers a wide range of topics from molecular biology and crop genetics to sustainable farming techniques and ecosystem-based management. It emphasizes both the need for deep specialized knowledge in subfields and the need for interdisciplinary collaboration to solve worldwide problems. Theoretical insights are complemented by practical case studies and policy considerations, offering readers a well-rounded understanding of current trends and future directions.

As editors, we hope this volume serves as a valuable resource for scholars, students, policymakers, and professionals seeking to engage with the complexities of modern agriculture and biological science. We also aim to inspire new research questions, interdisciplinary dialogues, and innovative solutions that will contribute to a more sustainable future.

We extend our deepest gratitude to our readers who will bring these ideas to their own work and to society, and to all those who have contributed for their dedicated work.



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# Applications of Embryo Culture in Grapevine 6

Berna Keles<sup>1</sup>

Emine Sema Çetin<sup>2</sup>

## Abstract

Plant tissue culture is a method that enables the regeneration or propagation of plant parts or whole plants by culturing plant protoplasts, cells, tissues, or organs under controlled conditions *in vitro*. Among these techniques, anther culture, meristem culture, shoot tip culture, ovule culture, and embryo culture are widely used *in vitro* techniques.

Embryo culture, defined as the cultivation of embryos isolated from seeds or seed primordia of higher plants on sterile nutrient media, is utilized for purposes such as the rapid development of new varieties, the creation of varieties more resistant to diseases, pests, and abiotic stresses, and enabling hybridization efforts hindered by incompatibility between plant genera and species.

In grapevine, embryo culture is employed for objectives such as developing new grape varieties, propagating seedless grape cultivars, and creating varieties resistant to diseases, pests, and diverse ecological conditions. However, despite extensive research on embryo rescue in viticulture, the number of newly developed cultivars remains limited. Breeding new grape varieties through embryo rescue is still a challenging and long-term technique, requiring a patient and dedicated approach from grape breeders.

This compilation will provide current and comprehensive insights into the embryo rescue technique in grape breeding, discussing its applications and practices.

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## 1. Introduction

Plant tissue culture is defined as the cultivation of plant cells obtained from various parts of the plant (root, stem, leaf, apical and meristem) under aseptic conditions and in an synthetic nutrient medium (Güven and Gürsul, 2014). Compared to traditional propagation methods, tissue culture is a modern vegetative propagation technique that provides high efficiency and quality. It is particularly suitable for mass production, ensures genetic homogeneity, and significantly shortens the juvenile period (Driver and Kuniyuki, 1986; Scaltsoyiannes et al., 1998).

In vitro propagation of various plant species using tissue culture techniques employs several types of cultures. These include shoot tip culture, node culture, callus culture, embryo culture, cell, and protoplast culture (Guney et al., 2020; Ahuja, 1986; Pierik, 1986; Srivastava and Steinbaver, 1981).

One of the earliest and most important applications of in vitro culture is embryo culture, also known as embryo rescue. It is an in vitro technique used to rescue zygotic embryos extracted from seeds and ovules in a nutrient medium, by identifying the essential requirements for embryo morphogenesis, differentiation, and continued development under aseptic conditions (Sharman and Kaur, 1996; Raghavan, 2003). Numerous studies have been conducted on various plants and species concerning the embryo rescue technique (Chen et al., 2025; Doygaci et al., 2024; Reddy et al., 2024; Yao et al., 2024; Yıldırım, 2012).

Grape is a fruit species of global significance. It has numerous uses, including as table grapes, raisin, and wine production. In grape cultivars, seedlessness is a highly valued trait for both table and raisin, ranking high among consumer preferences. Therefore, the breeding of seedless grape varieties has long maintained its importance and relevance (Alleweldt and Possingham, 1988; Wang et al., 2002; Ebadi et al., 2009).

Embryo culture plays a significant role in the breeding of seedless grapes. Many breeders have obtained new seedless grape varieties from stenospermocarpic varieties using as female parents by the embryo rescue technique (Spiegel-Roy et al., 1985; Gribaudo et al., 1993). Although seedless grape varieties are preferred by consumers, they tend to be more susceptible to diseases. Therefore, breeding seedless varieties with improved disease resistance has emerged as a separate and important objective (Nigar et al., 2024).

The embryo rescue technique has great potential in developing disease-resistant seedless grape varieties. Difficulties are encountered in traditional breeding methods due to the stenospermocarpic berry set in grapes. Stenospermocarpy refers to the degeneration of grape embryos before full development, which stems from abnormal development of the ovule and integuments. Thus, the embryo rescue technique offers a valuable and promising solution to overcome these limitations and develop desirable, disease-resistant, seedless grape varieties (Cui et al., 2017; Conner et al., 2018).

## 2.1. History of Embryo Culture

Embryo culture, which involves isolating embryos from seeds and seed primordia and cultivating them in specific nutrient media, is an important plant tissue culture technique. This technique is based on the principles of isolating the embryo without damage, establishing a suitable nutrient medium, and ensuring embryogenic development and transformation into a plant (Bridgen, 1994; Reed, 2005).

The embryo rescue technique is used to eliminate dormancy in seeds of certain plants, overcome incompatibility barriers in some hybrids, and initiate callus cultures.

Embryo rescue began to be used by plant breeders in the 18th century with Charles Bonnet's studies on *Phaseolus* and *Fagopyrum* (Schopfer, 1943; Sharma et al., 1996). The first successful experiment was conducted in the early 20th century by Hanning (1904), who used two radish species (*Raphanus* and *Cochlearia*). Mature embryos isolated from seeds were cultured in a nutrient medium containing macro and micro elements, and sugar, resulting in new plantlets (Narayanaswami and Norstog, 1964; Ramming, 1990). Later, in 1925, Laibach succeeded in cultivating hybrid embryos obtained from a cross between *Linum perenne* and *Linum austriacum*, which normally could not develop on the plant, thereby producing hybrid plants (Laibach, 1925).

Tukey's embryo culture study on cherries in 1933 was an important step in the application of embryo culture in fruit breeding (Tukey, 1993). Since then, embryo rescue has been widely applied in various fruit species. For example, it has been used in apple (Dantas et al., 2006), banana (Uma et al., 2011), citrus (Viloria et al., 2005), mango (Krishna and Singh, 2007), melon (Nunez-Palenius et al., 2006), peach (Anderson et al., 2002), persimmon (Hu et al., 2013), and watermelon (Taşkın et al., 2013; Li et al., 2014). Embryo rescue is the most commonly used method in seedless grape

breeding and is also applied to develop early-ripening and triploid grape varieties (Li et al., 2014).

Ovule culture is defined as the in vitro cultivation of ovules and the subsequent isolation of embryos developing from these ovules. After the first in-ovulo culture in grapevine was performed in 1982, embryo culture became widely used (Emershad and Ramming, 1982). Factors influencing the success of culture include genotype (Liu et al., 2003), the age of the ovule at isolation (Ji et al., 2013b), and the composition of the nutrient medium (Amaral et al., 2001).

Embryo culture is also used to overcome pre-zygotic and post-zygotic incompatibilities, which limit the development of sexual hybrids and result in sterility under natural conditions (George and Eapen, 1993). Additionally, It is also a valuable technique in preventing losses in the germination of hybrid seeds obtained from breeding studies under field conditions. Zygotic embryos are known as the best explant source for in vitro cultures due to their juvenile characteristics and high regeneration potential (Burgos and Ledbetter, 1993).

## **2.2. Advantages of Embryo Culture**

In a classical breeding study, the chance of obtaining a seedless individual in a seeded  $\times$  seedless cross is up to 49%, while in seedless  $\times$  seedless crosses with embryo culture, this rate increases to 92%.

Embryo rescue method is used because germination is generally poor in the embryos of early-ripening grape varieties. While in traditional breeding methods, early maturing grape varieties are used only as male parents, these varieties can also be used as female parents in embryo culture, and the chance of earliness in the resulting hybrids can be increased (Ramming et al., 1990).

In some cases, fertilization occurs after pollination, but divisions do not occur in the zygote or only a few-celled embryo is formed and development cannot continue. The endosperm may not support embryo growth, resulting in small, undeveloped embryos. In such situations, seeds containing embryos at a certain physiological maturity are sterilized, and embryos are isolated from surrounding tissues under sterile conditions. During isolation, small embryos should be isolated without damaging them, and the isolated embryos should be inoculated under appropriate conditions to ensure their transformation into full plants (Ergönül et al., 2021).

### 2.3. Disadvantages of Embryo Culture

Embryo culture, despite its many advantages, also has some disadvantages. These can be summarized as follows:

- i. Low success rates: Success rates in embryo recovery vary depending on the plant species and even the variety.
- ii. Genetic diversity: Genetic variations that affect plant traits may emerge during the culture process.
- iii. Environmental factors: Plants grown under controlled conditions may have difficulty adapting to natural environments.
- iv. High costs: Embryo culture is a costly technique that requires expert experience and a well-equipped laboratory infrastructure.

### 3. Applications of Embryo Culture in Viticulture

Due to its heterozygous genetic structure, significant genetic diversity is seen among grapevines adapted to various climatic conditions around the world. Therefore, seeds play an important role in grapevine breeding. In breeding programs, seeds with high germination potential are of particular significance (Elidemir et al., 1999).

Today, biotechnological approaches such as molecular techniques and tissue culture are integrated into traditional breeding efforts to enhance their effectiveness. Embryo rescue technique is a technique applied in cases where it is not possible to obtain hybrid plants with classical breeding methods. In recent years, embryo culture and embryo rescue techniques have significantly contributed to the improvement of seedlessness and polyploidy in grapevine breeding programs (Emershad and Ramming, 1982). This technique also provides significant benefits in the breeding of early-maturing grape varieties. In grapevine breeding where seedlessness is a priority, embryo culture is used to increase the likelihood of obtaining seedless individuals.

In addition to breeding for seedlessness and earliness, embryo culture is also integrated into programs aimed at producing haploid, triploid, and tetraploid plants. Moreover, it is utilized in interspecific hybridizations between *Vitis* species to combine seedlessness with other traits such as cold tolerance and resistance to fungal diseases (Ji and Wang, 2013; Li et al., 2015). Embryo and embryo rescue techniques are used in hybridization studies to develop varieties that are resistant to diseases and pests and produce high-quality grapes (Goldy et al., 1988).

Several factors influence the success of embryo rescue in grapes, with genotype being the most critical. Various researchers have reported that genotype plays a vital role in embryo development (Ji et al., 2013a; Razi et al., 2013). These studies have shown that genotype significantly affects the success of embryo rescue methods and that better results can be obtained from certain grape varieties. Therefore, optimizing genotype differences is of great importance for embryo rescue success.

Parthenocarpic seedless grapes (e.g., Black Pearl and White Corinth) are not suitable as female parents for breeding via embryo rescue. However, this technique can be applied using them as female parents in stenospermocarpic grapes (Singh et al., 2011).

Another factor affecting the success of embryo rescue is the time at which the embryo is isolated. While the early berry development stages of stenospermocarpic grapes similar to seeded varieties, they later undergo degeneration, limiting the transition of embryos into mature stages. Therefore, identifying the optimal period for embryo isolation plays a key role in improving success rates. Numerous studies have emphasized the importance of this period (Ponce et al., 2000; Roichev et al., 2007; Li et al., 2013). For instance, Gray et al. (1990) cultured ovules from Orlando Seedless grapes at 10, 20, 40, and 60 days after flowering. They reported that more embryos and plants were obtained from ovules cultured 40–60 days after flowering and the highest embryo recovery was from ovules cultured at 60–70 days. Other studies have also shown that genotype affects the sampling time of ovules (Ji et al., 2013b; Yang et al., 2007; Xu et al., 2005).

Xu et al. (2005) determined that the optimum sampling time in crosses between diploid and tetraploid cultivars depends on the maturation time of the female parent. This period was 6–9 weeks after pollination in early-ripening varieties, 7–10 weeks in mid-ripening varieties, and 9–12 weeks in late-ripening varieties.

The nutrient medium, which is part of the embryo rescue technique, is crucial for success. Since embryo culture involves multiple stages, different basal media and media solidity (liquid, solid, liquid-solid) are used. Examples include White (White, 1954), MS (Murashige and Skoog, 1962), B5 (Gamborg et al., 1968), NN (Nitsch and Nitsch, 1969), Smith (Smith et al., 1969), SH (Stewart and Hsu, 1977), Cain (Cain et al., 1983), ER (Emershad and Ramming, 1982), BD (Bouquet and Davis, 1989), and WPM (Woody Plant Basal Medium, Lloyd and McCown, 1980). These media contain macro and micro elements, sugars, and growth-promoting

substances. Liu et al. (2008) reported that increasing  $\text{CaCl}_2$  concentration in the medium enhanced success in embryo rescue (Liu et al., 2008).

In grape embryo culture, sucrose is the most commonly used sugar. While the most commonly used concentration in tissue culture studies is 20-30 g/L, this level is as high as 60 g/L for the culture of immature grape embryos. Sugar supports normal growth by balancing osmotic pressure and preventing premature germination of young embryos (Tian et al., 2008; Ji et al., 2013b). Many additives are also included in the culture medium, such as coconut water, casein hydrolysate, and malt extract—substances derived from endosperm tissues (Sharma et al., 1996).

Another factor affecting the success of the grape embryo culture technique is the type and concentration of plant growth regulators (PGRs). Success rates increase when plant growth regulators are added to the culture medium. Application before or during flowering can also be effective (Razi et al., 2013). Common PGRs in the culture medium include antigibberellins (e.g., chlormequat and uniconazole, which promote embryo germination when applied before flowering), cytokinins, and putrescine (Ledbetter and Shonnard, 1990; Tang et al., 2009).

#### 4. Studies on Applications of Embryo Culture in Viticulture

Grape (*Vitis vinifera* L.) is one of the most widely consumed fruits globally, available in many forms. Among consumer preferences, seedlessness has always been a prominent research area in grape breeding. Therefore, extensive breeding programs have been conducted to develop new seedless cultivars. Embryo rescue has been effectively applied by plant breeders to overcome the biological barriers encountered in crosses between seedless cultivars, aiming to recover immature embryos. For over thirty years, embryo rescue has been used in grape breeding. The most critical factors affecting embryo rescue success are genotype, the timing of embryo isolation, and the composition of the nutrient medium. Additionally, other factors such as culture techniques and the use of plant growth regulators also play significant roles. To date, this technique has been employed in wide hybridizations among various *Vitis* species to rescue weak embryos and to produce seedless and triploid lines. However, the development of new grape varieties through embryo rescue remains a challenging and long-term task, requiring consistent effort from breeders (Li et al., 2015).

To develop seedless and disease-resistant cultivars, Li et al. (2014) crossed stenospermocarpic *Vitis vinifera* cultivars with Asian grape species. They studied seven hybrid combinations in different media containing

growth regulators and evaluated embryo formation, germination, and plant development. The highest embryo germination and plant development rates were achieved using a medium composed of Woody Plant Medium (WPM) + 5.7  $\mu$ M indole-3-acetic acid (IAA) + 4.4  $\mu$ M 6-benzylaminopurine (BAP) + 1.4  $\mu$ M gibberellic acid (GA) + 2% sucrose + 0.05% casein hydrolysate + 0.3% activated charcoal + 0.7% agar. Adding proline increased embryo formation by 36.1%, germination by 64.6%, and plant development by 90.5%. The study established a high-efficiency protocol for hybridizing seedless *Vitis vinifera* with Asian species, significantly improving breeding efficiency for disease-resistant seedless grapes.

In breeding studies, it is of great importance to develop varieties that will meet consumer demands. De Menezes et al. (2014) emphasized the importance of developing new genetic materials for export-oriented fruit production in irrigated regions of Brazil. They highlighted that embryo rescue is effective in semi-arid regions to obtain such materials. They evaluated the in vitro development of intraspecific grape hybrids obtained by rescuing immature embryos from crosses between Brazilian clones “Superior Seedless” and “Thompson Seedless.” The culture medium contained 30 g/L sucrose, 0.1 g/L myo-inositol, 0.002 g/L glycine, 0.1 mg/L IAA, and 6.5 g/L agar, adjusted to pH 5.7. Parameters such as node number, leaf number, plant height, root number, and root length were evaluated over 90 days. Ovules cultured at 60 days showed the highest values, with approximately 50% embryo formation and a germination rate of 47.3%.

Embryo rescue is the most important in vitro method used in breeding seedless grapes. Breeding programs incorporating seedlessness along with other traits have also been studied. Moreira and Clark (2021) investigated the impact of embryo isolation timing and nutrient media on embryo rescue efficiency in cold-hardy hybrid grapes. Ovules were collected 5 to 9 weeks after flowering and cultured in four different media. The highest germination and plant development occurred from ovules collected eight weeks post-flowering. Lloyd & McCown WPM medium was found to support the best results. Although different growth regulators did not significantly differ in performance, the WPM plus medium containing BAP, IAA, GA, and casein provided the highest seedling yields. The authors concluded that ovule collection at eight weeks and culture in WPM plus is optimal for breeding programs.

Similarly, Doygaci et al. (2024) emphasized that genotype and embryo isolation timing are the most critical factors in achieving high success rates in embryo rescue. After four years of study, they identified the eighth week after

pollination as the optimal isolation time. They found that the combination using 'Yalova Seedless' as the female parent resulted in more plant recovery, especially when pollinated with Red Globe, Muscat Bailey A, or Exalta.

Giancaspro et al. (2022) noted that early-ripening grapes often experience embryo abortion, limiting traditional breeding. They applied a three-stage in vitro culture protocol—embryo development, germination, and rooting—to obtain viable plants from immature ovules of stenospermocarpic table grape hybrids. Factors such as parent genotype, ovule sampling time (30, 40, 50 days post-pollination), and duration of embryo germination induction (4, 6, 8 weeks) were evaluated. The optimized protocol involved isolating immature ovules 40 days after pollination and inducing germination for eight weeks. The best results came from hybrids of Thompson, Superior, and Regal, while the highest plant survival was from Luisa × Thompson crosses sampled at 50 days.

Chiaromonte et al. (2023), within the Italian Variety Club's 2017–2021 breeding program, validated an optimized embryo culture protocol across 39 cultivars and 41 hybrid combinations. They reported that sampling time (43–62 days after pollination) did not significantly affect success, but genotype did.

Chu et al. (2023) used embryo rescue to develop cold-resistant grape varieties. Using stenospermocarpic female parents and seeded, cold-hardy male parents, they evaluated genotype, sampling time, and culture conditions across 14 hybrid combinations. The highest embryo development (39.9%) and plant formation (21.5%) were observed in the cross of 'Ruby Seedless' × 'Beibinghong.' Optimal embryo isolation days for 'Yuehong Wuhe,' 'Ruby Seedless,' and 'Melissa Seedless' were 37, 55, and 52 days post-flowering, respectively. A sucrose concentration of 1.0–1.5% yielded the best results. They identified 91 seedless and 18 cold-tolerant hybrids using molecular markers.

In another study, different sampling times (20, 30, 40, and 50 days after pollination) were tested using NN medium with varying concentrations of BAP (0.1, 0.2, and 0.5 mg/L) and activated charcoal (1.5, 2, and 2.5 g/L). The best results across all parameters were achieved from ovules sampled at 40 days and cultured in NN medium supplemented with 0.5 mg/L BAP and 2 g/L activated charcoal. The study emphasized the importance of correct sampling timing and protocol standardization for efficient embryo rescue and seedless hybrid development (Nigar et al., 2024).

Li et al. (2024) aimed to explain the relationship between embryo rescue and hormonal changes in seedless grape breeding. They used four Eurasian seedless varieties: 'Thompson Seedless,' 'Flame Seedless,' 'Heshi Seedless,' and 'Ruby Seedless.' Endogenous hormone levels (3-IAA, GA, ABA) were measured at optimal embryo rescue times in both fruit and in vitro ovules. Exogenous application of these hormones during embryo rescue revealed significant variations in internal hormone levels among ovules from the same cultivar. This indicated a hormonal influence on ovule abortion and embryo development. Effective BDM concentrations were also determined:

For ovule development in fruit: 30 mg/L IAA + 30 mg/L ABA

For in vitro ovule development: 1.0 mg/L IAA + 2.0 mg/L 6-BA + 1.0 mg/L GA + 1.0 mg/L ABA

For embryo germination and seedling formation: 1.0 mg/L IAA + 2.0 mg/L 6-BA + 1.0 mg/L GA

The study concluded that hormonal changes significantly influence ovule and embryo development, closely linking embryo rescue success with hormone regulation. This research deepens the understanding of hormonal interactions in embryo rescue and supports the development of new seedless grape cultivars through advanced breeding technologies.

Embryo rescue, a fundamental technique in seedless grape production, has limited success due to the inadequacy of existing protocols. In one study, the 00-1-5 ('Muscat Hamburg' × 'Vitis amurensis'), a cold-tolerant seeded hybrid, was crossed with four stenospermocarpic seedless grape cultivars ('Flame Seedless', 'Qinhong No. 2', 'Qinhong No. 10', 'Ruby Seedless'). Application of 30–50 mg L<sup>-1</sup> IBA 14 days before flowering was found to significantly increase embryo development, germination, and seedling emergence. Embryo development was found to be higher on solid MM3 medium than on solid-liquid dual-phase medium. 'Ruby Seedless', 'Qinhong No. 2', and 'Qinhong No. 10' were reported to be the most suitable genotypes for embryo rescue as maternal parents. The most effective medium for ensuring normal development of deformed seedlings was 2×MS medium containing 0.2 mg L<sup>-1</sup> 6-BA, 0.1 mg L<sup>-1</sup> IAA, and 1.6 mg L<sup>-1</sup> ZnSO<sub>4</sub>. As a result of the study, a total of 311 embryos were successfully rescued, and these embryos were evaluated as cold-tolerant seedless hybrid candidates (Zhu et al., 2024).

The success of embryo rescue studies in seedless grapes depends on understanding the mechanism of egg cell abort. In a study conducted for this purpose, changes in 21 free amino acids and 9 mineral elements in egg

cells of four grape varieties ("Flame Seedless," "Ruby Seedless," "Muscat Hamburg," and "Pinot Noir") were examined across six developmental stages over two years. Metabolomic analyses revealed significant differences between seeded and seedless varieties. Multivariate statistical analyses revealed that 11 amino acids (e.g., glutamine, arginine, alanine, GABA) and 5 mineral elements (N, P, K, Ca, Mg) were associated with egg cell degeneration. Based on these data, 12 different culture media were designed for embryo development, and AM3 medium was found to increase embryo development rates by 8% and plantlet formation rates by 5%. The study provided important findings regarding the improvement of embryo rescue efficiency in seedless grape breeding (Wang et al., 2025).

In addition, the success rate of embryo rescue technique, widely used in seedless grape breeding, depends on many factors. In a study, the effect of 0.5 mg L<sup>-1</sup> thidiazuron application 10 days before flowering was investigated on two seedless cultivars of *\*Vitis vinifera\** ('Qinhong No. 2' and 'Qinhong No. 10'). Ovules obtained from open and self-pollinations were cultured in solid-liquid two-phase MM3 medium to evaluate the effect of thidiazuron on embryo development. The results showed that thidiazuron application significantly promoted ovule development and increased the success of embryo rescue in vitro. After the application, embryo development, germination, and seedling emergence rates ranged from 30.77%–42.62%, 29.36%–41.64%, and 22.02%–40.33%, respectively. Ovules obtained from open-pollinations showed higher success than those from self-pollinations. Additionally, MM3 medium was found to be more suitable for embryo development compared to NN and ER media. The germination rate of 'Qinhong No. 10' embryos obtained from open pollination on solid WPM medium was also higher than that of embryos from unpeeled ovaries on NN medium (Zhu and Zhang, 2025).

In a similar study, 22 hybrid combinations were investigated to increase embryo rescue efficiency in seedless grape breeding, and the effects of different parental genotypes and plant hormones on embryo development and germination were evaluated. Studies on the conversion of abnormal plantlets to normal were also conducted. Based on the results, 'Ruby Seedless', 'Delight', 'Huozhouheiyyu', 'Zitian Seedless', and 'Zhengyan Seedless' were identified as the maternal parents, while 'Zitian Seedless', 'Shennongxiangfeng', 'Hongqitezao', and 'Guibao' were identified as the paternal parents. The highest embryo rescue rates were obtained in the combinations 'Ruby Seedless × Shennongxiangfeng' (55.05%) and 'Ruby Seedless × Zitian Seedless' (59.76%). The addition of 1.0 mg·L<sup>-1</sup> zeatin to MM3 medium increased the development rate of 'Ruby Seedless × Zitian

Seedless' embryos to 64.73%, while the addition of  $0.2 \text{ mg}\cdot\text{L}^{-1}$  ZT +  $0.2 \text{ mg}\cdot\text{L}^{-1}$  IAA to WPM medium achieved the highest germination rate of 85.71% in 'Huozhouheiyu' × 'Shine Muscat.' Furthermore, 3,365 abnormal plants were successfully rescued, 1,234 normal plants were regenerated, and a total of 4,287 seedlings were transplanted by direct transformation and cotyledon induction methods (Chen et al., 2025).

## 5. Conclusion

Embryo culture is a tissue culture technique that can be successfully used for purposes such as developing new cultivars, using seedless grape varieties in breeding, rescuing degenerate embryos, and producing haploid plants. It can also be used to breed cultivars resistant to diseases, pests, and various stresses. In situations where conventional breeding methods are inadequate, embryo culture is an effective alternative, enabling rapid results. Integrating conventional breeding methods with embryo culture is crucial in this regard. Furthermore, factors such as genotype, the physiological developmental stage of the isolated explant, nutrient medium, and culture conditions influence the success of this technique. Embryo culture also requires significant technical experience and laboratory equipment.

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## Gene Expression Profiling as a Tool for Crop Improvement in Horticultural Biotechnology 8

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### Abstract

Gene expression profiling has become known as a crucial component of horticulture biotechnology, providing essential insights into the genetic and molecular pathways that influence crop yield, quality, and stress resilience. This overview emphasizes the ideas, applications, and integration of gene expression profiling to improve horticulture crops. High-throughput methodologies, including RNA-Seq, microarray analysis, and qRT-PCR, facilitate the discovery of differentially expressed genes associated with critical agronomic properties like as stress tolerance, fruit quality, and disease resistance. Progress in comprehending microRNA regulation, quantitative trait loci (QTL) mapping, and the discovery of molecular markers has expedited marker-assisted selection and genome editing techniques, including CRISPR/Cas9. The integration of transcriptomic data with other omics technologies—metabolomics, proteomics, and genomics—has enabled a systems-level comprehension of numerous plant processes, including secondary metabolite production and postharvest physiology. Although difficulties are associated with data complexity, elevated expenses, and restricted genetic resources in non-model species, novel methods such as single-cell RNA sequencing and artificial intelligence are improving analytical accuracy. Gene expression profiling ultimately connects molecular insights with applied breeding, facilitating the creation of better, climate-resilient, and nutritionally enhanced horticultural cultivars vital for sustainable agriculture.

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## 1. Introduction

Gene expression profiling has become an essential tool in horticultural biotechnology, facilitating crop improvement through the elucidation of genetic factors that determine plant traits and responses to environmental stimuli. This approach enables researchers to unravel the complex regulatory networks governing important characteristics such as stress tolerance, growth, and development in various horticultural species.

One of the primary benefits of gene expression profiling is its ability to identify differentially expressed genes (DEGs) under specific conditions. For instance, studies in banana (*Musa acuminata* L.) have identified genes related to stress response and hormone signaling pathways that can be targeted to enhance drought and cold tolerance, thereby improving crop productivity in water-limited environments and cold conditions (Xu et al., 2023). Similar results have been reported in tomato, where the transcription factor SIHY5 was found to regulate fruit ripening through complicated gene interactions linked to environmental signals (Wang et al., 2021). These insights are crucial for developing cultivars that can thrive under unfavorable conditions, hence enhancing horticultural practices (Guney et al, 2024a).

Moreover, gene expression analysis provides a foundation for the application of advanced genomic tools such as CRISPR/Cas9 technology. This technology allows for targeted editing of gene expressions and regulatory elements, thereby it makes the improving crops with desirable traits (Li et al., 2020; Ku & Ha, 2020). By understanding the specific genes involved in phenotypic variation, researchers can utilize integration of CRISPR methodologies and gene expression data to introduce new cultivars with improved nutritional profiles or increased resistance to pests and diseases (Guney et al, 2024b).

In addition to stress responses, gene expression profiling is also instrumental in addressing horticultural challenges such as fruit quality and yield. Research on peach (*Prunus persica*) has highlighted the crucial role of genomic analyses in elucidating the genetic basis of domestication traits, which can inform breeding strategies aimed at improving fruit characteristics such as size and sweetness (Cao et al., 2019; Bie et al., 2023). Notably, the production of antioxidant compounds, essential for maintaining fruit quality during storage, has also been linked to specific gene expressions influenced by various environmental factors, underscoring the potential for gene profiling in enhancing postharvest management practices (Özyalın, 2023; Guney et al., 2022; Ergun, 2021).

Moreover, numerous tools like dynamic expression atlases have been established for promoting functional genomics research in horticulture crops. For example, FEAtl is a web-based platform that catalogs gene expressions in tropical and subtropical fruit crops, aiding in the identification of genetic factors related to production challenges (Roy et al., 2024). Such databases enable researchers to perform large-scale analyses and promote collaboration across the field of horticultural biotechnology, emphasizing the importance of gene expression profiling in sustaining future crop improvement initiatives.

In conclusion, gene expression profiling stands as a basis of horticultural biotechnology, enabling detailed insights into the genetic underpinnings of plant traits and their responses to environmental conditions. By advancing our understanding of gene regulatory mechanisms and facilitating the application of biotechnological tools, gene expression analysis paves the way for sustainable agricultural practices and the enhancement of crop productivity.

## 2. Principles of Gene Expression Profiling

Gene expression profiling is a technique employed to assess the activity of numerous genes simultaneously, hence generating an extensive overview of cellular function. In horticultural biotechnology, this technique is vital for understanding the genetic basis of traits, enabling the enhancement of crop characteristics such as yield, disease resistance, and abiotic stress tolerance. By examining gene expression patterns, researchers can identify important molecular players involved in various physiological processes that directly affect plant performance.

A foundational aspect of gene expression profiling involves the use of high-throughput sequencing technologies, such as RNA-seq, which allows for the comprehensive analysis of transcriptomes in horticultural crops. RNA-seq has provided significant insights into the temporal and Tissue-specific expression of genes linked to important traits in a variety of species. For example, studies in avocado have identified transcripts associated with phenological events that correlate with seasonal changes, highlighting the relevance of gene expression data in predicting plant behavior and adaptability (Ahsan et al., 2023).

Understanding the molecular regulation of microRNAs (miRNAs) is another critical dimension of gene expression profiling in horticultural plants. miRNAs are small non-coding RNAs that modulate gene expression and have been implicated in plants' stress responses. Recent research has shown that specific miRNAs can enhance disease resistance in various crops,

illustrating the potential of miRNA profiling as a strategic tool in plant breeding programs (Zhang et al., 2023). The identification and validation of these microRNAs may lead to the development of crops that are better equipped to handle biotic stresses, thereby enhancing food production against pest invasions.

Another principle inherent in gene expression profiling involves the genetic mapping of quantitative trait loci (QTLs) to better understand heritable traits in crops. QTL analysis has successfully identified genetic markers associated with important horticultural traits such as fruit size, quality, and resistance to environmental stressors in crops like melon Zhao et al. (2023) and lettuce (Macias-González et al., 2021). Integrating gene expression data with QTL mapping enhances the precision of marker-assisted selection (MAS) strategies, thus accelerating the breeding process for desirable traits (Guney et al., 2018).

The development of robust molecular markers for breeding also relies on gene expression profiling. For instance, studies have resulted in the identification of functional genetic markers that can be utilized in the selection of clonal rootstocks for apple, demonstrating how genetic analyses can lead to improved horticultural practices (Shamshin et al., 2023). Marker-assisted breeding, boosted by gene expression data, facilitates the more efficient selection of plants with desirable traits, overcoming the limitations of traditional breeding techniques that often involve prolonged trial-and-error processes (Guney et al., 2021).

Methodically, gene expression profiling employs various bioinformatics tools to analyze expression data and identify significant differentially expressed genes (DEGs). These DEGs serve as potential targets for functional validation and further exploration regarding their roles in plant biology. For example, transcriptome analyses have provided insights into the genetic and physiological mechanisms underpinning abiotic stress tolerance in crops like Chinese cabbage Su et al. (2019) and litchi (Zhou et al., 2022). Such studies connect environmental responses to genetic expression pathways, offering a clearer understanding of resilience traits in horticultural species.

In conclusion, gene expression profiling serves as a dynamic and integral component of horticultural biotechnology, significantly enhancing our understanding of plant gene function and regulation. The interplay between high-throughput sequencing technologies, microRNA functionality, genetic mapping, and molecular marker development establishes a framework for advancing crop improvement strategies. With ongoing research, the

application of gene expression profiling will continue to empower scientists and breeders in their efforts to optimize and innovate horticultural practices.

### 3. Applications in Horticultural Crop Improvement

#### 3.1. Enhancing Stress Tolerance

Improving stress tolerance in horticulture crops is a vital goal in biotechnological interventions, particularly due to climate change and rising environmental stressors. Stress tolerance is crucial for maintaining crop productivity and quality under adverse conditions such as drought, salinity, extreme temperatures, and diseases. Advances in gene expression profiling and molecular biotechnology have provided significant insights into the mechanisms underlying plant stress responses, allowing for the development of varieties that can withstand these challenges.

One of the promising strategies for enhancing stress tolerance involves the application of phytohormones such as melatonin, tryptophan, salicylic acid, and abscisic acid. Melatonin, in particular, has demonstrated multifaceted roles in bolstering drought stress tolerance in crops like tomatoes by improving root architecture, enhancing photosynthesis, and fortifying antioxidant defense systems (Altaf et al., 2022; Kumari et al., 2023, Balci et al., 2023). It modulates stress responses through the regulation of redox homeostasis and increases the activities of various antioxidant enzymes, effectively scavenging reactive oxygen species (ROS) produced during stress (Altaf et al., 2022; Tiwari et al., 2021). Research has shown that melatonin application can also increase the expression of heat shock proteins, which are crucial in protecting cellular functions during temperature fluctuations (Zhang et al., 2017).

MicroRNAs (miRNAs) are another area of focus, as they play a vital role in mediating stress responses in plants. For instance, studies on bananas have elucidated the involvement of temperature-responsive miRNAs that target transcription factors associated with auxin signaling and redox homeostasis, revealing their potential for use in genetic improvement programs aimed at enhancing abiotic stress resistance (Zhu et al., 2019). Understanding the regulatory networks governed by miRNAs can offer avenues for precise genetic modifications that boost crop resilience.

Biotechnological applications also utilize genomic approaches, such as Genome-Wide Association Studies (GWAS) and marker-assisted selection (MAS), to identify key genes and quantitative trait loci (QTLs) responsible for stress tolerance. Research on apple has highlighted the potential of

integrating genomic methods with traditional breeding techniques to enhance abiotic stress tolerance (Dutta et al., 2022). Similarly, studies involving transgenic approaches have successfully discussed salt tolerance to various horticultural crops by transferring genes associated with stress responses, including those coding for glycine-betaine synthesis and regulatory transcription factors (Parmar et al., 2017; Wang et al., 2016). This genetic engineering can be pivotal in creating varieties with enhanced resistance to abiotic stresses such as salinity and drought.

Additionally, studies have documented the role of brassinosteroids in improving resilience against abiotic stressors. Brassinosteroids regulate various physiological and biochemical pathways that bolster stress tolerance, consequently supporting overall plant health under adverse conditions (Zhang et al., 2023). Their integration into crop management strategies can significantly improve the yield stability of horticultural crops subjected to environmental stress.

Furthermore, abiotic stress management in horticultural crops also emphasizes the importance of biostimulants, which are substances that enhance plant growth and resistance under stress conditions. These can include natural extracts such as seaweed, which is rich in phytohormones and essential nutrients, promoting growth and resilience in crops (Tejasree et al., 2024). Biostimulants contribute to stress mitigation by enhancing the plant's nutrient uptake and assimilation capabilities. Research has demonstrated that interventions like hydrogen-rich water and nitric oxide sprays can improve the stress tolerance of crops. These therapies are recognized for activating signaling pathways that regulate gene expression associated with stress responses (Sun et al., 2021; Gong et al., 2017). The control of ethylene levels has been shown to enhance cold tolerance in apple crops, highlighting the critical role of hormone regulation in stress adaptation (Wang et al., 2021).

### 3.2 Improving Fruit Quality and Postharvest Traits

The enhancement of fruit quality and postharvest traits is essential for maximizing the marketability and consumer acceptance of horticultural crops. A broad range of techniques, including the application of growth regulators, biostimulants, and novel postharvest treatments, have been systematically employed to enhance attributes of fruits such as flavor, texture, color, nutritional content, and shelf life (Ergun and Bozkurt, 2020;).

One notable approach in improving fruit quality involves the application of brassinosteroids, which are plant hormones that play a pivotal role in

various physiological processes. The gene SlCYP90B3 has been identified as a significant regulator of tomato fruit quality, influencing ripening processes, softening, and the accumulation of flavor volatiles, soluble sugars, and carotenoids. Manipulating the expression of SlCYP90B3 can lead to enhanced fruit quality attributes, including improved flavor and visual appeal, thereby increasing consumer acceptance (Hu et al., 2022).

Bioactive compounds, particularly flavonoids, are crucial for both fruit quality and resistance to postharvest diseases. The MADS-box protein SlTAGL1 in tomatoes regulates ripening-associated flavonoid biosynthesis, contributing to both enhanced fruit quality and resistance to pathogens like *Botrytis cinerea* (Wang et al., 2023). Similarly, the transcription factor SlbHLH95 has been implicated in the regulation of flavonoid metabolism, highlighting the potential for targeted genetic strategies to improve resistance to postharvest diseases while enhancing nutritional quality (Su et al., 2025). This underscores the importance of understanding the genetic and molecular mechanisms that underpin fruit quality traits.

Foliar application of compounds such as forchlorfenuron (CPPU) has been shown to significantly affect the biochemical indices and overall quality of crops. A study demonstrated that CPPU enhanced sugar transport from leaves to fruit, thereby improving fruit retention and quality in fig trees (Abdel-Azeem et al., 2023). Such chemical treatments can effectively enrich fruit quality by addressing physiological aspects related to sugar accumulation and metabolic processes.

Beyond chemical applications, the role of nitric oxide (NO) during postharvest management is also worthy of attention. Research indicates that exogenous applications of NO not only delay fruit senescence but also enhance fruit quality attributes, increase antioxidant activity, and mitigate chilling injuries during storage (Wei et al., 2024). The metabolic pathways stimulated by nitric oxide enhance fruit quality preservation, offering strong strategies to combat postharvest decline.

Innovative techniques for fruit preservation, including the use of ethylene scavengers and protective coatings, significantly contribute to maintaining postharvest fruit quality. The combination of potassium permanganate with ultraviolet light has been shown to preserve sensory attributes of peaches, enhancing both taste and aroma which are important factors that influence consumer preferences (Alonso-Salinas et al., 2023). Techniques that focus on preserving the initial aroma and sensory quality of fruits extend their market potential and consumer appeal.

The application of bagging techniques in fruit production has emerged as an effective method for enhancing the external appearance and quality of fruits. Preharvest bagging has been associated with improved coloration, size uniformity, and reduced rates of fungal infections (Buthelezi et al., 2023). Moreover, bagging not only protects fruits from environmental stressors but also promotes secondary metabolite biosynthesis, thereby enriching the nutritional composition and flavor profiles of fruits. In addition to bagging, the innovation of using biodegradable films for fruit preservation has gained traction. Recent developments in green bioactive films have shown promise in protecting fruit quality by minimizing exposure to pathogens and controlling respiration rates, thus prolonging shelf life (Ahmed et al., 2022,). The incorporation of natural extracts into these films provides added health benefits, enhancing the marketability of fresh produce. Therefore, Transcriptomic analyses of bagged fruits have demonstrated notable differential expression in genes associated with secondary metabolism, especially those implicated in flavonoid biosynthesis, hormonal regulation, and defense mechanisms, suggesting that bagging modifies fruit microenvironments and initiates molecular reprogramming.

Non-destructive testing technologies, such as near-infrared spectroscopy and advanced image analysis, are instrumental in assessing fruit quality parameters without damaging the product. These techniques offer rapid and precise evaluations of attributes like fruit maturity, sweetness content, and external appearance, enabling improved decision-making in postharvest processing and marketing (Kusumiyati et al., 2019; Kim et al., 2024). Understanding and applying these analytical approaches can significantly enhance the efficiency of the fruit supply chain, ensuring that only high-quality produce reaches consumers. Although non-destructive approaches do not directly quantify gene expression, they record phenotypic characteristics such as color, hardness, and biochemical composition—that are ultimately governed by underlying gene activity (Hernández-Hierro et al., 2022).

### **3.3. Disease Resistance**

The enhancement of disease resistance in horticultural crops is a critical focus of research aimed at ensuring sustainable agricultural practices and enhancing food security. Various approaches have been developed to improve disease resistance, utilizing both traditional breeding techniques and modern biotechnological methodologies.

One of the promising strategies involves the application of biostimulants like melatonin, which has been shown to enhance resistance against various

pathogens. Studies reveal that exogenous melatonin can improve the resistance of apple (*Malus domestica*) to the fungal pathogen *Marssonina*, which causes significant foliar damage. This protective role is attributed to the ability of melatonin to modulate oxidative stress responses, leading to enhanced reactive oxygen species (ROS) generation that can inhibit pathogen growth (Yin et al., 2013). Melatonin also plays a crucial role in enhancing the general stress tolerance of horticultural crops, contributing to their resilience against biotic stress (Hao et al., 2025).

Natural plant extracts have gained attention as a means of improving disease resistance in horticultural crops. For instance, research has demonstrated that specific extracts can effectively control fungal pathogens, thereby improving productivity in crops like zucchini (*Cucurbita pepo*). Flavonoids, which are abundant in these extracts, exhibit antimicrobial properties and can play dual roles as defense signals as well as UV protectants (Hassan et al., 2021). The exploration of these natural compounds as biopesticides provides an environmentally friendly alternative to synthetic pesticides, which are often associated with chemical residues and ecological concerns.

Chitosan, a biopolymer derived from crustacean shells, has been reported to induce systemic resistance against pathogens like *Fusarium oxysporum* in tomato. The mechanism involves the upregulation of specific pathogenesis-related (PR) proteins and antioxidants, which enhance the plant's defensive abilities. Chitosan acts as both a physical barrier and a biochemical stimulus, fostering an innate immune response that can be utilized in integrated disease management systems (Carmona et al., 2021; Guney et al., 2024c).

A contemporary advancement in enhancing disease resistance involves the use of genome editing technologies, particularly CRISPR/Cas9. This powerful tool allows for precise modifications of plant genomes to improve resistance against specific pathogens. For instance, transgenic crops expressing resistance genes or gene-editing constructs can improve resilience to diseases caused by fungi, bacteria, and viruses (Tyagi et al., 2021). Such genetic engineering techniques enable the development of varieties that are tailored for enhanced disease resistance while maintaining desirable agronomic traits.

The NPR1 gene, known for its role in initiating systemic acquired resistance, has been successfully integrated into various horticultural crops to boost their defense mechanisms against pathogens. Studies show that overexpression of NPR1 enhances the disease resistance responses of plants, particularly against pathogens such as *Xanthomonas citri*, responsible for citrus canker (Zhang et al., 2010). This approach illustrates how biotechnological

strategies can lead to significant improvements in the health and survival of crops under pathogen pressure.

The use of light quality manipulation has also emerged as an innovative method to enhance disease resistance. Research indicates that exposure to specific wavelengths of light, particularly the red and far-red spectrum, can improve the resistance of cucumber seedlings to powdery mildew. This physiological defense mechanism is linked to enhanced antioxidant activity and the accumulation of signaling molecules involved in stress responses (Shibuya et al., 2011).

Fungal elicitors, such as those derived from pathogens, have shown potential in activating defense responses in crops. The application of elicitors can stimulate the innate immunity of plants, conferring resistance to various pathogens, including *Botrytis cinerea*, which causes significant losses in multiple horticultural crops (Perato et al., 2020; Li & Cheng, 2023). The mechanisms underlying these defense responses involve complex signaling pathways that can be harnessed for developing resilient crop varieties.

Moreover, even traditional approaches such as grafting have proven effective in improving disease resistance. Grafting horticultural plants onto resistant rootstocks has been widely practiced to manage soil-borne diseases and enhance overall plant health. This technique allows for the combination of a robust root system with a productive scion, significantly enhancing resistance against pathogens and improving yield outcomes (Goldschmidt, 2014).

In conclusion, the quest for improving disease resistance in horticultural crops encompasses a multitude of strategies ranging from the application of natural biostimulants and elicitors to advanced genetic engineering techniques. By integrating these methods, researchers can develop sustainable solutions to combat plant diseases, ensuring the viability and productivity of important horticultural species in an era of increasing agricultural challenges.

### **3.4. Understanding Secondary Metabolism**

The study of secondary metabolism in horticultural plants is crucial as it encompasses the biochemical pathways leading to the synthesis of a wide variety of compounds that play essential roles in plant defense, flavor, aroma, and color. These metabolites greatly influence the nutritional, medicinal, and economic value of horticultural crops. Advances in molecular biology, proteomics, and genomics have provided significant insights into the regulatory mechanisms that govern the production of secondary metabolites.

Phenolic compounds, including flavonoids and phenolic acids, have been recognized for their important roles in plant defense mechanisms and human health (Özyalın and Yaman, 2023). In particular, recent studies have demonstrated that environmental factors such as light exposure and stress can dramatically influence the accumulation of these compounds (Zeng et al., 2011). Instead, studies have shown that manipulation of light quality through LED technology can increase the concentration of beneficial secondary metabolites in various crops (Arif et al., 2024).

Moreover, the interaction between environmental stresses and secondary metabolism has been the subject of considerable research. For instance, exposure to ozone stress in *Brassica campestris* has been shown to significantly impact the growth and secondary metabolite production of plants (Han et al., 2023). Such responses illustrate how secondary metabolism is intricately linked to the adaptive strategies under stress conditions in plants.

In apple, studies have explored the regulation of secondary metabolite biosynthesis by bHLH and MYC transcription factors. Xie et al. (2012) reported that the antagonistic relationship between SmbHLH60 and SmMYC2 plays a crucial role in the regulation of phenolic acids and anthocyanins, two important classes of secondary metabolites known for their health benefits. This regulation highlights the complexity of molecular networks that control secondary metabolite synthesis and indicates potential targets for metabolic engineering to enhance the nutritional value of crops.

Melatonin, a small molecule with significant roles in plant development, has also been involved in secondary metabolism. While the current literature reviews the impact of melatonin on antioxidant responses, there is insufficient evidence linking melatonin directly to enhanced secondary metabolites in postharvest horticultural products (Zhang et al., 2022). Further research is required to clarify these connections.

Additionally, phenylpropanoid metabolism, which is pivotal in producing various secondary metabolites such as flavonoids and lignin, has gained attention for its role in environmental interactions. Cold stress, for instance, has been noted to activate the phenylpropanoid pathway, increasing the synthesis of valuable compounds that enhance plant resilience (Kang et al., 2025). Understanding these metabolic shifts is essential for developing strategies to improve crop productivity and resilience against climate-related challenges.

Compounds like carotenoids are another class of secondary metabolites that are crucial not only for plant health but also for human nutrition. They

contribute to the color of fruits and vegetables and have essential antioxidant properties. Recent advancements highlight the intricate regulation of carotenoid biosynthesis through various transcription factors, thus opening avenues for biofortification efforts aimed at increasing provitamin A content in crops like tomato and watermelon (Zheng et al., 2020; Moreno et al., 2020).

The integration of high-throughput omics technologies, including transcriptomics, proteomics, and metabolomics, has significantly advanced the understanding of secondary metabolism in horticultural crops. These technologies enable researchers to elucidate metabolic pathways, identify regulatory genes, and explore the functional genomics of secondary metabolite production (Xu & Xian-pu, 2025). For example, studies combining metabolomics with transcriptomic data have revealed the mechanisms by which phenolic acids are synthesized in grape varieties, providing insight into potential breeding targets for enhanced phytochemical content (Cheng et al., 2023).

#### 4. Integration with Other Omics Technologies

The combination of diverse omics methods—such as genomics, transcriptomics, proteomics, and metabolomics has transformed the comprehension of intricate biological processes in horticulture plants. This multi-omics methodology enables researchers to investigate the complex interconnections of genes, proteins, and metabolites, resulting in a comprehensive comprehension of plant development, stress responses, and secondary metabolite synthesis.

One significant application of integrating metabolomics and transcriptomics is the study of anthocyanin biosynthesis in horticultural crops. For instance, research by Juxian et al. (2022) highlighted that the combination of transcriptomic and metabolic data reveals how light conditions influence anthocyanin accumulation in *Brassica* plants. This study illustrated that different light regimes directly impact the expression of genes involved in anthocyanin synthesis, subsequently affecting metabolite levels (Juxian et al., 2022). Such findings underline the importance of light as a regulatory factor and demonstrate how integrating omics data can elucidate the mechanisms governing secondary metabolite production, further enhancing fruit quality.

In blueberries, the dual analysis of small RNA and transcriptomic data has unveiled the regulatory roles of microRNAs during fruit development. In a study, specific miRNAs and their target genes implicated in ripening

processes, emphasizing the significance of small RNAs in modulating developmental pathways that contribute to fruit quality (Hou et al., 2017). This approach showcases how integrating transcriptomic data with small RNA studies provides a more comprehensive understanding of the regulatory networks underpinning fruit ripening.

The metabolic profiling of herbaceous plants, such as *Paeonia lactiflora*, together with transcriptomic data, aids in identifying key genes involved in oil biosynthesis within seeds. Xu et al. (2024) utilized this integrated analysis to reveal the genetic framework contributing to triacylglycerol accumulation, crucial for optimizing oil content in horticultural crops. Such studies exemplify how multi-omics strategies can facilitate targeted breeding studies aimed at enhancing specific agriculturally important traits.

In kiwifruit (*Actinidia chinensis*), an integrative analysis of the metabolome and transcriptome was employed to uncover gene regulatory networks associated with flavor formation. Their findings highlighted the contributions of specific transcription factors in controlling the biosynthesis of flavor compounds, illustrating the potential of omics integration to enhance flavor profile optimization in horticultural crops (Wang et al., 2021).

Additionally, the integration of transcriptomics with metabolomics has been pivotal in understanding flower color differentiation in various plants. Another study discussed how a combined approach revealed the relationship between anthocyanin metabolism and the specific genetic controls that influence petal pigmentation in *Camellia reticulata*. This analysis provides insights into color regulation and aids in selecting traits for ornamental plant breeding (Geng et al., 2022).

The outcome of integrating these omics technologies extends beyond basic research. For example, studies have demonstrated that high-throughput phenotyping methods, used in conjunction with omics analyses, can enhance the efficiency of breeding programs. This integration allows for a better selection of traits based on phenotypic and genotypic data, facilitating the development of new horticultural varieties that meet market demands while maintaining desirable characteristics (Cembrowska-Lech et al., 2023; Zhang et al., 2022).

Furthermore, the application of multi-omics approaches has profound implications for addressing abiotic stresses in horticultural crops. By integrating transcriptomics and metabolomics, researchers can identify physiological and biochemical responses to stressors such as heat, drought,

or cold. For instance, the use of omic strategies to enhance crop resilience to heat stress reported how this integration could reveal the underlying mechanisms by which plants adapt to temperature fluctuations, guiding breeding for climate resilience in horticultural plants (Zhou et al., 2022).

## 5. Challenges and Future Prospects

The application of advanced biotechnology methods in horticulture, including multi-omics, genome editing, and precision agriculture, has demonstrated considerable potential for improving crop yield and sustainability. Nonetheless, numerous problems remain that must be resolved to effectively harness the advantages of these technologies in the horticultural sector. A major challenge facing the horticultural sector relates to the integration of multi-omics data.

While the collective application of genomics, transcriptomics, proteomics, and metabolomics offers a comprehensive understanding of plant biology, the complexity of data integration poses significant problems. Research indicates that discrepancies in data compatibility, quality control issues, and the lack of standardized protocols create barriers to effectively consolidating multi-omics data sets (Cembrowska-Lech et al., 2023). The effective use of artificial intelligence and machine learning may provide solutions for better handling and interpreting large and complex omic data. Future efforts should focus on developing frameworks that enhance data interoperability among different omics technologies, facilitating a holistic understanding of plant phenotypes.

Another critical challenge is the adoption of genome editing technologies, such as CRISPR/Cas9, in the horticultural sector for crop improvement. Despite its potential to generate crops with desirable traits like disease resistance and abiotic stress tolerance, public acceptance and regulatory patterns remain significant obstacles (Bhavanee et al., 2024; Priyanka et al., 2025). Genetic modification techniques face intense analysis and varying regulations across different countries, which can slow down research translation into practical applications in horticulture (Bhavanee et al., 2024). Future research must focus on developing frameworks for transparent communication regarding the safety and benefits of genome-edited crops to bolster public trust.

Moreover, while advances in protected cultivation practices offer opportunities to enhance fruit quality and yield, challenges such as the high cost of initial setup and energy requirements persist (Jain et al., 2023). Achieving sustainability in protected horticulture requires comprehensive

evaluation of resource management techniques and the adoption of renewable energy sources to mitigate operating costs and environmental effects (Jain et al., 2023). Research should concentrate on innovative methodologies that enhance resource utilization efficiency and diminish economic obstacles for producers, particularly smallholder farmers.

Climate change also poses an ongoing challenge for horticultural crop production, affecting yields and fruit quality across various regions (Deori et al., 2024). Horticulture is particularly vulnerable to the changing climate due to its dependence on specific environmental conditions for optimum growth. Future research should prioritize developing climate-resilient varieties through breeding and genetic engineering, as discussed in studies exploring the adaptation of horticultural crops to climate unpredictability (Deori et al., 2024). Additionally, integrating climate-smart agricultural practices into the horticultural sector can help mitigate these challenges and enhance long-term sustainability.

In light of the rapid advancements in technology, another crucial prospect involves the integration of automation and robotics for efficiency in horticulture. While autonomous systems can significantly improve productivity, challenges related to equipment adaptability, crop variability, and the delicate handling of horticultural produce need to be addressed (Sharma et al., 2024). Ongoing research in developing resilient, intelligent automation systems that can adapt to various horticultural conditions will be crucial for optimizing operations and improving labor efficiency.

Furthermore, the lack of skilled horticulturists and researchers to utilize emerging technologies is a barrier to innovation in the horticultural landscape (Pitt, 2021). Educational institutions must adapt curricula to include training in new technologies and sustainable practices. Prospects must emphasize the importance of developing a skilled workforce capable of navigating and utilizing the latest advancements in horticulture.

## 6. Conclusion

The integration of gene expression profiling with advanced biotechnological tools has revolutionized horticultural science, offering precise and innovative strategies to address key challenges in crop improvement. By enabling the identification of differentially expressed genes (DEGs), regulatory microRNAs, and key transcription factors, gene expression profiling lays a robust foundation for understanding the complex genetic networks that govern traits such as stress tolerance, disease resistance, fruit quality, and postharvest performance.

High-throughput technologies like RNA-seq have empowered researchers to uncover the molecular basis of plant responses to environmental signals, while genome editing tools such as CRISPR/Cas9 have allowed for the precise modification of target genes, accelerating the development of improved cultivars. The regulation of secondary metabolites by transcription factors like MYC and bHLH further highlights how gene expression influences nutritional and commercial traits, with external factors such as melatonin, brassinosteroids, and light quality offering practical means of modulation.

Emerging tools, including non-destructive phenotyping methods and multi-omics approaches (e.g., genomics, transcriptomics, metabolomics), are enhancing the accuracy and efficiency of breeding programs by providing a comprehensive understanding of crop physiology and biochemical composition. Platforms like expression atlases and integrated databases also enable large-scale data analysis and global collaboration, supporting more informed decisions in cultivar development.

The future of horticultural biotechnology relies on an integration of omics technologies, computational tools, and sustainable practices. However, obstacles persist in aligning regulatory frameworks for genome editing, maintaining data compatibility across platforms, mitigating climate change impacts, and enhancing competencies in the advanced technical workforce. Resolving these difficulties necessitates synchronized efforts in research, education, and policy.

Continuous investment in research and innovation enables horticulture biotechnology to make substantial contributions to food security, environmental sustainability, and agricultural resilience. As global demands increase, the significance of cultivating resilient, high-quality horticulture crops capable of thriving in diverse and evolving conditions also rises.

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# Histological Responses of Horticultural Plants to Abiotic Stress Conditions

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## Abstract

Plants are exposed to various environmental stress conditions throughout their life cycles. These stress factors cause significant changes in the morphological, physiological, biochemical, and histological characteristics of plants. Abiotic stresses include environmental factors such as drought, salinity, and flooding. Histological adaptations developed in horticultural plants to these stress conditions are an important part of plant survival strategies. Drought stress causes water loss and plasmolysis in cells, leading to thickening of the cell wall, denser cuticle, and tightening of the leaf mesophyll tissue. Under salinity stress, plants often thicken their epidermal cell walls to prevent water loss from leaves. During flooding, aerenchyma tissue develops in roots due to hypoxic conditions. This tissue facilitates oxygen transport within the plant, reducing the negative effects of anaerobic respiration in root tissues. Consequently, the histological responses of horticultural plants to abiotic stresses are key indicators of plant resilience. These responses vary depending on genetic makeup and species characteristics. Understanding the cellular and tissue changes that occur under stress conditions significantly contributes to the development of highly stress-tolerant varieties and the sustainable production of horticultural crops.

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## 1. INTRODUCTION

Plants are exposed to numerous stress factors throughout the growing season. These stress factors are generally divided into two categories: abiotic and biotic. Biotic factors consist of living factors such as bacteria and fungi, while abiotic factors arise from nonliving factors such as drought, salinity, and low temperatures. These stress factors cause various symptoms and damage to plants, leading to yield and quality losses.

Plants respond differently to stress factors. These responses can be morphological, physiological, biochemical, or histological. Responses to stress are crucial for establishing and/or triggering plant defense mechanisms. Changes at the anatomical level are crucial in plant responses to stress. Anatomical structures, particularly vascular bundles, cortical cells, spongy and palisade parenchyma, and stomata, play important roles in defense mechanisms. Histology, also known as microscopic anatomy, is the science of tissue. Specific parts of histology are stained with special stains to examine anatomical structures. This section will examine the anatomical structures that can be examined histologically and how these structures respond differently to stress conditions in horticultural plants.

## 2. ABIOTIC STRESSES IN HORTICULTURAL PLANTS

Plants are directly affected by environmental conditions throughout their life cycle. Changes in environmental factors such as soil, water, temperature, light, and atmospheric conditions largely determine plant growth, development, and productivity (Mondal et al., 2016). Adverse changes in these environmental factors are called abiotic stresses. The term “abiotic” refers to non-living environmental factors. These stresses lead to both yield loss and quality decline, particularly in economically important crops such as horticultural crops.

Abiotic stresses are generally classified as drought (water stress), salinity, temperature extremes (extreme heat and cold), light stress, heavy metal toxicity, radiation, air pollution, and nutrient deficiencies. Each stress factor causes specific changes in the plant’s physiological, biochemical, and histological structure. These changes determine the plant’s survival strategies and its ability to adapt to environmental conditions.

Drought stress occurs when the balance between water uptake and water loss in plants is disrupted. This typically occurs due to a decrease in soil water potential or an increase in atmospheric vapor pressure deficit (Bodner et al., 2015; Çopur Doğrusöz ve Gülmser, 2024). Drought stress in horticultural crops causes serious problems, especially during summer months, under

conditions of high temperature and low humidity. Under drought stress, plant stomata close, transpiration decreases, and photosynthetic activity declines. As cells lose water, turgor pressure decreases, leading to growth retardation. Histologically, epidermal cell shrinkage, reduced spaces between mesophyll cells, thickened cuticle layers, and increased xylem lignification are observed (Manokari et al., 2021). Furthermore, the accumulation of osmotic regulating compounds such as proline, sugar alcohols, and betaine helps maintain intracellular water balance (Singh et al., 2015; Aykoç et al., 2025). Morpho-anatomical adaptations such as reduced leaf surface area, reduced stomata, and thickened cell walls are evident in drought-tolerant species. These structural changes minimize water loss, thus ensuring plant survival.

Salinity is an abiotic stress factor that occurs particularly when irrigation water or soil contains high levels of NaCl (Güleç Şen et al., 2023). Salt stress creates both osmotic pressure and ion toxicity (Arif et al., 2020). High salt concentrations impede plant water uptake and disrupt ion balance. In horticultural crops, histological responses to salt stress include increased suberinization and lignification in roots, vacuole growth, and epidermal thickening. Deeper burial of stomata in leaves, thickening of the cuticle, and accumulation of phenolic compounds are important defensive responses to salt stress. Additionally, plants store  $\text{Na}^+$  and  $\text{Cl}^-$  ions in vacuoles to keep them out of the cytoplasm. This reduces the harmful effects of ion toxicity. Some salt-tolerant plants (halophytes) have developed salt glands or salt vesicles to excrete excess salt. These anatomical structures allow the plant to maintain growth under stress conditions by maintaining ion balance.

While the optimum temperature for plant growth and development varies by species, exposure to temperatures above or below these limits creates stress (Aras and Özyalın, 2023). Excessive heat results in protein denaturation, membrane disruption, and disruption of chloroplast function (Wang et al., 2018). Low temperature, on the other hand, causes intracellular water freezing, membrane ruptures, and metabolic slowdown. Histologically, high-temperature conditions distort chloroplast shape, increase vacuolation in the cytoplasm, and anthocyanin accumulation in the epidermis (Dúranová et al., 2023). Under cold stress, protoplasm condenses, ice crystals accumulate in intercellular spaces, and cell wall lignification increases. Furthermore, meristematic activity in the root apex decreases, and growth stalls.

Horticultural plants protect their cellular structures against such stresses by synthesizing heat shock proteins (HSPs) and antifreeze proteins. These

proteins play critical roles in maintaining membrane integrity and preventing protein denaturation.

Plants require light for photosynthesis, but excessive or insufficient light conditions are also stress factors. Under insufficient light, leaf surface area expands, chlorophyll content decreases, and photosynthetic efficiency decreases. Excessive light, in turn, leads to the accumulation of reactive oxygen species (ROS) and photooxidative damage. Histologically, under shade conditions, the palisade parenchyma thins, the number of chloroplasts decreases, and mesophyll cells enlarge. Under excessive light conditions, the epidermis thickens, the cuticle expands, and anthocyanin and flavonoid pigments increase. These pigments mitigate the harmful effects of UV radiation and protect the plant from oxidative stress.

Heavy metals (Cd, Pb, Ni, Zn, Cu) accumulated in the soil due to industrial activities, mining, and chemicals used in agriculture are toxic to plants (Alengebawy et al., 2021). Heavy metals disrupt the ionic balance in the cell membrane, inhibit enzyme activity, and reduce chlorophyll synthesis. Histologically, symptoms such as parenchymal degeneration, plasmolysis, and mitochondrial and chloroplast deformation are observed in the root cortex. Lignin and cutin accumulation increases in the xylem and phloem walls; this is a defense mechanism developed to limit the transport of metal ions. Furthermore, pectin and phenolic compounds accumulate in the cell wall, which bind metal ions and reduce their intracellular toxicity. In tolerant species, heavy metals are stored in complex form in vacuoles, thus protecting the cytoplasm.

Deficiencies of macronutrients (N, P, K, Ca, Mg, S) and micronutrients (Fe, Mn, Zn, Cu, B, Mo), which are essential for healthy plant development, are also important abiotic stress factors. Deficiencies in each element cause specific physiological and histological changes. For example, nitrogen deficiency results in small and pale leaves and a decrease in chloroplast numbers. Phosphorus deficiency results in poor root system development, while potassium deficiency results in necrosis of leaf margins and stomatal dysfunction. Calcium deficiency results in weakened cell walls, and necrotic areas form in meristematic tissues. Many of these changes manifest microscopically as cell wall thinning, vacuole shrinkage, and parenchymal tissue deterioration.

With increasing industrialization, air pollution has become a serious source of abiotic stress on plants. Ozone ( $O_3$ ), sulfur dioxide ( $SO_2$ ), and nitrogen oxides ( $NO_x$ ), in particular, cause oxidative damage to leaf tissues. These gases oxidize lipids in the cell membrane, disrupting membrane

permeability. Histologically, degeneration of epidermal cells, destruction of chloroplasts, widening of vacuoles in the mesophyll tissue, and necrotic lesions are observed in leaves. Radiation stress (e.g., UV-B) can also cause DNA damage and cell death. In response, plants attempt to mitigate the harmful effects of radiation by producing phenolic compounds and anthocyanin pigments.

Horticultural plants develop similar defense strategies against different types of stress. These can be summarized as follows:

Osmotic regulation: Intracellular water balance is maintained through the accumulation of compounds such as proline, sugar, and polyamines.

Antioxidative defense: Reactive oxygen species are neutralized by the enzymes superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD).

Cell wall strengthening: Tissue durability is increased through the accumulation of lignin, suberin, and cutin.

Stomatal and cuticle changes: Stomatal density decreases and the cuticle thickens to reduce water loss.

Pigment accumulation: Anthocyanins, carotenoids, and phenolic compounds protect against stress-induced oxidative damage.

These structural and biochemical adaptations are key factors determining the resilience of horticultural plants to environmental stresses.

### **3. ANATOMICAL STRUCTURES OF HORTICULTURAL PLANTS**

Horticultural plants are classified as higher plants and include the categories of fruit, vegetable, vine, and ornamental plants. The tissues of these plants contain specialized anatomical structures. Horticultural plants have two vascular bundles: xylem and phloem. These vascular bundles are responsible for the transport of many substances, such as mineral nutrients, proteins, hormones, and sugars. Xylem is a dead tissue that cannot regenerate and consists of a phenolic compound called lignin (Meinzer et al., 2001; Liu et al., 2015). Xylem is primarily responsible for the unidirectional (bottom-up) transport of water and nutrients (Aras et al., 2021). The phloem vascular bundle, on the other hand, facilitates both bottom-up and top-down transport and is particularly capable of transporting phytochemicals such as sugars and hormones (Lee et al., 2020).

In horticultural plants, every organ and/or living cell consists of cortex cells. These cortex cells possess the ability to divide in the initial developmental stage of the tissue in which they are located. In the subsequent stages, this division phase is completed and they acquire the ability to grow (Gonzalez et al., 2012). Cortex cells have been reported to contain many substances, such as water, nutrients, and proteins (Singh et al., 2013).

When leaves are examined in general, the outermost layer consists of the cuticle and epidermal cells (Jetter et al., 2020). This cuticle layer is coated with wax. The middle part of the leaf contains palisade and spongy parenchyma (Gonçalves et al., 2008). The palisade parenchyma contains color pigments such as chlorophyll, which are involved in photosynthesis, and is capable of photosynthesis. The spongy parenchyma, on the other hand, has air spaces and plays a major role in gas storage. Stomata are located on the underside of the leaves of horticultural plants, and gas exchange and transpiration occur in this section (Bai et al., 2013).

#### **4. HISTOLOGICAL RESPONSES TO ABIOTIC STRESSES**

Horticultural plants exhibit different histological responses to abiotic stressors (Aras and Özyalın, 2024). When examining histological responses, each stress factor must be evaluated separately. Because plants live in fixed locations, they cannot develop behavioral responses such as escape or hiding in response to changes in environmental conditions. Therefore, abiotic stress factors—such as drought, salinity, temperature extremes, heavy metal toxicity, light deficiency or excess, radiation, and air pollution—produce direct physiological and morphological effects on plants. Many of these effects manifest as histological changes observable at the tissue level. In horticultural plants, these histological responses are important adaptation mechanisms that increase resistance to stress conditions or limit damage.

Drought stress is one of the most common abiotic stresses. In plants exposed to water deficit, epidermal cells shrink, the cuticle thickens, and stomatal density may decrease. In some species, stomata are buried deeper to minimize water loss. The intercellular spaces between mesophyll cells decrease, increasing water-holding capacity in tissues. Increased lignification and narrowing of vessel diameters are observed in xylem elements, reducing the risk of cavitation in water transport. At the same time, cell wall thickening increases mechanical strength.

Under salinity stress, plasmolysis and protoplast shrinkage are frequently observed at the cellular level. Ion imbalances occur as cell membrane permeability is impaired. Plants attempt to maintain cytoplasmic ion balance

by storing salt ions in vacuoles. Histologically, vacuole enlargement in parenchymal cells, salt crystal accumulation in epidermal cells, and increased suberinization in the root cortex are evident. The suberin layer limits water and ion transport, preventing excessive salt intrusion.

Extreme heat stress alters the fluidity of cell membranes and leads to protein denaturation. Histologically, chloroplasts are deformed, the granules are disorganized, and increased vacuolation is observed in the cytoplasm. High temperatures can also cause anthocyanin accumulation in the epidermis; these pigments protect cells from oxidative damage during light and heat stress. During cold stress, the accumulation of ice crystals in the intercellular spaces mechanically damages the plasma membrane. In response, plants mount defensive responses such as cell wall lignification and protoplasm condensation.

Heavy metal toxicity (e.g., cadmium, lead, nickel) directly affects cell structure in horticultural crops. Lignin and cutin accumulation increases in the cell walls of xylem and phloem tissues, limiting the transport of metal ions. Histological symptoms such as cortical parenchymal degeneration, mitochondrial and chloroplast deformations, and nuclear condensation are observed in root cells. Additionally, structural defenses such as phenolic compound accumulation and sclerenchymal tissue thickening are also activated.

Under light insufficiency (shade stress), the palisade parenchyma in leaf tissue thins, spongy parenchyma cells enlarge, and the number of chloroplasts decreases. Conversely, under excessive light, epidermal thickening, wax accumulation in the cuticle, and an increase in antioxidative pigments are observed. These changes are photoprotective adaptations designed to reduce the damaging effects of light.

As a result, horticultural plants have evolved a wide variety of histological responses to abiotic stress conditions. These responses include structural adaptations such as cell wall thickening, lignification, cuticle proliferation, stomatal modification, vacuolization, suberinization, and pigment accumulation. The purpose of these changes is to maintain cell integrity, maintain water and ion balance, protect vascular tissues, and sustain metabolic activities. These histological defense strategies are among the fundamental factors determining horticultural plant tolerance to environmental stresses.

## 5. HISTOLOGICAL RESPONSES TO LIME STRESS

Lime stress is one of the most significant stress factors encountered both in our country and world. This stress is caused by high levels of lime in

the soil and/or irrigation water where horticultural crops are grown. Lime stress causes an increase in soil pH, negatively affecting plant growth. High pH reduces the availability of iron (Fe), thus restricting plant growth. The histological effects of lime stress on horticultural crops have not been extensively studied; the effects of Fe deficiency have been studied more extensively. It has been reported that the growth of cortical cells in leaves of peach saplings grown under iron deficiency is inhibited (Aras et al., 2022). Cortical cells are rich in nutrients and can also serve as reserves for these nutrients. It has also been determined that xylem vascular bundles fail to develop due to Fe deficiency. Because xylem is responsible for the transport of water and nutrients, its failure to develop can also lead to problems with nutrient transport. Another study examined the leaf stomata of peach saplings under Fe deficiency (Aras et al., 2021). Fe deficiency led to a decrease in stomatal conductance and area. Stomata are important as openings responsible for both gas exchange and transpiration and also play a major role in the plant's uptake of certain nutrients, such as Fe (Rios et al., 2016).

A study examining the quality of cucumber seedlings in calcareous soil reported that calcareous soil negatively affects the seedling's cortical cells and xylem, reducing seedling quality (Çoban and Aras, 2023). The study determined that lime stress limits xylem development and inhibits cortical cell growth. Fernández et al. (2008) determined that epidermal cells enlarge and stomatal guard cells remain small in size in pear and peach trees under Fe deficiency. They also reported that Fe deficiency negatively affects the anatomical structures that serve as leaf barriers, reducing leaf water potential.

## 6. HISTOLOGICAL RESPONSES TO FLOOD STRESS

Flooding is a significant environmental factor that limits plant growth and development. This stress negatively affects plant survival, biomass, and plant height (Loreti et al., 2016). Flooding stress causes oxygen depletion (hypoxia and anoxia) and root suffocation (asphyxia) in plant roots (Jackson and Colmer, 2005). This phenomenon is generally called asphyxia. Complete submersion of plants due to flooding can lead to oxygen deficiency in aboveground organs (Voesenek et al., 2006). When oxygen becomes limiting for respiration, plants experience hypoxia, while complete oxygen deprivation (anoxia) can lead to plant death. Horticultural plants are generally among the species susceptible to flooding stress (Blanke and Cooke, 2004).

Horticultural plants exhibit diverse histological responses to flooding stress. Horticultural plants can increase and/or structurally enhance aerenchyma, which are gas spaces, to facilitate gas storage (Takahashi et al., 2014). Plants can normally tolerate asphyxia stress through histological responses. Aerenchyma formation is an important indicator of a plant's tolerance to asphyxia (Liang et al., 2008). Aerenchyma is a tissue responsible for gas storage in plants (Evans, 2004) and can develop well in roots, leaves, and main leaf veins (Takahashi et al., 2014). Aerenchyma formation in tissues is crucial and can occur in leaves and roots (Takahashi et al., 2014). In addition, the apoplastic spaces between cells also play a role in gas storage, and for this purpose, the apoplastic spaces increase by increasing the size of the cortex cells (Nishiuchi et al., 2012).

In a study examining the histological responses of strawberry plants exposed to flood stress, it was reported that after 2 days of flooding, aerenchyma formation in the roots did not occur, but the area of the sponge parenchyma in the leaf mesophyll increased, creating more space for gas storage (Kaya et al., 2025). Pimentel et al. (2014) reported that the histological responses of rootstocks commonly used in stone fruit species to flood stress increased the size of lenticels, adventitious roots, and aerenchyma tissue. In another study, it was reported that a cavity formed in the main vein of a peach leaf under flooding, increasing gas storage space (Xu et al., 2022).

## 7. HISTOLOGICAL RESPONSES TO SALT STRESS

Salt stress results from increased salinity in soil and/or irrigation water due to factors such as coastal farming and high doses of fertilization. While horticultural crops are generally known to be sensitive to salt stress, salinity exceeding 2.0 EC (electrical conductivity) can be quite damaging.

Plants have developed various adaptation mechanisms to the abiotic stress factors they encounter in their habitats. Among these stresses, salinity (salt stress) is one of the most significant environmental problems, particularly in agricultural areas, where soil salinity increases. High salt concentrations create both osmotic stress and ion toxicity in plants. Salt stress leads to a series of structural changes at the cellular and tissue levels; these changes are clearly observable in the plant's histological structure.

Salinity stress primarily affects root tissues. Increased  $\text{Na}^+$  and  $\text{Cl}^-$  ions cause plasmolysis in root cells, resulting in water loss resulting in the separation of the cell membrane from the cell wall. This reduces cell turgor and limits root growth. In plants tolerant to salt stress, the root epidermis thickens, the spaces between cortical cells decrease, and suberinization

increases in the endodermis. Suberin accumulation limits the permeation of ions into the xylem, preventing the transport of toxic ions. Furthermore, lignin and cutin accumulation is observed in the exodermis, which reduces water and ion exchange between the root and the external environment, creating a protective barrier.

Vacuole organization in roots also changes under salt stress. Plants store harmful ions in vacuoles to direct them away from the cytoplasm. This manifests histologically as vacuole enlargement and cytoplasmic volume reduction. Plasmodesmata, which provide intercellular communication, can partially close under salt stress, limiting ion transport.

Significant histological changes also occur in leaf tissues. In leaves exposed to salt stress, epidermal cells thicken and the cuticle layer expands, a process designed to reduce water loss. Furthermore, stomata are either reduced in number or buried deeper, minimizing water loss through transpiration. Within the mesophyll tissue, air spaces between cells are reduced, and cells become more tightly packed. This is an adaptation to increase the cells' water retention capacity under osmotic stress.

Salt stress also produces significant changes in vascular tissues (xylem and phloem). Xylem elements narrow in diameter, and secondary wall thickening and lignification in vascular tissues increase. These structural changes reduce the risk of cavitation during water transport and stabilize the plant's hydraulic conduction system. However, under extreme salinity, xylem elements collapse and phloem transport slows.

Another histologically significant response is cell wall thickening. Wall thickening, particularly in parenchyma and collenchyma cells, increases mechanical resistance to changes in intracellular osmotic pressure. Microscopically, accumulation of phenolic compounds and flavonoids can also be observed. These compounds strengthen the cell wall and protect against oxidative stress.

Some halophyte plants have developed histological adaptations specific to salt stress. For example, excess salt is excreted in specialized epidermal structures called salt glands or salt vesicles. The presence of these structures maintains physiological balance by preventing ion accumulation in the plant's internal tissues. Furthermore, water-storing cells (hydrenchyma) can develop in leaves, which contribute to maintaining osmotic balance.

Salt stress limits the development of horticultural plants. This limitation stems primarily from the negative effects of salinity on cells and xylem vascular bundles. Cortex cell growth and xylem development have been

reported to be inhibited in strawberry plants grown under salt stress (Aras, 2025). Histological responses in cucumber plants exposed to salt stress revealed increased palisade and spongy parenchyma thickness, and leaf structural integrity was impaired (Yuan et al., 2015). When tomato plant responses to salt stress were examined, it was reported that the size of the palisade and spongy parenchyma increased and stomatal aperture decreased to prevent water loss (Albaladejo et al., 2017). In pepper plant responses under salt stress, there were delays in cortex cell growth and xylem development (Coban, 2023). Problems in the development of xylem vascular bundles under salt stress have also been reported in tomato plants (Hoffmann et al., 2021). When grapevine leaf histological responses were examined, the epidermis and spongy parenchyma thicknesses and main vein width increased as a result of salt stress (El-Banna et al., 2022).

## 8. HISTOLOGICAL RESPONSES TO DROUGHT STRESS

Drought stress is one of the most common environmental stress factors, significantly reducing fruit yield and quality. Horticultural crops generally contain species that are susceptible to drought (Klamkowski and Treder, 2008; Razavi et al., 2008; Jiménez et al., 2013).

Drought stress is a significant factor limiting plant growth. When plants lose or cannot find water, it creates numerous problems in both vegetative development and physiology. This is reflected in the plant's fruit yield and quality. Some plants can develop resistance to drought stress through various defense mechanisms. Examples of these mechanisms include stomatal control, increased root development to access water, and the accumulation of various osmoprotectants (Singh et al., 2015; Pirasteh-Amosheh et al., 2016). Histological responses also play important roles in these defense mechanisms. Under drought stress, the number of sponge parenchymal cells increased and stomatal density decreased in almond (Rajabpoor et al., 2014). In a study examining the histological responses of different citrus rootstocks to drought, significant increases in epidermal cell thickness, vascular bundle length, and xylem thickness were found in drought-tolerant rootstocks (Shafqat et al., 2021). In a study on cucumber, the length and width of palisade cells decreased under drought (Liu et al., 2018). The thickness of the sponge parenchyma also decreased.

One of the first responses to drought stress is changes in stomatal density, size, and aperture (Anjum et al., 2011). Plants regulate stomatal movements to conserve available water, triggering their defenses against drought. Decreased stomatal size and density and/or narrowing of stomatal apertures

under drought have been reported in many plants, including tomato (Liang et al., 2020), melon (Kusvuran, 2012), and apple (Aras and Keles, 2019).

## **9. HISTOLOGICAL RESPONSES TO EXTREME TEMPERATURE STRESS**

Horticultural plants can be exposed to both low and high temperatures throughout the growing season. Histological responses within plants are crucial in triggering defenses (Miranda et al., 2025).

A study examining peach tree responses to low temperatures reported that increased xylem vascular bundle thickness plays a significant role in cold adaptation (Niu et al., 2023). A study examining cucumber histological responses determined that abscisic acid levels increase under low temperatures, closing stomatal apertures and thus providing defense (Ikkonen et al., 2012). This decreases root hydraulic conductivity, reducing water flow to shoots and preventing freezing.

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## The Application Models of Chromosomal Techniques in Agriculture

Halil Erhan Eroğlu<sup>1</sup>

### Abstract

The chromosomal techniques in agriculture, especially in breeding studies, are progressing in parallel with developments in the field of cytogenetics in biology. These techniques are used in crop improvement studies (e.g., more resistant, more productive, more nutritionally valuable etc.) using genetic information from plant genomes, particularly in economically important plants. In short, the chromosomal techniques are important in obtaining agricultural products with desirable improved traits. The areas using chromosomal techniques in agriculture and their detailed examination will form the basis of this section. These can be listed as cytogenetics, hybridization and polyploidy, chromosome engineering, chromosome-specific breeding programs, functional genomics, genetic mapping, genome sequencing, polymerase chain reaction (PCR), clustered regularly interspaced short palindromic repeats (CRISPR-CAS9) technology, and marker-assisted selection (MAS). The classical cytogenetic methods are the oldest strategies used in agricultural crop improvement. The hybridization, polyploidy, chromosome engineering, and chromosome-specific breeding programs are the continuation of these strategies, the molecular cytogenetics represents the cutting edge. In the present chapter, application models of chromosomal techniques in agriculture are evaluated from the oldest to the newest. The first of these, cytogenetics, is a sub-branch of genetics that deals with the number, shape and structure of chromosomes. The hybridization is a process in which two complementary single-stranded molecules combine to form a double-stranded, while polyploidy is the presence of more than two sets of chromosomes in somatic cells. The chromosome-specific breeding programs are artificial selection methods used to obtain agricultural products with desired traits. The molecular cytogenetics is a more advanced model of classical cytogenetic methods combining molecular biology and cytogenetics techniques.

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## 1. Overview of Chromosomal Techniques

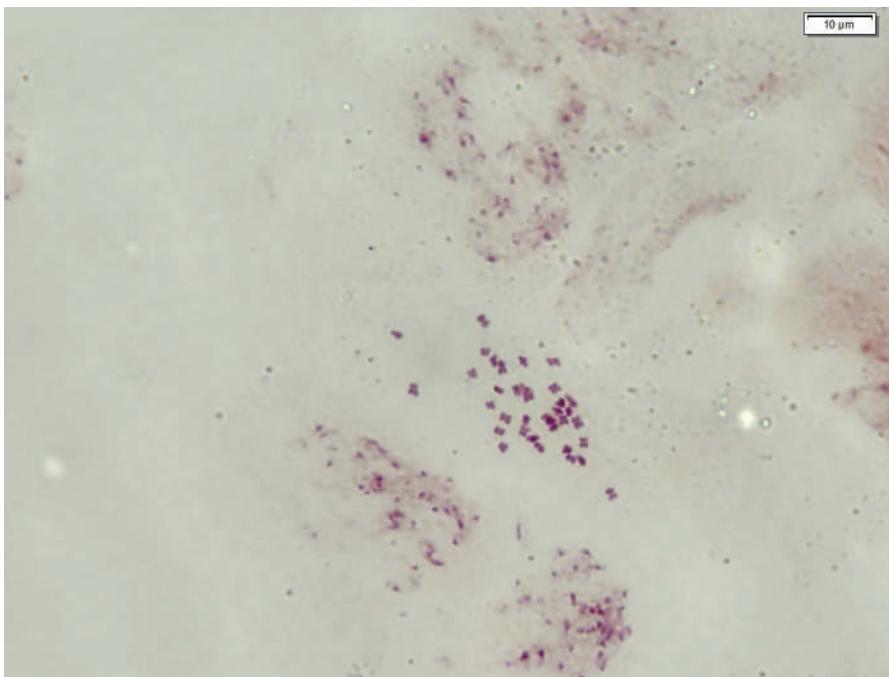
### 1.1. Cytogenetics

The cytogenetics, is a sub-branch of genetics that deals with the number, shape and structure of chromosomes. Although cytogenetics first emerged as a discipline in the early 1900s, the first cytogenetic applications in agriculture were Barbara McClintock's (1902-1992) studies on maize (McClintock and Creighton, 1931). Since then, the cytogenetics were used in crop domestication and development studies (e.g., more resistant, more productive, more nutritionally valuable etc.) using genetic information from plant genomes, particularly in economically important plants.

The cytogenetic techniques developed rapidly, particularly after Belling introduced the crushing method (Belling, 1921). The classical cytogenetics includes the function and movement of chromosomes during cell division, determination of chromosome structure and number (basic and diploid number), and karyotype analysis (Singh, 2018). The most appropriate situation to determine these chromosomal parameters is to observe the chromosomes in the metaphase stage of cell division (Figure 1). For this purpose, the plant seeds were germinated between moist Whatman papers in Petri dishes. Then, the root tips were pretreated in hydroxyquinoline,  $\alpha$ -mono-bromonaphthalene, or colchicine; fixed with fixative solution (ethanol:acetic acid); stored in ethanol (70%); hydrolyzed with 1N HCl; stained with carmine, orcein, or fuchsin; and squashed for preparation and observation. At least 10 metaphase plates with well-distributed, uncontracted chromosomes, distinct chromosome morphology, and chromosomes lying in a plane are evaluated for observation. After imaging, the chromosomal measurements are performed using a karyotype computer software program. After the total chromosome lengths, long arm lengths, short arm lengths, and chromosome arm ratios are determined; the karyotype formulae, relative lengths, centromeric index values, and symmetry/asymmetry index values are calculated. The chromosomal types are determined based on chromosome arm ratios and centromere location according to the criteria proposed by Levan et al. (1964). The ideograms are drawn in order of chromosome type from largest to smallest (Figure 2).

The molecular cytogenetics is a more advanced model of classical cytogenetic methods combining molecular biology and cytogenetics techniques. Thus, it is a discipline that expands the scope of classical cytogenetic techniques consisting of routine chromosome analysis and increases the value of their intended use in agriculture. Molecular

cytogenetic applications of agricultural products began in the early 1970s with the advanced characterization of somatic chromosomes by C-banding and fluorescence in situ hybridization (FISH) techniques (Gill and Kimber, 1974) and progressed with DNA sequencing and mapping studies. The concepts related to classical and molecular cytogenetics are briefly mentioned in this section, as they will be covered in detail in other topics.



*Figure 1. The metaphase plate used to observe chromosomes. It is important that metaphase areas have well-distributed, unshrunken chromosomes, distinct chromosome morphology, and chromosomes lying in one plane.*

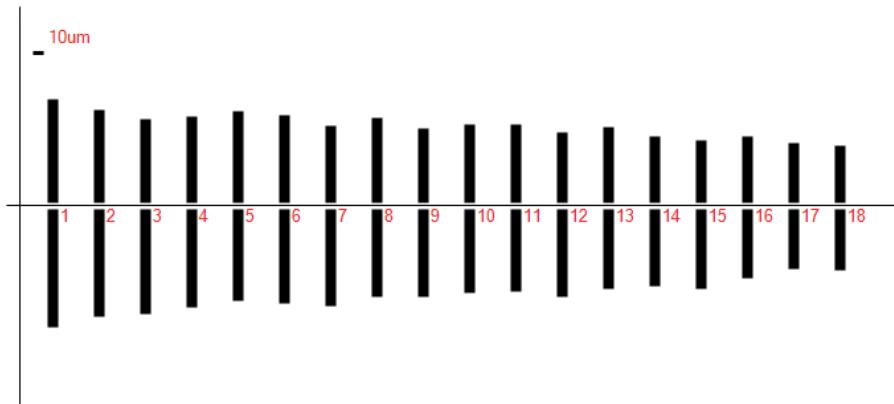
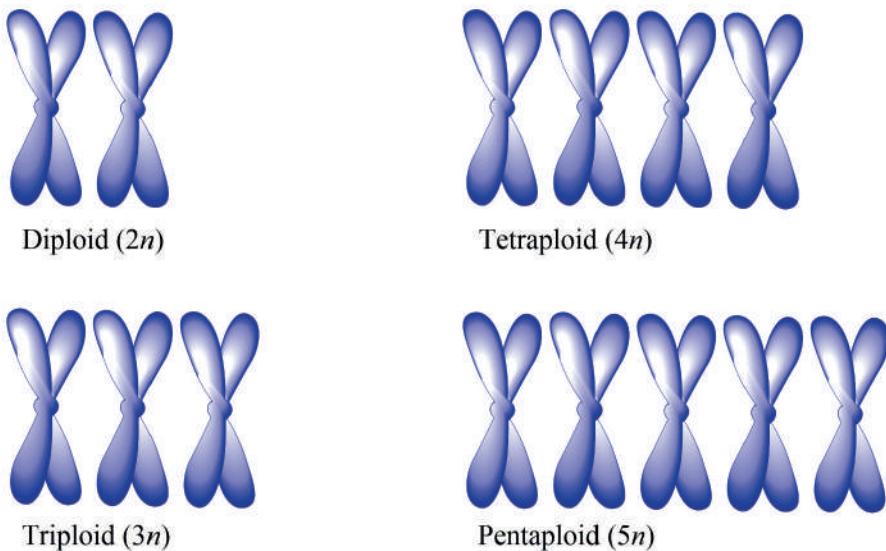


Figure 2. An example ideogram with diploid ( $2n = 36$ ) chromosomes. The chromosomes are arranged from largest to smallest based on centromere position.

## 1.2. Hybridization and Polyploidy

The hybridization is a process in which two complementary single-stranded molecules combine to form a double-stranded, while polyploidy is the presence of more than two sets of chromosomes in somatic cells. In agriculture, hybridization enables the emergence of new genotypes and hybrids by crossing two genetically different plants. The artificial hybridization that a controlled crossover or artificial selection model, is one of the most important mechanisms in plant breeding. Important consequences of this mechanism are self- or cross-pollinated plant development and heterosis (hybrid vigour). The hybrid vigour is the display of superior or higher characteristics (biomass, growth rate etc.) in hybrid offspring compared to their parents (Chen, 2010).

Organisms may have one (monoploid,  $n$ ) or two sets (diploid,  $2n$ ) of chromosomes, but they may also have more than two sets (polyploid) of chromosomes. This mechanism, called general polyploidy, also has special definitions such as triploidy ( $3n$ ), tetraploidy ( $4n$ ), and pentaploidy ( $5n$ ) (Figure 3). If the extra genome resulting from polyploidy comes from the same species, it is called autopolyploidy; if it comes from different species, it is called allopolyploidy (Eroğlu, 2022). The allopolyploidy is a mechanism that leads to the fixation of hybrid vigour. Some agricultural crops are grown as hybrids (*Oryza sativa*, *Zea mays*, *Brassica juncea* etc.), while others (*Gossypium hirsutum*, *Triticum aestivum*, *Arabidopsis suecica* etc.) are grown as allopolyploids (Chen, 2010).



*Figure 3. The general view of diploid (2n), triploid (3n), tetraploid (4n), and pentaploid (5n) chromosome sets. Here, autoploidy is present for polyploidy is expressed on only one homologous chromosome pair.*

The polyploids can arise naturally or artificially. The natural polyploids arose spontaneously due to various factors and progressed towards speciation over a long period of time (Wood et al., 2009).

### 1.3. Chromosome Engineering

Increasing population growth and limited resources have made it necessary to achieve high efficiency in agriculture. (Gerland et al., 2014). The study of manipulating chromosomes to develop crops resistant to diseases, pests, or environmental stresses forms the basis of chromosome engineering. The chromosome manipulation can be achieved through mechanisms such as CRISPR/Cas, which allows for the specific introduction of mutations to a specific chromosome region, or directly through genotoxic agents (Pacher and Puchta, 2017). The chromosome engineering in agriculture was first used in wheat (*Triticum aestivum*) chromosomes using X-ray irradiation to insert foreign chromosome fragments (*Aegilops umbellulata*) into them (Sears, 1956). Although X-ray irradiation is still preferred in breeding studies, many modern chromosome engineering techniques have been developed and used today. The modern chromosome engineering techniques, starting with methods based on specific DNA sequences using endonucleases, include the engineering of plant mini-chromosomes, the

engineering of plant centromeres, the insertion of genes of interest into engineered chromosomes, centromere engineering to produce haploid inducers, the redirection of meiotic recombination, the breaking of genetic linkages through chromosomal translocations, engineered apomixis, and aspects of meiotic recombination (Puchta and Houben, 2024).

The mini-chromosomes have been generated by telomere-mediated chromosomal excision in *Arabidopsis thaliana*, maize, barley, rice, wheat, and rapeseed (Puchta and Houben, 2024). Creating centromeres from scratch in agriculture is a highly useful chromosome engineering technique, but direct transformation of centromere sequences is not feasible in plants. Therefore, in most plants, it resorts to epigenetic marking of the centromere with CENH3 (histone H3 variant) (Liu et al., 2023).

#### 1.4. Chromosome-Specific Breeding Programs

The chromosome-specific breeding programs are artificial selection methods used to obtain agricultural products with desired traits. Advances in molecular genetics have enabled plant breeders to select desired genes throughout the genome in agriculture and develop targeted agronomic traits. The chromosome-specific breeding programs have been proposed to control allelic variation in genes associated with agriculturally desirable traits (Peleman and van der Voort, 2003). The chromosome-specific breeding programs, or “breeding by design,” have evolved into their current form after going through various stages. The techniques began with “selection breeding,” then progressed to “hybridization breeding,” “marker-assisted breeding,” and “breeding by design” (Zhang, 2021). While the selection breeding only allows the selection of agricultural plants that show spontaneous variation and the selected agricultural products are the result of years of experience-based selection by agricultural producers (Zeven, 1998), the chromosome-specific breeding programs allow the selection of desired genes from the entire genome in three stages. The first stage involves mapping all agronomically important loci; the second stage involves examining allelic variation at mapped loci; and the third stage involves performing chromosome-specific breeding with the desired alleles (Peleman and van der Voort, 2003).

The chromosome-specific breeding programs have been used in the improvement of many agricultural crops such as rice (*Oryza sativa*, *Oryza glaberrima*, *Oryza nivara*, *Oryza rufipogon*, *Oryza barthii*, *Oryza meridionalis*, and *Oryza glumaepatula*), wheat (*Triticum aestivum*), cotton (*Gossypium*

*hirsutum*, *Gossypium tomentosum*, and *Gossypium barbadense*), maize (*Zea mays*), and *Aegilops tauschii* (Zhang, 2021).

### 1.5. Functional Genomics

Many genes are known that affect the characteristics such as durability, resistance, yield, quality etc. of agricultural products, and focusing on these genes is very important for breeding studies. The functional genomics applications, which investigate the connections between genes and their effects, reveal valuable data about genes that control vital characters such as durability, resistance, yield, and quality (Li et al., 2025). The functional genomics makes significant contributions to the development of agricultural products together with different disciplines (especially computer technology). The functional genomics applications are technologies that investigate critical genes and their quantitative loci (**QTL**, quantitative trait loci) that show rapid adaptation and response to changing environmental conditions. Thus, the development of agricultural products that are more durable, more resilient, and higher yield and nutritional value (Yang, 2025).

The genome studies of agricultural products are generally divided into three genomic models: structural, quantitative and functional genomics. The integration of these models in the form of structural-quantitative, quantitative-functional, and structural-functional makes it more convenient to determine the desired properties (Yang, 2025). There are many agricultural examples of applications of functional genomics (Table 1).

**Table 1. Functional genomics applications in agriculture. Agricultural products are listed alphabetically.**

Agricultural product	Characteristic	References
Carrot ( <i>Daucus carota</i> )	Fertility Abiotic stress	Duran and Ipek, 2022 Hao et al., 2020
Cotton ( <i>Gossypium</i> sp)	Fiber yield and quality Drought tolerance	Naoumkina and Kim, 2023 Sharif et al., 2024
Maize ( <i>Zea mays</i> )	Cold tolerance Heat stress Salt stress Drought	Farooqi et al., 2022 Waqas et al., 2021
Peanut ( <i>Arachis hypogaea</i> )	Biological stress Pathogen resistance	Cai et al. 2023
Rice ( <i>Oryza sativa</i> )	Disease resistance Heat tolerance	Yang et al., 2024
Soybean ( <i>Glycine max</i> )	Biological stress resistance Oil yield Protein content	Du et al., 2023 Liu et al., 2023
Wheat ( <i>Triticum aestivum</i> )	Yield Disease resistance Drought tolerance Nutritional quality	Boehm Jr and Cai, 2024 Roychowdhury et al., 2024

## 1.6. Genetic Mapping

Genetic mapping, which determines the linear connection of chromosomes as relative distance, uses recombination and genetic markers for these processes. The genetic mapping is a technique that facilitates advanced genomic studies in the improvement of agricultural products. Linkage maps allow calculation of the relative distance between two genes based on the crossover rate or recombination frequency. These maps are very important in agriculture as they provide an introduction to fields such as comparative genomics, candidate gene detection, quantitative trait loci (QTL) mapping, detection of trait-marker relationships, and marker-assisted selection. In genetic markers, it is essential to establish both quantitative trait loci mapping and high-quality linkage maps. The genetic markers closely associated with a gene or locus that expresses a desired trait are widely used in the breeding of agricultural products. The mapping product population, selection of appropriate genetic markers, genotyping and polymorphism screening of the population, and construction of linkage maps are the basic requirements for genetic mapping (Begna and Yesuf, 2021).

While a high-resolution map yields markers with the same order as the physical map, there is no linear relationship between the number of base

pairs (**kb**) and the distance (**cM**, centimorgan) in terms of measurement. The relative distances in linkage maps reveal different distance profiles in agricultural products. For example, 1 cM distance corresponds to a length of 30 to 550 kb in *Arabidopsis thaliana*, 258.5 kb in rice, and 118 to 22.000 kb in wheat (Schmidt et al., 1995; The Rice Genome Sequencing Project, 2005).

### 1.7. Genome Sequencing

Genome sequencing is crucial for identifying genes that express desired traits in agricultural crops. The genomes of many plants, especially agricultural foods, have been sequenced (Table 2). Because breeding improved varieties requires a thorough understanding of the crop genome and this is possible with information from the genome, transcriptome, proteome, and epigenetics (Thottathil et al., 2016).

*Table 2. The genome sequences of the important agricultural products (alphabetically).*

Agricultural product	Genome size (mb)	References
Bean ( <i>Phaseolus vulgaris</i> )	587	Schmutz et al., 2014
Cabbage ( <i>Brassica oleracea</i> )	630	Liu et al., 2014
Maize ( <i>Zea mays</i> )	2.300	Schnable et al., 2009
Pigeon pie ( <i>Cajanus cajan</i> )	833	Varshney et al., 2011
Rapeseed ( <i>Brassica napus</i> )	1.130	Chalhoub et al., 2014
Rice ( <i>Oryza sativa</i> )	430	Yu et al., 2002
Soybean ( <i>Glycine max</i> )	1.115	Schmutz et al., 2010
Sugar beet ( <i>Beta vulgaris</i> )	758	Dohm et al., 2014
Tobacco ( <i>Nicotiana tabacum</i> )	4.500	Sierro et al., 2014
Wheat ( <i>Triticum aestivum</i> )	17.000	Brenchley et al., 2012

### 1.8. Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) is a technique that facilitates the detection and analysis of trait with reliable, rapid, and accurate results to the feature of interest by amplifying a specific sequence. This technique is widely used in agriculture for purposes such as quality and yield control, agricultural product authenticity, pathogen detection and resistance, resistance, and genetically modified organism (**GMO**) analysis (Chavan, 2002).

Many different PCR models for different purposes in agricultural studies, such as conventional PCR, real-time PCR (RT-PCR), nested PCR, colony PCR, asymmetric PCR, thermal asymmetric interlaced PCR (TAIL-PCR),

reverse transcription PCR, multiplex PCR, assembly PCR, nanoparticle assisted PCR (Nano-PCR), single specific primer PCR (SSP-PCR), allele specific PCR, methylation specific PCR, inverse PCR, linear after the exponential PCR (LATE-PCR), degenerate PCR, and hot start PCR. is used (Chavan, 2002).

### **1.9. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR-CAS9)**

Genome editing systems, which modify the genome in a desired, predictable, rapid, and precise manner, are widely used methods in agriculture. Four families of directed nucleases such as CRISPR-associated protein (CRISPR-Cas), homing endonucleases (HEs), transcription activator like effector nucleases (TALENs), and zinc finger nucleases (ZFNs) are involved in genome editing. According to the site-directed nuclease, CRISPR/Cas, which works with the complementarity of the guide RNA to a specific sequence, is a simpler cheaper, faster and more efficient system (Cong et al., 2013).

The working mechanism of the CRISPR/Cas9 system in agriculture consists of the following steps:

- (1) Selection of genes to be regulated and determination of gene-specific spacers.
- (2) Prepare ribonucleoprotein or transformation carrier.
- (3) Deliver foreign proteins or nucleotides into the agricultural product cells.
- (4) Identify edited lines in T0 generation with next generation sequencing.
- (5) Select null agricultural products by the gene edited in T1 and confirm them with next generation sequencing in T2 generation.
- (6) Obtaining the lines edited homozygous and evaluation of expression of the gene.
- (7) Using null lines at new agricultural breeding variety (Liu et al., 2021).

In agriculture, studies are being carried out to improve traits such as grain size, length and width, slender grain shape, fruit size, shape and colour, seed colour, root colour, leaf colour, flower longevity and colour, long shelf life, low or high amylose content, fragrant, super sweet and waxy product, high  $\beta$ -carotene content, low Cd accumulations, high oleic acid proportion,

low phytic acid content, high lycopene content, low phytic acid content, high oil production, low gluten content, and low tartaric acid content using the CRISPR/Cas system. Thus, this improves the agricultural product's physical appearance and texture quality, increases palatability, and enriches its nutritional elements (Liu et al., 2021).

### **1.10. Marker-Assisted Selection (MAS)**

Marker-assisted selection (MAS) is the selection of agriculturally important traits using DNA markers. The markers can significantly increase durability, productivity, nutritional value, sensitivity, etc. The marker approach involves selecting and transferring loci expressing desired traits. The development of molecular maps and genetic markers has facilitated the applicability of marker-assisted selection. Because the success of marker-assisted selection depends on many factors, such as the distance between genes underlying the gene maps, the number of related genes, and genetic markers. Trait improvement has been achieved in many agricultural products by the applications such as marker-assisted evaluation of breeding material, marker-assisted backcrossing, marker-assisted pyramiding, early generation marker-assisted selection, and combined marker-assisted (Collard and Mackill, 2008).

For marker-assisted selection to achieve full success in achieving desired agronomic traits, it is necessary to utilize its advantages over traditional methods. The applications such as preliminary studies and computer simulations can facilitate this success (minimize costs and maximize genetic gain) (Kuchel et al., 2005).

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## Phytoremediation

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### Abstract

With the rapid growth of the global population, demands for food, water, and energy have led to significant environmental stress. Heavy metals, organic pollutants, waste (oil, solvents), military activities (explosives, chemical weapons), agricultural chemicals (pesticides, herbicides), and industrial waste (chemicals, petrochemicals) resulting from agricultural activities and industrialization cause serious environmental pollution. Phytoremediation is a technique that uses plants to remove, transform, evaporate, or fix these pollutants found in air, soil, water, sludge, and sediments. Phytoremediation is an effort to remediate contaminated areas using nature's own mechanisms. Pollutants can be taken up by plants, degraded within the plant or in the plant root zone, retained through conjugation, or vaporized.

Phytoremediation offers a green alternative to traditional physical and chemical cleaning methods with its low cost, direct use of solar energy, and ability to restore habitats. Thanks to these natural mechanisms, plants not only contribute aesthetically and ecologically to human life but also play an active role in combating pollution from industrial activities. This review provides general information about phytoremediation.

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## 1. Introduction

### What is Phytoremediation?

With the rapid increase in the global population, demands for food, water, energy, and other ecosystem services have led to serious stresses on the environment (Kamran et al., 2021; Saleem et al. 2020; Zaheer et al., 2020). Agricultural activities (Mózner et al., 2012), industrialization (Cherniwchan, 2012; Wu et al., 2016), heavy metals (Anyakora et al., 2013; He et al., 2015; Su, 2014), radionuclides (Hu et al., 2010; Prakash et al., 2013), organic compounds (Afzal et al., 2014), chemicals used in agriculture, chemical fertilizers (Malik et al., 2017; Sunitha et al., 2012), and oil spills (Ron and Rosenberg, 2014) cause environmental pollution (Kafle et al., 2022). Heavy metals play a major role among these.

Metals such as copper, zinc, iron, manganese, molybdenum, nickel, and cobalt are defined as micronutrients that play an important role in the growth and development of plants and animals. Some heavy metals, such as arsenic, mercury, cadmium, and lead, are not essential for life development. Whether essential for plants or not, heavy metal levels above the threshold set by nature are a serious problem that leads to environmental pollution, reduces soil quality, and reduces crop yields.

Plants can directly absorb these pollutants through their roots, rendering them harmless, or stabilize the environment by reducing the mobility of toxic substances in soil and water. Phytoremediation is a sustainable strategy that uses plants to remove, transform, volatilize, or fix pollutants found in air, soil, water, sludge, and sediments (Munive Cerron et al., 2018; Santana Flores et al., 2020). Phytoremediation is an environmentally friendly pollution removal method that uses the natural abilities of plants to clean environmental pollutants (Basharat et al., 2018).

The use of phytoremediation is not limited to soil; it is also used in water purification. Phytoremediation is an economically and environmentally advantageous technique because it uses green plants to capture, trap, or detoxify contaminants in contaminated soil and water (Ashraf et al., 2019). These plants are capable of absorbing heavy metals, accumulating them at high levels in their tissues and neutralizing them after various processes. These plants are called hyperaccumulators. Hyperaccumulators are plants that can uptake one or more contaminant elements at very high rates and accumulate 50-500 times more metals in their leaves, branches, and trunks than in the soil. Many plants, such as *Thlaspi caerulescens*, *Arabidopsis thaliana*, *Brassica juncea*, *Lycopersicon esculentum*, *Zea mays*, *Hordeum vulgare*, *Oryza sativa*,

*Pisum sativum*, and *Sedum alfredii*, are known to have phytoremediation potential for various heavy metals. *Thlapsi caerulescens*, known as the most common hyperaccumulator plant, accumulates over 26.000 ppm of zinc, while most plants found in nature have been found to accumulate around 100 ppm (Lasat, 2000). These characteristics make plants ideal for both phytoremediation and phytomassing activities (Basharat et al., 2018).

Phytoremediation, which has become widespread in many countries in recent years, is considered a passive technology for cleaning heavy metal-contaminated soils, offering advantages such as being relatively inexpensive, aesthetically pleasing, easy to implement, and quick to implement (Glass, 1999). Phytoremediation encompasses many different technologies, each serving different purposes. These can be categorized as phytoextraction, rhizofiltration, phytostabilization, phytovolatilization, phytodegradation, rhizodegradation, and phytodesalination (Pivetz, 2001).

## 2. Types of Phytoremediation

### 2.1. Phytoextraction

This technique is performed in areas with low or moderate metal pollution using natural hyperaccumulator plants (Baker et al., 1994; Padmavathiamma and Loretta, 2007). Plant growth cannot be sustained in more heavily polluted areas. It involves the uptake of contaminants by plant roots, their accumulation in above-ground organs, and the subsequent harvesting and destruction of the plants. This technique is used to remove actively absorbed nutrients such as copper and zinc, as well as non-nutrient heavy metals such as cadmium, nickel, and lead (Padmavathiamma and Loretta, 2007). Chelating agents can also be added to this technique to increase the solubility of metals with low solubility in the soil solution and promote their uptake (Evangelou et al., 2007).

### 2.2. Rhizofiltration

Rhizofiltration is the removal of toxic substances using plant roots and the uptake of pollutants by the roots of metal-tolerant aquatic plants or other underwater organs (Jadia and Fulekar, 2009). This method is often effective in purifying soil and water heavily contaminated with nutrients such as nitrogen and phosphorus (Mithembu, 2012). Hyperaccumulator plants selected for rhizofiltration undergo specific stages of adaptation before being planted in the original environment. First, the plants are kept in clean water instead of soil until their roots develop to the desired level. They are then transferred to an artificial, contaminated water source for adaptation.

Once acclimated, they are planted in the original contaminated area where rhizofiltration will be applied. Once the roots are saturated, harvesting begins and they are safely disposed of.

### 2.3. Phytostabilization

Phytostabilization is a method often used to prevent erosion in erosion-prone areas, prevent pollutant leaching into groundwater, and prevent direct contact with the soil (Bert et al., 2005). Hyperaccumulator plants used in phytostabilization are plants that can grow and thrive in soils contaminated with heavy metals and convert toxic metals into less toxic forms. These plants have extensive root systems that can alter the physical, chemical, and biological properties of the soil (Berti and Cunningham, 2000; Rizzi et al., 2004). For example, ryegrass can take up the herbicide trifluralin and convert it into a bound residue (Li et al., 2002).

### 2.4. Phytovolatilization

This method, known as phytovolatilization, is applied primarily to groundwater, soil, sludge, and sediment, and is taken up by plant roots, converting the majority of contaminant-containing water into less pollutant and volatile forms before being released into the air. This technique is effective when evaporated contaminants have less toxic effects when transferred from the soil to the atmosphere. The evaporation method is generally effective for organic contaminants (Limmer and Burken, 2016). Trifluralin compounds have been reported to be bound to residue and excreted by rye grass through transpiration from the leaves (Li et al., 2002).

### 2.5. Phytodegradation and Rhizodegradation

Phytodegradation is the metabolism of pollutants within plant tissues. Organic pollutants are degraded by the rhizospheric interaction between plant metabolic processes and soil microorganisms. Organic pollutants, such as pesticides, can be removed by various plant parts through degradation or transformation. Because plants lack active transporters, these organic pollutants are absorbed through passive uptake.

Chlorinated hydrocarbons can be dechlorinated and converted into non-toxic chemicals by plants such as *Tegetes patula*, *Ipomea balsamina*, and *Mirabilis jalapa*. Reductive dehalogenation of DDT has also been observed in the aquatic plant Elodea and the terrestrial plant Kudzu (Garrison et al., 2000). *I. balsamina* and *P. nil* have been found to absorb and degrade petroleum hydrocarbons (Liu et al., 2017). When pollutant degradation occurs in the

rhizosphere, this process is called rhizodegradation. Rhizodegradation is the decomposition of organic pollutants by microorganisms in the root zone. While sugars, amino acids, organic acids, fatty acids, sterols, nucleotides, and enzymes released from the roots influence microbial activity in the root environment, contaminants are also present.

## 2.6. Phytodesalination

Some salt-tolerant plants can extract significant amounts of salt from the soil, allowing saline soil to be reclaimed (Arif et al., 2020). As a biological and clean approach, halophytic plants are used for desalination. Halophytic plants tolerate higher halogen levels in soil or groundwater. These plants take in  $\text{Na}^+$  and  $\text{Cl}^-$  and accumulate these salt ions in plant roots and shoots (Kafle et al., 2022).

Phytoremediation is another practice used to remove pollutants flowing into streams by planting strips of suitable plants along the banks. This prevents pollution from spreading into the environment and contaminating groundwater. Furthermore, erosion is controlled, reducing transport. Studies in Canada have shown that this technique reduces soil erosion by 90% and herbicide runoff by 42-70%. In addition, sediment in the water can be reduced by 71-91%, nitrogen by 67-96%, phosphorus by 27-97%, pesticides by 8-100% and fecal coliforms by 70-74% (Gabor et al., 2001; Hamutoğlu et al., 2012).

## 3. Advantages of Phytoremediation

- It is more economical than other remediation technologies. With its direct use of solar energy and its ability to restore habitats, it offers a green alternative to traditional physical and chemical cleaning methods.
- It does not require a new plant population to re-invade the site.
- It does not require an additional site for waste disposal.
- Compared to other methods, it creates an aesthetically pleasing appearance that is well-received by the public.
- Its in-situ remediation feature prevents the spread of contaminants without the need to relocate the contaminated area.
- It can simultaneously address multiple contaminants beyond a single type of contaminant, enabling site remediation (Basharat et al., 2018).

#### **4. Disadvantages of Phytoremediation**

- Phytoremediation is not effective under all conditions. First, contaminants must be present in the plant root zone; it may not be directly effective for deep underground contamination.
- Environmental factors such as climatic conditions, plant growth process, and soil structure also directly affect success. The success rate depends on the plants used in the area's adaptation to the site's edaphic and biotic factors, as well as the plant's resistance to the contaminant.
- In areas heavily exposed to contaminants, plants may have difficulty growing, which can slow down the remediation process. Therefore, phytoremediation is generally most effective in low- to moderately contaminated areas.
- Contaminants accumulated in leaves may re-enter the soil with leaf fall in the fall.
- Contaminants may accumulate in the tissues of plants used for firewood.
- Remediation time may be longer compared to other methods. Phytoremediation for the treatment of industrial wastewater also requires a large area (Abdullah et al., 2020). These problems are closely related to the rate of degradation of pollutants by plants during treatment. Biological treatment has a different reaction pattern compared to chemical treatment (Imron et al., 2020). Many researchers recommend using phytoremediation as a secondary or tertiary treatment technique to treat wastewater before discharge into water bodies (Yuliasni et al., 2023).
- If pollutants are not safely harvested and disposed of after being accumulated by plants, environmental risks may persist. Therefore, phytoremediation applications must be carefully planned and managed. The likelihood of pollutants leaching into the soil may increase (Basharat et al., 2018). As plants grow during treatment, plant biomass is produced, and this amount can be considered abundant. If phytoremediation is applied to treat toxic substances (usually heavy metals), the produced plant biomass must be processed according to standard toxic substance treatment procedures (Kwoczynski and Čmelík, 2021). If phytoremediation is applied to treat organic or nutrient-rich wastewater, various conversion options can be selected. Various biomass utilization studies have been successfully implemented to convert biomass into animal feed (Kadir et al., 2020), biochar (Das et al., 2021), adsorbent (Alshekhli et al., 2020), biofuel (Correa et al., 2019; Rezania et al., 2020), and even fertilizer (Diacono et al., 2019; Kurniawan et al., 2020). With these conversion options, wastewater

treatment using phytoremediation can lead to a cleaner production strategy through the utilization of treatment byproducts.

## 5. Some Studies in Phytoremediation

In a study on phytoremediation, grass plants (*Lolium perenne* L.) were used in soils contaminated with nickel (Ni) and cadmium (Cd) heavy metals at different concentrations (1000, 4000, and 8000 ppm). The experiments were conducted in greenhouses using pots. Twenty-five kilograms of soil was prepared for each heavy metal. To obtain results closer to real-field conditions, no additives (such as chelate) were used in the soil; instead, completely natural field soil was used. Throughout the experiment, samples were taken at regular intervals when the grasses reached 10 cm in height, and heavy metal accumulation was measured over four periods. The results indicated that the grasses continuously absorbed heavy metals from the soil. However, in the cadmium experiments, a decrease in the amount of metal absorbed by the grasses was observed over time. While they initially took up more metals, this amount decreased in later periods (Arikan and Bağdatlı, 2021).

Plant species such as *Brassica napus* L. (canola), *Chenopodium quinoa* Willd. (quinoa), and *Allium cepa* L. (onion) were used to remove lead contamination using chelate-assisted phytoremediation. During the research, chelating agents such as EDTA and humic acid, as well as microbial fertilizers, were added to the soil to improve plant performance. It was determined that the plants retained more lead, especially when supplements such as humic acid and EDTA were used. In the study, it was determined that onion and quinoa plants transported lead more effectively from the root to the shoot. Quinoa, in particular, performed quite well when nitro chelate was used, while canola did not always exhibit the expected hyperaccumulator properties (Kılıç and İpek, 2019).

Sunflower, corn, and canola plants were used in a study aimed at cadmium stabilization through sequential application of phytoremediation and pyrolysis. To reduce cadmium pollution, first phytoremediation with plants and then pyrolysis were applied. High removal rates ranging from 89.6% to 93.5% were achieved through phytoremediation. The harvested plants were then pyrolyzed in a special reactor at 500°C. It was determined that the accumulated cadmium from this process was stabilized in the solid products. This technique both cleaned the soil and controlled the metal risk in the plants (Özkan et al., 2015).

The phytoextraction capacity of the cocklebur (*Xanthium strumarium* L.) plant was evaluated in soils artificially contaminated with copper. The plant was effective in soil cleansing by accumulating copper (Eren, 2018). The potential of ornamental plants such as petunia, ice plant, mustard, cabbage, and honeysuckle to remove contaminants such as arsenic, chromium, and textile dyes was investigated. *Alyssum maritima*, in particular, demonstrated high chromium accumulation capacity (Özay and Mammadov, 2013).

The removal of pollutants from wastewater was investigated using duckweed (*Lemna minor*) and floating fern (*Salvinia natans*) in constructed wetlands. Both plants were evaluated as effective phytoremediation agents in aquatic environments (Kaya and Yıldız 2017).

In recent years, it has been discovered that genetic engineering techniques, particularly next-generation genome editing tools like CRISPR-Cas9, can increase the potential for phytoremediation. Researchers are working to make plants more efficient in polluted environments by modifying genes that control metal tolerance, pollutant uptake, and detoxification mechanisms. For example, by increasing the production of metal transporter proteins, metal chelators (such as metallothioneins and phytochelatins), or growth hormones (auxins and cytokinins), plants can be made more resilient to pollutants (Basharat et al., 2018).

Phytoremediation is not limited to plants; it has also begun to be supported by the genetic engineering of beneficial bacteria (PGPR - Plant Growth Promoting Rhizobacteria) that live in the root zone of plants. These bacteria can provide numerous benefits, such as accelerating plant growth, reducing toxic effects, and facilitating the mobilization of pollutants. CRISPR technology is also enhancing the phytoremediation capabilities of these bacteria, making environmental cleanup processes faster and more effective. As a result, it is anticipated that phytoremediation technologies will continue to evolve, and gene editing techniques like CRISPR will allow us to precisely optimize plant traits, resulting in greater pollutant tolerance and higher uptake capacity. This will enable cleaning of larger areas in shorter periods of time, while also providing economic benefits such as the recovery of precious metals. Innovative approaches such as combining phytoremediation with energy production also hold great promise for sustainable environmental management (Basharat et al., 2018).

## 6. Conclusion

Phytoremediation is one of the oldest techniques used to remove pollutants, particularly those found in water and soil, from the environment.

The basic principle of phytoremediation is to degrade, remove, transform, or immobilize toxic compounds found in soils, groundwater, and surface water by exploiting the interaction between plant roots and root microorganisms. Phytoremediation offers several advantages over traditional techniques, including its low cost and environmental friendliness. Disadvantages include climatic and geological limitations, the phytotoxicity of the pollutant, its potential for entry into the food chain, and its longer processing time compared to other technologies. Phytoremediation of wastewater or polluted water can remove organic and inorganic pollutants, but it can also release unknown contaminant derivatives into the environment. Therefore, compared to phytoremediation of polluted soils, further research is needed on the removal of pollutants from wetlands.

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# Greenhouse Gas Emissions and Mitigation Strategies in Dairy Cattle Farms<sup>1</sup>

Ertuğrul Kul<sup>1</sup>

## Abstract

Global warming results from the accumulation of greenhouse gas (GHG) in the atmosphere, which trap heat and raise the Earth's temperature. The emission of these gases and their effects on the global climate have become a major international concern. The carbon footprint (CF) primarily arises from the release of GHGs such as carbon dioxide (CO<sub>2</sub>), produced through various biological and anthropogenic activities. On farms, significant sources of emissions include enteric methane (CH<sub>4</sub>) from ruminants, CO<sub>2</sub> and nitrous oxide (N<sub>2</sub>O) from manure during storage and land application. The dairy industry is recognized as one of the largest contributors to the global GHG emissions. Nonetheless, there are considerable opportunities to mitigate climate change by reducing emissions from dairy cattle. Addressing livestock-related emissions is therefore critical to significantly curbing their impact on global warming. Reducing dairy cattle-derived GHG emissions remains a central objective in efforts to lower agriculture's environmental footprint. Potential mitigation strategies include housing feeding management, grazing and pasture management, manure storage and treatment, manure application to soil, disease control and genetic selection. Although these approaches show great promise, further research is needed to validate their effectiveness and to determine their practical potential for reducing CH<sub>4</sub> emissions in dairy cattle. This review aims to investigate GHG and reduction strategies in dairy farms.

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## 1. Introduction

Climate change has had a significant impact on agriculture, livestock production, and the overall food supply, leading to serious global environmental and economic challenges. Research indicates that the relationship between climate change and the livestock industry is bidirectional (KumAŞ and Akyüz, 2024). Alongside the global climate crisis, food insecurity has emerged as a critical issue, posing a major threat to human societies and becoming more severe in many regions due to increasingly unfavorable weather conditions (Kyriazakis et al., 2024).

There is clear evidence that climate change is occurring, primarily driven by greenhouse gas (GHG) emissions (Jose et al., 2016). The release of GHGs into the atmosphere and their potential effects on the global climate have become pressing concerns worldwide (Chianese et al., 2009). In recent years, policymakers have shown growing interest in reducing agricultural GHG emissions. This heightened attention stems from the agricultural sector's significant share in both global and national emissions, as well as the relatively cost-effective opportunities it offers for mitigation compared with other industries (Glenk et al., 2014).

Over the past five decades, the discussion regarding the anthropogenic influence on climate change has intensified, as the harmful consequences of rising global temperatures have become increasingly acknowledged by both scientific and non-scientific communities (Ozlu et al., 2022). The livestock industry not only plays a major role in driving climate change through its GHG emissions but also faces direct impacts from it. Rising atmospheric temperatures, extreme weather events, and overall climate instability pose serious risks to livestock systems. These changes influence the sector directly, by affecting animal health, growth, and productivity, and indirectly, through reduced water availability, declining feed quality, and diminished soil fertility. Consequently, the livestock sector is both a contributor to climate change and one of its victims (KumAŞ and Akyüz, 2024).

A variety of human activities such as burning fossil fuels, clearing forests, transportation, industrial processes, farming, and livestock production play a major role in driving climate change (Symeon et al., 2025). Considerable efforts have been made to measure GHG emissions from key sources on dairy farms; however, monitoring and quantifying all emissions from an entire farm or production system simultaneously remains nearly impossible and prohibitively costly (Rotz, 2018). In recent years, interest in the GHG effect closely linked to the rise in global temperatures has grown substantially. The three most important GHGs are carbon dioxide ( $\text{CO}_2$ ), methane ( $\text{CH}_4$ ),

and nitrous oxide ( $\text{N}_2\text{O}$ ) (Smith et al., 2007; Burbi, 2014; Króliczewska et al., 2023). These gases are largely emitted through processes such as enteric fermentation, feed production, dietary management, and total farm output (Singaravelan et al., 2023). They trap heat in the atmosphere by absorbing solar energy and slowing its release back into space (Króliczewska et al., 2023). Although the majority of emissions originate from fossil fuel use, agriculture and livestock production account for roughly 12% of global emissions (Symeon et al., 2025). Among livestock sources, cattle are responsible for approximately 65% of these emissions (KumAŞ and Akyüz, 2024).

While the global agricultural sector is dispensable for sustaining human livelihoods and supporting economies worldwide, it is also a major source of GHG emissions, highlighting the need for sustainable farming practices particularly through smart farming approaches. The adoption of information and communication technologies in agriculture holds considerable promise for enhancing both sustainability and productivity. Nevertheless, a substantial gap persists in our understanding of how these technologies influence overall GHG emissions. This gap emphasizes the complexity of agriculture's environmental role and the necessity of developing more comprehensive approaches to assess the net effects of technological interventions (Polymeni et al., 2024). Although climate change poses the greatest long-term challenge, farmers face more immediate difficulties in managing GHG emissions at the farm level (Clark et al., 2011).

In recent years, there has been growing policy interest in reducing GHG emissions from agriculture. This attention stems from the agricultural sector's significant contribution to GHG emissions at both global and national levels, as well as the relatively cost-effective opportunities for mitigation it offers compared to other sectors. Policymakers face the complex task of designing and implementing effective GHG abatement strategies for agriculture. This involves identifying mitigation practices that are both cost-efficient and capable of delivering substantial emission reductions, followed by selecting appropriate policy instruments to encourage their adoption (Glenk et al., 2014). While climate change presents the most significant long-term challenge, farmers must address the immediate task of managing GHG emissions at the farm level (Clark et al., 2011). The livestock sector, in particular, should focus its mitigation efforts on practical management strategies that can be applied directly in field conditions (Jose et al., 2016).

## 2. Climate Change and Dairy Farming

Dairy farming is a major contributor to GHG emissions throughout the life cycle of milk and dairy products. According to Thoma et al. (2013), roughly 72% of total emissions associated with the national fluid milk supply chain are generated prior to the milk leaving the farm. This highlights the importance of identifying emission sources at the farm level and understanding the processes that generate them. A deeper understanding of these sources and mechanisms provides valuable opportunities to develop effective mitigation strategies (Rotz, 2018).

As shown by recent data from the U.S. Department of Agriculture in Figure 1, the agricultural sector contributes roughly 10% of total emissions, highlighting its significant role in influencing climate patterns (Polymeni et al., 2022).

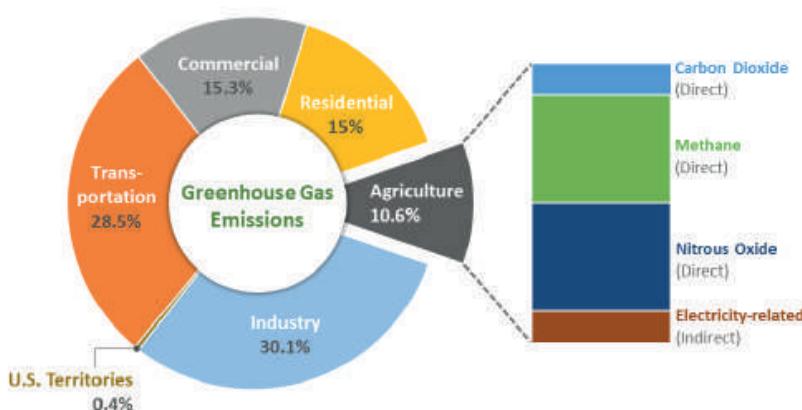


Figure 1. Global GHG emissions (Polymeni et al., 2022)

The main contributors to GHG emissions on dairy farms are CH<sub>4</sub> released through enteric fermentation and manure, N<sub>2</sub>O emitted from soils, crop residues, manure, and fertilizers, and CO<sub>2</sub> generated from soil processes, fuel combustion, on-farm energy consumption, and the production of agricultural inputs (Le, 2018). Although these sources are often analyzed independently, interactions between them can influence the total amount of emissions (Rotz, 2018). Feed management plays a key role in reducing enteric CH<sub>4</sub> production, which is the largest single contributor to GHG emissions on dairy farms (Boadi et al., 2004; Le, 2018). According to a life

cycle assessment by McGeough et al. (2012), 64% of emissions originate from lactating cows, 20% from dry cows and pregnant heifers, and 10% from animals less than one year old.

Extensive efforts have been made to measure GHG emissions from individual sources on dairy farms; however, monitoring and simultaneously quantifying all emissions from an entire farm or production system is both technically challenging and prohibitively expensive. As a result, the use of modeling approaches is necessary to estimate and evaluate GHG emissions from dairy production systems. These models can range from simple emission factor calculations to highly detailed process-level simulations (Rotz, 2018). Identifying effective mitigation strategies is crucial to reducing farm-level emissions, especially as agricultural production continues to intensify (Chianese et al., 2009). Dairy farming plays a significant role in driving climate change, largely because of  $\text{CH}_4$  released through enteric fermentation and  $\text{N}_2\text{O}$  generated during manure management. However, implementing sustainable practices such as optimized grazing systems and soil conservation strategies can enhance carbon sequestration, partially offsetting these emissions and highlighting the sector's potential contribution to reducing its environmental footprint (Neethirajan, 2024).

The different livestock related activities and their contribution to existing GHG pool are described in Figure 2.

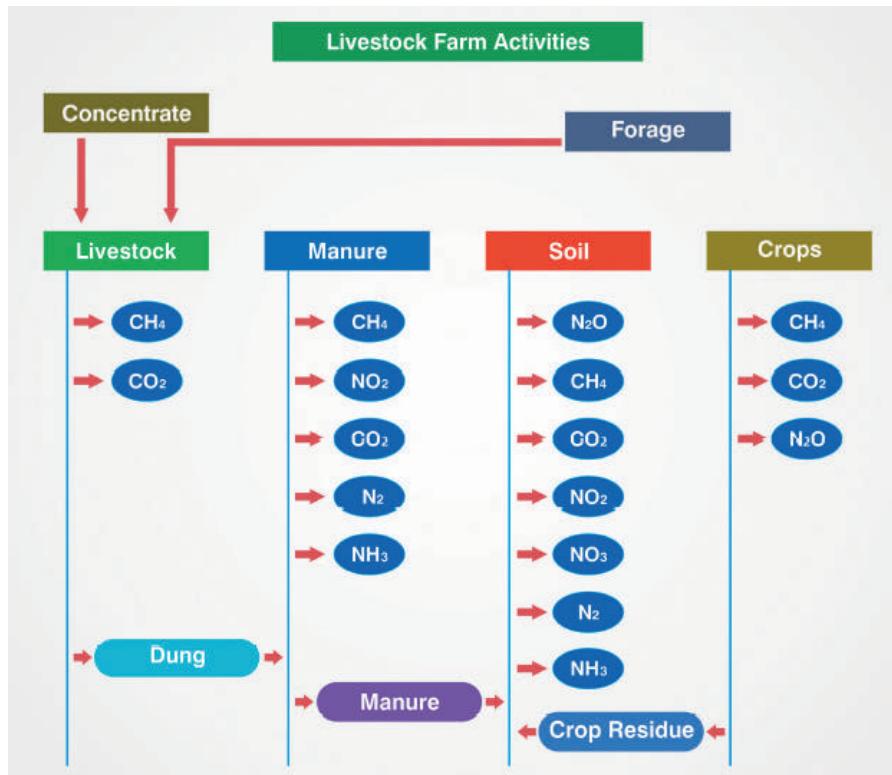


Figure 2. Pictorial representation of different livestock related activities and their contribution to GHG pool (Jose et al., 2016)

### 3. Emission Sources

The primary GHGs emitted from agriculture are  $\text{CH}_4$ ,  $\text{CO}_2$ , and  $\text{N}_2\text{O}$ . These gases vary in their global warming potential, or their capacity to retain heat in the atmosphere.  $\text{CH}_4$  is approximately 20 times more potent than  $\text{CO}_2$ , while  $\text{N}_2\text{O}$  is nearly 300 times more effective at trapping heat. Together,  $\text{CH}_4$  and  $\text{N}_2\text{O}$  account for more than 95% of total agricultural GHG emissions.  $\text{CO}_2$  is primarily released through soil cultivation, electricity consumption, and fuel combustion;  $\text{CH}_4$  originates from enteric fermentation and manure decomposition; and  $\text{N}_2\text{O}$  is emitted during the breakdown of fertilizers, manure, and crop residues (Le, 2018). Many of these emission pathways are regulated by microbial processes, which are influenced by the optimal temperature range of the specific microorganisms involved (Chianese et al., 2009).

The various sources of GHGs from livestock farms are described in Figure 3.

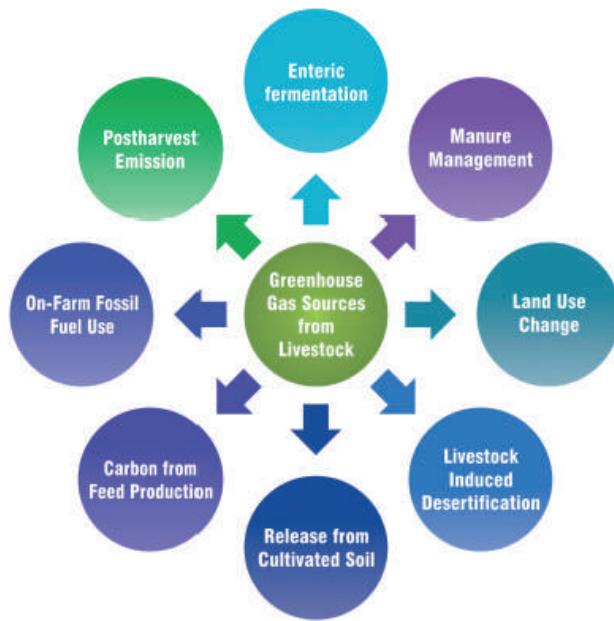


Figure 3. Different sources of GHGs from livestock farms (Jose et al., 2016)

### 3.1. Methane (CH<sub>4</sub>)

CH<sub>4</sub> is the second most important GHG after CO<sub>2</sub> and absorbs significantly more energy than CO<sub>2</sub>. Over a 100-year time horizon, its global warming potential is approximately 28 times higher than that of CO<sub>2</sub>. In addition, CH<sub>4</sub> contributes to indirect climate effects as a precursor to ozone, another potent GHG (Króliczewska et al., 2023). The primary source of CH<sub>4</sub> emissions is the anaerobic decomposition of organic matter. Interestingly, recent studies suggest that plants may also release small amounts of CH<sub>4</sub>, although the exact mechanisms behind this process remain unclear (Chianese et al., 2009).

CH<sub>4</sub>, one of the most potent GHGs, is a significant byproduct of ruminant digestion and manure management (Neethirajan, 2024). Methanogenesis is a key component of the global carbon cycle, contributing to both CO<sub>2</sub> and CH<sub>4</sub> emissions and thus shaping the overall carbon footprint (CF) of GHG-emitting systems (Ozlu et al., 2022). Domesticated ruminants including cattle, sheep, and goats naturally produce CH<sub>4</sub> during digestion,

with cattle and dairy cows accounting for nearly 72% of total CH<sub>4</sub> emissions from the sector in 2020 (Króliczewska et al., 2023), while buffalo and small ruminants accounted for 8.7% and 6.7%, respectively. Ruminants are unable to digest cellulose on their own; however, rumen microorganisms break down cellulose from forage, releasing hydrogen as a byproduct. Symbiotic enteric methanogens utilize this hydrogen to reduce CO<sub>2</sub> to CH<sub>4</sub>, preventing hydrogen accumulation (Chianese et al., 2009). Lactating cows emit roughly twice as much CH<sub>4</sub> as dry cows or heifers, primarily due to their higher feed intake, although diet composition and body size also play a role (Chianese et al., 2009). Consequently, researchers and livestock breeders worldwide are actively seeking strategies to simultaneously enhance animal productivity and mitigate CH<sub>4</sub> emissions, particularly from ruminants (Króliczewska et al., 2023). Additionally, manure applied to cropland surfaces can serve as an additional emission source (Sherlock et al., 2002).

In ruminant production systems, CH<sub>4</sub> represents the primary GHG emitted, with enteric fermentation in the rumen being the main contributor (Crosson et al., 2011). This process, driven by methanogenic archaea in the ruminant stomach, facilitates the breakdown of ingested feed but simultaneously produces CH<sub>4</sub> as a byproduct, which is mostly expelled into the atmosphere through eructation. As this mechanism is a fundamental aspect of ruminant physiology, it constitutes a consistent and largely unavoidable emission source within livestock systems (Polymeni et al., 2024). The overall CH<sub>4</sub> balance in agricultural systems is determined by the interplay between methanogenesis (CH<sub>4</sub> production) and methanotrophy (CH<sub>4</sub> oxidation). The relative strength of these processes dictates whether net CH<sub>4</sub> emissions are positive or negative. While methanogens are the microorganisms responsible for CH<sub>4</sub> production, methanotrophs are bacteria that oxidize and utilize CH<sub>4</sub> as an energy source (Bhattacharyya et al., 2020).

The amount of CH<sub>4</sub> generated by ruminants is influenced by multiple factors, such as animal species and body size, feed digestibility, and the intake of dry matter, total carbohydrates, and digestible carbohydrates (Chianese et al., 2009). Among these factors, feed quality plays a particularly critical role: diets high in fiber, especially cellulose, tend to increase CH<sub>4</sub> emissions. In addition to enteric fermentation, N<sub>2</sub>O is released during manure decomposition. When manure is stored in liquid systems, the organic material is subjected to anaerobic microbial activity, resulting in GHG emissions (Jose et al., 2016). During storage, CH<sub>4</sub> is produced through pathways similar to those in the rumen, as microbial cellulose degradation creates substrates for methanogenesis (Monteny et al., 2001; Chianese et

al., 2009). Therefore, strategies to mitigate CH<sub>4</sub> emissions must focus on reducing CH<sub>4</sub> generation without disrupting hydrogen removal, which is essential for maintaining efficient digestion (Clark et al., 2011).

### 3.2. Carbon Dioxide (CO<sub>2</sub>)

CO<sub>2</sub> is the primary GHG emitted from soils, originating from the decomposition of active organic matter that accumulates carbon through crop residues, roots, and root exudates, and is broken down by soil microorganisms (Ozlu et al., 2022). CO<sub>2</sub> enters agricultural systems via photosynthesis and is subsequently released through the respiration of plants (both shoots and roots), animals, and microorganisms decomposing organic matter from manure and crop residues (Chianese et al., 2009). In addition, CO<sub>2</sub> emissions in agricultural practices are largely linked to the use of fossil fuels in farm machinery and equipment, where combustion generates substantial amounts of CO<sub>2</sub> into the atmosphere (Polymeni et al., 2024).

Both livestock and manure management are notable sources of CO<sub>2</sub> emissions on dairy farms. CO<sub>2</sub> is naturally released through animal respiration, forming a crucial component of the carbon cycle that begins with the photosynthetic fixation of carbon by plants. When animals consume plant-derived feed, much of the carbon captured by crops is returned to the atmosphere as CO<sub>2</sub> through respiration (Kirchgessner et al., 1991). Additionally, long-term manure storage can contribute significantly to CO<sub>2</sub> emissions, with production levels largely influenced by aerobic microbial activity. Solid manure stored in piles allows for greater oxygen penetration, which enhances microbial respiration and results in higher CO<sub>2</sub> emissions compared to high-moisture manure stored under anaerobic conditions in tanks. Research indicates that tillage systems and crop management practices can strongly influence carbon sequestration in agricultural soils (Chianese et al., 2009). Moreover, farming practices directly and indirectly affect CO<sub>2</sub> emissions by altering soil health and structure. For instance, the use of both organic and inorganic fertilizers has been identified as a significant source of GHG emissions in agricultural systems (Ozlu et al., 2022).

CO<sub>2</sub> emissions tend to increase with higher temperatures, reaching their peak during the summer, while decreasing at lower temperatures and falling to their lowest levels in snow-covered winter periods, indicating higher CFs during the growing season (Ozlu et al., 2022). In addition to temperature, soil moisture has been identified as a key factor influencing CO<sub>2</sub> emissions. Other important drivers of CO<sub>2</sub> release from soil to the atmosphere include

soil pH, temperature, and management practices such as tillage, drainage, fertilization methods, crop rotation, crop type, residue management, and the use of soil amendments (Bhattacharyya et al., 2020).

### 3.3. Nitrous Oxide ( $\text{N}_2\text{O}$ )

$\text{N}_2\text{O}$  is a significant GHG, even at low concentrations, due to its much higher global warming potential compared to  $\text{CH}_4$  and  $\text{CO}_2$ , and it also contributes to stratospheric ozone depletion (Ozlu et al., 2022).  $\text{N}_2\text{O}$  emissions occur primarily through two microbial pathways: nitrification and denitrification (Bhattacharyya et al., 2020). Nitrification is an aerobic process in which ammonium ( $\text{NH}_4^+$ ) is oxidized to nitrate ( $\text{NO}_3^-$ ), with  $\text{N}_2\text{O}$  and  $\text{N}_2$  produced as intermediate compounds. Therefore,  $\text{N}_2\text{O}$  emissions are influenced by both nitrification and denitrification processes. Denitrification can act as either a source or a sink for  $\text{N}_2\text{O}$ , as it is an intermediate product in the reduction pathway (Chianese et al., 2009; Soussana et al., 2010).

Recently, the agricultural sector has promoted the replacement of chemical inputs with organic or biodynamic growing methods. The use of chemical fertilizers in agriculture has been shown to increase GHG emissions, particularly  $\text{N}_2\text{O}$ , thereby exacerbating climate change concerns. Consequently, agricultural practices need to be reviewed and adapted to become more environmentally sustainable (Ozlu et al., 2022).  $\text{N}_2\text{O}$  emissions are closely associated with the wetting of dry soils and the application of nitrogen via manure or fertilizers (Chianese et al., 2009). These emissions may originate directly from stored manure, organic or inorganic fertilizers applied to soil, or nitrogen deposition by grazing animals (Crosson et al., 2011). In most agricultural soils, the biogenic formation of  $\text{N}_2\text{O}$  is enhanced by increased availability of mineral nitrogen, which stimulates both nitrification and denitrification. Therefore, the addition of nitrogen through fertilizers, manures, or other nitrogen-rich wastes generally promotes  $\text{N}_2\text{O}$  emissions, with the magnitude influenced by soil conditions at the time of application (Soussana et al., 2010).  $\text{N}_2\text{O}$  emissions from manure depend on its nitrogen and carbon content, the duration of storage, and the type of treatment (Jose et al., 2016).

$\text{N}_2\text{O}$  emissions from livestock systems are generally low but not negligible. Annual emissions from manure storage are typically between 0 and 0.1 kg  $\text{N}_2\text{O}$  per cubic meter of stored manure, with most emissions arising from stacked solid manure or slurry with a surface crust or other conditions that encourage aerobic microbial activity. In contrast, liquid or slurry manure without a crust generally produces minimal  $\text{N}_2\text{O}$  emissions.

Livestock housing also contributes to  $\text{N}_2\text{O}$  release, primarily from manure on barn floors, with average annual emissions estimated at around 0.3 kg  $\text{N}_2\text{O}$  per livestock unit (Chianese et al., 2009). When nitrogen fertilizers are applied to soils, particularly for crops with high nitrogen requirements, they undergo complex microbial transformations. During nitrification, soil microorganisms convert ammonia-based fertilizers to nitrate ( $\text{NO}_3^-$ ) under aerobic conditions, releasing  $\text{N}_2\text{O}$  as a byproduct. Under anaerobic conditions, denitrification further reduces nitrate to gaseous forms, including  $\text{N}_2\text{O}$ . As agricultural production expands to meet rising global food demand, the use of synthetic fertilizers has increased significantly, contributing to elevated soil  $\text{N}_2\text{O}$  emissions (Polymeni et al., 2024). These emissions are often concentrated in localized “hot spots,” such as urine patches or areas with fertilizer granules and crop residues, even when nutrient applications are evenly distributed (Flechard et al., 2007).

#### 4. Enteric Fermentation

Enteric fermentation is a complex biological process that cannot be fully described by a single equation. To predict  $\text{CH}_4$  emissions from ruminants across different diets, models have been developed that simulate nutrient digestion, absorption, and passage through the rumen and hindgut (Ellis et al., 2001). This process is a significant contributor to GHG emissions in milk production, accounting for roughly 37% of total anthropogenic  $\text{CH}_4$  emissions (Rotz and Thoma, 2017). These emissions are inevitable, as they result from the normal digestive processes of dairy cows. The quantity and composition of farm animals, particularly those not used for milk production, influence enteric  $\text{CH}_4$  emissions; a higher proportion of non-lactating animals leads to greater GHG emissions per litre of milk produced. Additionally, the type of feed, the physiological state of the animals, and their level of production all significantly affect the amount of enteric  $\text{CH}_4$  generated (Singaravelan et al., 2023).

$\text{CH}_4$  is generated during enteric fermentation as a byproduct of carbohydrate breakdown by methanogenic microorganisms in the rumen. The produced  $\text{CH}_4$  is released by the animal primarily through eructation, contributing to GHG emissions (Jose et al., 2016). Estimates of enteric  $\text{CH}_4$  from livestock are generally based on average daily feed intake, expressed as gross energy (GE; MJ/d), and corresponding  $\text{CH}_4$  conversion rates. In livestock reporting, cattle are typically categorized into dairy cows and other types, with the latter further classified by sex, age, and feeding regimen (Crosson et al., 2011). One nutritional strategy with the potential to reduce enteric  $\text{CH}_4$  emissions is partially replacing grass silage with maize silage in

the diet. Dijkstra et al. (2018) reported that substituting 50% of grass silage with maize silage in a diet consisting of approximately 70% grass silage and 30% concentrates can decrease enteric CH<sub>4</sub> production by around 8%. At the individual animal level, this strategy provides a practical approach for mitigating GHG emissions.

Enteric CH<sub>4</sub> mitigation has been extensively studied, and several well-established strategies have been developed for practical use on farms. These include the supplementation of ionophores, incorporation of dietary lipids, improvement of forage quality, and increased inclusion of grains in ruminant diets (Boadi et al., 2004). For instance, feeding monensin an ionophore has been shown to lower enteric CH<sub>4</sub> emissions by approximately 20% in dairy cattle (Sauer et al., 1998). These mitigation approaches work primarily by inhibiting methanogenic archaea or by redirecting hydrogen ions away from methanogenesis pathways, thereby reducing CH<sub>4</sub> formation (Boadi et al., 2004).

Enteric fermentation and urinary nitrogen are the primary sources of CH<sub>4</sub> and N<sub>2</sub>O emissions in ruminant systems (Waghorn, 2008). Enteric CH<sub>4</sub> contributes approximately 78–86% of total CH<sub>4</sub> emissions, with the remainder originating from manure management (McGeough et al., 2012). The model developed by Mills et al. (2003), which represents enteric CH<sub>4</sub> production as a function of metabolizable energy intake and the starch-to-fiber ratio, has proven effective for predicting CH<sub>4</sub> emissions across a wide range of forage-based diets (Stackhouse-Lawson et al., 2012). In addition to CH<sub>4</sub>, cattle also produce enteric N<sub>2</sub>O as part of their normal nitrogen metabolism (Hamilton et al., 2010).

## 5. Carbon Footprint (CF)

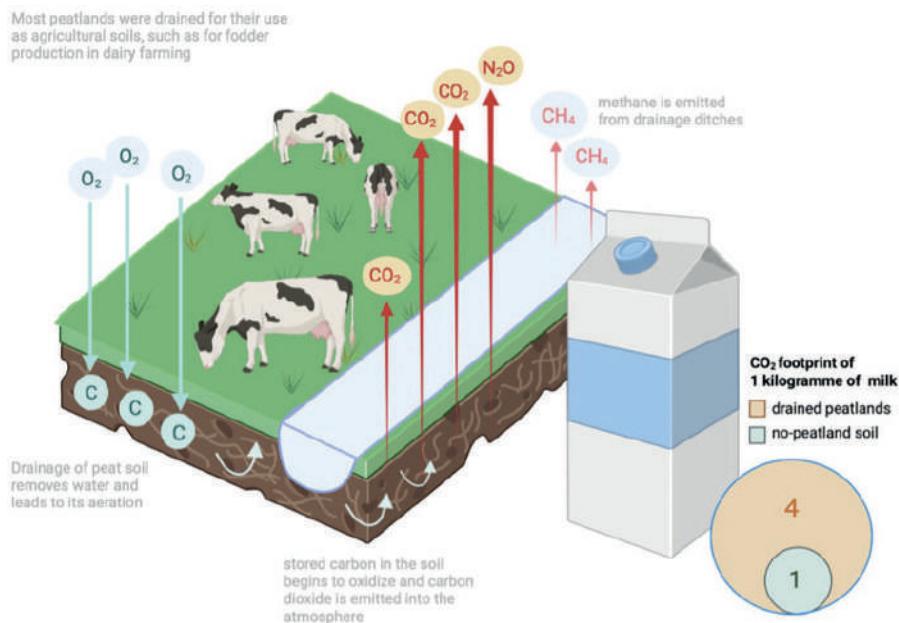
More specifically, the CF quantifies the total amount of GHGs produced both directly and indirectly through agricultural activities or throughout the entire life cycle of a product. It provides a comprehensive measure encompassing all GHG emissions linked to production, including CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O, originating from sources such as fuel use, livestock rearing, fertilizer application, and the decomposition of crop residues (Polymeni et al., 2024).

The emission sources from agricultural and dairy farming drained peatlands and their potential impacts on dairy products are illustrated in Figure 3.

The farming stage plays a critical role in determining the CF of most food products, as approximately 70–90% of total emissions occur before the

products leave the farm gate (Hermansen and Kristensen, 2011). Evaluating agricultural CFs can serve as a valuable tool for managing farming practices to mitigate GHG emissions and, consequently, climate change. However, efforts to reduce GHG emissions should be implemented while ensuring that soil health and quality are maintained or enhanced (Ozlu et al., 2022).

To determine whether dairy cow nutrition can significantly reduce the CF, it is first necessary to define what constitutes the CF and clarify what is considered a “meaningful” reduction. Subsequently, the potential impact of implementing GHG mitigation strategies related to animal nutrition must be evaluated. The primary GHGs contributing to CF on dairy farms are CH<sub>4</sub> originating from enteric fermentation and manure management and N<sub>2</sub>O resulting from manure handling and feed production. Since nutritional interventions are expected to mainly influence ruminal fermentation, this analysis focuses on strategies for mitigating enteric CH<sub>4</sub> in relation to CF (Hristov et al., 2013).



*Figure 4. Sources of emissions from drained peatlands used for agriculture and dairy farming and their potential influence on the CF of milk (Müller et al., 2025)*

Increasing milk yield, optimizing diets and feed efficiency, as well as improving manure and land management, are frequently highlighted as key strategies for mitigating the CF of milk production (Figure 4).



*Figure 4. Widely adopted strategies for mitigating the carbon footprint associated with milk production (Mueller et al., 2025)*

According to Sahu and Agarwal (2021), the dairy industry represents a significant source of anthropogenic GHG emissions when assessed through CF analysis. Studies conducted under different international standards and methodologies consistently identify enteric CH<sub>4</sub> as the primary contributor to emissions, followed by manure management and fertilizer production. Recommended strategies for reducing these emissions include providing balanced feed rations, minimizing the use of nitrogen-based fertilizers, and adopting alternative energy sources such as biogas. Ibidhi and Calsamiglia (2020) estimated the GHG emissions and CF of twelve dairy farms in Spain, reporting that CH<sub>4</sub> emissions were the largest component of total GHG emissions, with an average CF of 0.84 kg CO<sub>2</sub>e per kilogram of milk produced. Their findings indicated that management-based interventions were more effective than dietary modifications, achieving up to a 27.5% reduction in the CF. Hermansen and Kristensen (2011) observed that the CF per kilogram of milk declines substantially as milk production per cow increases, particularly up to 3,000–4,000 kg per cow per year. The higher CF at lower yields was primarily attributed to reduced feed efficiency in such systems, where a greater proportion of feed is allocated to non-productive uses such as maintenance, replacement stock, and draught power.

## 6. Mitigation Strategies for GHG Emission

### 6.1. Housing

Livestock housing management plays a significant role in influencing both CH<sub>4</sub> and N<sub>2</sub>O emissions. CH<sub>4</sub> output varies across production stages; for example, emissions from farrowing units are typically lower than those from fattening units because they are cleaned approximately every 27 days compared to 90–100 days in fattening houses (Sommer et al., 2009). In straw-flow housing systems, improved litter aeration can result in higher temperatures, which may increase CH<sub>4</sub> emissions. Nevertheless, implementing daily scraping practices has been shown to reduce CH<sub>4</sub> emissions by up to 50% (Philippe et al., 2007).

The design of animal housing facilities is an important factor when evaluating emissions from livestock operations (Chianese et al., 2009). In freestall barns, emissions from housing typically account for less than 5% of total farm emissions. By contrast, systems such as slatted floors or bedded pack facilities effectively combine housing with long-term manure storage, resulting in substantially higher emissions (Rotz and Thoma, 2017). Emission levels also depend on the manure management system employed. In freestall barns, manure is generally removed every few hours or at least once daily through scraping or flushing, which limits the time available for CH<sub>4</sub> and N<sub>2</sub>O formation, thereby keeping emissions relatively low (Rotz, 2018).

Freewalk housing systems represent an important step toward sustainable dairy farming by providing cows with a more natural, welfare-oriented living environment. Compared to conventional stall-based housing, cows in freewalk systems show reduced incidences of health problems such as lameness and mastitis. From an environmental perspective, these systems support natural cow behavior, which results in more even manure distribution and improved management, ultimately helping to lower ammonia emissions. Additionally, the installation of cooling systems in bedding areas can slow the decomposition of organic matter, thereby reducing CH<sub>4</sub> and ammonia emissions. The integration of automated bedding management technologies further enhances farm hygiene and supports emission mitigation by maintaining a clean, controlled environment for the animals (Neethirajan, 2024).

The adoption of renewable energy sources in farm operations plays a vital role in lowering GHG emissions and enhancing agricultural sustainability, not only by reducing carbon output but also by improving

energy independence (Ayaz et al., 2019). Furthermore, the implementation of precision agriculture technologies offers a significant opportunity to both mitigate GHG emissions and boost overall farm productivity (Balafoutis et al., 2017). In terms of animal management, strategies for reducing GHG emissions generally include improving animal productivity and health, shortening the time to puberty, reducing days on feed, and selecting animals with greater fertility through genetic improvement (Hristov et al., 2013). Enhancing per-animal production is another effective approach, as Gerber et al. (2011) reported that although higher milk yield per cow increases total emissions per animal, it simultaneously lowers GHG emissions per kilogram of fat-corrected milk.

## **6.2. Feeding Management**

The inclusion of concentrates in cattle diets can be an effective strategy to reduce nitrogen losses, as they lower ruminal pH and alter rumen activity (Klevenhusen et al., 2011). Another approach to mitigating nitrogen excretion involves reducing the overall protein intake of cattle. To ensure a minimum intake of 14% dry matter, oilseed meals can be incorporated to balance crude protein levels when legumes are not included in the forage component of the ration (Beauchemin et al., 2008). A primary objective of grassland management is to enhance the nutritional quality of forage consumed by grazing ruminants. Since  $\text{CH}_4$  production is strongly associated with fiber digestion in the rumen, decreasing the fiber content of forages is expected to lower  $\text{CH}_4$  emissions (Clark et al., 2011).

The implementation of the Total Mixed Ration (TMR) system or the use of ration formulation software can significantly enhance nutrient management, minimize dietary nitrogen surpluses, and ensure that energy requirements are properly balanced for different animal groups particularly monogastrics across various production stages (Basely and Hayton, 2007). Diets containing higher levels of concentrates, greater proportions of legumes, tannin-rich forages, or elevated lipid concentrations have all been associated with a reduction in  $\text{CH}_4$  emissions when expressed per kilogram of dry matter intake (Clark et al., 2011). In a comprehensive review, Hristov et al. (2013) recommended feed management practices for mitigating GHG emissions, including the use of tannins, lipids, increased grain proportions, high-quality forage, and processed feeds. Tannins plant-derived bioactive compounds have gained recognition for their ability to reduce enteric  $\text{CH}_4$  emissions in dairy cattle by binding to rumen proteins, thereby reducing protein degradation and subsequent ammonia production. However, their inclusion must be carefully managed to prevent potential negative

effects on feed intake and nutrient utilization (Neethirajan, 2024). Fat supplementation in ruminant diets not only lowers CH<sub>4</sub> emissions but has also been shown to decrease ammonia (NH<sub>3</sub>) emissions from fresh manure by approximately 14% (Machmüller et al., 2003). Nevertheless, since fat and grain levels in North American dairy rations are already high, further increases have limited potential for additional GHG reduction (Lee and Sumner, 2014). Moreover, excessive dietary protein contributes to elevated nitrogen excretion and ammonia emissions. Optimizing protein levels to meet but not exceed the physiological requirements of cattle can help mitigate CH<sub>4</sub> production and reduce the environmental burden associated with nitrogen losses (Neethirajan, 2024).

An effective alternative for reducing farm GHG emissions is the use of high-quality forages, which are less expensive than grain and require less fuel for cultivation, thereby potentially lowering production costs and net emissions (Johnson et al., 1996). Diets with higher fiber content increase acetate production in the rumen, which is associated with greater CH<sub>4</sub> output. Incorporating grain into a forage-based diet and selecting forages with lower fiber content increases starch intake and decreases fiber intake, leading to lower ruminal pH and a shift in fermentation toward propionate rather than acetate, ultimately reducing CH<sub>4</sub> emissions (Burbi, 2014). Conversely, feeding diets high in cellulosic material has been shown to increase enteric CH<sub>4</sub> emissions (Króliczewska et al., 2023). In pasture-based systems, grazing on younger pastures, especially those containing legume-grass mixtures with lower fiber content, increases the rate of passage and consequently reduces CH<sub>4</sub> production (Robertson and Waghorn, 2002).

Feeding rations with lower fiber and higher energy content can significantly influence enteric fermentation; however, excessive reductions in fiber may compromise the economic and physiological benefits of proper rumination. The inclusion of concentrates lowers ruminal pH, which in turn decreases CH<sub>4</sub> production. High-fiber dried grains, such as barley, contain more fiber compared to maize or wheat and therefore have a lower capacity to reduce ruminal pH and CH<sub>4</sub> emissions, though they are generally more affordable (Chianese et al., 2009). Lee et al. (2004) reported that enteric CH<sub>4</sub> emissions can be significantly reduced when diets contain a high proportion of white clover, but under practical conditions where white clover typically comprises less than 20% of the diet, the effect is negligible. Silage quality defined by its fermentation profile and nutrient composition plays a crucial role in shaping ruminal fermentation dynamics. Thus, improving silage production and management practices is essential for minimizing the environmental footprint of dairy farming (Neethirajan,

2024). Moreover, incorporating fat supplements into cattle diets can lower total GHG emissions by approximately 8–14% (Petersen et al., 2013). Finally, efforts to reduce energy and water consumption not only contribute to emission reduction but also promote sustainable and cost-effective farm management (Pretty et al., 2018).

### **6.3. Grazing and Pasture Management**

Grasslands represent the natural climax vegetation in regions where rainfall is insufficient to support forest growth. In contrast, in areas with higher precipitation, grasslands are not the dominant climax vegetation and tend to be more productive. Rangelands, which are found on every continent, are characterized by low-growing vegetation shaped by temperature and moisture limitations (Soussana et al., 2010). Livestock play a crucial role in maintaining these grassland ecosystems, thereby preventing land-use changes that could lead to increased carbon emissions. Moreover, they may contribute to soil carbon sequestration: studies on temperate, unfertilized grasslands where cattle are reared without supplemental feed or fertilizers indicate that the carbon sequestered in the soil can offset their CH<sub>4</sub> and N<sub>2</sub>O emissions (Garnett, 2009). Thus, the carbon sequestration potential of grasslands and rangelands offers an opportunity to partially mitigate GHG emissions from the livestock sector (Soussana et al., 2010).

Grazing livestock, especially those consuming legume-based pastures, excrete a greater proportion of ruminal ammonia (NH<sub>3</sub>) as urea in their urine (Arelovich et al., 2003). This ammonia originates from the breakdown of non-protein nitrogen during digestion. Once excreted, the nitrogen in urine is transformed into nitrate (NO<sub>3</sub><sup>-</sup>) through soil nitrification processes, which can then be converted to N<sub>2</sub>O via denitrification. This biochemical pathway explains the strong link between urine deposition by grazing ruminants and elevated N<sub>2</sub>O emissions (Menner et al., 2005).

The implementation of rotational grazing affects both the duration and intensity of grazing. Specifically, relocating animals every 1–2 days can significantly influence CH<sub>4</sub> and N<sub>2</sub>O emissions. Shortening grazing periods offers multiple benefits: it encourages animals to feed on younger, lower-fiber pasture, potentially reducing CH<sub>4</sub> emissions by up to 22%; additionally, when combined with decreased fertilizer application, it limits nitrogen availability in the pasture, thereby reducing N<sub>2</sub>O emissions by up to 10% (DeRamus et al., 2003; Schils et al., 2006). Furthermore, lowering grazing density helps prevent overgrazing and soil compaction, supporting overall pasture health (Saggar et al., 2007).

#### 6.4. Manure Storage and Treatment

Livestock manure represents both an asset and a challenge in agricultural systems. It provides essential nutrients to the soil; globally, approximately 22% of applied nitrogen and 38% of phosphate originate from animal manure, with over half contributed by beef cattle (Garnett, 2009). However, manure also accounts for up to 10% of total GHG emissions from livestock worldwide (Symeon et al., 2025). Typically, manure is excreted in slurry form, and separators can be employed to partition solids from liquids, enabling separate storage and handling. When manure is deposited on open lots, it dries and forms solid material. These solids may be stored in stacks without treatment or periodically turned to encourage composting (Rotz, 2018).

Anaerobic conditions in liquid or slurry manure favor  $\text{CH}_4$  production, while  $\text{N}_2\text{O}$  emissions remain low unless a crust develops on the surface. Particles of bedding and undigested feed can rise to the surface, forming a crust in which alternating aerobic and anaerobic conditions facilitate incomplete denitrification, leading to  $\text{N}_2\text{O}$  formation (Rotz, 2018). Manure contains considerable amounts of inorganic nitrogen, microbially available carbon, and water, making it a valuable resource if managed correctly. At the same time, these components serve as substrates for microbial generation of  $\text{CH}_4$  and  $\text{N}_2\text{O}$ , two potent GHGs contributing to climate change (Symeon et al., 2025). The magnitude of emissions from manure storage and treatment is influenced by the quantity produced, its carbon and nitrogen content, the proportion decomposing anaerobically, and the storage duration, temperature, and method. Generally, liquid (primarily anaerobic) systems emit more  $\text{CH}_4$ , whereas solid systems tend to produce more  $\text{N}_2\text{O}$  (Crosson et al., 2011).

Manure management is a key aspect of farm nutrient budgeting and is closely linked to fertilizer application. Livestock manure consists primarily of organic material and water. In anaerobic environments, the organic fraction of cattle manure is decomposed by facultative and anaerobic bacteria, resulting in the production of  $\text{CH}_4$ ,  $\text{CO}_2$ , and stabilized organic matter as byproducts. On-farm GHG emissions are influenced by manure management practices, the volume of manure produced, and whether the manure is in solid or liquid form (Singaravelan et al., 2023). Manure is produced daily by farm animals, with composition and quantity varying according to animal type, production stage, production system, and diet. For instance, a cow typically generates around 29 kg of manure per day. Manure can be classified based on its dry matter content into liquid, slurry, and solid

forms. The main nutrients contained in manure include carbon (C), nitrogen (N), phosphorus (P), sulfur (S), and potassium (K), the concentrations of which depend heavily on the animal's diet and physiological characteristics (Symeon et al., 2025).

There are several strategies available to mitigate manure-related emissions. Manure not only represents a significant portion of GHG emissions from dairy farms, but it also contains a large fraction of nutrients from feed. For instance, only about one-third of the nitrogen in feed is incorporated into the protein of animal products, while the remainder is excreted in urine and manure (Kirchgessner et al., 1994). Consequently, the energy content in manure can be harnessed through biogas production for electricity generation instead of being released into the atmosphere.

Recommended manure management practices include reducing dietary protein, separating solids, acidifying manure, matching application rates to crop needs based on soil and manure testing, and avoiding spreading during late fall, winter, or adverse weather conditions such as heat, wind, or rain (Hristov et al., 2013). Treatment methods for solid and liquid manure significantly influence emission rates: low temperatures, aeration, and composting help reduce  $\text{CH}_4$  emissions, while adding straw, using covers, and treating solid and liquid fractions separately can minimize  $\text{N}_2\text{O}$  losses. Furthermore, the frequency of manure removal, the type of bedding, the floor design, and regular flushing and cleaning of housing units all contribute to reducing  $\text{CH}_4$  and  $\text{N}_2\text{O}$  emissions from livestock facilities (Burbi et al., 2016).

It is crucial to note that controlling gaseous emissions from manure depends on regulating the compost pile's temperature and maintaining a balance between aerobic and anaerobic conditions. Compaction can reduce ammonia ( $\text{NH}_3$ ) emissions by up to 90% and, to a lesser extent,  $\text{N}_2\text{O}$  emissions by approximately 30%.  $\text{CH}_4$  emissions are generally lower during colder months but may increase substantially in warmer periods (Chadwick, 2005). Adding water can decrease the free air space by 20–60%. Separating the liquid and solid fractions of manure offers several advantages for storage management. Storing these fractions separately enables better control of factors influencing emissions, such as temperature, moisture content, aeration, and covering. Additionally, separation can enhance slurry storage capacity and result in fractions with higher nutrient concentrations. However, the reduction of ammonia and  $\text{CH}_4$  emissions particularly from the solid fraction is most effective when slurry separation is combined with lower storage temperatures and solid-cover solutions. Storing the liquid

fraction under anaerobic conditions prevents the nitrification of NH<sub>3</sub> to nitrate (NO<sub>3</sub><sup>-</sup>), thereby minimizing denitrification processes that produce N<sub>2</sub>O (Fangueiro et al., 2008).

### 6.5. Manure Application to Soil

When applying manure to soils, careful consideration of both timing and application rates is essential to prevent excessive nitrogen input. Different application techniques have also been shown to help reduce soil emissions (Burbi et al., 2016). Manure can either be injected beneath the soil surface or incorporated into the soil immediately after application through tillage. Incorporating manure into the soil minimizes emissions during application and can be estimated using an emission factor or process-based simulation for any manure remaining on the surface (Rotz et al., 2011). In general, adding nitrogen from fertilizers, manure, or other readily mineralizable sources stimulates N<sub>2</sub>O emissions, with the magnitude influenced by prevailing soil conditions. N<sub>2</sub>O losses under anaerobic conditions are typically more significant than those from nitrification under aerobic conditions (Skiba and Smith, 2000). Soil N<sub>2</sub>O emissions often occur in localized 'hot spots' such as urine patches or fertilizer and residue particles, even when manure and fertilizers are applied diffusely (Flechard et al., 2007). CH<sub>4</sub> emissions from well-drained grassland soils, however, are generally negligible (Hendriks et al., 2007).

GHG emission processes continue after both manure and inorganic fertilizers are incorporated into the soil. Three key processes influence GHG emissions from cropland and pasture: oxidation, nitrification, and denitrification (Rotz, 2018). In most soil conditions, atmospheric CH<sub>4</sub> is oxidized to CO<sub>2</sub>, thereby acting as a net sink for CH<sub>4</sub> (Boeckx et al., 1997). A review of published data for crops commonly grown on dairy farms indicated an average annual CH<sub>4</sub> absorption of approximately 1.5 kg CH<sub>4</sub> per hectare (Chianese et al., 2009).

### 6.6. Disease Control

Animal health plays a crucial role in determining how efficiently animals utilize resources such as feed and water. Poor health typically leads to increased resource inputs, including medications, while reducing productivity. Consequently, there is a direct positive relationship between good animal health and production efficiency, which is expected to result in lower GHG emissions (Kyriazakis et al., 2024).

Mastitis has been identified as a condition that can increase GHG emissions in the dairy sector, primarily due to reduced production and higher rates

of involuntary culling. Lowering somatic cell count (SCC), an indicator of mastitis, from 800,000 to 50,000 cells/ml has the potential to decrease GHG emission intensity by 3.7% (Gülzari et al., 2018). Additionally, a reduction of 18% in subclinical mastitis and 17% in clinical mastitis (CM) incidence was shown to reduce herd-level GHG emissions by 2.5%. However, there remains a significant knowledge gap regarding how different animal health conditions affect feed intake and utilization, which limits accurate estimation of GHG emissions from feed conversion (Mackenzie and Kyriazakis, 2021).

### **6.7. Genetic Selection**

Genetic improvement in livestock represents a highly effective approach, producing permanent and cumulative enhancements in performance (Wall et al., 2010). One key strategy involves selecting or genetically adapting breeds that are better suited to heat stress. Breeds that sustain productivity under warmer conditions and exhibit traits such as heat tolerance and disease resistance may provide viable pathways for climate change adaptation (Neethirajan, 2024). Reducing GHG emissions in livestock production offers clear societal benefits by helping to mitigate the impacts of climate change. However, these benefits will only materialize if producers themselves perceive tangible advantages. For genomic selection strategies designed to enhance feed efficiency and decrease CH<sub>4</sub> emissions to be widely adopted, the economic incentives for farmers must be evident. In the past decade, genomic advancements have facilitated more precise selection of animals for improved feed efficiency and lower GHG emissions, as identifying these traits at the genotypic level is relatively cost-effective (Hailu, 2018).

Animal selection is inherently a long-term process, and choosing animals with lower CH<sub>4</sub> emissions represents a promising strategy for the future (Króliczewska et al., 2023). In dairy cattle, a slightly different approach has focused on selecting animals with reduced residual feed intake, as lower feed consumption generally results in decreased enteric CH<sub>4</sub> emissions due to the direct correlation between feed intake and CH<sub>4</sub> production (Clark et al., 2011). Consequently, improving the productivity of low-producing animals tends to have a relatively greater impact on reducing emissions, whereas enhancing productivity in high-producing animals has a smaller effect (Króliczewska et al., 2023). Genetic selection has also led to physiological modifications that influence the rumen, feeding behavior, rumen outputs, and overall body composition of livestock (Rowe et al., 2013). Conducting a genetic selection program requires the collection of thousands of measurements, ideally on a weekly basis. The complexity of this process is further heightened by the diversity of grazing systems, which

vary according to climate, plant species, soil type, and livestock management practices, including continuous grazing, rest-rotation grazing, deferred rotational grazing, and intensive grazing strategies. Therefore, developing reliable biomarkers through animal selection is essential to accurately estimate CH<sub>4</sub> production across diverse farm systems (Manzanilla-Pech et al., 2021).

Integrating data from multiple studies and countries can facilitate the creation of an accurate genomic reference database and enable the development of precise genetic parameters for CH<sub>4</sub> traits. Achieving this requires genotyping and phenotyping a sufficiently large population of animals, with the resulting data made publicly accessible (de Haas et al., 2021). Basarab et al. (2013) indicate that improving feed efficiency through selective breeding provides an indirect means of lowering enteric CH<sub>4</sub> emissions in both beef and dairy cattle. In a similar vein, Goddard et al. (2016) show that adopting genomic selection to enhance feed efficiency not only offers economic benefits for the beef industry but also generates environmental advantages for society. Biotechnological approaches, such as genomic selection targeting improved feed efficiency and reduced CH<sub>4</sub> emissions, have substantial potential to achieve these objectives within the livestock sector. For these strategies to be successfully implemented, the economic and environmental benefits must be clearly established, and widespread adoption is crucial (Hailu, 2018).

## 7. Conclusions

Climate change has profoundly affected nearly all aspects of agriculture, particularly the livestock sector, resulting in significant global environmental and economic challenges. Animal-derived foods generally have a substantially higher carbon footprint compared to plant-based foods, making livestock production a key focus for climate mitigation efforts. As a result, policies targeting GHG reductions from livestock have become increasingly common in recent years. Dairy farming is a major contributor to GHG emissions across the entire life cycle of milk and dairy products. Therefore, reducing emissions from dairy cattle remains a central objective in minimizing the agricultural sector's overall carbon footprint. Potential mitigation strategies include improved feed and barn management, optimized grazing and pasture systems, enhanced manure storage and treatment, efficient manure application, disease prevention, and genetic selection. Given the complexity of dairy farming systems, it is essential to account for emissions at the whole-farm level rather than focusing solely on individual components, as emission reductions in one area may inadvertently increase emissions elsewhere.

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## Sustainability and Environmental Impacts in Dairy Cattle Farming in Türkiye

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### Abstract

The dairy cattle sector in Türkiye is a strategically important component of animal production and therefore requires the establishment of sustainability in economic, social, and environmental dimensions. While the average lactation yield per cow in European Union countries is approximately 6.5 tons, this value in Türkiye has remained around 3 tons for many years. However, due to the increase in the number of purebred and crossbred cows over the last decade, the average milk yield has improved; in 2012, the yield was 2.5 tons/year, rising to around 3.2 tons/year in 2019. Although this trend is positive in terms of reducing the environmental load, it remains well below EU averages. The main constraints on the sector's sustainability performance are the small-scale farm structure, high feed costs, fluctuations in raw milk prices, lack of environmental infrastructure, and limited access to modern technology. The inability of small farms to invest in modernization, the rapid increase in feed prices compared to inflation, and the decline in the number of dairy cows threaten the economic sustainability of the sector. From an environmental perspective, manure management and pasture utilization remain significant problems, especially in small-scale farms. To achieve sustainability, it is essential to promote economies of scale through cooperatives and producer organizations, increase forage crop production, expand pasture rehabilitation programs, encourage the adoption of environmentally friendly technologies, and strengthen training and extension activities. Integrated policies in this regard will not only enhance producer welfare but also support the sector's long-term competitiveness.

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## **1. Introduction: The Importance of Dairy Cattle Farming in Türkiye**

Dairy cattle farming holds strategic importance in Türkiye's agricultural sector and provides the rural economy with high-value-added food and employment opportunities. For many rural households, dairy production is the main source of income, and it contributes notably to the national economy (Turan et al., 2017). Türkiye ranks among the top ten countries in the world for annual raw milk production; for example, total milk output reached 22.96 million tons in 2019, and more than 90% of this was cow's milk (Semerci et al., 2020). In 2022, raw milk production was recorded at about 21.56 million tons (Demirel, 2025). The dairy sector is considered critical not only for nutrition, but also for rural development and food security. In Türkiye, dairy cattle farming is carried out largely by family farms, most of which are small-scale: in more than 75% of dairy farms, there are 1–6 milking cows (Bulut, 2025). While this structure highlights the sector's significant contribution to rural employment, it also presents certain challenges to productivity and competitiveness (Güven, 2024).

Dairy cattle production in Türkiye has grown significantly in recent years. According to official statistics, annual cow's milk production rose from 8.9 million tons in 2000 to 16.9 million tons in 2014, an 87.7% increase; during the same period, the total cattle inventory increased from 11.2 million to 14.1 million. This upward trend continued through the 2010s, and the number of milking cows steadily increased from the early 2010s to 2019. However, the sector faced fluctuations entering the 2020s. Especially in 2021–2022, rising input costs and market volatility led to many dairy cows being withdrawn from production and sent to slaughter, resulting in a decline in the dairy cattle population. The year-by-year change in cattle numbers is presented in Table 1 (TEPGE 2023; TUIK, 2024).

Table 1. Cattle numbers in Türkiye by year (TUİK, 2025)

Türkiye Cattle Inventory (Head)						
Years	Cattle - Purebred	-	Cattle - Crossbred	-	Cattle - Native	Total
1991	1.253.865		4.033.375		6.685.683	11.972.923
1995	1.702.000		4.776.000		5.311.000	11.789.000
2000	1.806.000		4.738.000		4.217.000	10.761.000
2005	2.354.957		4.537.998		3.633.485	10.526.440
2010	4.197.890		4.707.188		2.464.722	11.369.800
2015	6.385.343		5.733.803		1.874.925	13.994.071
2018	8.419.204		7.030.297		1.593.005	17.042.506
2019	8.559.855		7.554.625		1.573.659	17.688.139
2020	8.838.498		7.594.127		1.530.274	17.962.899
2021	8.824.784		7.641.100		1.384.659	17.850.543
2022	8.295.825		7.324.866		1.231.265	16.851.956
2023	8.070.159		7.303.667		1.047.430	16.421.256

The place of dairy cattle in Turkish agriculture matters not only for production volume but also for productivity and quality. However, there is still room for improvement in the sector's efficiency. For example, while the average lactation yield per cow in European Union countries is approximately 6.5 tons, in Türkiye it remained around 3 tons for many years (Demircan et al., 2006). Low yield raises the cost per unit of product, limits competitiveness, and increases environmental impact. In recent years, government policies have expanded beyond simply raising productivity to focus on the sector's sustainable development (Yilmaz and Ata, 2016). As a result of the support provided, both milk output and the number of dairy cows have increased significantly (Torgut et al., 2019).

Today, with growing production volume but productivity factors that still need improvement, Türkiye's dairy cattle sector requires a complete treatment of sustainability in all its dimensions. In this section, within the concept of sustainable agriculture, the environmental impacts of dairy cattle, the practices carried out in Türkiye, and strategies for the future will be evaluated.

## 2. The Concept of Sustainable Agriculture and Its Implications for Dairy Farming

Sustainable agriculture is an approach that aims to meet today's needs without harming the resources of future generations and while protecting

the natural balance. In general, sustainable agriculture focuses on producing sufficient, high-quality food at a reasonable cost while safeguarding soil, water, biodiversity, and the well-being of farmers (Ülger et al., 2024). This approach seeks to develop farm systems that are economically viable in the long term, environmentally sustainable, and socially equitable. Sustainability is not a single method; it encompasses various practices, including organic farming, good agricultural practices, and regenerative agriculture (Çukur and Saner, 2005). The ultimate goal is to maintain agricultural productivity while minimizing environmental damage, enhancing the quality of life for those working in farming, and ensuring the sustainability of rural communities.

The dairy sector is a crucial area for applying the concept of sustainable agriculture. This is because dairy cattle farming have a strong impact on sustainability in both economic terms (as a source of rural income and jobs) and environmental terms (greenhouse gas emissions, water, and land use). Sustainable dairy farming aims to meet the growing demand for animal protein while reducing its environmental footprint and protecting animal welfare (Çukur and Saner, 2005; Şengül et al., 2022). In this context, sustainability in dairy cattle is addressed under three main pillars:

- **Environmental sustainability:** Minimizing the negative effects of milk production on soil, water, and air, and aligning with nature's cycles. For example, managing pastures to avoid overgrazing, properly storing and using manure wastes, and adopting methods that reduce greenhouse gas emissions fall under this pillar.
- **Economic sustainability:** Keeping farms profitable and financially stable in the long run. The sustainability of dairy farms depends first on profitability; this requires lowering costs, increasing productivity, and building a structure that can withstand market volatility (e.g., reducing feed costs, investing in productivity-enhancing technologies) (Latruffe et al., 2016).
- **Social sustainability:** Improving the quality of life of farmers and workers, raising levels of training and organization, and ensuring animal welfare. Sustainability in dairy also encompasses protecting the health and well-being of individuals working in the sector and preserving the social fabric of rural communities (Lebacq et al., 2013).

From a sustainability perspective, increasing productivity in dairy cattle is a key strategic goal. Having milking cows that yield more milk per animal means fewer animals are needed for the same amount of milk; this

brings economic benefits and lowers the environmental impact per animal. In Türkiye, state policies have recently shifted from purely yield-focused support to building a competitive and sustainable dairy sector (Yılmaz and Ata, 2016; Başer, 2021). In line with this goal, measures such as promoting modern barns and milking systems, utilizing high-quality breeding stock, and supporting the production of feed crops have gained prominence. The sustainable agriculture mindset encompasses not only improving traditional methods with more eco-friendly practices but also utilizing innovative technologies. For example, solar-powered irrigation systems, smart herd management devices, and automatic milking and cooling systems can improve resource efficiency and support long-term sustainability of production (Keskinkılıç, 2019; Kalkan, 2019; Van Heurck et al., 2020; Şengül, 2020).

In short, sustainable dairy farming is a forward-looking approach that tries to balance production with the environment. Its success is closely tied to proper management of environmental impacts and making the current production system more resilient.

### 3. Environmental Impacts of Dairy Cattle Farming

Dairy cattle activities have significant environmental impacts because of natural resource use and waste outputs. The main areas are greenhouse gas emissions (carbon footprint), water use, land use, and waste management.

**3.1. Carbon footprint and greenhouse gas emissions:** The environmental side of sustainability looks at the total effects of human activity on ecosystems and is grounded in the natural sciences (Arvidsson et al., 2020). For livestock farms, discussions on environmental sustainability focus on greenhouse gas emissions, water and energy use, and waste management. Recent estimates put global greenhouse gas emissions at about 49 Gt CO<sub>2</sub>e, of which roughly 5.4 Gt CO<sub>2</sub>e comes from agricultural activities. About 3.9 Gt CO<sub>2</sub>e of this is from livestock and animal manures. In other words, around 11% of total global emissions stem from agricultural production, and 7.9% directly from livestock and manure-related processes (Rotz, 2020).

The main greenhouse gases from animal production are methane, nitrous oxide, and carbon dioxide. In the livestock sector, key sources of emissions are animal housing and manure storage areas. When manure is removed from the facility daily or at short intervals, greenhouse gas emissions stay quite low. In contrast, long-term manure storage leads to substantial emissions (Rotz et al., 2019). For this reason, manure management is a critical stage

of production, especially for cutting methane and nitrous oxide emissions (Galioto et al., 2017). Dairy farming can also harm aquatic ecosystems through nitrogen and phosphorus pollution caused by manure application to soil and by overgrazing (Arvidsson et al., 2020). These issues demonstrate that implementing better feeding strategies, utilizing feed additives, and enhancing manure management can help reduce the carbon footprint. In summary, the dairy sector's impact on climate change mainly comes from methane emissions, and lowering this impact is a key sustainability goal.

**3.2. Water use and water footprint:** Milk production is water-intensive. Beyond the drinking water cows need, large amounts of water are used for growing feed crops, cleaning barns, and processing milk. According to water footprint accounting, producing 1 liter of cow's milk uses about 1,020 liters of water (De Vries and De Boer, 2010). This notable figure covers all stages, including rainwater used in feed production (green water), irrigation water (blue water), and the water needed to dilute pollution (gray water). In Türkiye, dairy cattle farming puts a two-way pressure on water resources: on one side, feed crops (corn silage, alfalfa, etc.) require high agricultural irrigation; on the other, intensive farms consume a lot of water for drinking and cleaning. In arid regions, the growing need for feed production makes the sustainable use of groundwater and surface water especially critical. From a sustainability perspective, improving water-use efficiency (e.g., using drip irrigation for feed crops, implementing closed-loop water systems) and protecting water resources are crucial. Otherwise, rising drought risk with climate change may make it harder to secure the water needed for milk production. In this context, adopting better water management practices in dairy farming is essential for both maintaining production and protecting ecosystems (Chriki and Hocquette, 2020; Leroy et al., 2022).

**3.3. Land Use and Ecological Footprint:** Raising animals for meat, leather, wool, and milk requires using feed grown on croplands, fish feed produced in natural waters or on farms, and pasture areas set aside for grazing. Worldwide, there are about 3.5 billion hectares of natural and semi-natural rangelands. When calculating the rangeland footprint, the amount of animal feed available for use in a country is compared with the annual amount of feed needed for all animals that is obtained from rangelands (Kitzes et al., 2007).

Dairy cattle farming needs large land resources for feed production. Feeding a dairy cow depends mostly on quality forages and concentrates, and producing these feeds uses agricultural land. In Türkiye, a significant share of field-crop patterns is allocated to forage crops (e.g., corn, alfalfa,

vetch, sainfoin). In regions with intensive dairy activity (especially Marmara, Aegean, and Central Anatolia), pressure on pastures and croplands is increasing. Overuse and uncontrolled grazing can weaken plant cover, leading to soil erosion. On the other hand, monoculture and heavy inputs (irrigation, fertilization) in feed-crop production can affect ecological balance. To prevent overgrazing and improper land use, it is essential to implement pasture management plans and promote sustainable techniques in feed-crop farming, such as rotation and intercropping.

Because feed from croplands, such as grains, is used more widely instead of herbaceous plants from rangelands, Türkiye's rangeland footprint ratio is trending down. This reduces the impact of animal product consumption on rangelands, yet increases the cropland footprint (Dinç, 2015). Using high-yield feed varieties and preservation methods such as silage can also increase feed produced per unit of land. These steps make it possible to get the same output with less land and reduce the ecological footprint.

**3.4. Waste Management and Pollution:** The main waste from dairy farms is animal manure. On large farms, properly storing and utilizing the high volume of manure is critical to both preventing environmental pollution and enable recovery. However, studies show manure management is often insufficient. For example, in a study of 100 dairy farms in Kütahya province, only 20% had suitable manure storage structures, while 80% kept manure in open piles (Şahin et al., 2001). The same research reported that nearly half of the farms (47%) were located very close to water sources, creating a risk of water pollution through manure leakage (Peypazar and Kılıç, 2021).

Without proper infrastructure, stored manure can carry nitrates and phosphorus into groundwater via rainwater, causing water pollution. Uncontrolled manure accumulation also creates odor and fly problems and can harm quality of life in residential areas. Solutions include locating farms far enough from settlements and sensitive water bodies, as well as promoting the use of leak-proof manure storage facilities. Managing manure in ways that do not harm the environment is also an opportunity: manure can be used in biogas plants to produce renewable energy, or composted and used as organic fertilizer. This can reduce fossil-fuel use and lower the need for chemical fertilizers. In Türkiye, some large-scale farms and integrated companies have begun producing biogas from manure to meet their energy needs, providing an example of a circular economy. However, waste management remains a significant challenge for small family farms and is a priority issue for environmental sustainability (Atilgan et al., 2006).

In summary, the environmental impacts of dairy farming are multidimensional. Elements such as carbon footprint, water and land use, and waste management need integrated improvement. The success of sustainability practices in the sector depends on correctly managing and reducing these impacts.

#### **4. Sustainability Practices in Türkiye and Current Situation Analysis**

In recent years, both the public and private sectors in Türkiye have taken various steps on sustainability in the dairy cattle sector. In this section, we assess the current situation and discuss the good practices already in place, as well as the overall level of sustainability in the industry.

**4.1. Legislation and supports:** From an environmental sustainability standpoint, there are several legal regulations for livestock farms in Türkiye. Especially for large-scale farms, the Environmental Permit and License Regulation sets conditions such as manure storage and wastewater treatment. The Ministry of Agriculture and Forestry provides training and grant support through programs like “Environmentally Friendly Agriculture” and “Good Agricultural Practices.” For example, within rural development supports, new barn projects above a certain capacity are required to include manure pits and storage facilities, and grants are provided for these. Through IPARD (EU Rural Development) funds, investments for modernizing dairy farms (milking parlors, cooling tanks, biogas units, etc.) have also been supported. Thanks to this, many farms have improved their infrastructure and installed systems that increase energy and water use efficiency. However, there is still room to improve full enforcement of the rules and to bring small-scale farms under the same umbrella (Can and Esengün, 2007).

**4.2. Training and extension:** Applying sustainable farming principles in the field depends critically on farmers' awareness and knowledge. Provincial agriculture directorates and chambers of agriculture occasionally organize trainings on eco-friendly practices in dairy farming. For instance, seminars and demonstrations have been held on composting animal wastes, using manure in biogas plants, pasture management techniques, and organic milk production. Through dairy producer unions and cooperatives, efforts are also made to help members access sustainable production techniques (Pezikoglu, 2006). These training activities are particularly important for helping young farmers adapt to new technologies and transition from traditional practices to greener methods. Still, there is a need to scale such training nationwide and reach farms of all sizes. In particular, raising sustainability awareness

among small family farms will have positive long-term effects across the sector.

**4.3. Good Examples and Pilot Projects:** In Türkiye, several good practices have encouraged progress toward sustainable dairy farming. Some large integrated dairy companies (those that adopt a “farm to table” model) have built circular systems on their own farms. Firms like Sütaş, for example, convert crop residues and animal manure into energy in biogas plants and use that energy on their farms and in their factories; the solid fraction left after fermentation is returned to fields as organic fertilizer (Ata and Yılmaz, 2015). Such models serve as an example for waste recovery and reducing fossil-fuel use. Some large farms have also turned to renewable energy by generating their own electricity with solar panels and using solar power for irrigation wells.

In terms of water efficiency, some feed producers have transitioned from closed or sprinkler systems to drip irrigation. There have also been notable advances in animal welfare and productivity, including the introduction of automatic milking systems and cooling tanks, which have improved milk quality and reduced losses. Modern barns use ventilation and cooling (fans, misting/showering systems) to reduce heat stress (Sejian et al., 2018). In regions of Türkiye where summers are very hot, dairy farms report measures such as installing shade structures and using showering systems to lower heat stress (Dikmen and Hansen, 2009). By limiting productivity losses, these practices bring both economic and environmental gains.

**4.4. Analysis of the Current Situation:** Despite ongoing improvement efforts, the overall sustainability performance of dairy cattle farming in Türkiye has not yet reached the desired level. From an economic standpoint, rapidly rising feed costs and volatile raw milk prices in recent years have seriously constrained farm profitability. Along with steep feed prices, raw milk prices have often failed to keep up with inflation, putting many producers under pressure. As a result, a notable number of breeding cows were sent to slaughter, the milking herd shrank, and short-term milk output declined. This is a critical warning sign for the sector’s sustainability.

On the environmental side, partial improvements have been achieved in large-scale farms, but problems persist among smaller ones. Many small farms still lack proper manure storage structures; manure is left in the open or spread randomly on land, keeping pollution risks for local water and soil ecosystems alive. Likewise, overgrazing and unplanned pasture use remain issues in some areas; therefore, pasture rehabilitation and management need to be adopted more widely.

There are however, positive trends, most notably in yield. With more purebred and crossbred dairy cows, the average milk yield per cow in Türkiye has risen steadily over the last decade. The average was about 2.5 tons per cow per year in 2012 and around 3.2 tons in 2019. In line with changes in herd size and yield, raw milk output fell in 2022 from 23.2 million tons in 2021 to 21.6 million tons in 2022, a 7.1% drop. Of total raw milk, 92.3% is cow's milk, 7.5% is sheep and goat milk, and 0.2% is buffalo milk (TEBGE, 2023). It appears that yields continued to rise into the 2020s. Farm-level evidence points in this direction even if official figures are not yet complete. Higher yield lets fewer animals produce the same volume of milk, which lowers the long-term environmental impact. Even so, average yields remain well below EU levels, so there is still ample room to improve.

In short, while awareness and effort around sustainable dairy are growing in Türkiye, structural and on the ground problems continue. On one side, integrated and modern farms operate with circular, eco-friendly methods; on the other, some small family farms still use traditional practices that are low-yield and relatively burdensome on the environment. This dual structure means sustainability policies and supports must both reach a broad base and be targeted by scale. The next section will discuss the challenges in this direction and propose solutions.

## 5. Challenges and Proposed Solutions

Türkiye's dairy sector faces a range of hurdles to becoming truly sustainable. These appear across economic, social, and environmental dimensions, and they interact with one another. Below are the main problem areas and practical ideas to address them:

**5.1. Small scale and weak organization:** A large share of dairy farms are very small (as few as 1–5 cows), which keeps them from benefiting from economies of scale. Smallholders struggle to build capital for modernization and to gain bargaining power. The result is lower productivity and, proportionally, a heavier environmental load (Uçum and Gülcubuk, 2018).

**Suggested remedy:** Encourage small producers to organize in cooperatives or producer unions. Shared milking parlors, cooling tanks, and manure processing units can be set up. Group purchasing can help lower input costs. In one district, for example, several small farms could jointly invest in a biogas or compost plant. Such an investment would be unrealistic for a single farm to make. Public support should also be redesigned so small farms can access it more easily (lower own-capital requirements, simpler applications and similar measures).

**5.2. High feed costs and feeding challenges:** Feed is the dominant cost in sustainable milk production. Producers in Türkiye are hit by rising prices for concentrate and forage. Weather variability can cut pasture productivity, and drought years reduce forage output. Buying feed from outside the farm, especially for smallholders, puts economic sustainability at risk (Alçıçek, 2021).

**What might help:** Widen the use of local feed resources. Promote on farm feed production, such as silage making and sowing forage crops. The state should continue to support strategic forage crops and distribute equipment that reduces post-harvest losses, such as silos and balers. Provide hands-on advice for ration optimization to raise efficiency. Identify region-specific least-cost rations and share them widely. Also invest in research and development on alternative feed sources, such as selected industrial byproducts and the smarter use of grazing, and deliver the results to farmers through extension.

**5.3. Instability in milk prices:** Sustainability depends on the producer selling milk at a profit. In recent years, price swings and periods when raw-milk prices lagged behind inflation have squeezed farmers, pushing many to cull animals (Ayyıldız et al., 2021). This creates a damaging loop: low prices shrink the herd, future supply tightens and volatility persists.

**Policy options:** Make sure the National Milk Council's reference price mechanism functions effectively. Update market prices regularly to reflect rising costs, and, when needed, use intervention purchases or skim milk powder supports to steady the market. A floor price or premium scheme that guarantees a reasonable margin would also keep producers in the sector and help maintain the national herd over time.

**5.4. Compliance with environmental rules and gaps in enforcement:** Türkiye imposes environmental duties on large farms, yet practice does not always match the rules. Small and mid-sized farms are often exempt from parts of the legislation or simply fall outside routine inspections. That leaves room for off standard practices in manure management and wastewater treatment.

**Proposed fix:** Pair tighter inspections with smart incentives to spread eco-friendly production. Raise the frequency of audits so that all farms above a given size meet minimum manure storage standards, while also creating voluntary compliance programs for smaller holdings. A label such as an “Environment Friendly Farm Certificate” could grant recognition and modest support to farms that meet defined criteria, building both awareness

and social pressure. Region-level manure solutions can also help: as seen in parts of Europe, manure collected across a district can be processed in a central biogas or compost facility. In this model, environmental investment happens at a regional scale, and smallholders can join the system (Ermetin and Bayramoğlu, 2010).

**5.5. Climate change and adaptation issues:** Rising temperatures, shifting rainfall, and extreme weather affect dairy directly. High heat and humidity in summer cause heat stress in cows, lowering milk yield and harming reproduction (Ermetin et al., 2023). Drought reduces forage output and pushes feed prices up. In short, climate change is a long-term test for dairy sustainability.

**Suggested response:** Put adaptation strategies to work at the farm level. Start with better housing: ensure adequate ventilation, fans, and sprinkler systems for cooling, along with sufficient shade. Studies show that proper shade and showering systems clearly reduce heat stress in dairy cows. Next, promote climate tolerant forages (e.g., drought tolerant corn varieties and alternative feeds) and expand their cultivation. With early-warning tools and meteorological guidance, farmers can prepare for extremes. For instance, ahead of a heatwave, increase access to water and, if needed, shift feeding to the cooler night hours. Over the long term, build climate resilience into breeding programs by supporting the use of heat tolerant local genotypes or crosses (Dağdemir, 2005).

**5.6. Barriers to technology and finance:** Many sustainability tools (biogas plants, solar panels, automatic milking and smart sensors) require sizable up front investment. For small and medium-sized farms, access to these technologies can be financially challenging, and limited technical expertise slows adoption (Özkan and Gürbüz, 2019; Özkan, 2023).

**Ways forward:** Offer farmer friendly finance models. Low-interest loans, leasing options, and grant schemes can make green technologies, such as biogas and solar, more attainable. Create long-term credit lines through state banks and the private sector to back these investments. On skills, provide hands-on guidance through provincial advisors and veterinarians. Young farmers adopt digital tools faster, so highlight successful cases with contests, prizes or demo farms. In selected regions, set up pilot sustainable farms that demonstrate integrated setups, such as sensor-based feed and milk yield optimization, energy from manure, and similar solutions to inspire and train others.

Taken together, these proposals form parts of a multi-layered approach to the sector's sustainability challenges. Improvements along the axes of organization, training, support, and oversight can help dairy move toward a model that is both environmentally and economically sound; however, local context still matters. Eastern Anatolia's small, pasture-based farms will prioritize different fixes than intensive operations in Marmara. Policies and projects should reflect these differences to utilize resources effectively and achieve meaningful impact.

## 6. Policy Recommendations and Forward-Looking Strategies

Strengthening sustainability in the dairy sector calls for broad, future-minded policies. Below are practical proposals and strategic directions tailored to Türkiye:

- **Develop an integrated sustainability strategy:** Within the Ministry of Agriculture and Forestry, prepare a “Sustainable Livestock Strategy Document” covering both dairy and beef. Define clear indicators, targets, and action plans for instance, five-year goals for GHG reductions, water savings, and the number of farms with organic/good-agriculture certification. Draft and monitor this strategy with all stakeholders (public bodies, universities, producer groups, private sector).
- **Use environmental incentives and taxation:** Encourage eco-friendly production with smart rewards. Offer extra milk premiums or tax relief to farms that send manure to biogas plants or use solar energy. Conversely, after a fair transition period, consider environmental taxes for large farms that fail to invest in required systems. As carbon markets grow, evaluate livestock carbon credits. Create a national mechanism that lets producers earn credits when they use methane reducing feed additives or carry out pasture improvement.
- **Boost innovation and R&D:** Fund university and institute projects that deliver durable fixes. Priority topics include methane-reducing feed additives, low-emission dairy genotypes, low-water-use forages, and converting manure into bioplastics/energy, with dedicated calls open via TÜBİTAK and TAGEM. Support start-ups with grants and incubators so made-in-Türkiye solutions can scale globally.
- **Embed sustainability in curricula:** Highlight sustainable livestock in agricultural high schools, veterinary schools, and faculties of agriculture. Ensure that new graduates are familiar with climate-smart

husbandry, lifecycle assessment, and water/carbon footprinting. Field staff who carry this mindset will guide farmers more effectively.

- **Advance animal-welfare and certification schemes:** Treat sustainability as a whole that includes welfare. Build a National Animal Welfare Certification for milk from herds with good housing, free movement areas, proper feeding, and veterinary care. Position certified products as a distinct market segment. With consumers placing more value on ethical, sustainable food, this both rewards producers and lifts the sector's image.
- **Roll out digital monitoring and early warning tools:** Create an integrated platform that combines meteorology and farm data to deliver real-time advice. Send alerts (SMS/app) before heat-stress events so farms can add shade/cooling and shift feeding to cooler hours. Track roughage stocks and prices; when shortages are anticipated, implement prompt policies (such as imports or alternative feed support). Encourage voluntary reporting of environmental metrics (manure output, energy use) from farms to inform data-driven policies.
- **Raise consumer awareness and steer the market:** Grow domestic demand for sustainable milk. Run campaigns that explain why eco-friendly production matters. Use labels like “ecological milk” or “carbon neutral milk” to open new channels. Let sustainable producers capture a modest price premium, encouraging others to follow suit. Encourage big retailers and processors to include sustainability criteria in supplier programs. In parallel with the EU Green Deal, prepare for tools such as carbon footprint labels or sustainability declarations in the food sector.
- **Pursue international cooperation and alignment:** As part of the global climate effort, set agriculture and livestock emission reduction pledges and a roadmap to meet them. As noted in the 2024–2030 Climate Change Action Plan, most farm sector emissions come from livestock and need targeted rules (Demir, 2025). Study effective policies abroad (e.g., methane charges in some countries, California's methane programs) and adapt them to Turkish conditions. Join FAO/World Bank initiatives in sustainable livestock to access technical and financial support.

These policies collectively form a long-term vision for a resilient dairy sector. The core idea is to balance incentives with obligations, include every

stakeholder in the process, and base decisions on sound evidence. The sector is dynamic, economic and environmental pressures shift—so strategies must stay flexible and be updated through regular monitoring and evaluation. Ultimately, real success hinges on a shared culture, where producers, consumers, and the public sector collaborate towards sustainability.

## 7. Conclusion

Dairy cattle farming in Türkiye are essential for nutrition and the economy, and it must be addressed from several angles under the lens of sustainability. This review shows that sustainability in dairy is not only an environmental matter but also an economic and social one. The introduction underlined the sector's national importance and noted that it relies largely on small family farms. Production has grown in recent years, yet some structural issues remain. Within sustainable agriculture, it is vital to keep resources intact while maintaining productive output, and to treat environment, economy, and society as a single framework.

Considering environmental impacts, there is clear room for improvement in carbon footprint, particularly in terms of methane, as well as water use, land pressure, and waste management. Cutting livestock emissions, handling manure and other wastes safely, using water efficiently, and protecting pasture ecosystems should be top priorities. Türkiye has already taken steps toward sustainability. Even so, the current picture reveals a dual structure. Large and modern farms have made partial gains, while many small holdings still face both economic strain and environmental risk. In recent years, rising costs led to the loss of breeding stock. That outcome shows the economic pillar is as critical as the environmental one.

Under the challenges section, key problem areas were set out with practical responses. The list includes small farm size, high feed costs, unstable milk prices, weak compliance with environmental rules, climate stress, and limited access to technology and finance. The common thread is the need for sector-wide transformation and capacity building. Cooperatives can unlock scale, lower input costs, and enable shared environmental investments. Smart public support can protect and guide producers. Training and extension can raise awareness and improve day-to-day practice.

At the policy level, broader measures can speed the shift. A national sustainability strategy for livestock, incentives and taxes aligned with environmental goals, R&D and innovation, stronger coverage of sustainability in education, and learning from international best practice can help the sector move faster. In the twenty-first century, when climate action

is a global necessity, dairy will need to aim for a smaller carbon and water footprint.

In summary, achieving sustainability in Turkish dairy farming requires a holistic approach involving multiple stakeholders. Farmers, public authorities, the private sector and consumers all have roles to play. Sustainable practices may bring initial costs and changes, yet they strengthen competitiveness and food security in the medium and long term. A more sustainable dairy sector means higher-yielding cows, a cleaner environment, more prosperous rural producers, and healthier generations. The following steps should not be postponed. National agricultural policy should give priority to dairy-specific sustainability programs so that Türkiye can align its deep livestock tradition with future needs and provide enough balanced food for today's citizens and tomorrow's.

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## The Effect of Renewable Energy Sources on Animal Production 8

Mehmet Akif Boz<sup>1</sup>

### Abstract

The growing world population and the associated demand for energy are increasing the importance of renewable energy sources. Due to the limited nature of fossil fuels, their environmental damage, and energy security risks, there has been a shift towards renewable energy sources such as solar, wind, geothermal, hydraulic, and biomass. However, the negative effects of these sources, particularly on crop production and livestock farming, are being debated. Solar and wind power plants, which are built on large areas, lead to the reduction of agricultural land and pastures, as well as the decrease and loss of grazing areas. This situation particularly affects nomadic livestock farming and small producers. Furthermore, the noise, vibration, and electromagnetic fields generated by turbines can cause stress in animals, reduced productivity in some cases, and orientation problems. While solar panels create shaded areas that may benefit animals in some situations, they can negatively affect plant growth and forage quality. Infrastructure development for renewable energy makes it difficult for animals to access water and pastures. Wind turbines also have a negative impact on bees and other pollinating insects. Turbulence, pressure differences, and electromagnetic fields cause bee deaths. This indirectly threatens agricultural production, even if not directly. In conclusion, although renewable energy production is necessary from an environmental perspective, it must be planned in a way that does not disrupt agricultural activities.

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## 1. INTRODUCTION

The world's population continues to grow as the years progress. Naturally, humanity's needs also increase in line with this population growth. The most important of these needs is energy. There is a direct relationship between energy consumption, which is a fundamental element of economic development, and living standards (Karaca, 2017). In response to the increasing demand for energy, the limited nature of fossil fuel resources, as well as their impact on the environment and climate change, has brought the use of different energy sources to the forefront (Gürbüz et al., 2021). This situation has shown a high rate of increase in recent years, and almost every country has turned to renewable energy sources (Kaya et al., 2019; Khanlari et al., 2020a; 2020b).

Countries around the world meet a large part of their energy needs from fossil fuels. However, energy sources such as natural gas, coal, and oil have limited reserves. These resources are at risk of depletion. Furthermore, the use of limited resources threatens energy security and causes environmental problems (Yaşar et al., 2025).

It is very important to produce energy using renewable, clean, and sustainable sources. However, production must be carried out taking into account both positive and negative environmental factors (Gürbüz et al., 2021). Energy obtained from renewable sources is also important in terms of increasing national energy security, reducing imports, and lowering greenhouse gas emissions (Lopez et al., 2024).

The renewable energy sources we will examine for their impact on livestock are solar and wind energy. In addition to these, geothermal, hydraulic, and biomass energy should also be noted (Gürbüz et al., 2021).

## 2. THE NEGATIVE EFFECTS OF RENEWABLE ENERGY SOURCES ON LIVESTOCK FARMING

Most of the land used in many regions of our country is for agricultural purposes. From this perspective, the agricultural sector has significant potential for renewable energy. The vast expanses of land are well-suited for wind and solar energy. In addition, residues from both plant and animal production provide raw materials for biofuel or biomaterial. (Gürbüz and Kadağan, 2019; 2022).

Due to the continuous growth of the world population, the demand for food is increasing day by day. To meet this growing demand for food, new agricultural lands and high-yield soils are needed. Unfortunately, however,

the world's agricultural lands are on a downward trend (Karakuş et al., 2019).

Given current economic developments and population growth rates, it is reported that by 2030, global food demand will increase by 50%, energy demand by 40%, and water demand by 30%. According to these reports, the world will face difficult times in terms of basic needs such as food and energy. Along with the growing population, many needs are also emerging. While these needs are important, they also lead to the use of agricultural land for purposes other than its intended use (Aksoy, 1997; Bıçakçı et al., 2023). Examples of these include:

- Regulation and growth of urbanization,
- Industrialization and the increasing development of industry,
- The need for and development of the mining sector,
- Construction of highways,
- Continued construction of airports,
- Construction of railways such as high-speed trains,
- Expansion of recreational areas,
- Need for tourist areas and continuous expansion of these areas,
- Creation of protected areas,
- Creation of infrastructure related to urbanization and industrialization,
- Construction of dams for energy needs and irrigation,
- Construction of canals,
- Creation of pipelines for natural gas, fuel, etc.,
- Construction of power plants for energy needs.

Türkiye is home to some of the world's most important bird migration routes. Therefore, to minimize risks related to GES projects and birds, monitoring studies should be conducted at least twice a year (spring and fall) during the periods when bird migration occurs. This is particularly important after solar panels have been installed. This is because, after the panels are installed, a still water surface appears on the panels when viewed from above. In this case, there is a possibility that birds may mistake the panels for a still water surface while flying (Turan et al., 2023).

Naturally, measures can be taken to prevent this situation by using audible, visual, and moving deterrents, thereby mitigating the risks. There

are a limited number of scientific studies conducted and published on these topics. It is crucial to be sensitive to this issue in solar power plant projects in our country and to implement the necessary measures (Turan et al., 2023).



*Figure 1. A vulture killed by the impact of wind turbine blades (Photo: Atabey, 2022)*

The presence of wind turbines in bird migration areas poses significant problems. Birds living in forests and wetlands (including bats) are negatively affected by wind turbines, leading to an increase in harmful insects not only within the forest but also in surrounding agricultural areas, olive groves, and orchards (Kantarci, 2015).

Research conducted to determine why birds, especially bats, are drawn to wind turbines has identified some possible reasons, although these are not definitive (Atabey, 2022). These include:

- Sound-directed movement,
- Loss of sense of direction due to electromagnetic fields,
- Approach due to heat effect,
- For roosting and night-time activity,
- Linear corridor (farms built on ridges may be a good option for covering distance),
- Orientation may occur due to the presence of a high area on the migration route for mating behavior. Wind turbine-related deaths may occur for these reasons.



Figure 2. Blade hub on a wind turbine (Photo: <https://khosann.com>)

The health issue known as “wind turbine syndrome” caused by the low-frequency sound and shadow flicker effects emitted by wind turbines, is one of the most significant and rare effects observed in humans (Pierpont,

2009). To overcome this problem, buffer zones between wind turbines and residential areas have been established in many countries in recent years through the enactment of laws and regulations. Nevertheless, wind energy remains the renewable energy source that causes the least environmental problems (Kadioğlu and Tellioğlu, 1996).

Solar power plants built on large areas can negatively affect the ecosystem and biodiversity of their surroundings. They can restrict the vital movements of living creatures in these areas. The ecosystem and biodiversity are negatively affected by factors such as light reflections, high temperatures on the surface of the panels, the magnetic field created, changes in the microclimate, and the reduction of vegetation and water resources (Tsoutsos et al., 2005; Sarsıcı, 2020).

Although renewable energy projects generally prioritize environmental benefits, some negative effects on livestock activities are anticipated. These effects may vary depending on the type, size, and location of the energy facility, the type of animal raised, the current land use, and the management practices applied.

## **2. 1. Land use and loss of grazing areas**

Large-scale solar farms and wind turbine infrastructure cause land loss in pasture and grazing areas. This situation poses a significant problem, particularly for small-scale producers and farmers engaged in nomadic livestock farming (Karadeli, 2001).

Renewable energy projects can reduce the boundaries of areas such as pastures or grazing lands where animals need to graze. Land reduction is likely to occur, especially in systems where animals are fed seasonally in a nomadic manner (Karadeli, 2001).

Facility construction on pasture areas can restrict grazing activities in those areas. Clearing operations in forested areas or areas that are not actually forested cause significant damage to these areas. Ground clearing operations in areas such as scrubland, heathland, marshes, and reed beds can cause permanent damage to the flora, fauna, and land. Any intervention in such areas may also result in the loss of these areas (Biçakçı et al., 2023).

Despite this, a counterargument is also defended. According to this view, wind power plants occupy more space than other power plants. This is because these turbines are spaced far apart so that they do not block each other's wind. Consequently, for example, 20 turbines, which is a large number, cover an area of approximately 1 km<sup>2</sup>. However, only 1-1.5%

of this area is actually occupied by the turbines. The vast area outside the turbine installation zone can be easily used as agricultural land or for various livestock activities (pasture-meadow), provided that no structures are built that would block the wind (Hayli, 2001).

Following the enactment of Law No. 4342 on Pastures in 1998, various beneficial applications required by law began to be implemented on pastures. The practices outlined in the law are essentially grouped under four main headings: the identification, delimitation, and allocation of pastures, as well as pasture improvement projects planned and implemented to restore pastures that have become worn out and unproductive for various reasons to their former productive state. Law No. 4342 also includes regulations and instructions for the allocation of certain pasture areas to the public and private sectors for the purpose of providing public services that meet the basic needs of society, primarily our national security, natural disasters, and our country's energy needs. According to these regulations, if it is considered that establishing a solar power plant (GES) or wind power plant (RES) on a pasture area is the best solution under current conditions, low-yield pasture areas should be preferred (Bıçakçı et al., 2023).



*Figure 3. Goat herds in the field of solar energy (Photo: <https://www.piagrid.com/rehber/hayvancilik-icin-gunes-enerjisi>)*

## **2. 2. Noise, vibration, and visual - public interaction stress**

Wind turbines can generate noise and low-frequency sounds from sources such as rotational movements, tower foundations, and generator noise. Such sounds are predicted to cause effects on animal behavior, including stress, loss of appetite, and decreased milk production. However, studies in this area have often failed to produce clear results. There are also findings suggesting that the effects are low or limited in the context of animal husbandry (Anses, 2021; Energy Savings Lab, 2025; ELC, 2025).

In particular, vibrations from wind turbines or construction work during infrastructure development can be a source of disturbance for animals. For example, activities such as road construction and foundation excavation for large wind farms, although temporary, can cause some negative effects (Chiu et al., 2020).

To prevent this and take precautions in advance, the necessary measures should be presented in the EIA reports prepared.

## **2. 3. Shading, microclimate changes, and heat stress**

Solar panels can potentially have positive effects on animals by creating shade. They can also play an important role in protecting animals from heat stress during hot weather. However, plant growth rate and quality may change in shaded areas. Solar energy facilities can disrupt the homogeneity of plant cover. In this case, negative effects may arise in terms of quality and price in animal nutrition (Andrew et al., 2021; Faria et al., 2023).



*Figure 4. A flock of sheep in a solar panel field (Photo: <https://temizenerji.org>)*

Depending on shading and panel configuration, the photosynthetic capacity of plants benefiting from sunlight may decrease. This situation may be felt more particularly in regions with sunny climates where plant growth is closely related to light (Velilla et al., 2021).

#### **2. 4. Transportation, access issues, and infrastructure barriers**

The foundations of turbines, the installation areas for solar panels, maintenance and service roads, and roads constructed for grid connections can divide pasture or grazing areas, potentially restricting the free movement of animals (Marsh, 2023).

When areas are separated, animals' access to water sources or shaded areas may be reduced. In this case, risks to individual animal health may arise, especially in hot weather (Chiu et al., 2021).

#### **2. 5. Social and cultural dimensions**

Nomadic pastoralism is a production system carried out on pastures and summer pastures. Renewable energy projects can sometimes affect local communities' pasture traditions or reduce common use areas (Chiu et al., 2021).

Failure to involve local people in project planning and site selection, as well as practices such as fencing and infrastructure development, can limit animals' access to grazing and water.

### **3. THE NEGATIVE EFFECTS OF RENEWABLE ENERGY SOURCES ON BEEKEEPING**

Bees play a crucial role in agricultural sustainability. Since bees help ensure pollination, a large bee population is essential for the continuity of agriculture (Corten et al., 2001; Pustkowiak et al., 2017). Approximately 90% of plants require the assistance of pollinators. Bees are also the most effective pollinators.

More than half of the wind hitting the blades of wind turbines is converted into kinetic energy. The remaining wind can be scattered at high speeds. This can cause moist air on the ground to rise, causing significant damage to the surrounding bee population. Furthermore, the turbulence and pressure differences created around the turbine cause permanent physical damage to bees (Kantarcı, 2015).

Rotating wind turbine blades can be a deadly mechanism for insects and bees. In addition to this lethal effect, the turbulence and pressure differences

around the turbine can also cause permanent physical damage to bees. Insect and bee deaths caused by turbine blades result in the scattering of fragmented insects and the formation of odors. This odor formation will also attract other winged animals. Thus, this situation continues to escalate (Gürbüz et al., 2021). Due to the noise generated by wind turbine blades and the stray voltage caused by leakage currents, bees become disoriented and lost. The electromagnetic field effect of wind turbines can prevent honeybees from finding their direction, food source, or hive (Atabey, 2022).

The presence of insects and bees can also affect wind turbine performance. The incompatibility of flying insects with wind turbines is a significant problem. Insect remains on wind turbine blades can cause reductions in wind turbine performance. Additionally, insect and other animal deaths cause cleaning problems on the blades.

#### **4. CONCLUSION AND RECOMMENDATIONS**

The allocation of first and second class agricultural land for the purpose of developing public services or meeting public needs leads to the use of these areas for non-agricultural purposes. Although the regulations and instructions of Law No. 4342 on Pastures, Law No. 2872 on the Environment, and Law No. 5403 on Soil Protection and Land Use apply to these allocated lands, the demand for energy and food is increasing along with the growing population. Unfortunately, this situation is an inevitable reality. At this point, it is necessary to ensure that agricultural production is sufficient for the growing population and that the energy needs of the growing population are met. It is essential to act in a planned manner in the use of land resources and to carefully select areas for energy production, especially those to be used for purposes other than food. If land is used for purposes other than its intended use and this situation is not controlled, countries may be driven into disaster in the near future.

While using our country's renewable energy potential in the most efficient way, it is of great importance not to disrupt agricultural activities, to ensure the production of feed necessary for animal production, and to protect our pasture and grazing areas, which are the cheapest source for meeting the high-quality roughage needs of farm animals.

In today's world, where agricultural land, one of the limited and almost fully utilized resources, is becoming increasingly important, the misuse of these areas must be prevented. This is because our animal protein needs will become a significant problem as the years progress. The decline in agricultural areas such as pastures will lead to a decline in animal husbandry.

Significant effort must be made to ensure that the ecological benefits of renewable energy production translate into benefits for rural areas as well. Technological developments at the global and national levels will have both positive and negative effects on plant and animal production when implemented in rural areas. Therefore, we can make renewable energy production sustainable not through a centralized approach, but as a process that also includes rural areas. It is believed that progressing in this manner will lead to more applications for renewable energy production and raise awareness among people living in rural areas.

Pasture grazing areas, transportation corridors, and shaded areas must be taken into consideration when selecting project locations. Noise, vibration, and shading effects should be assessed according to animal species, and adjustments should be made to panel/turbine placement, distance, and protective fencing. Shaded areas, access to water sources, observation paths, etc. should be planned with the needs of animals in mind. The opinions of local communities engaged in animal husbandry should be sought, and local cultural practices should be taken into account.

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# The Importance of Breeding Soundness Examination of the Mares

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## Abstract

Reproductive efficiency is an essential factor for producing a healthy foal every year from broodmares. However, the reproductive physiology and seasonal pattern of mares restrict the reproductive efficiency and cause a limited breeding season. Moreover, there has been a universal birthday for all thoroughbreds on 1 January in the Northern Hemisphere. Thus, the owners aim to breed mares and birth foals as early as possible in the year. Breeding soundness examination is a systematic and effective procedure to assess the health and fertility status of the mares. An annual breeding soundness examination can save time, budget, and the welfare of the animal.

## 1. Introduction

The horses are one of the most valuable livestock animal species, used for agriculture, transportation, military operations, sports, and cultural activities for thousands of years. The accepted knowledge of the domestication of the horse occurred in Central Asia by the Turks and Mongols (Genç et al., 2022; Crossley, 2023; Peker et al., 2024). Selective breeding has significantly enhanced the evolution and development of current horse breeds worldwide, particularly in racing horses. The selection of broodmares for traits such as strength, racing performance, morphological structure, temperament, conformation, and other factors has improved the performance of different disciplines, including racing, showing, and dressage (Perdomo-Gonzales

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et al., 2024). The horse breeders' purpose is to produce one healthy foal each year from broodmares. However, the reproductive physiology and seasonal pattern of mares restrict the reproductive efficiency and cause a limited breeding season. Moreover, there has been a universal birthday for all thoroughbreds on 1 January in the Northern Hemisphere. Thus, the owners aim to breed mares and birth foals as early as possible in the year to give their foals a developmental advantage, enabling them to compete in their first race or be sold at higher prices (Crabtree, 2021). The *Breeding Soundness Examination* (BSE) procedure assesses the broodmare's fertility status, diagnoses, and solves the problems before breeding season that may interfere with breeding, conception, maintaining pregnancy, and foaling. Breeders can have healthy foals and save money owing to a thorough BSE procedure (Giffin and Darling, 2007; Usman et al., 2023).

## 2. Reproductive Physiology of the Mares

The equine species has a seasonal polyestrous long-day breeding pattern. Mares evolved to ensure that their foals are born into a favorable environment with optimal nutritional conditions, thereby maximizing their survival rate in nature. The breeding season in the Northern Hemisphere begins in the spring with an increase in daylight and temperature; the reproductive efficiency declines with a decrease in the amount of daylight. The official breeding season begins from 15<sup>th</sup> February to 15<sup>th</sup> June to support an equal official age for foals born in the same breeding season. However, almost 30 percent of the mares exhibit reproductive activity throughout the year (Nagy et al., 2000; Blanchard et al., 2003; Yüksel and Saat, 2017).

A circannual endogenous rhythm in mares characterizes the seasonality regulated by environmental factors, including photoperiod, nutrition, body condition, and temperature. Photoperiod is the primary factor that increased light signals, perceived by the retina, transmit to the pineal gland via the optic nerves, thereby decreasing the secretion of melatonin (Scott, 2020). Melatonin has an inhibitory function on the gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus, and this inhibitory effect disappears with the increased daylight. Pituitary gland, stimulated by increased pulsatile GnRH secretion, begins to release follicle-stimulating hormone (FSH), which induces follicular growth in the ovaries, and luteinizing hormone (LH), responsible for follicular maturation, estrogen secretion from follicles, ovulation, and luteinization. The estrogen produced in the follicles, particularly from the dominant follicle, promotes LH secretion through a positive feedback effect; meanwhile, estrogen and inhibin suppress FSH release from the pituitary gland via a negative feedback effect. The

ovulation of dominant follicle occurs with the LH surge, and progesterone secretion begins from the luteal cells of the corpus luteum (CL). The regular estrous cycles continue approximately every  $21 \pm 2$  days during the breeding season following the first ovulation of the year (Brinsko et al., 2011; Ekici, 2017).

The primary regulating factor of the seasonality is daylight length, which controls the duration of melatonin secretion. In the meantime, there are additional endogenous neuroendocrine mechanisms that affect the gonadotropic activity. The neurotransmitters function as suppressor agents on GnRH and/or gonadotropin secretion. Endogenous opioids inhibit gonadotropin secretion and control LH release during winter anestrus. Catecholaminergic neurons and dopamine also participate in the mare's reproductive activity by suppressing GnRH secretion (Nagy et al., 2000).

Nutrition has a significant impact on fertility, folliculogenesis and the timing of first ovulation in mares. The feed regime of the mare should be implemented depending on her age, breed, and reproductive status. Studies reveal that mares fed high-quality protein, vitamins, and minerals, and those with a positive energy balance, ovulate earlier after winter anestrus than mares fed a poor diet and those with a negative energy balance (Nagy et al., 2000). Nutritional supplementation of omega-3 fatty acids, L-arginine, and essential vitamins improves fertility (Khan et al., 2025). Pasture grazing also positively affects the ovarian activity and first ovulation in broodmares (Dugdale et al., 2010). Body condition score (BCS) is a valuable method for monitoring nutritional efficiency, energy intake, and reproductive performance by assessing overall and regional adiposity in animals (Henneke et al., 1983). The field trials documented that low BCS has a negative effect on fertility. The optimum BCS for entering the breeding season is accepted with a score of greater than 5 (on the scale 1 to 9) with a body fat content  $> 15\%$  for early onset of ovulation. Inadequate energy intake and low BCS also adversely affect embryonic development and pregnancy. Studies reported that broodmares with a BCS between 6.5 and 8.0 may continue to ovarian activity through winter, and have a higher conception rate than mares with a  $\leq 5$  (Morley and Murray, 2014; Scott, 2020).

## 2.1. Seasonality in Mares

The reproductive rhythm of the mares is categorized into four seasonal periods: anestrus, vernal (spring) transition, breeding, and autumn transition phases. The reproductive year of the mare is gradually divided into those

four seasons in accordance with day length (Blanchard et al., 2003). In the *winter anestrus* phase, the mares are reproductively inactive after the shortest day of the year (winter solstice). During the deep anestrus period, ovaries are small, firm, and inactive; follicular development is minimal, with < 20 mm in diameter. The GnRH and LH secretions are at baseline levels; FSH concentration may be high due to the lack of negative feedback control of estrogen and inhibin. The plasma estradiol concentration is undetectable (< 5 pg/ml), and progesterone concentration is under 1.0 ng/ml. The mare is passive or unreceptive to the stallion and is disinterested in other mares. However, some mares may exhibit estrous behaviors and reproductive activity during the anestrus period, depending on the breed, nutrition, and other individual factors. The mare enters an anovulatory receptivity period with the vernal equinox, called the *spring (vernal) transition* period. With the increase in day length, the melatonin concentration decreases, and the suppressive effect of melatonin on the hypothalamus disappears, allowing GnRH secretion to increase. Ovarian activity begins with follicular development, and follicles greater than 20 mm in diameter may be detected on routine examinations. In the late transition phase, the follicular diameters reach  $\geq 30$  mm, but they don't ovulate because of inadequate LH synthesis and become regressing follicles. In the spring transition period, the GnRH release amplitude and frequency are gradually increased. Plasma FSH concentration is also increased early in this period, and it decreases one to two weeks before the first ovulation at the end of the period. LH concentration increases slowly and peaks just before the first ovulation of the year. Plasma estrogen levels are low during the early transitional phase, but begin to increase with growing dominant follicles 5-7 days before the first ovulation. The progesterone concentration is also low until after the first ovulation. The spring transitional period lasts one to two months, characterized by prolonged or irregular estrous signs (England, 2005; McCue et al., 2007).

The *breeding season* (i.e., the ovulatory phase or peak fertility period) of the year begins with the first ovulation following the spring transition period. The estrous activity occurs after the end of the luteal phase of the first ovulation. The estrous cycle of the mares lasts  $21 \pm 2$  days during the breeding season, thus described as seasonal polyestrous animals. The cycle can be divided into two phases: 1) estrus (follicular phase, sexual receptivity period with estradiol dominance), 2) luteal (early luteal phase forming corpora haemorrhagica, and late luteal phase having an active CL with progesterone dominance) phases. The length of estrus stage varies during the beginning and end of the breeding season. The shortest length generally occurs in late spring and summer (approximately 4-7 days) and may exceed 10 days in early spring

and autumn. The FSH stimulates follicular growth during diestrus stage, and estrogen concentration begins to rise following growth of recruited follicles. Pulsatile LH release stimulates the dominant follicle and oocyte maturation during the estrus phase, and ovulation occurs. LH concentration peaks two days after ovulation, and progesterone concentration increases along with the luteinization of granulosa cells. The diestrus stage lasts for 14-15 days, characterized by a high plasma progesterone concentration (>2 ng/ml). Endometrial release of prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) between days 13-16 post-ovulation causes luteolysis; subsequently, a rapid decline in plasma progesterone concentration occurs within 4 hours. The diestrus stage ends, and a new estrus cycle begins following progesterone decline. During the estrus phase, the mare is generally placid. Mares in heat straddle their hind legs and raise their tails when approached by the stallion, urinate, and evert their clitoris persistently. Some mares may show aggressive behaviors toward the stallion. In the luteal phase, the estrus signs terminate abruptly following ovulation. The aggressive response exhibit to the stallion (England, 2005; Ekici, 2017).

The peak breeding season ends with the decrease of day length, and the last ovulation of the year signals the beginning of the *autumn transition* period. The hypothalamic-pituitary-ovarian axis function declines due to the increase in melatonin secretion from the pineal gland. Inadequate gonadotropin secretion, suboptimal follicular and luteal development cause irregular estrous signs initially, and then acyclicity in mares (Irvine et al., 2000). Individual variations may be observed during the autumn transition period. Some mares display non-ovulatory follicular waves in decreasing magnitude, while others exhibit prolonged diestrus following luteinization (England, 2005).

### **3. Breeding Soundness Examination of the Mare**

The purpose of the BSE is to check the mare's general and reproductive health, condition, and mating ability, potential of being pregnant, carrying, and delivering a healthy foal. If any morphological or infectious problem is diagnosed, it may be treated immediately before the breeding season. Examinations should be performed annually, preferably during the spring transition period, 50-60 days before the planned breeding season. Thus, the mare could be treated if she has any uterine infections, etc., and can breed as early as possible in the season (Giffin and Darling, 2007; Usman et al., 2023). The open mares (non-pregnant), mares have a reproductive pathology in the breeding season, barren mares after season, and mares with

embryonic loss, abortion, or different infertility history should also be added to the BSE practice (McCue, 2021).

Breeding soundness examination includes a systematic set of steps of breeding history evaluation, general physical examination, perineal conformation assessment, rectal palpation, transrectal ultrasonography, vaginal speculum examination, digital examination of cervix, uterus, and clitoral/vestibular cultures, and endometrial biopsy. Detailed diagnostic methods, such as transcervical endoscopic examination, hormone and chromosome analyses, and oviductal flushes, may be planned in addition to routine BSE tests for undiagnosed subfertile mares (Blanchard et al., 2003; Kılıçarslan and Uçar, 2017; Crabtree and Pycock, 2020).

### **3.1. Breeding History**

The detailed breeding history assessment should begin with a proper identification of the mare. The breed, age, date of birth, registration name and number, color/markings, and microchip number should be recorded. The temperament, body condition, previous illness, surgeries, treatments, and vaccination administrations should be evaluated. The reproductive history should also be examined with questions about whether barren, maiden, pregnant, lactating, number of previous foals, abortions, dystocia, and number of previous breeding (stallion, artificial insemination, or embryo transfer) (Crabtree and Pycock, 2020; Usman et al., 2023). Young maidens may signify irregular cyclicity during their first estrous cycles or aggressiveness to the stallion. Barrens bred over two times but did not get pregnant, diagnosed as infertile, generally due to chronic uterine infections. The old mares (>15 years old) usually have low fertility because of multiple foaling and breeding, which causes uterine infections. The mares having a history of recurrent pregnancy losses should be screened in detail (Giffin and Darling, 2007).

### **3.2. General Physical Examination**

The general physical examination is performed primarily to assess the ability of the mare to carry the foal to term. Any chronic disease history, including heart failures, and heaves, or heritable/genetic diseases such as patella luxation, degenerative joint disease, inguinal or umbilical hernia, laryngeal hemiplegia, and wobbler syndrome, is investigated during this procedure. Oral cavity, eyes, respiratory, musculoskeletal, and cardiac systems should be evaluated. Urine, hematology, serum biochemical, and fecal egg count analyses should be assessed (Brinsko et al., 2011; Usman et al., 2023). The serological tests of Coggins (equine infectious anemia) and equine

viral arteritis should be performed. The body condition score is a reliable indicator of a horse's health, indicating whether the horse is at an ideal weight, underweight, or overweight. It is usually hard to conceive of obese or overweak mares. Thus, the overweak mares should be supported with a high-energy diet that includes adequate protein, vitamins, and minerals. Obese mares should be put on a diet and an exercise program. Some barren and maidens exhibit abnormal winter hair-coat (hirsutism) at the beginning of the season. The winter coat will be lost before the regular cycles start in healthy mares. However, mares with pituitary tumors may exhibit hirsutism (Giffin and Darling, 2007).

### 3.3. Perineal Conformation Assessment

The perineal conformation abnormalities directly affect fertility due to ascending genital canal infections. Therefore, the examination of the external genitalia primarily focuses on perineal conformation, as the anus and vulva should be completely vertical in this. The muscular tone of the vulva should be sufficient to close the vulvar entrance and to prevent air aspiration, which causes windsucking and pneumovagina in mares (Dascanio and McCue, 2021). The length and angle of declination of the vulva measure (Caslick's Index) to detect any necessity for surgical treatment (usually with Caslick's vulvoplasty technique) has been designed by Pascoe (1979).

*Caslick's Index = Distance between dorsal commissure and pelvic brim (cm) x Vulva angle*

The index  $<100$  marks normal vulvar conformation, and no need to perform treatment; index between 100 and 150 marks to careful evaluation of the mare; the index  $>150$  marks the mare obviously needs Caslick's vulvoplasty (Pascoe, 1979).

Any vulvar discharge, lateral or dorsal tears, Caslick's operation sutures to prevent pneumovagina, depigmented areas, and urine stain on the ventral commissure of the vulva should be noted. The clitoris examination shouldn't be forgotten in terms of clitoral enlargement, which indicates testosterone administration, pseudohermaphroditism, ovarian androgen-secreting tumor, or sex chromosome anomaly (Kılıçarslan and Uçar, 2017).

### 3.4. Rectal Palpation

Rectal palpation is an essential stage of BSE, in which a veterinarian may palpate the ovaries, uterus, and cervix in mares. The mare should be adequately restrained before examination to protect both animal and veterinarian. The ovaries are bean-shaped in mares. The size, presence or absence of follicles, and the stage of the estrous cycle can be confirmed between the fingers and

thumb. In the anestrous period, the ovaries are small in size and inactive; hypoplastic ovaries are also very small. The different-sized ( $>20$  mm in size) follicles began to palpate during the transition period, and  $\geq 35$  mm in size dominant follicles can be examined during the breeding season. Increased ovarian size may indicate an ovarian tumor, hematoma, or parovarian cysts (Blanchard et al., 2003). The non-pregnant uterus is T- or Y-shaped, and its size, position, and tone can be evaluated by palpation. During the deep anestrous phase, the uterus is thin-walled and difficult to palpate; tonus begins to increase during the transition period. The uterus is edematous, thick, and heavy in the estrus stage of the cycle. Uterine pathologies, such as endometrial fold atrophy, lymphatic cysts, uterine neoplasia, and large purulent fluid in the lumen, can be detected by rectal palpation. The cervix examination per rectum reveals the stage of estrus. In the estrus stage, the cervix is dilated, soft, and flaccid; in the diestrus stage, it is tightly closed and thick-walled (Kılıçarslan and Uçar, 2017).

### **3.5. Transrectal Ultrasonography**

Ultrasonography examination is beneficial for diagnosing reproductive tract pathologies, follicular dynamics, ovulation and development of CL, pregnancy, and twins. Doppler ultrasound is an advanced technology of grey-scale ultrasound. The uterine and ovarian blood flow can be evaluated during estrous cycle and pregnancy (Ortega-Ferrusola et al., 2022). Fluid-filled, anechoic, various-sized follicles may be observed in the ultrasound. A conic or pear-shaped preovulatory follicle with a diameter of 40-50 mm can be observed 24 hours before ovulation. Ovulation can be detected as the absence of a preovulatory follicle in the ovulation fossa. Corpus hemorrhagicum is seen as a homogeneous, grey image, while CL has a more echogenic appearance in ultrasonography. The estrus stage has a dramatic ultrasonography image with a small amount of free fluid in the uterine lumen and oedema due to elevated estrogen. The cross-section of the uterine horn is described as a “sand dollar” or “spoke wheel” image (McCue, 2021).

### **3.6. Vaginal Speculum Examination**

Visual vaginal examination is a useful method to evaluate the entire vagina and the external opening of the cervix. The determination of the stage of the estrous cycle, cervix or vaginal wall lacerations, accumulation of purulent discharge or urine in the vagina, vaginal or cervical inflammations, persistent hymen, adhesions can be performed by visual examination per vaginam. However, the vaginal examination has an important risk of genital

tract contamination by air and pathogens into the uterus. Thorough cleaning of perineum with proper disinfectant solutions, and wrapping tail before examination should not be forgotten (Brinsko et al., 2011).

Vaginal examination using a sterile speculum helps to evaluate various physiological and pathological conditions in mares. In the estrus stage, the vaginal mucosa is pink, hyperemic, bright, and moist. The cervix is lying on the vaginal floor, relaxed, soft, and pink colored during estrus. In the diestrus stage, the vaginal mucosa is dry, grey to pale pink, and the cervix is located well off the vaginal floor, closed, and firm. In the anestrus, the vaginal mucosa and cervix are dry and pale; the cervical os is closed and located high on the vaginal vault (Kılıçarslan and Uçar, 2017; Dascanio, 2021a).

A complete or partial persistent hymen may be detected between vestibule and in the maiden mares during vaginal examination. The persistent hymenal tissue blocks the drainage of endometrial secret and may lead a uterine infection, it should be removed manually or surgically two or three weeks before breeding (Dascanio, 2021b). Various colored and consistent (white, grey, watery, or purulent) fluid on the floor of the vagina coming from the cervix reveals a uterine infection. Yellow, urea-like odour fluid pooled near the cervical opening is urine, which is diagnosed as urovagina, another cause of infertility (Giffin and Darling, 2007).

### **3.7. Digital Examination of the Vagina and Cervix**

Manual examination of the vagina and vulva is performed following a complete vaginal speculum exam during BSE. The digital examination should be done using maximum sterile technique and gently to avoid ascending contamination and damage. Postpartum diagnosis of vaginal and cervical trauma, laceration due to dystocia or uneventful delivery, retained fetal membranes, and adhesions can be performed. The cervical lumen may be controlled by gently dilating and palpating with a finger to explore lacerations and adhesions (Sitters, 2021).

### **3.8. Endometrial Biopsy**

An endometrial biopsy is used to diagnose the cause of pregnancy loss and uterine diseases, usually for barren mares. The uterine tissue sample is mostly collected prior to the onset of breeding season, in the diestrus or early estrus stage. The tissue samples may also be used for uterine culture, cytological evaluation or molecular analyses such as reverse transcription polymerase chain reaction (RT-PCR). Histopathological analysis of the tissue sample

reveals pathological changes in the endometrial glands, including luminal and glandular cells, as well as inflammatory or degenerative changes, and the accumulation of inflammatory cells, such as polymorphonuclear leukocytes (Crabtree and Pycock, 2020; McCue, 2021; Usman et al., 2023).

### **3.9. Endometrial Cytology**

Cytological sampling from the uterus is a simple, practical, and helpful method for the detection of endometritis. Uterine culture should be performed in conjunction with endometrial cytology. Uterine cytology samples may be obtained by swab, brush, or a low-volume uterine lavage method from the uterine cavity and endometrial surface. The sampling should be under sterile conditions to avoid contamination of the genital tract. The stained cytology preparations are investigated under a microscope for neutrophils, microorganisms, and epithelial cells. Samples from mares having acute and subacute endometritis contain increased neutrophils and degenerated epithelial cells. An excessive number of macrophages, lymphocytes, and plasma cells is observed in the mares having chronic endometritis. The moderate to severe content of debris is also associated with bacterial endometritis (Dascanio and Ferris, 2021). The infections caused by yeast and fungi may be diagnosed with uterine cytology, due to the proliferation of these organisms in the luminal epithelium and uterine lumen (Brinsko et al., 2011).

### **3.10. Clitoral and Uterine Culture**

Clitoral culture is mostly used to diagnose venereal diseases caused by *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Taylorella equigenitalis*. The stallion could be contaminated with those pathogens from an asymptomatic mare during breeding (Giffin and Darling, 2007). Uterine cultures may be obtained via uterine cytology swabs, brushes, lavages, or biopsy samples. The most common pathogens of the uterus are *Streptococcus zooepidemicus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Candida* spp., and *Aspergillus* spp (Kılıçarslan and Uçar, 2017; Dascanio, 2021).

### **3.11. Other Diagnostic Tests**

The advanced diagnostic procedures can be performed in addition to the routine breeding soundness examination in undiagnosed infertile mares. Karyotype analyses in maiden mares, gonadal hormones (progesterone, estrogen, and testosterone) for the detection of ovarian structure functions or granulosa cell tumor, hysteroscopy, and oviductal flush or PGE2 application

to evaluate any blockage of the oviduct could be performed in broodmares (Blanchard et al., 2003; Crabtree and Pycock, 2020).

#### **4. Conclusions**

The broodmares should be healthy and reproductively productive to meet the demands of breeders. The general health and the reproductive system organs should be controlled annually before the expected breeding season. Breeding soundness examination is a systematic and effective procedure to assess the health and fertility status of the mares. Annual breeding soundness examination can save time and budget as well as the welfare of the animal.

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## Live Weight and Animal Welfare in Small Ruminants

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### Abstract

Animal welfare is becoming an increasingly important component of global animal husbandry. Animal welfare is an important dimension that shows whether animal-based systems are sustainable. A husbandry system that results in low animal welfare is not sustainable and is considered unacceptable by many consumers. Animal welfare is assessed from the point of view that the animals' biological functions function optimally under the husbandry conditions and that they can maintain positive emotional states that correspond to their natural living conditions. The quality of food is determined by the welfare status of the animals from which the food originates and the quality and safety of the final product. Therefore, there is a need for strategies to improve animal welfare and for reliable monitoring systems on farms to assess animal welfare, manage potential risks and meet societal concerns and market demands. There is also a need for practical and standardised methods to assess animal welfare.

Live weight is used as an important selection criterion for improving the productivity of livestock. Changes in live weight in small ruminants are used to monitor animal health. It is also known that body condition scores based on live weight are more readily accepted by breeders when monitoring animal performance, health and welfare. By regularly monitoring body weight, body reserves can be identified and growth, reproductive performance, meat and milk production can be brought to the desired level. This study aims to emphasise the importance of live weight and welfare practises in small ruminants.

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## 1. Introduction

The rising global demand for animal-based foods poses significant challenges to sustainability and animal welfare, especially in intensive production systems (Odeon et al., 2025). Animal welfare is a crucial aspect of the food chain, as factors such as an animal's well-being, health, and stress levels prior to slaughter directly impact the quality of products derived from it (Blokhuis et al., 2008). Intensive farming techniques that prioritize high yields and production often subject animals to stress (Broom, 1986). While intensifying livestock farming is one way to meet the growing global demand for animal products, it raises concerns about animal welfare and the sustainable use of feed resources (Herrero et al., 2021; Resare Sahlin et al., 2024). Specifically, intensification in ruminant meat production focuses on increasing liveweight gain and improving resource efficiency through enhanced feed management (Wezel et al., 2015). Animal welfare remains a vital component of sustainability, as it both influences and is influenced by natural resource conservation, public health, and the economic sustainability of production systems (Paranhos da Costa, 2010). Given the ongoing global climate challenges, farm and land management decisions must balance climate concerns, economic profitability, animal welfare, and ecosystem health (Lanzoni et al., 2025).

Many sheep and goat breeds worldwide are intensively selected to improve meat, milk, and fiber production (Visser, 2025). Goats are present on five continents, with a global population exceeding one billion. Of these, 55.4% are located in Asia and 38.7% in Africa (Mazinani and Rude, 2020). This distribution corresponds with the expected pattern for animals well adapted to harsh environments and typically raised in extensive production systems (Amills et al., 2017). Similarly, the global sheep population, estimated at 1.1 billion, is distributed with 43.6% in Asia and 30% in Africa (Mazinani and Rude, 2020).

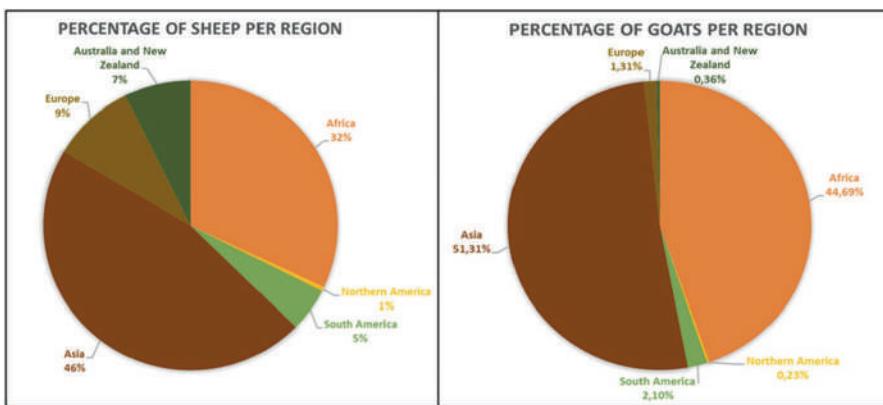


Figure 1. Worldwide distribution of small ruminants (Visser, 2025; FAOSTAT, 2024).

Sheep and goats are among the most versatile livestock species globally, thanks to their strong adaptability and ability to thrive in a wide range of ecological zones, including deserts, mountainous areas, and tropical climates (Amills et al., 2017). Although small ruminants are raised in diverse agricultural and geographical contexts, their populations are concentrated primarily in tropical and subtropical regions. In Africa and Asia, locally adapted breeds of sheep and goats are commonly raised due to their superior resilience to challenging environmental conditions. These animals are often kept in low-resource settings and marginal production areas. In contrast, in developed countries such as France and Italy in Europe, and Canada and the United States in North America, small ruminants are typically managed under intensive dairy production systems (Visser, 2025).

The welfare of animals is assessed from the point of view that the biological functions of the animals function optimally under the husbandry conditions and that they can maintain positive emotional states that correspond to the natural living conditions. The quality of food is determined by the welfare status of the animals from which the food is obtained and the quality and safety of the final product (Broom, 2010). Therefore, strategies to improve animal welfare and reliable on-farm monitoring systems are needed to assess animal welfare, manage potential risks and address societal concerns and market demands (Blokhuis et al., 2003; Caroprese et al., 2016). Animal welfare should be monitored in a way that is appropriate to farm conditions and does not impose significant additional burdens (Bozkurt et al., 2019). Animal welfare is a multidimensional concept based on the subjective experiences of animals (Broom, 2001). Therefore, animal welfare cannot be assessed directly; instead, measurements and assessments of

parameters that strongly reflect animal welfare can be used (Broom, 2010; Richmond et al., 2017). Although a more comprehensive assessment that includes physiological, behavioural and emotional states is necessary for the assessment of animal welfare, low body weight or body condition score values are highly correlated with low animal welfare.

Changes in live weight in small ruminants are used to monitor animal health (Broom, 2001; Richmond et al., 2017). In livestock farming, live weight and body condition are important factors influencing the productive period of animals (Robinson, 1990). The most practical indicator of the feeding level of sheep and goats is expressed by live weight and body condition score (Kenyon et al., 2014). Animals respond to unfavourable environmental conditions during their production period and even throughout their lives by using the fat and protein reserves in their bodies (Butler-Hogg, 1984; Fattet et al., 1984). Animals must have sufficient live weight and body condition before they reach physiological stages such as breeding, gestation and parturition (Özder et al., 1997). Live weight plays an important role in determining various traits, especially economic traits, of livestock. Parameters such as birth weight, early development, feed utilisation and growth rate can only be determined by knowing the live weight at different stages (Riva et al., 2001). By regularly monitoring body weight, body reserves can be determined and growth, reproductive performance, meat and milk production can be brought to the desired level. This study aims to emphasise the importance of live weight and welfare practises in small ruminants.

## **2. Live Weight And Animal Welfare in Small Ruminants**

The welfare of farm animals is becoming an increasingly important part of global animal husbandry. Animal welfare is an important dimension that shows whether animal-based systems are sustainable. A husbandry system that results in low welfare is not sustainable and is considered unacceptable by many consumers (Broom, 2010; Miranda de Lama et al., 2019). There is also a need for practical and standardised methods to assess animal welfare (Blokhuis et al., 2003; Fraser, 2008; Grafton et al., 2015). However, the methodology for the scientific assessment of animal welfare has developed rapidly in recent years and animal welfare has become an important scientific discipline (Broom, 2010). An important question is how animal welfare can be measured. Live weight is used as an important selection criterion when selecting to improve the productivity of farm animals. Changes in live weight in small ruminants are used to monitor animal health. In addition, body condition score has been reported to be more readily adopted by

farmers than live weight to monitor animal performance, health and welfare (Brown et al., 2015; Richmond et al., 2017).

Brown et al (2015) reported in their study that the effect of sheep live weight on sheep performance and more recently on lamb performance has been the focus of numerous studies. The most recent of these studies were conducted by Behrendt et al. (2011); Ferguson et al. (2011); Oldham et al. (2011); Thompson et al. (2011); Hickson et al. (2012); Kenyon et al. (2012) and Schreurs et al. (2012). It has been shown that ewe live weight in relation to potential mature live weight and changes in live weight before and during pregnancy are reliable indicators of ewe and lamb production outcomes (Behrendt et al. 2011).

Live weight is a very important parameter for determining the market price of small ruminants and evaluating the economic efficiency of the farm. In fact, economic modelling shows that farm profitability can be increased if live weight of sheep and goats is maintained at an optimal level (Young et al., 2011). By regularly determining and monitoring live weight in sheep and goats, feed, labour, health and other costs can be kept at the lowest possible level (optimal), thereby increasing farm profitability (Brown et al., 2015). In addition, this approach, which ensures good monitoring of animal welfare, can prevent economic losses associated with poor animal welfare and enable the production of high quality and safe sheep and goat meat, resulting in higher priced product sales (Brown et al., 2015; Young et al., 2011).

Live weight is a widely accepted indicator of the energy status of sheep and goats at a given time. In small ruminants, live weight and live weight change influence animal productivity and optimisation can increase the profitability of the whole farm. This is due to the direct relationship between live weight and energy balance, so that an increase in live weight means an increase in fat and protein content. The live weight of sheep and goats is an important physiological parameter that needs to be monitored as it is directly related to the internal energy balance of the animals. In adult animals, the increase in live weight is accompanied by an increase in fat and protein tissue, while weight loss is inversely related. The lack of acceptance of live weight data collection among small ruminant producers can be attributed to the labour, time and associated costs required to regularly weigh a herd (Van Burgel et al., 2011).

A practical and effective way to help farmers identify sheep at risk or with compromised welfare is to closely examine a representative sample of the flock while the animals are on pasture during routine management tasks

such as vaccination, parasite control, or weaning. Since seasonal variations impact welfare, these thorough examinations should be conducted multiple times per year to ensure accurate assessments. Targeting critical periods, such as mid-pregnancy and weaning, focuses attention on times when welfare issues are most prevalent (Stubsjøen et al., 2011; Phythian et al., 2011). Providing farmers with simple and accessible tools to address common welfare problems may also increase the frequency of care given to sheep needing extra attention. These methods should be practical to perform while sheep remain on pasture or easy to implement in paddocks. For example, decision trees and checklists, which are user-friendly and readily available, can assist farmers in managing animals requiring additional care. Furthermore, sensor technologies offer promising alternatives. GPS tracking devices have been developed to support farmers in monitoring flocks and detecting sick animals (Fogarty et al., 2018; Umstätter et al., 2018). However, more research is necessary to improve the usability and wider application of these technologies (Munoz et al., 2019).

Animal welfare organizations are increasingly demanding more time and workforce to be allocated for monitoring sheep to ensure adequate welfare standards (Cronin et al., 2002). However, Petherick and Edge (2010) argue that fulfilling these requirements is becoming more difficult for the industry due to limited labor availability and economic constraints. Consequently, most sectors within the livestock industry have adopted automated technologies that allow producers to remotely measure and monitor animal production (Morris et al., 2012).

Liveweight is an important measure of an animal's current physical condition and its changes over time, providing valuable insight into how the animal responds to its environment (Baker et al., 1947). Factors such as growth, nutrition, health, stress, pregnancy, and genetics all influence liveweight (Brown et al., 2015; Coates and Penning, 2000), making it widely used in studies of these aspects in small ruminants. Globally, liveweight is one of the most commonly used measurements in livestock research because it is easy to collect and interpret, comparable across and within animals, sensitive to various influences, and provides quantitative data that can be flexibly used in statistical analyses. Additionally, methods for monitoring liveweight can be effectively applied in commercial farm management (Brown et al., 2015; Coates and Penning, 2000). Recording liveweight and making management decisions based on it are recognized as key factors in improving productivity and efficiency on commercial sheep farms (Brown et al., 2015; Wishart et al., 2015; Young et al., 2011). Advances in weighing technology have recently enabled new practical applications. The use of radio-frequency

identification (RFID) chips embedded in ear tags, combined with automated readers at weighing stations, allows for the easy collection and utilization of individual liveweight data (Morgan-Davies et al., 2006). Research and implementation in the field of Precision Livestock Farming (PLF), which employs technology for precise individual animal management, is on the rise (Banhazi et al., 2012). These weighing technologies offer promising opportunities to develop management systems that leverage liveweight data as a critical decision-making tool in sheep production (Brown et al., 2014).

The majority of research and commercial applications involving liveweight data rely on comparing measurements taken at different time points, either within individual animals or across groups. To obtain accurate and comparable liveweight values, it is crucial to recognize and control for the inherent variability and potential sources of error in such measurements (Wishart et al., 2017). Short-term liveweight variations in ruminants are influenced by several factors, including feed and water consumption (Whiteman et al., 1954), the duration since the last meal (Hughes, 1976), the quality and quantity of available forage (Hughes and Harker, 1950), the age of the animal and surrounding temperature (Lush et al., 1928), as well as individual differences in grazing behavior (Hughes and Harker, 1950).

Advancements in technology and the declining cost of electronic devices have enabled the creation of various sensor-based tools in livestock farming (Caja et al., 2016; Halachmi et al., 2019). These sensors can automatically collect real-time data, allowing for early identification of important issues such as production losses, decreases in liveweight, health problems, and risks to animal welfare at both the group and individual levels (Caja et al., 2016; Krueger et al., 2020; Maltz, 2020). This approach, commonly known as Precision Livestock Farming (PLF), is defined by Berckmans (2008) as “measuring variables, modeling data to extract useful information, and using these models to monitor and control animals in real time.” A core aspect of PLF is its emphasis on the “sensor-equipped individual animal,” which represents the smallest management unit in these systems (Halachmi et al., 2019). With increasing concern for animal welfare among consumers and producers (Alonso et al., 2020), sensor technology is expected to play an important role in improving welfare monitoring and management. These technologies support a move away from traditional manual assessments at the farm level (Krueger et al., 2020) toward automated or semi-automated continuous monitoring of individual animals (Maroto-Molina et al., 2020). There is strong agreement that PLF systems can substantially improve the profitability and sustainability of livestock production, including small

ruminant farming, under a variety of production settings (Bocquier et al., 2014; Rutter, 2017).

### **3. Conclusions and Recommendations**

Live weight is a very important parameter for determining the market price of small ruminants and evaluating the economic efficiency of the farm. In fact, economic modelling shows that farm profitability can be increased if live weight of sheep and goats is kept at an optimal level. By regularly determining and monitoring the live weight of sheep and goats, the costs of feed, labour, health and other costs can be kept at the lowest possible (optimal) level, thereby increasing the profitability of the farm. In addition, this approach, which ensures good monitoring of animal welfare, can prevent economic losses associated with poor animal welfare and enable the production of high quality and safe sheep and goat meat, leading to the sale of products at higher prices.

Changing global consumption trends require innovations and changes in production strategies. Small ruminants are the easiest to adapt to strategies to produce high quality, safe food with a low carbon footprint to meet this demand. To be in line with global food production strategies, small ruminants must be reared in healthy and species-appropriate conditions. Overcoming the challenges associated with monitoring animal welfare in extensive sheep and goat farming which should be continued as a benefit is essential. Advances in technology and the decreasing cost of new electronic technologies have enabled the development of numerous sensor-based solutions for livestock farming. In this context, there is a need for new and practical solutions to better utilise live weight, which is highly linked to animal health and welfare.

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## Lamb and Kid Raising Practices and Advanced Techniques 8

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### Abstract

Lamb and kid rearing is a critical process for herd productivity and economic profitability in small ruminants. Care, feeding, and health management practices implemented from birth to weaning directly impact lamb development and survival. In recent years, the use of advanced techniques, in addition to traditional methods, has played a significant role in increasing lamb and kid rearing success. In this context, ensuring timely and adequate colostrum intake is crucial for early immune development. Artificial lamb and kid rearing systems offer an effective alternative in cases of motherlessness or inadequate lactation in multiple births. Automatic lamb and kid feeding systems, combined with fixed ration planning, optimize growth performance while reducing the risk of stress and disease. Furthermore, supporting rumen development with early provision of concentrated feed and high-quality forage facilitates post-weaning adaptation. Among advanced technological applications, sensor-based monitoring systems that allow monitoring of body temperature, activity level, and feed consumption offer significant advantages for early disease diagnosis and intervention. Furthermore, selecting individuals with high lamb and kid rearing potential through genetic selection programs increases growth and survival rates across the flock. Consequently, integrating modern management strategies and advanced techniques into lamb and kid rearing not only improves individual animal performance but also ensures the sustainability of productivity across the flock. Therefore, increasing producers' technical knowledge and developing infrastructure will increase the effectiveness of modern lamb and kid rearing practices. This study evaluates studies on the effective parameters and advanced techniques for offspring growth in small ruminant farming and offers recommendations.

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## 1. Introduction

Lamb and kid sales are among the primary sources of income for small ruminant farms and play a critical role in the economic sustainability of this production line. Lamb and kid breeding, in particular, is a strategic process that directly impacts not only individual animal performance but also herd health and farm efficiency. In this context, effective management of the period from birth to weaning increases survival rates and ensures the development of healthy, fast-growing individuals.

In modern animal husbandry practices, the lamb and kid rearing process should be based on scientific foundations and supported by developing technologies. The methods employed in this process are among the primary determinants of lamb and kid growth performance, health status, immune system development, and the future production potential of the herd. Lamb rearing encompasses a multifaceted management process, beginning with pre-birth preparation and extending through the post-weaning period.

This study examines the interaction of lamb and kid rearing practices with physiological, behavioral, and environmental factors, emphasizing the importance of science-based approaches for sustainable small ruminant farming.

### 1.1. Prenatal and Early Postnatal Period in the Growth of Lambs and Kids

In small ruminant farming, raising healthy lambs and kids is a fundamental process that directly impacts herd productivity and economic gain. The success of this process depends on scientifically based care and nutrition practices, starting from prenatal to postnatal. Adequate and balanced nutrition of sheep and goats, especially during pregnancy, is one of the most important factors determining the birth weight and viability of lambs and kids (Castillo-Gutierrez et al., 2022). Maternal nutritional strategies during the last third of pregnancy play a decisive role in offspring development and postnatal performance. Inclusion of essential amino acids, particularly methionine, in the maternal diet can support fetal development and increase birth weight. Methionine supplementation during pregnancy has been reported to positively affect birth and growth parameters but does not significantly alter milk yield and composition (Castillo-Gutierrez et al., 2022).

Rapidly establishing a bond between the lamb and its mother immediately after birth is critical for lamb health. This bond is supported by the lamb's acquisition of the suckling reflex and contact with the mother. Furthermore,

feeding colostrum in the first hours after birth provides a protective barrier against infection, laying the foundation for the immune system. Passive immunity depends on the absorption of colostrum-derived immunoglobulins (Gökçe et al., 2022; Zamuner et al., 2024). Feeding kids colostrum or milk replacer containing 80 mg/ml IgG is expected to provide twice the immune effect compared to feeds with low IgG levels. For optimal performance, colostrum consumption of at least 10% of birth weight within the first 6 hours after birth is recommended (Koşum et al., 2018). It has been determined that each hour of delay after birth significantly reduces IgG absorption, and absorption efficiency decreases significantly, especially with 12-hour delays (Zamuner et al., 2024). Colostrum intake positively affects intestinal health (Zhu et al., 2022). Timely and adequate intake of colostrum directly affects lambs' survival and growth performance. Adequate body reserves in sheep and goats are critical for both the healthy progression of pregnancy and supporting postpartum offspring development (Tozlu Celik et al., 2021; Cimpean, 2025). It has been suggested that offspring born heavier and larger grow faster in the postpartum period; this may increase reproductive performance by triggering the earlier onset of puberty (Castillo-Gutierrez et al., 2022). Therefore, maternal nutrition plans should be optimized not only for milk yield but also for offspring survival and long-term productivity goals. In this context, planning the lamb rearing process in accordance with physiological, immune-based, and environmental requirements is indispensable for the sustainability of small ruminant farming.

### **1.1.1. The Effect of Mother-Offspring Relationship on Growth**

Maternal behaviors in sheep and goats are among the fundamental biological processes that increase the offspring's chances of survival, and the expression of these behaviors is influenced by various environmental and physiological factors (Fonsêca et al., 2016). Reproductive parameters such as parity, litter size, and offspring sex are thought to play a determinant role in maternal behaviors exhibited in the early postpartum period (the first 7 days). Understanding the neuroendocrine mechanisms underlying these behaviors is important for both animal welfare and production efficiency. In this context, maternal behaviors such as suckling, care, following, tending, and udder rejection were observed in Han sheep, and the body weights of the lambs were monitored for 35 days. Additionally, serum estradiol, oxytocin, norepinephrine, dopamine, nitric oxide (NO), and  $\gamma$ -aminobutyric acid (GABA) levels in the dams were determined using ELISA and correlated with behavioral data. Study findings showed that ewes with multiple births exhibited less udder rejection and tending behavior, while higher lamb body

weights were achieved. While ewes with twin lambs exhibited more frequent lactation, ewes giving birth to ewe lambs exhibited significantly higher levels of lactation and caregiving. Neuroendocrine assessments revealed a positive correlation between the quality of maternal behaviors and oxytocin and norepinephrine levels. This study demonstrates that parity, litter size, and litter sex are important factors in the expression of maternal behaviors and that these behaviors are closely linked to neuroendocrine underpinnings (Wang et al., 2021).

### **1.1.2. Season of Birth**

Lamb and kid mortality occurring before weaning in small ruminants causes significant economic losses for producers. To reduce these losses, understanding the effect of calving season on lamb and kid development and survival rates is crucial. A study investigating the influence of calving season on lamb performance demonstrated that lambs born during the winter season exhibited significantly greater live weight and enhanced growth metrics compared to those born in other seasons. These findings indicate that calving season exerts a substantial impact on the growth trajectory and developmental patterns of lambs. In this context, it was concluded that winter lambing provides an advantage in terms of growth performance and can be considered an alternative production strategy in sheep farming (Yilmaz et al., 2007). A study conducted by Ceyhan and Kozaklı (2023) reported statistically significantly higher survival rates for lambs born in summer and autumn. The same study suggested that closer monitoring of male, twin, low birth weight (<3.5 kg) lambs, and those born in winter, could increase survival success (Ceyhan and Kozaklı, 2023).

Another study on Norduz lambs reported that lambs born in winter reached 0.5 kg, 1.6 kg, and 1.7 kg higher live weights at birth, 90, and 180 days, respectively, compared to those born in spring, and these differences were statistically significant ( $P < 0.01$ ) (Yilmaz et al., 2007). According to data by Ceyhan and Kozaklı (2023), the overall pre-weaning survival rate was 75.7%, and the mean survival time was calculated as 62.25 days. In the post-weaning period, birth season and sex had no significant effect on average daily live weight gain (ADG), with single-born lambs having ADG values 16 g/day higher than twin lambs ( $P < 0.01$ ). These findings suggest that birth season has a significant effect on lamb growth patterns and that winter lambing, in particular, provides an advantage in terms of growth performance. In this context, production strategies based on birth season should be evaluated in order to increase productivity and reduce lamb and kid losses in small ruminant farming.

### 1.1.3. Effect of Birth Weight, Sex and Birth Type

An increase in birth weight stands out as a factor that directly affects neonatal survival. Because low-birth-weight offspring have limited metabolic and physiological adaptation capacities, they are more vulnerable to stress factors encountered in extrauterine life. It has been noticed that offspring with an ideal birth weight tend to have better survival rates and more consistent growth until they are weaned. Lamb birth weight and the difference in birth weight between lambs born in the same litter are among the main parameters affecting survival and growth performance in small ruminants (Juengel et al., 2018). Genetic analyses performed in Texel sheep assessed both direct and maternal genetic influences on birth weight, weaning weight, and post-weaning weight. The estimated direct heritability values for these traits were 0.11, 0.37, and 0.31, respectively. The results underscore the presence of considerable genetic variability in growth-related traits, highlighting the potential for genetic enhancement through targeted selection strategies (Canaza-Cayo et al., 2024). Juengel et al. (2018) conducted a study on 7033 lambs born on pasture, and it was reported that birth weight was significantly affected by factors such as lamb sex, birth type, maternal weight, and parental breed; however, embryo loss and ovulation patterns had no effect on birth weight. Birth weight is considered to be a moderately heritable trait, with a heritability estimate of  $h^2 = 0.20$  (Juengel et al., 2018). Maternal age, sex, month of birth, and year of birth were also reported to affect average birth weight, weaning weight, and daily live weight in Kilis goats (Gül et al., 2021).

Male lambs have been reported to show higher live weight gains than females (Rabaa et al., 2025). In Norduz sheep, it has been emphasized that sex and birth type have statistically significant effects on lamb growth, and these factors should be considered in productivity planning (Yilmaz et al., 2007). Another study reported that birth type affects lamb weaning weight (Hızlı et al., 2022). Genotype, birth type, and sex were found to have statistically significant effects on live weights of kids from birth to 6 months of age ( $P < 0.05$ ). Furthermore, dam age was found to have a significant effect on live weights, particularly at birth and at 2.5 months of age ( $P < 0.05$ ) (Tozlu Çelik and Olfaz, 2018). These results demonstrate that growth performance is closely related to genetic and environmental factors, and herd management strategies should be optimized by considering these variables. Focusing on improving body weight through nutritional, genetic, and management solutions is thought to be beneficial for lamb survival and growth of twins and triplets (Juengel et al., 2018).

## **2. Postpartum Growth of Lambs and Kids**

### **2.1. Housing and Environmental Effect**

Housing conditions in lamb and kid farming are among the key environmental factors that directly impact animal welfare and growth performance. It is vital that lambs, who are susceptible to hypothermia, especially in the first weeks after birth, have a housing environment that is dry, clean, draft-free, and well-ventilated (Figure 1) (Broster et al., 2017). One study reported that reducing the flock size of triplet ewes by 10 during lambing increased the survival of triplet lambs by 1.5% ( $P < 0.001$ ). Lambing triplet ewes in smaller flocks can significantly increase the survival of triplet lambs (Lockwood et al., 2023). Crowded housing increases the risk of disease transmission; therefore, adequate space planning is necessary for lamb and kid per lamb. Significant increases in lamb mortality are observed during periods of concurrent rainfall, low temperatures, and high winds. However, under these stressful environmental conditions, providing shelters that lambs can effectively utilize can play a significant protective role in reducing mortality rates (Broster et al., 2017). It has been reported that flock size (10-150 head), sheep body condition score (2.8-3.5), and feed supplementation (800-2500 kg dry matter/ha) during lambing can have an impact on lamb survival rates (Thompson et al., 2023).

Welfare-focused management systems are implemented in modern lamb and kid shelters, and technological solutions such as high hygiene standards, mechanical ventilation and heating systems, and intra-herd behavioral monitoring reduce stress factors and limit negative effects on growth performance (Altinçekici and Koyuncu, 2012). It is emphasized that stress negatively affects vital functions in animals, such as the immune system, growth rate, and disease resistance; therefore, shelter-related stress factors must be carefully managed (Atkin-Willoughby et al., 2022).

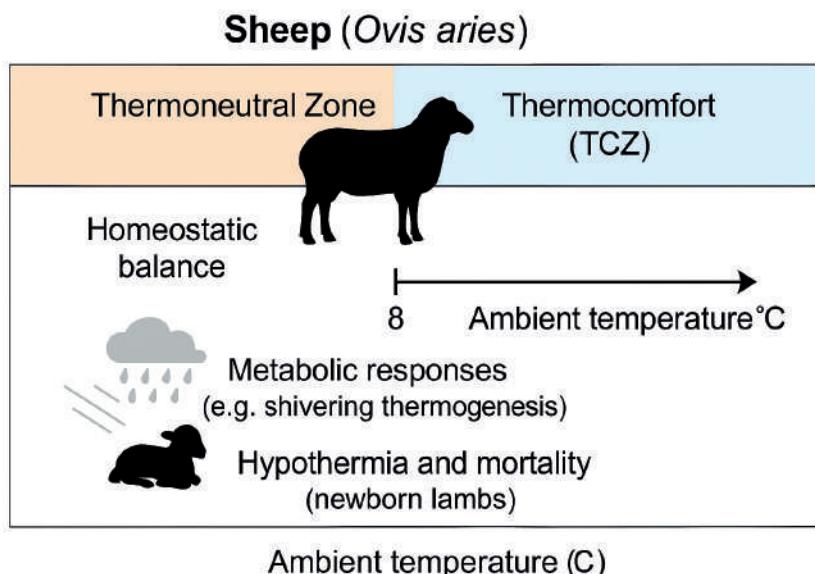


Figure 1. Thermal Zones and Survival Dynamics of Newborn Lambs

Barn ventilation systems play a critical role, particularly in controlling temperature, humidity, and harmful gases ( $\text{CO}_2$ ,  $\text{NH}_3$ ,  $\text{H}_2\text{S}$ ). One study reported a 15% increase in live weight and increased thyroxine hormone levels in lambs in fan-cooled barns (Koluman and Daskiran, 2011). Consequently, designing and managing lamb and kid barns to meet physiological needs both improves animal welfare and supports production efficiency. In this context, integrated management of elements such as barn hygiene, ventilation, and stress management is a fundamental requirement for sustainable small ruminant farming.

## 2.2. Feeding Effect

Sheep and goat farming in developing countries is critical for climate resilience and socioeconomic contributions. However, global heat stress causes significant economic losses in production systems and negatively impacts sheep's productivity, reproductive performance, and growth performance. These negative effects can be mitigated through management, genetics, and, particularly, nutritional strategies. Nutritional interventions stand out as one of the most cost-effective methods for alleviating heat stress. In this context, antioxidant supplements (e.g., vitamins B and E, selenium, zinc, naringin, seaweeds, etc.), electrolyte supplements (sodium bicarbonate, potassium bicarbonate, sodium hydroxide), mineral mixtures

(chromium, zinc, mineral blocks), and probiotics (various bacterial and yeast strains) support animal physiological responses, immunity, and production efficiency. Furthermore, prebiotics and various herbal supplements (e.g., rosemary, cinnamon, turmeric, and moringa) have positive effects on growth performance, feed intake, antioxidant capacity, and reproductive health. Effective implementation of these nutritional strategies can contribute to the sustainability of sheep production under changing climatic conditions (Rebez et al., 2025).

Feed additives (prebiotics and probiotics) are used to stabilize a healthy gut microbiome by supporting beneficial microorganisms, thus improving animal growth rate. One study found that increasing prebiotics to 0.15% increased average daily gain and feed efficiency compared to the control group, and prebiotic supplementation was reported to improve nutrient digestibility and nutritional value (Shoukry et al., 2023). Furthermore, further research is needed to investigate the effects of prebiotics, probiotics, and synbiotics as feed additives on productivity and reproductive performance in ruminants (Shoukry et al., 2023).

Feeding strategies in lamb farming aim to promote rumen development, leading to early weaning and healthy growth. Providing lambs fed milk in the first week of life with highly digestible, energy- and protein-rich starter feeds and clean water from the second week onward is critical for early activation of rumen functions. Weaning usually occurs between 6 and 10 weeks of age. This process should be planned individually based on the lambs' feed intake and developmental stage. Nutritional programming implemented early in the lambing period shapes later-term productivity characteristics and has lasting effects on the immune system, digestive capacity, and growth performance. In this context, functional foods such as natural plant extracts (e.g., garlic, thyme, and propolis), functional oils (especially omega-3 fatty acids), microminerals, and vitamin supplements offer supportive contributions to physiological development (Atılgan and Karabiyıklı Çiçek, 2021).

Individual monitoring and performance optimization can be achieved with technologies such as automated feeding systems developed to meet individual care needs in large herds and RFID-supported feeders that monitor milk consumption and weight gain per lamb (Kavurur, 2023; Tüfekci and Tozlu Çelik, 2024). These systems, as a reflection of digitalization in herd management, increase animal welfare and production efficiency.

A study conducted in subtropical climate conditions found that the use of eubiotics (probiotics and prebiotics), either alone or in combination,

increased feed efficiency and dietary energy utilization in Pelibuey × Katahdin crossbred lambs, was effective in reducing the negative effects of heat stress, and improved fattening performance. A probiotic-prebiotic combination was reported to significantly improve daily live weight gain (Estrada-Angulo et al., 2021). Similarly, a study by Mohamed et al. (2022) found that 5 g of daily probiotic supplementation positively affected live weight gain, digestibility, and feed conversion rates.

In a study conducted on the Mediterranean islands of Spain, the meat quality of Mallorquina and Roja Mallorquina male lambs was evaluated depending on the rearing system. Lambs fed continuous breast milk had higher lactic acid and milk aroma notes in their meat, while lambs fed concentrates had higher undesirable fatty acids. These results suggest that prolonged access to breast milk before weaning is a determining factor in sensory quality in traditional Mediterranean lamb production (Gutiérrez-Peña et al., 2022).

### 3. Factors Affecting Survival in Lambs and Kids

Survival and growth performance in lamb and kid farming are closely linked to preventive health practices against infectious diseases, biological factors, and environmental stressors. Diarrhea and respiratory infections, particularly common during the growing season, can negatively impact lamb and kid development; therefore, regular vaccination programs, parasite control, and hygiene practices are crucial (Hatami et al., 2022; Alavedra et al., 2025). Biosecurity measures aim to minimize the risk of disease transmission, and preventive measures such as barn cleaning, navel disinfection, and antiserum applications support lamb and kid health (Sejian et al., 2021).

One study found a positive correlation between lamb survival and birth weight; when the birth weight difference between lambs born in the same litter was  $>1.3$  kg, the survival rate decreased to 73.3%, while this rate ranged from 82.8% to 85.7% in lambs with lower birth weight differences (Juengel et al., 2018). Birth weight has also been reported to be positively correlated with the growth performance of twin and triplet lambs, with lambs with moderately high birth weight differences having approximately 3% higher growth rates. In a survival analysis conducted by Ceyhan and Kozakli (2023) using data from 11523 lambs, the overall pre-weaning survival rate was 75.7%, and the average survival time was 62.25 days. It has been determined that male lambs have a higher risk of mortality than females, singleton lambs have a lower risk of mortality than twins,

and lambs with a birth weight over 3.5 kg have a higher probability of survival (Ceyhan and Kozaklı, 2023). On the other hand, heat stress, one of the environmental stress factors for small ruminants in semiarid regions, challenges thermoregulatory capacity and can limit growth performance. In a study conducted by Silveira et al. (2025), the relationships between thermoregulatory responses and hematological, behavioral, morphometric, and carcass characteristics in 4-month-old male lambs were evaluated. Thermoregulatory variables were reported to be significantly correlated with non-carcass components ( $P = 0.002$ ), carcass performance ( $P = 0.027$ ), commercial meat cuts ( $P = 0.032$ ), and morphometric measurements ( $P = 0.029$ ), while a trend toward behavioral responses ( $P = 0.078$ ) was observed. These findings suggest that lamb survival and growth performance are shaped not only by genetics and feeding strategies but also by environmental stressors and physiological adaptability. Therefore, developing climate-resilient and welfare-focused breeding programs should be considered a strategic imperative for the sustainability of small ruminant farming.

#### **4. Advanced Techniques in Lamb and Kid Raising**

In addition to traditional rearing methods, with the advancement of livestock technologies, awareness is rising regarding the use of advanced techniques to increase growth performance, reduce environmental stress, and improve animal welfare. New technologies are improving animal welfare, health, and the sustainability of production through the monitoring and management of animal behavior. Increasing awareness of solutions developed for these systems can encourage adoption. The use of technologies for sheep and goat farming focuses on thermal stress, colostrum intake, passive immunity, offspring survival, metabolic disease biomarkers, and parasite resistance (Silva et al., 2022).

Sensor and imaging technologies, including wearable sensors that monitor parameters such as body temperature, mobility, and eating and drinking behaviors within the scope of Precision Livestock Farming (PLF), thermal cameras for early disease detection, image processing, and artificial intelligence-based weight estimation systems enable continuous monitoring of the growth process (Kavurur, 2023; Tüfekci and Tozlu Çelik, 2024). Early weaning and functional rumen development have been promoted to reduce feed costs and achieve more efficient growth. In this process, the digestive system is supported by rumen development-promoting starter feeds, probiotic and prebiotic additives, and inert yeast cultures (Estrada-Angulo et al., 2021; Mohamed et al., 2022). The success of early slaughter is directly related to the provision of high-quality starter feeds and access

to clean water. The risk of disease in lambs and kids is high during the growing period. Therefore, disease resistance is being increased by using new-generation vaccines (recombinant, subunit) and natural immune stimulants ( $\beta$ -glucans, inactivated yeast cell walls). Vaccination schedules are being tailored to specific pathogens. Advanced biotechnological applications such as genomic selection (using SNP chips), embryo transfer, and early-age sex determination, which identify genetic factors affecting growth rate, enable the reproduction of superior genotypes. These methods accelerate the process of genetic advancement. The primary goals of these technologies are to reduce labor costs, minimize the need for supplemental feeding, and increase production performance on an individual animal basis. However, extensive sheep farming systems are influenced by numerous external factors, such as local and global market conditions, government policies, and cultural dynamics, which can limit the adoption of innovative technologies (Odintsov Vaintrub et al., 2021). As noted in a qualitative study conducted by Kaler and Ruston (2019), the financial sustainability of farms, producers' level of trust in technology, and openness to new ideas stand out as the primary constraints preventing the widespread adoption of PLF technologies. However, current economic and environmental trends affecting the agricultural sector have the potential to alter these dynamics. In this context, the widespread adoption of PLF technologies may be facilitated by farmer profiles that are more open to innovation and have a higher capacity for adaptation (Odintsov Vaintrub et al., 2021).

The use of advanced, intelligent analysis methods is recommended to reveal correlations between physiological and morphological parameters associated with thermoregulation in production animals and to clarify the underlying biological mechanisms driving these relationships. The identified phenotypic biomarkers are of particular importance in the context of animal welfare and environmental adaptation. In this context, longitudinal studies examining changes in thermoregulatory responses across different environmental conditions and timeframes can provide important information for assessing the adaptive capacity of animal populations to climate change. These findings highlight the need to combine phenomic data and computational modeling techniques to guide genomic selection strategies within the context of breeding programs prioritizing climate resilience and animal welfare (Silveira et al., 2025).

## 5. Conclusion

The study demonstrates that the integration of new technologies into widespread sheep and goat farming systems contributes to improved animal

welfare by enabling more accurate monitoring and prediction of health, lambing, nutrition, and management problems. These technologies have been successfully implemented in species such as poultry and dairy cattle and also hold promise for small ruminants. The findings highlighted in the review highlight the need for a scientific and technical approach to developing sustainable solutions for widespread production systems, emphasizing the need for a holistic and global perspective. The success of this process depends on a high level of integration and collaboration among not only the research community but also all stakeholders, including farmers, technicians, veterinarians, animal production specialists, consumers, and policymakers (Silva et al., 2022).

Advanced techniques used in lamb and kid rearing positively impact not only individual development but also herd health, economic profitability, and environmental sustainability. These scientifically based practices increase production efficiency, particularly in commercial enterprises, shaping the future of modern small ruminant farming. However, the successful implementation of these techniques requires a combination of trained personnel, technical equipment, and appropriate management strategies.

Lambs and kids should be weighed regularly to monitor growth performance, weight gain should be monitored, and individuals exhibiting developmental delays should be identified early. Practices such as early weaning, group separation, individual or group-based feeding, and castration of male lambs and kids should be planned in line with production goals. The adoption and economic support of new practices in this area will enable the implementation of modern techniques in small ruminant farming, thereby increasing breeders' incomes.

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## Problems and Advanced Techniques in Herd Management Practices in Small Ruminants

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### Abstract

Small ruminant farming, particularly through sheep and goat breeds, plays a strategic role in rural development, food security, and sustainable animal production. This production model offers advantages such as biodiversity conservation, low input requirements, adaptability to challenging environmental conditions, and support for economic and ecological sustainability, particularly in mountainous and semi-arid regions. However, multiple structural and technical problems observed in its field negatively impact the sector's productivity and animal welfare. Ineffective utilization of genetic resources, inadequate breeding programs aimed at protecting and improving native breeds, seasonal fluctuations in feed supply, and a lack of quality forage increase production costs and limit animal performance. Furthermore, regional inequalities in health services make early diagnosis and control of diseases difficult, and substandard housing conditions threaten animal welfare and production sustainability. In addition to these problems, limited access to information by breeders, lack of technical training, and climatic variability reduce the sector's adaptive capacity.

Climate change is another critical factor directly impacting small ruminant farming. Rising temperatures, depleting water resources, and pasture degradation threaten both forage production and animal health. In this context, protecting indigenous breeds resilient to environmental stressors and developing climate adaptation strategies are vital for the future of the sector. Significant challenges also exist in terms of marketing and the value

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chain. Producers struggle to deliver their products directly to the market and earn low incomes in a system dominated by intermediaries. Effective cooperatives, the use of digital marketing tools, and local branding strategies are among the potential solutions that can increase producers' incomes. Finally, digitalization and data-based management systems have the potential to increase productivity in small ruminant farming. Innovative approaches such as animal tracking systems, health and production monitoring through mobile applications, and artificial intelligence-supported decision-making mechanisms both guide breeders and facilitate the production of scientific data. This study aims to develop sustainable and innovative solutions by addressing the current problems of small ruminant farming with a multidimensional approach. Improvements at both the technical and socioeconomic levels will ensure the sector achieves a resilient and competitive structure.

## 1. Introduction

Herd management is the proper execution of all animal husbandry-related tasks to create a healthy and long-lived herd. Housing animals in an environment that meets their natural behavioral needs, providing a balanced diet, ensuring access to clean and adequate water, combating diseases and pests, and timely and correct intervention are the most important factors affecting animal health and productivity. The goal of herd management is to ensure that all tasks related to animal husbandry are carried out in a timely and orderly manner. A profitable livestock enterprise is only possible through herd management. Increasing production efficiency is one of the most important concepts that contribute to competitive advantage. Consequently, agricultural enterprises engaged in livestock farming must focus on modern and professional husbandry.

Achieving good and high-quality yields in sheep and goat farming operations, as well as the continuation of the herd, depends on well-organized herd management. The concept of herd management refers to maximizing the income generated by a sheep-goat enterprise and all practices that will or should be implemented regarding the herd.

Herd management practices involve the following processes: first, all operations within the farm are defined. Second, decisions are made and implemented. Then, the farm's development is monitored, and finally, future plans are made (Öz and Bilgen, 2002). The primary goal of herd management is to manage the flock professionally, taking into account the comfort and well-being of the animals raised. Regardless of the number of animals in these farms, information on various yields obtained from these animals is collected and evaluated according to their purpose, and decisions

are made and implemented for the farm. This cycle continues, repeating itself annually. The decisions of the herd management manager regarding goals, resource allocation, planning, implementation, evaluation, and review will determine the success of the farm (Göncü, 2023).

The systematic use of herd management practices minimizes the physical and psychological burden on the farmer, increases the farm's success, reduces risks, ensures the most efficient use of farm resources, ensures the highest level of adaptation of all input elements to the needs of the animals, provides human support in herd management and early diagnosis of diseases, minimizes medication use through early diagnosis and preventative measures, maximizes the use of individual animals' potential (Bergfeld, 2006; Bewley, 2008), utilizes more reliable data in animal selection, generates herd projections, and enables more accurate planning for future periods. This study aims to highlight the challenges encountered in herd management practices in small ruminants and highlights the importance of these issues.

## 2. Genetics and Breed Selection

Genetic diversity in small ruminant farming is crucial for the continuity of animal food production and the preservation of biodiversity. One of the most effective strategies for protecting small ruminant genetic resources is the "conservation through use" approach. Rational utilization of indigenous breeds that demonstrate high adaptability to local environmental conditions significantly contributes to both strengthening food security and the environmental sustainability of agricultural production. The use of these breeds reduces dependence on external inputs, enabling the establishment of lower-cost production systems, particularly in terms of feed supply and health management. Therefore, the integration of indigenous genetic resources into production systems has the potential to increase the economic profitability of small-scale livestock enterprises (Kosgey et al., 2006). Intra-breed selection is a selection strategy frequently employed in genetic improvement programs and applied within a given population. This method aims to increase the average genetic merit (genetic merit) of a population by assessing phenotypic and genetic variation among individuals within the same breed. Intra-breed selection practices are often based on measurements and evaluations of production traits (e.g., milk yield, growth rate, reproductive performance) and contribute to sustainable genetic progress (Kosgey et al., 2006). Lack of knowledge and widespread record keeping among small ruminant breeders are among the factors that complicate selection practices. Farm size can influence the acceptance of selection practices. It is hoped that assessing the socio-economic status of small ruminant breeders in the

implementation of breeding programs will influence their success (Kosgey et al., 2006).

## **2.1. Breeding Stock and Inbreeding**

In small ruminant farming, breeding stock is sourced from within the herd, neighboring herds, or markets. Prolonged use of breeding stock within the herd can lead to problems stemming from inbreeding. Controlling breeding stock use and monitoring the duration of breeding stock within the herd are important for ensuring the sustainability of small ruminant farming. Problems arising from inbreeding are more common in small herds (Kosgey et al., 2006). In a study aimed at defining the population structure of Romanov sheep raised in the Czech Republic and assessing the impact of inbreeding on offspring production, the average inbreeding coefficient was 5.5% and the annual inbreeding rate was 1%. The same study reported a decrease in genetic diversity and the emergence of inbreeding depression (Vostry et al., 2018). This suggests that genetic variation will continue to decline unless changes are made to current breeding strategies. In a separate study, inbreeding levels and genetic diversity trends in six different purebred sheep breeds (Belclare, Charollais, Galway, Suffolk, Texel, and Vendeen) were analyzed to guide breeding programs. Based on pedigree data from a total of 472,612 individuals, the analysis revealed effective population sizes ranging from 116.0 (Belclare) to 314.8 (Charollais). The Charollais population has the highest genetic diversity, with the largest number of effective founders, effective ancestors, and effective founder genomes, while the Belclare population has the lowest values for these parameters, representing the most limited genetic diversity. Inbreeding has been reported to increase between 0.05% (Charollais) and 0.17% (Belclare) per year (Rafter et al., 2022). It has been stated that current breeding strategies are generally sufficient to maintain genetic diversity, but monitoring and intervention are still necessary, particularly for low-diversity populations (Rafter et al., 2022). Monitoring these parameters is critical, as a decrease in genetic diversity can have negative effects on both sustainable genetic progress and performance (inbreeding depression) (Bahrami et al., 2020; Cortellari et al., 2022).

## **3. Feeding and Feed Management**

Livestock production occurs in a variety of system types, which can vary greatly. Some systems are pastoral or pasture-based, while others exist in more intensive systems where livestock can be raised on smaller plots (Herrero et al., 2012). One of the biggest challenges in these

livestock systems is the high cost of animal feed. Feed costs in livestock production systems have been reported to account for up to 70% of total costs (Alqaisi et al., 2017). This significant financial burden highlights the need for more sustainable approaches to livestock production. A promising solution lies in the availability of grazing land, which offers farmers the opportunity to develop extensive livestock production. By utilizing grazing pastures, farmers can reduce their reliance on purchased feed for livestock production. Furthermore, improving livestock productivity without using additional land can be achieved through improved grazing management and pasture production practices (Thornton et al., 2010; Webb and Erasmus, 2013). Consequently, understanding the economics of various grazing management and pasture production strategies is important, particularly as their feasibility can vary significantly depending on the costs associated with implementation (Godfray et al., 2010). Furthermore, techniques such as cultivated pastures and effective grazing management systems can help reduce feed costs on farms.

In an era of high-cost inputs, pasture-based livestock production systems offer an effective way to maintain soil and plant integrity while promoting ruminant growth. In recent years, significant research has focused on the interactions between ruminants and pasture vegetation (Dickhoefer et al., 2010; Ogel and Gul, 2018). Rangeland productivity is typically assessed by measuring animal yield per acre or per capita during the grazing season. This productivity reflects the combined effects of forage growth and the efficiency with which animals convert forage into animal products. Investments in rangelands have primarily aimed to increase livestock production by increasing forage availability and grazing area (DelCurto et al., 2023). Pasture rental, feed costs, and insufficient grazing are among the problems seen (Ogel and Gul, 2018; Tüfekci, 2020; Acıbuca and Bostan Budak, 2021; Tozlu Çelik and Tüfekci, 2024).

### **3.1. Different Physiological Periods**

The proper development of the fetus and newborn lambs and kids in the womb requires adequate transport of nutrients to the placenta and mammary gland. Approximately two-thirds of a developing fetus's birth weight is gained in the last six weeks of gestation. Therefore, a balanced diet in late pregnancy is crucial for fetal development and survival at birth. The diet must contain sufficient energy and protein, which influence the development of pregnant sheep, embryonic and fetal growth, the maintenance of metabolic processes, mammary gland growth, colostrum, and milk production. Adequate transport of nutrients to tissues also affects

fetal ovarian development, postnatal growth, reproductive performance, and metabolism (Mahoub et al., 2013).

The nutrition of sheep and goats from pregnancy to birth is known to be a crucial factor in the health and survival of lambs (Hinch and Brien 2014; Rooke et al., 2015). Nutrition in sheep and goats directly affects birth weight, milk yield, the establishment of the mother-offspring bond, postpartum lamb growth, and sheep mortality. Rooke et al. (2015) reported that maternal malnutrition during the last third of pregnancy is critical, with maternal malnutrition affecting calf birth weight and survival rate from weaning to 50-85%. It has been reported that the nutrition of small ruminants during the breeding season also affects the physiology and behavior of lambs and kids (Kleemann et al., 1993). It has been reported that undernourished animals during this period give birth to smaller, less active lambs and kids.

Other side effects of undernourishment in sheep and goats include decreased udder development and decreased colostrum production and quality. Feeding supplements during mid- or late pregnancy can be used to reduce calf mortality by increasing lamb and kid birth weight, colostrum, and milk production. Furthermore, feeding sheep and goats throughout pregnancy affects maternal behavior at birth. Undernourished animals take longer to interact with their calves, exhibit more aggression, spend less time licking their calves, and spend more time feeding after birth (Nowak and Poindron, 2006).

Seasonal feed resource inadequacy leads to feeding problems, especially during dry periods. Lack of knowledge about ration preparation leads to unbalanced nutrition and production losses. Incorrect ration preparation and seasonal feed shortages in small ruminants reduce production performance. Roughage availability is a critical issue, especially during droughts.

#### **4. Health and Disease Control**

Parasitic infections, zoonotic diseases, and epidemics remain a widespread problem. Inadequate preventive veterinary services make early diagnosis and intervention difficult. Parasitic infections and zoonotic diseases are widely reported in small ruminants (Tüfekci, 2020). Regional inequalities in veterinary services make early diagnosis and treatment difficult. Irregular vaccinations and errors in medication use threaten herd health (Yıldız and Aygün, 2021).

Selecting genetically resistant animals is a more reliable option among sustainable parasite control strategies and can yield results when integrated

with other strategies such as grazing management and anthelmintic applications (Zvinorova et al., 2016). A study in Ethiopia demonstrated that veterinary and genetic improvement interventions significantly increased the number of sheep and goats offered to market by smallholders. Producers utilizing community-based veterinary services achieved higher economic returns per animal; it was determined that they reached higher levels in terms of purchasing power, annual income, and earnings per animal unit. In the same study, producers participating in the Community-Based Improvement Program were able to offer a higher number of small ruminants to the market and demonstrated a more advantageous economic performance in terms of total income (Kassie et al., 2021). In livestock farms in Greece, quarantine practices, feed analysis, the use of ultrasound for pregnancy control, and the frequency of veterinarian visits were positively correlated with milk production, milk protein content, and calf number (Lianou and Fthenakis, 2021). A study conducted in Nigeria indicated that health management practices were at a low level (Kalu et al., 2021). As awareness of health practices has increased, the number of practices to be implemented in this direction has increased in recent years (Tozlu Çelik and Tüfekci, 2024). In addition to increasing the accessibility of vaccines, encouraging activities to raise public awareness is of vital importance for the effectiveness of strategies for disease prevention and control at the national level (Tüfekci, 2020; Win et al., 2021).

## 5. Shelter and Management Conditions

Inadequate shelters in terms of hygiene, ventilation, and space requirements reduce animal welfare. The lack of modernization of herd management techniques reduces labor productivity. The inadequacy of shelters, particularly the lack of humidity and temperature control, is among the primary factors negatively impacting animal welfare. Compared to farming practices in Europe and America, the need for modernization of shelter systems in Turkey becomes evident. It has been determined that adobe pen structures constitute the majority (Yıldız and Aygün, 2021).

Housing conditions are a key parameter affecting the survival of newborn calves in small ruminant farming (Pritchard et al., 2021). Studies have indicated that shelter conditions are inadequate and primitive (Ogel and Gul, 2018). However, in recent years, shelters have been equipped with windows necessary for ventilation and light (Tüfekci, 2020).

Community-Based Breeding Programs (CBBPs) have emerged as a sustainable and participatory approach to breeding indigenous livestock on

smallholder farms. Goat CBBP models implemented in Malawi and Uganda demonstrate the potential to achieve the dual objectives of conserving and improving local genetic resources and supporting rural livelihoods. These programs promote and strengthen smallholders' access to feed resources for small ruminants through the sustainable management of existing communal pastures, increasing feed production capacity, and effectively utilizing agricultural residues and by-products (Kaumbata et al., 2021). Housing conditions need to be updated to meet changing climate conditions. Some of the practices that address climate change include ventilation, fans, and cooling devices in the shelters.

## **6. Access to Education and Information**

A study conducted in Malaysia found that the majority of farmers were between 40 and 50 years of age (23.5%). The majority of farmers were male (92.8%), while the female rate was 7.2%. A significant portion of farmers were educated, with 36% having less than five years of professional experience. However, a small proportion of participants (1.2%) exhibited insufficient literacy (Melissa et al., 2016). A recent study reported that 67% of small ruminant farmers in Malaysia were between 21 and 40 years old, and the majority were male (Mazlan et al., 2023). In Nigeria, 68.7% of farmers were between 31 and 50 years of age, and 96.0% were literate (Adetarami et al., 2020). The average age of farmers participating in the study in Şırnak was 49.98. Of the participants, 75.78% were only literate, 23.44% were primary school graduates, and 0.78% were high school graduates. The average professional experience in goat farming was 13.32 years, while this figure was 13.37 years in sheep farming. Access to information and communication technologies was generally low; while all farmers owned a mobile phone, only 31.25% had a computer, and 21.88% had internet access (Ogel and Gul, 2018). In Karaman province, 61.9% of farmers were between the ages of 41 and 55, and primary school graduates constituted the majority (63.5%) (Demirbük, 2021). Various literatures indicate that breeders are, on average, 40 years old or older, and the majority are male (Tüfekci, 2020; Acibuca and Bostan Budak, 2021; Yıldız and Aygün, 2021; Lianou and Fthenakis, 2021; Kalu et al., 2021; Tozlu Çelik and Tüfekci, 2024).

Breeders' lack of technical knowledge prevents scientific practices from being implemented in the field. Limited extension activities hinder knowledge transfer and the adoption of innovations. Lack of knowledge in small ruminant farming is one of the main causes of application errors. Limited extension activities hinder the transfer of scientific knowledge to the

field. Access to and acceptance of information is influenced by breeders' age, region, educational status, and economic opportunities.

## 7. Climate and Environmental Factors

Environmental stress factors such as extreme heat, humidity, and drought reduce production performance, particularly in breeds with low adaptability. Overgrazing and erosion of pastures threaten sustainable forage supply. In migratory livestock farming, climate variability challenges the adaptability of animals. Extreme heat and drought, in particular, complicate herd management. Overgrazing and erosion of pastures threaten the sustainability of forage resources.

As part of agricultural adaptation strategies for climate change, the use of concentrated feed, promotion of forage crop production, increasing diversity in production patterns, and breeding breeds more resilient to environmental stressors such as disease and drought stand out as key practices. However, the most significant obstacle to climate change adaptation has been identified as a lack of knowledge. Regular communication of climatic impacts on agricultural production and possible future scenarios to producers should be made possible through planned training activities in this context (Demirbük, 2021; Kaygisiz et al., 2023). Importance should be given to providing awareness training to growers regarding climate change and to dissemination activities through the media.

## 8. Adoption of New Technologies

The potential for integrating precision animal husbandry (HH) technologies into dairy sheep farming systems, particularly those commonly implemented in the Mediterranean region, has been examined. Mediterranean countries (France, Italy, Greece, and Spain) account for approximately 40% of global sheep milk production, and dairy sheep farming is an important production model in these regions, both culturally and economically. In developed countries in the region, dairy sheep farming has evolved into highly specialized systems through improved animal breeding, increased feeding strategies, and increased production intensity. However, extensive systems remain important due to their low input costs and resilience to market fluctuations. HH technologies include components such as electronic identification systems (ear tags, rumen bolus, subcutaneous RFID), on-animal sensors (accelerometers, GPS, activity monitors), and stationary management systems (weight measuring devices, automatic drafting machines, virtual fences, and milking technologies). The suitability of these technologies for integration into widespread dairy

sheep production systems has been evaluated. However, adoption of these technologies in small and medium-sized farms remains limited. The high average age of breeders, conservative attitudes toward technological change, limited financial resources, and a low risk-taking tendency are among the main factors slowing this process. However, global trends such as global warming, animal welfare, antibiotic resistance, and changes in European Union agricultural policies are increasing the importance of HH technologies and creating an environment that could encourage their wider adoption in the future. Consequently, the adoption of HH technologies in dairy sheep farming offers significant opportunities in terms of production efficiency, animal welfare, and sustainability. The dissemination of these technologies requires overcoming socioeconomic barriers and developing targeted support policies (Odintsov Vaintrub et al., 2021).

To collect baseline data from New Zealand sheep farmers on lamb tail docking and castration techniques, their perceptions of the level of pain experienced by lambs after these procedures, and their views on the use of analgesia. Methods: Descriptive statistics for quantitative study variables were provided from a cross-sectional survey of New Zealand sheep farmers administered to volunteers. Thematic analysis was conducted using free-text comments. Univariate logistic regression was used to assess factors associated with farmers indicating they would consider using a device that allows analgesia simultaneously with castration and tail docking. Results: There were 432 survey responses with sufficient data for analysis. Of the 340 (77.5%) who always or sometimes castrated ram lambs, 242 (72.2%) used a rubber ring for complete castration, 23 (6.9%) used the short scrotum method for cryptorchid castration, and 75 (22.4%) used a combination of both methods. Of the 423 (97.9%) respondents who reported always or sometimes performing tail docking, 245 (57.9%) used only a hot iron, 148 (35.0%) used only a rubber ring, 26 (5.8%) used both methods, 3 (0.7%) used a surgical blade, and 1 (0.2%) did not provide any response. Less than 2% of respondents always or sometimes used painkillers for these procedures. Of the 432 respondents, 139 (32.2%) and 180 (41.7%) strongly agreed that castration and tail docking, respectively, did not cause enough pain to warrant the use of painkillers. Time and cost were identified as the biggest barriers to providing painkillers. In unadjusted logistic regression analyses, participants who were female, had a higher level of education, had been farming for less than 20 years, believed lambs experienced high levels of pain after the procedures, and believed the pain lasted longer than 6 hours were more likely to express a desire to use pain control devices. Conclusion and clinical relevance: Our findings suggest that very few sheep farmers in New

Zealand provide pain relief to lambs after tail docking or castration. This is likely due to the perception that the procedures are not painful enough to warrant pain relief and to concerns about time and cost. This highlights the need for farmers to be educated about lamb pain and distress after tail docking and castration and the negative impact this can have on animal welfare. Farmers also need pain relief techniques and devices that can be applied simultaneously with these procedures to save time and labor costs (Kongara et al., 2023).

## 9. Marketing Products

It is crucial that animal products are transported from production areas to the market and safely transferred from producer to consumer. Products derived from small ruminants, in particular, are highly valuable. Due to the increasing demand for natural products in recent years, they must be marketed at a value price.

Small ruminant farming contributes significantly to the rural economy through the production of various animal products, such as meat, milk, wool, and leather. However, despite the strategic importance of marketing these products, the process of marketing them still faces numerous structural problems in Turkey. Disorganized production structures, lack of organization, inadequate marketing channels, and price instability are among the main obstacles directly impacting producers' incomes. Furthermore, the lack of product standardization in line with consumer demands and the lack of branding limit the competitiveness of small ruminant products. In this context, addressing marketing issues from a multifaceted perspective is a critical necessity for both improving producer welfare and the sustainability of the sector.

A study conducted in Şırnak province identified insufficient use of mass media channels. It has been reported that private veterinarians, veterinary consultants, and other breeders are the primary sources of information on sheep farming and marketing. It has been noted that breeders in this region are unable to market their products at value (Ogel and Gul, 2018). Producers struggle to deliver their products directly to the market and earn low incomes in a system dominated by intermediaries. Effective cooperatives, the use of digital marketing tools, and local branding strategies are among the potential solutions that can increase producers' incomes.

## 10. The Problem of Finding Shepherds

Small ruminant farming plays a critical role in economic sustainability and food security, especially in rural areas. However, this sector has faced serious challenges in accessing a qualified workforce in recent years. The availability of shepherds, in particular, has become one of the main problems directly affecting the continuity of the production process and animal welfare. The aging rural population, the shift of young people to non-agricultural sectors, and the perceived unattractiveness of shepherding due to social status are exacerbating this problem. In this context, examining the effects of a lack of shepherds on small ruminant farming is crucial for increasing sectoral productivity and developing sustainable livestock policies. Various studies have indicated that small ruminant farmers face difficulties finding shepherds (Ogel and Gul, 2018; Tüfekci, 2020; Acıbuca and Bostan Budak, 2021; Yıldız and Aygün, 2021; Tozlu Çelik and Tüfekci, 2024). To ensure the sustainability of small ruminant farming, problems must be addressed not only from a technical perspective but also from a socio-economic perspective. Active participation of farmers in decision-making processes, leveraging local knowledge, and integrating young people into the sector are critical to sustainable production. Difficulties in recruiting shepherds, in particular, directly impact both workforce continuity and animal welfare.

## 11. Advanced Technology Applications

Manually tracking livestock data is both time-consuming and insecure. This highlights the need for electronically based livestock management systems to quickly and accurately record and back up data and to make future decisions by leveraging previous data when necessary (Tsipis et al., 2022; Ariff et al., 2014).

Flock management in livestock farms is complex, requiring technical knowledge, ensuring animal health and welfare, ensuring quality and product safety, monitoring worker productivity and health, carefully evaluating various data sources within its own logic, and requiring a professional approach to making sound decisions. Therefore, herd management systems, automation, and artificial intelligence applications have gradually begun to be used in livestock farms. These applications are of great importance because they form a never-ending cycle for the continuity and profitability of production in the short and long term (Kopuzlu, 2023). Although farm management may show some differences on a country and regional basis, considering that it serves within a global economic structure, it must know and follow what is happening in different parts of the world, both locally and

globally, sectorally. In such farms, every process, from herd management and insemination matching to heat stress and manure management, from udder health to estrus and fertility monitoring, and from animal behavior to animal selection, is carried out digitally. In modern animal husbandry, all types of data related to productivity, behavior, and disease are collected. Electronic identification devices (IRFDs) attached to the ears, ankles, and necks of animals, along with sensors and cameras, record their rumination count, movements, estrus, live weight, calving times, lying and standing times, the amount of factory feed consumed, body condition scores, milk yield and characteristics, length of time spent in the feedlot, body temperature, and many other parameters digitally (Kopuzlu, 2023).

Herd management can transition from a group-level approach to individual-focused herd management thanks to technological applications (Bewley, 2008). Technological applications in herd management allow for the effective use of automatic animal identification, detection, measurement, and information processing technologies to continuously monitor the production process, achieving optimal results in profitability, health, quality, product safety, animal protection, and environmental protection. These systems aim to increase the effectiveness of the production control process and, by doing so, positively impact the management of yield, quality, feeding, health, and reproduction within the farm (Uzmay et al., 2010).

Within the herd management system, records are kept for animals' birth and various life-cycle weights, productivity traits, various health-related data, daily feeding principles, and information on various environmental impacts. These records allow for the creation of a herd projection encompassing health practices, feed supply, feeding programs, animal breeding programs, quality milk production, monitoring worker performance, and operating income and expenses to ensure the continuity of a productive and healthy herd in the future. These systems not only save on labor but also minimize human errors.

Barcodes and tags, found on suitable low-frequency RFID (Radio Frequency Identification) devices, are used for animal tracking and ensure the most efficient and effective tracking. Using RFID antennas, reader modules, and tag technologies, animals are quickly and securely identified. Furthermore, this allows for automated data collection. RFID tags are divided into three groups based on their functions: active, passive, and semi-active (Doğan et al., 2016). Active tags communicate via a built-in battery. They offer excellent reading range but are expensive. Therefore, they are used for identifying and tracking expensive items (Domdouzis et al., 2007). Passive

tags lack a power source. Therefore, they derive the necessary energy from readers. The reader sends a radio signal to the antenna. The tag receives this signal via the antennas, providing the power needed to operate the chip. In other words, the tag uses the energy from the signal to perform its function. The communication range is relatively short. They can be used in many systems due to their cost-effectiveness (Roberts, 2006). Semi-active tags, on the other hand, have their own power source, but this source is used only to power the chip. Like passive tags, they can read via electromagnetic fields emitted from the reader, but they cannot broadcast like active tags (Karaca, 2010). If the sole purpose is animal identification or tracking, passive tags are sufficient. However, if sensor-based applications are desired, active or semi-active tags must be used (Chandrud et al., 2008). RFID tags can be injected into the body. Rumen-type tags are more sensitive to external factors but have a relatively more complex structure (Hong, 2012). There is a possibility that injected tags may become contaminated with animal products after slaughter. Alternative electronic tags have been designed to mitigate this risk (Doğan et al., 2016). These tags, called rumen bolus tags, can be made of ceramic or steel (Fallon, 2001). Numerous studies by Hong (2012) and Varese et al. (2008) have demonstrated the superior properties of rumen bolus tags over other tags. Additionally, RFID systems have some drawbacks, such as mechanical damage, environmental damage from dust and extreme heat or cold, difficulty reading tags over time, and problems arising from the effects of other electronic devices in the environment (Mennecke and Townsend, 2005).

Herd management in small ruminants is a complex process that requires coping with challenges such as climate change, antimicrobial resistance, and pathogen control. While traditional methods are valuable for parasite management and disease diagnosis, the integration of modern technologies can provide significant improvements in this process. Innovative tools such as new sensor technologies and smart monitoring systems offer significant opportunities to improve herd health and welfare. These technologies have the potential to increase productivity through applications such as early disease detection, determination of thermal stress through temperature measurements, and monitoring animal behavior. However, the dissemination of these innovations is limited by barriers such as cost and difficulty in collecting accurate data. Providing small ruminant producers with access to these new technologies is critical for the adoption of animal welfare practices and improving overall production efficiency (Tüfekci and Tozlu Çelik, 2024). The effective adoption of a technology in agricultural production systems depends primarily on its compatibility with the needs of farmers

and existing production conditions. In this context, proposed technologies should be relatively simple, economically accessible, and present low risks during implementation. The success of breeding programs depends not only on technical parameters but also on a holistic approach to evaluating the production system and integrating producers' traditional knowledge, behaviors, and value systems into the process. Active participation of producers in the planning, implementation, and evaluation of breeding programs increases their sustainability and acceptability. However, as with all innovative initiatives, the possibility of failure in animal breeding programs must be considered. Rather than operating with unrealistic expectations of success, accepting a certain level of failure as a natural part of the development process allows for the development of more flexible and adaptable strategies that support long-term progress (Kosgey et al., 2006).

As a result, the use of innovative technologies in animal production will contribute to animal health and welfare while also contributing significantly to the economic sustainability of animal production through its long-term cost-reducing effect as a result of obtaining more efficient and higher-quality animal products.

## **12. Conclusion and Recommendations**

Small ruminant farming is a production area that provides animal food and income. Identifying the problems faced by small ruminant farmers and developing solutions is crucial for the sustainability of small ruminant farming and ensuring the safe production of the resulting food. Small ruminant farming is a widespread practice in various parts of the world. To address these challenges, training should be provided to farmers to facilitate their needs and adapt to innovations. Planning should consider genetic diversity, regional production areas, herd sizes, and the farmers' opinions when setting short-, medium-, and long-term goals. Evaluating the support provided to small ruminant farmers by considering regional conditions and socioeconomic structures is crucial for sustainability.

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## Occupational Health and Safety Practices in Animal Production 8

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### Abstract

Occupational accidents and diseases are among the most significant issues in the workplace worldwide and in our country. The concept of occupational health and safety encompasses all systematic scientific studies aimed at protecting workers from potential hazards and factors that may harm their health while performing their jobs in the workplace, as well as reducing risks and improving the workplace environment. Workers in the agricultural sector mostly perform their jobs alone and work without protection for long periods of time in terms of occupational health and safety. Almost all activities carried out within agricultural production, whether plant or animal production, are classified as hazardous. Occupational health and safety in animal production is very important, as it is in many other areas. Animal husbandry is a hazardous activity that involves many factors that can potentially contribute to injury and even death among workers.

Occupational health and safety in animal husbandry must be clearly defined. It must be consistent with animal development policies covering both commercial and smallholder farming. Specific programs and strategic action plans emphasizing the prevention of occupational risks for workers in animal production must be developed. Furthermore, occupational health practices must be integrated into the basic health service structure.

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## 1. Introduction

Occupational accidents and diseases are among the most significant problems in the working life worldwide and in our country. Occupational disease is defined as “diseases resulting from exposure to risk factors in the workplace” within the framework of the International Labor Organization’s Occupational Health and Safety Convention No. 155 and the ILO protocol prepared in 2002 (Şener et al., 2024). Occupational health and safety is one of the most important components of efforts to protect individuals’ fundamental rights in working life and to create a safe environment in the workplace. Organizations such as the International Labor Organization (ILO) and the World Health Organization (WHO) define occupational health and safety as a discipline that supports the mental, physical, and social well-being of individuals. Approaches developed since the industrial revolution to prevent occupational diseases and accidents have become more comprehensive and systematic today. Technological advances and workplace dynamics have necessitated the continuous development of occupational health and safety practices. Legal regulations such as the Occupational Health and Safety Law (No. 6331) in Türkiye clearly define the responsibilities of employers and employees in this area. In this context, the obligation of employers to reduce occupational risks, protect employees, and provide safe working environments at workplaces emerges as both an individual and a social responsibility (İnci et al., 2024). The purpose of occupational health and safety is to eliminate risks and hazards that threaten individuals’ right to life and to provide a safe working environment by taking the necessary measures for previously identified hazards (Dizdar and Önder, 2023). In this context, occupational health and safety aims to protect the life, physical, and mental health of employees. By preventing occupational accidents and diseases, workforce losses are reduced and production safety is ensured (Karabal, 2021).

Agriculture continues to be important worldwide in terms of meeting food needs, providing inputs to the industrial sector, exports, and the employment opportunities it creates. If the characteristics of agriculture are analyzed properly, it can be seen that it differs from other economic sectors. Agriculture, which differs significantly from the other two main economic sectors, industry and services, in terms of production methods and factors, has a structure in which production takes place in both open and closed areas, unlike other industries. In addition to plant and animal production in open areas, livestock activities in closed areas and production activities in greenhouses demonstrate the diversity and scope of agricultural production. These characteristics also deepen the differences in its economic and social

structure. The variability of supply and demand in agricultural production significantly distinguishes the economic aspect of this sector from other sectors. In addition to these characteristics, factors such as dependence on the season, year, and natural conditions, the limited production period, the abundance of product varieties, the diversity of working conditions, and the lack of division of labor and specialization make regulations and studies in this field difficult in every respect. These differences also affect working conditions and the conditions, rights, and responsibilities of workers in agriculture (Yurtlu, 2015).

The concept of occupational health and safety encompasses all systematic scientific studies aimed at protecting workers from potential hazards and factors that could harm their health while performing their duties in the workplace, as well as reducing risks and improving the workplace environment (Balkır, 2012). Workers in the agricultural sector mostly perform their jobs alone and work unprotected for long periods of time in terms of occupational health and safety (Akpinar and Özyıldırım, 2015). Long working hours and weekend work prevent workers in the agricultural sector from having opportunities to rest. At the same time, inadequate working and living conditions also cause various health problems among workers (Özel and Güğercin, 2020).

The importance of the agriculture and food sector has come to the forefront in many countries as a result of the COVID-19 crisis. Measures taken to slow down the pandemic have also highlighted the sector's problems in meeting demand, generating income, and ensuring safety and health conditions for millions of agricultural workers and producers. In our country, the sustainability of agriculture is possible through ensuring the health and safety of agricultural workers and farmers in their workplaces. The agricultural sector is of concern to the entire country in terms of food production and nutrition, its share of the active population and workforce, its contribution to national income, and the raw materials and capital it provides to the industrial sector, as well as the creation and protection of a healthy environment, the establishment of ecological balance, and sustainability. Therefore, it retains its characteristic as an economic and social sector. When viewed from both an economic and employment perspective, occupational health and safety in the agricultural sector is seen to be an issue of paramount importance (Onan Erdal, 2020).

Agricultural activities carried out to meet the needs of the growing world population and the food requirements to satisfy it always retain their importance. These activities are carried out in open, closed, or semi open

areas within plant and animal production. Activities in closed areas also include agriculture and agriculture-based industries. Agricultural activities encompass all hazard classes, including highly hazardous, hazardous, and low-hazard. When agricultural activities are examined separately according to NACE (Nomenclature of Economic Activities) codes within all job categories, it is understood that they harbor many different hidden risks and hazards within themselves, due to factors arising from the materials used and environmental conditions (Alanyurt and Tekin, 2023). Due to the exposure of workers in the agricultural sector to various chemicals, pesticides, particulate matter, zoonotic diseases, and work accidents, occupational diseases and work accidents are more common in the agricultural sector than in other sectors. For this reason, it is considered one of the most dangerous industries worldwide (Molina-Guzmán and Ríos-Osorio, 2020).

The agricultural sector is mechanized in developed countries, while it is labor intensive in developing countries. In developing countries, it is mostly run by small businesses or family businesses, and workers' wages are also quite low (Bilir, 2019). According to 2021 data from the International Labor Organization (ILO), approximately 1.1 billion people are engaged in agricultural work. This report states that a large number of temporary workers are employed by small and large growers. In addition, family members work for free in agriculture to support informal agricultural work or small-scale family farming (ILO, 2023). According to European Union data, approximately 8.7 million people were working in agriculture in European Union member countries in 2020. It is stated that 9 out of every 10 workers (86.1%) are family businesses. In our country, according to TÜİK data, 16.8% of workers were employed in the agricultural sector in 2021 (TÜİK, 2023, Babaoğlu, 2023).

As a result, the rapidly growing world population has made the agriculture and livestock sectors, which provide people with basic foodstuffs, one of the most important sectors. In order to ensure sustainability in these sectors, it is essential that workers benefit from occupational health and safety services and that working conditions are improved. Timing is one of the main factors that complicate agricultural activities. Over a period of approximately one year, it is important to adapt to changing climate and natural conditions at every stage of production and to take the necessary precautions. For this reason, work must be done at the right time to reduce losses and prevent wasted effort. When work is rushed to meet deadlines, the protection and prevention activities that form the basis of occupational safety cannot be carried out properly. Considering factors such as lack of information, carelessness, and negligence, various studies have revealed the

serious extent of accidents and injuries related to occupational health and safety in agriculture. This study aims to highlight occupational health and safety practices in animal production and the importance of this issue.

## 2. Occupational Health and Safety in Livestock Farming

Livestock farming is a hazardous activity involving many factors that can potentially contribute to worker injuries and even death. However, farm workers generally do not perceive livestock farming as a source of danger (Lindahl et al., 2013; Doğan and Demirci, 2012). Working in the livestock sector continues to be among the most dangerous jobs across all occupations (Mitloehner and Schenker, 2007). Occupational health and safety in this sector is of great importance due to workers' exposure to physical, chemical, biological, and ergonomic risks. Aggressive animal behavior, improper handling techniques, and poor hygiene lead to workplace accidents, while zoonotic diseases and manure gases can cause serious health problems. The indiscriminate use of chemical disinfectants and medications causes respiratory and skin diseases, while heavy lifting and repetitive movements lead to an increase in musculoskeletal disorders. A large proportion of work accidents occur due to direct contact with animals, incorrect use of equipment, and poor hygiene. It is vital to raise awareness among workers about the use of personal protective equipment, provide ergonomic working environments, and establish emergency plans. Furthermore, risks should be minimized by providing regular training on safe working techniques with animals, hygiene rules, and emergency procedures (Serap and İkbal, 2020).

Occupational health and safety in animal production is very important, as it is in many other areas. Most occupational health and safety accidents and incidents occurring in workplaces in Türkiye are not reported. The most common hazards in animal production in Türkiye are zoonotic diseases, ergonomics, noise, climate control, chemicals, animal attacks, bites, injuries, transport accidents, psychological stress, and diseases transmitted through the skin. In particular, animal collisions and zoonotic diseases are very important in animal husbandry (Aygün et al., 2014; Aygün et al., 2019).

Agricultural workers are exposed to attacks by wild animals such as snakes, scorpions, and spiders in the areas where they carry out agricultural activities, resulting in poisoning, injuries, and even deaths. Injuries caused by animals result from agricultural workers who raise animals working in close proximity to them, and are caused by factors such as hitting a hard surface, being kicked, the farmer's incorrect behavior, being bitten, standing in a blind spot, and exposure due to triggering behaviors.

## 2. 1. Zoonotic (zoonosis) diseases

Diseases that can be transmitted from humans to animals and from animals to humans, and that are observed in both humans and animals, are referred to as zoonosis (Weissenböck et al., 2010). Due to their potential for pandemic and endemic spread, zoonotic diseases pose significant risks as they directly impact human health. Diseases transmitted from animals to humans (zoonosis) are caused by various agents, including viruses, bacteria, and parasites. These agents can be transmitted to humans through animal feces, urine, saliva, blood, milk, animal-derived foods, contact with animals, the mouth, skin, and respiratory tract. (Anonymous 2015; Anonymous, 2016; Anonymous, 2017). While there are approximately 250 diseases transmitted from animals to humans worldwide, this number is around 50 in Türkiye. The main diseases found in Türkiye that are transmitted from animals to humans are anthrax, brucellosis, salmonella, influenza, and rabies (Anonymous, 2004). Common zoonosis, their transmission routes, and effects are presented in Table 1 below.

*Table 1. Zoonosis, transmission routes and effects (Taş, 2018)*

Zoonosis	Transmission Route	Effects
Brucellosis	Transmitted by touching tissues such as the placenta of infected farm animals.	Causes fever in humans.
Anthrax	Transmitted by contact with tissues of infected animals.	Causes skin lesions.
Leptospirosis	Transmitted when the skin comes into contact with dirty water contaminated by rodents or farm animals.	Causes fever in humans.
Campylobacter, Cryptosporidium	Transmitted from farm animals through contaminated water and food.	Causes gastrointestinal diseases such as diarrhea.
Rabies	Transmitted by bites from wild animals and virus-carrying dogs.	Causes severe nervous system disorders that can lead to death.
Psittacosis	Transmitted by inhaling dust contaminated with droppings from birds and poultry.	Causes pneumonia.
Tuberculosis	Transmitted by inhaling airborne droplets or by drinking unpasteurized milk.	Causes fever, cough, weight loss, fatigue, and night sweats.

Various measures must be taken to protect against zoonotic diseases. Regular vaccination of animals and humans, ensuring hygiene in the workplace, and using food in safe and healthy conditions will significantly reduce the risk of transmission of zoonotic diseases. Furthermore, it is crucial to educate employees about the transmission routes of these diseases and prevention methods through occupational health and safety training (Ağar, 2025).

## 2. 2. Respiratory Diseases and Skin Diseases

According to research, 25% of workers in the livestock sector suffer from some form of respiratory disease. This rate places respiratory diseases among the most common illnesses affecting workers in the livestock sector. The most significant causes of these illnesses are dust, gases, agricultural chemicals, and infectious agents. Dusts can be divided into two groups. The first group consists of dusts composed of organic components, while the second group consists of dusts composed of inorganic components. The most important source of inorganic dusts is pastures and green areas. The most important source of diseases, however, is agricultural organic dusts that harbor microbes. Bronchitis and asthma are the most common disorders. Gases are also a major cause of respiratory diseases encountered in the livestock sector. High ammonia levels in large enclosed areas and poultry houses where ruminant animals are fed pose certain dangers (Anonymous, 2004; Demirhan et al., 2016).

Workers in the animal production sector are exposed to various agents, including the influenza virus, *Escherichia coli*, and drug-resistant *Staphylococcus* species, which are zoonotic diseases. Some of these agents cause acute or chronic respiratory symptoms and pose a significant public health problem (Klous et al., 2016; Nordgren and Charavaryamath, 2018). These diseases caused by biological agents can lead to serious labor force losses among workers. This reduction in the workforce causes disruptions in animal care and production processes, leading to significant economic losses in the livestock sector (Ağar, 2025).

Living in areas with a high concentration of livestock farms has been associated with adverse effects on respiratory health in some studies. In their study, van Dijk et al. (2016) reported that individuals with chronic obstructive pulmonary disease (COPD) and asthma are at higher risk from these environmental effects due to their already compromised respiratory function and chronic airway inflammation. The use of personal protective equipment is critical for protecting against disease, particularly for the

health and well-being of workers in the livestock sector. Considering that transmission in this sector is mostly through respiratory and skin contact, appropriate masks and work clothing must be provided and used correctly. Furthermore, installing local and general ventilation systems for workers in enclosed spaces will significantly reduce dust exposure and contribute to protecting respiratory health (Demirhan et al., 2016; Ağar, 2025).

Workers in plant and animal production within agricultural production and other rural residents are frequently in contact with agents that can cause skin diseases. The main causes of skin disease in the agricultural environment are plants, insects, insecticides, sunlight, heat, and infectious agents. Farm and rural-related skin diseases can be divided into five main categories: contact dermatitis; infectious dermatitis; dermatitis caused by arthropods; skin conditions caused by the sun and skin disorders related to heat, cold, and humidity. This section describes the type, diagnosis, treatment, and prevention of skin diseases in each of these five categories. Contact dermatitis can be divided into the following categories: irritant contact dermatitis; allergic contact dermatitis; photoirritant contact dermatitis; and photoallergic contact dermatitis. The three most important and most common infectious dermatoses among agricultural workers are zoonotic dermatophyte fungi and two zoonotic viral diseases, contagious ecthyma of sheep and goats and pseudocowpox of cattle (Donham and Thelin, 2016). Skin diseases can be classified as contact dermatitis; sun-induced, infection-induced, or insect induced. It is estimated that nearly 70% of diseases encountered in the agricultural sector are skin-related. Contact dermatitis is common. Manure, plants, feed, pesticides, and antibiotics added to feed also cause skin diseases. Prolonged exposure to the sun also causes quite serious skin diseases. Actinic keratosis and skin cancers are the most important of these skin diseases (Ağar, 2025).

## **2. 3. Occupational Accidents**

Occupational accidents and diseases arising from various causes result in numerous losses of life and property, ultimately causing economic and social harm to individuals, employers, and the country. Many factors contribute to the occurrence of occupational accidents and diseases. These can be listed as: 1. Natural conditions (climate), 2. Individual reasons, 3. Unsafe environment, and 4. Unsafe behaviors (Dursun, 2013). According to ILO data, an average of 313 million work accidents occur worldwide each year, and 2.7 million people lose their lives in these accidents. Similarly, two million people contract occupational diseases. The agricultural sector also presents a bleak picture in terms of occupational accidents. For example, according to

the ILO, 250 million people worldwide are affected by agricultural accidents each year, with 170,000 agricultural workers losing their lives in a total of 335,000 fatal accidents. Undoubtedly, these figures are also debatable. This is because a significant portion of workers are unregistered, while another portion are involved in production as members of family businesses. For this reason, not all accidents are reported to the relevant institutions (Gürgencin and Baytorun, 2018).

In our country, work accidents and occupational diseases have emerged as an increasingly problematic issue in recent years. Work accidents are particularly common in sectors with intensive industrial activity. Unfortunately, the measures taken and regulations implemented to prevent these accidents are insufficient, and it is not possible to completely prevent deaths and injuries (Özbakır and Akşit, 2023). A study examined the total number of occupational accidents and diseases that occurred on a sectoral basis between 2008 and 2021. The study reported that accidents caused by plant and animal production ranked 35th (42.906), while occupational diseases ranked 51st (26). The study considered 88 different sectors (Özbakır and Akşit, 2023).

A significant portion of work accidents in the livestock sector stem from direct physical contact with the animals being raised. Animals kicking, pushing, becoming aggressive and attacking, and biting cause injuries. According to the National Traumatic Injury Surveillance of Farmers (NIOSH) in the US, in all agricultural sectors, including livestock farming, a significant portion of injuries stem from physical contact with animals, and incidents caused by cattle and sheep alone account for 18% of the total. At the same time, these injuries are the events that cause the most workday loss in terms of temporary inability to work (Demirhan et al., 2016). (Demirhan ve ark., 2016).

### **3. Conclusions and Recommendations**

Nearly all activities carried out within agricultural production, both plant and animal production, are classified as hazardous. The obligations specified by law to eliminate risks cannot be fulfilled. This situation is primarily caused by the informal nature of agriculture and the fact that most businesses are small family businesses operating on their own behalf and account. Furthermore, there is a need for policies to prevent the informal employment of workers and for measures to include them in the social security system. It is clear that any legislative work undertaken without taking these requirements into account will have no practical effect. Furthermore, considering the serious

relationship between agriculture in Türkiye and the country's economy in terms of exports, employment, food safety, and food security, for the "Agricultural Occupational Health and Safety Law No. 6331" to be effective in practice, those living in rural areas must share in and feel the benefits of prosperity and development. If this happens, the Law will gain validity and prevalence.

In our country, there are serious deficiencies in occupational health and safety in the livestock sector, and there is a lack of awareness. Providing training to farmers engaged in livestock farming, developing protective approaches against animal-related risks in large-capacity livestock farms, and conducting risk assessments can help eliminate animal-related risks. In addition, protective measures such as wearing gloves when approaching and handling animals and animal products, getting protective vaccinations, etc., should be taken, paying attention to the transmission routes of zoonotic diseases. Along with these, regular health monitoring, vaccination measures, pest control, protective creams, and good pesticide use practices are extremely important in terms of occupational health and safety practices. Livestock farming can bring various health problems. Occupational health and safety in livestock farming must be clearly defined. It must be in line with animal development policies covering both commercial and smallholder. Specific programs and strategic action plans emphasizing the prevention of occupational risks for workers in animal production must be prepared. In addition, occupational health practices must be integrated into the basic health service structure.

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## Medicinal And Aromatic Plants Used As Functional Feed Additives 8

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### Abstract

Medicinal and aromatic plants are increasingly recognised as valuable functional feed additives in modern animal production. Owing to their high content of phytochemical compounds, these plants have long been utilised in food and medicine, and are now considered promising alternatives to synthetic feed additives and antibiotic growth promoters. Plant metabolism is generally classified into primary and secondary pathways; while primary metabolites are indispensable for vital plant functions, secondary metabolites—referred to as phytochemicals—are responsible for a wide range of bioactivities. Major groups of phytochemicals include phenolic compounds, terpenes, nitrogenous compounds, and sulphurcontaining metabolites, all of which exhibit antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory effects. When incorporated into animal diets, these bioactive compounds can enhance feed efficiency, support growth, strengthen the immune system, and improve overall health and product quality. Furthermore, their use contributes to more sustainable and environmentally friendly livestock systems by reducing reliance on synthetic additives. However, the beneficial effects of phytochemicals are dose-dependent, and excessive levels may cause

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adverse outcomes. A deeper understanding of their mechanisms of action and optimal inclusion levels is therefore essential to fully exploit the potential of medicinal and aromatic plants as functional feed additives.

## 1. Introduction

Throughout human history, plants have constituted the foundation of traditional medicine and nutrition, serving not only as sources of essential food but also as therapeutic agents. Their use has taken multiple forms, including the direct consumption of fresh leaves, extraction of essential oils, and preparation of plant-based remedies. Medicinal and aromatic plants are particularly valued for their multifunctional properties: they are widely employed as spices due to their aromatic compounds, utilised as natural pigments and colourants, and applied in the preservation of foodstuffs. Moreover, their bioactive constituents provide the basis for the development of pharmaceutical products. In recent decades, interest in these plants has expanded beyond the field of medicine into diverse industrial sectors in Europe, America, the Middle East, and Asia, where they are increasingly integrated for enhancing product quality, safety, and sustainability (Fakim, 2006; Gurgu et al., 2025; Güldiken et al., 2018; Bharadvaja, 2023).

Plants naturally synthesise a wide spectrum of organic compounds during their life cycle, which are generally classified into primary and secondary metabolites. Primary metabolites—including nucleic acids, amino acids, carbohydrates, lipids, and proteins—are indispensable for fundamental physiological processes such as photosynthesis, glycolysis, electron transport, and energy regulation. Secondary metabolites, on the other hand, are biosynthetically derived from primary metabolites. Although not essential for the survival of plants, they play crucial roles in ecological adaptation, including defence against pathogens, tolerance to abiotic stress, and allelopathic interactions with competing vegetation (Tiring et al., 2020; Fakim, 2006; Bakır, 2020).

Secondary metabolites are commonly categorised into phenolic compounds, terpenes, nitrogenous compounds (alkaloids), and sulphur-containing compounds. These phytochemicals have been widely studied for their biological activities, which include antimicrobial, antioxidant, anti-inflammatory, anti-diabetic, and anti-carcinogenic effects. Their applications extend across pharmaceutical and agricultural sectors, where they are utilised in the development of drugs, natural herbicides, and insecticides. In addition, they play a significant role in the food and cosmetic industries by contributing to flavour, aroma, and colour. However, the health-promoting effects of phytochemicals are strongly dose-dependent, as excessive intake may lead

to toxic effects, including neurotoxicity, genotoxicity, and gastrointestinal disturbances (Özay and Pehlivan, 2024; Bakır, 2020; Ülger and Ayhan, 2020; Güldiken et al., 2018).

In the context of animal nutrition, medicinal and aromatic plants have gained growing attention as functional feed additives. Their incorporation into diets has been associated with enhanced nutrient utilisation, improved feed efficiency, stimulation of digestive enzyme secretion, modulation of gut microbiota, and reinforcement of immune responses. Additionally, their antioxidant and anti-inflammatory properties contribute to reducing oxidative stress and improving overall animal health and welfare. Importantly, the use of phytogenic feed additives provides an effective alternative to synthetic additives and antibiotic growth promoters, thus addressing consumer demand for sustainable, residue-free, and environmentally friendly livestock production. Nevertheless, their efficacy and safety are contingent upon plant species, bioactive composition, dosage, and administration method, highlighting the necessity of continued research to optimise their utilisation in modern production systems.

### **1.1. Medicinal And Aromatic Plants Used As Functional Feed Additives**

Medicinal and aromatic plants contain valuable bioactive compounds that occur naturally and have numerous health effects in humans and animals. These substances are employed extensively in traditional medicine, the pharmaceutical industry, and the food and feed industries. In the contemporary context, distrust of artificial additives and a growing demand for natural products have led to an increased focus on aromatic plants. This phenomenon can be better elucidated by data from the World Health Organisation (WHO) which indicates that approximately 80% of the global population uses herbal medicines. In the field of animal husbandry, the utilisation of extracts and essential oils derived from aromatic plants, through various extraction methodologies, has emerged as a preferred feed additive. This preference is attributed to the numerous benefits these substances offer, including their role as a natural substitute for antibiotics, as well as their use as a replacement for artificial additives. This practice is in alignment with the European Union's decision, which has effectively prohibited the use of artificial additives as feed additives. The utilisation of feed additives has been demonstrated to enhance productivity in animals, thereby facilitating the production of high-quality and reliable animal products. This has led to an increased emphasis on the incorporation of aromatic plants within the

domain of animal husbandry (Hasan et al., 2024; Christaki et al., 2012; Adawy et al., 2020).

### 1.1.1. Thyme (*Thymus vulgaris*)

*Thymus vulgaris* is a perennial herbaceous, semi-shrubby aromatic plant belonging to the *Lamiaceae* (mint) family. Despite its Mediterranean provenance, where it is found in approximately 400 different species, it is a plant that has been widely used across the world, particularly in traditional medicine and nutrition, due to its aromatic properties and chemical composition. The species under consideration is able to thrive in conditions characterised by elevated temperatures, aridity, and abundant sunlight. The plant possesses a stem that is both woody and hairy, with a variability in height ranging from approximately 10 to 40 centimetres. The leaves constitute the edible portion of the plant and are arranged circumferentially around the stem. The specimens are green and greyish in colour, ranging in length from 2 to 6.5 mm, and exhibit diverse morphologies, predominantly linear, oval, spear-shaped, with pointed tips and curved edges. An aromatic scent is also present. The flowers are predominantly purple or white in colour and possess a hairy, tubular, and bilabiate structure, located in the leaf axils (see Figure 1). Thyme has been observed to demonstrate a high level of resistance to various stress factors, including drought, salty soil, and frost. Propagation can be achieved through cuttings, seeds, or layering (Pirbalouti et al., 2013; Silva et al., 2021; Halat et al., 2022; Jain and Choudhary, 2022).



Figure 1 *Thymus vulgaris* above-ground parts (Cianfaglione et al., 2022)

The above-ground parts of the thyme plant are particularly significant due to their high content of flavonoids and phenolic antioxidants, making them a valuable component in herbal teas, spices, and medicinal products. The plant's leaves are characterised by a high concentration of valuable minerals, including potassium, iron, calcium, magnesium, manganese and selenium, in addition to vitamins B, C, A, E and K. Analysis of its volatile components reveals their classification into multiple categories, namely monoterpenes, phenols, ketones, aldehydes, ethers and esters. The essential oil is comprised of a number of volatile components, including two phenolic compounds of particular value: thymol and carvacrol. These compounds belong to the monoterpene group, which is responsible for the antioxidant activity of the oil. These components possess a range of biological activities, including antimicrobial, antioxidant, anti-inflammatory, and antifungal properties. Consequently, they have a wide range of applications, including in the treatment of respiratory tract infections, parasitic diseases, sprains, coughs, expectorants, disinfectants, and food preservation. However, studies have indicated that the essential oil may also exert cytotoxic effects due to its increasing concentration, damage intestinal cells, irritate the skin, and cause rare allergic reactions (Dauqan and Abdullah, 2017; Nikolic et al., 2014; Kowalczyk et al., 2020).

### 1.1.2. Rosemary (*Rosmarinus officinalis* L.)

*Rosmarinus officinalis* L., a member of the *Lamiaceae* family, is an aromatic plant species that contains a plethora of valuable bioactive compounds. Its utilisation in culinary practices and as a medicinal agent dates back to antiquity, with contemporary applications extending to various domains such as cosmetics, aromatherapy, pharmacy, and food preservation. Despite its Mediterranean provenance, it has been observed to thrive in a variety of environments, including rocky, sandy, and coastal regions across Europe, Africa, and Asia. The classification system under consideration comprises approximately 236 genera and 6,900 to 7,200 species. Rosemary (*Rosmarinus officinalis*) is a shrubby plant that can grow up to 1 metre in height. The plant's inflorescences exhibit a colour spectrum ranging from blue to white, with a stemless structure that is characterised by a curved, linear or lanceolate morphology. The leaves are distinguished by a medium vein configuration, as illustrated in Figure 2. The presence of an aromatic scent is attributable to the accumulation of essential oils in the trichomes present on the leaves and flowers of the plant (Malayoğlu, 2010; Moore et al., 2016; Santos et al., 2015; Hassani et al., 2016; Andrade et al., 2018).



Figure 2 The morphological appearance of the rosemary plant (Diass et al., 2021)

The bioactive components of rosemary are primarily classified as phenolic and volatile compounds. In particular, the following substances were identified: diterpenes, triterpenes, ursolic acid, betulinic acid, phenolic acids such as carnosic acid and rosmarinic acid, and volatile compounds, namely the essential oil's main components, including camphor, eucalyptus, 1,8-cineole,  $\alpha$ -pinene and  $\beta$ -pinene, borneol, limonene, and camphene. *Rosmarinus officinalis* is employed in various forms, including fresh, dried, extracted, or essential oil, derived from various plant parts such as flowers, buds, leaves, stems, and bark. Of particular interest are rosmarinic acid and carnosic acid, which are phenolic compounds, and which have been used in traditional medicine due to their antioxidant, antiviral, anti-inflammatory, and antibacterial properties (Malayo\u0111lu, 2010; Moore et al., 2016; Santos et al., 2015; Hassani et al., 2016; Andrade et al., 2018).

### 1.1.3. Laurel (*Laurus nobilis*)

*Laurus nobilis* L., the plant under discussion is a green, aromatic shrub belonging to the *Lauraceae* family, which comprises between 2,500 and 3,500 different species. The natural habitats of this species are countries such

as Turkey, Spain, Greece, Portugal, Mexico and Morocco, which account for 97% of production. These countries are endemic to regions with temperate and warm climates, such as southern Europe and the Mediterranean. In the context of domestic gardens, the maximum recorded height of Laurel is 2 to 6 metres, whereas in its natural habitat, it has been observed to reach heights of up to 20 metres. The bark is characterised by a smooth texture, with a colour spectrum ranging from olive green to reddish tones. The leaves of the plant under scrutiny are characterised by their pointed and spear-like morphology, their absence of hair, and their notable glossiness. The colouration of these leaves ranges from olive green to brown, with the presence of veins on the underside and an alternate arrangement of veins and veins. The leaves are also characterised by a bitter taste and an aromatic scent. The flowers, which bloom in spring, are characterised by a yellowish-white hue and a pleasant scent. The fruits, which bear a resemblance to olives, are characterised by a black and purplish pigmentation. Volatile oils have been identified within these fruits (Figure 3). Propagation can be achieved through seeds, cuttings, or in vitro culture. The species exhibits a high degree of adaptability to a wide range of climatic conditions, but it has been observed to thrive in moist, well-drained soils with a pH range of 4.5 to 8.2 and in humid regions with a maritime climate (Awada et al., 2023; Caputo et al., 2017; Kaurinovic et al., 2010; Paparella et al., 2022; Sırıken et al., 2018; Khodja et al., 2023).



Figure 3 Bay leaf and fruit (Awada et al., 2023)

*L. nobilis* the extract is characterised by the presence of flavonoids, phenolic acids, and tannin-class compounds. The essential oil components obtained from the plant's flowers, seeds, and stem contain a high proportion of monoterpenes group compounds, with 1,8-cineole being

the predominant component present in the highest proportion, followed by sabinene,  $\alpha$ -terpinyl acetate, linalool, limonene,  $\alpha$ -pinene,  $\beta$ -pinene, camphene, cadinene, caryophyllene, and terpinol. In addition to these prominent components, the plant possesses various bioactive compounds with antimicrobial, antioxidant, anticarcinogenic, digestive and immune-supporting properties. In addition, it is the components eugenol, methyl eugenol and elemicin that are responsible for the quality and aroma of the spice. These valuable components have been utilised in various sectors throughout history, including the pharmaceutical industry due to its antibacterial, antifungal, and antioxidant properties, the food industry as a preservative and spice, and the cosmetics sector in creams and soaps due to its pleasant aroma (Caputo et al., 2017; Kaurinovic et al., 2010; Paparella et al., 2022; Siriken et al., 2018; Khodja et al., 2023).

#### 1.1.4. Fennel (*Foeniculum vulgare*)

*Foeniculum vulgare* Mill. is an aromatic, herbaceous plant belonging to the *Apiaceae* (*Umbelliferae*) family, which grows as an annual, biennial or perennial. Despite its provenance in temperate and tropical regions, such as the southern Mediterranean, it has also been identified in various other parts of the world, including Asia, North America and Central Europe, particularly in areas with high levels of coastal erosion and in arid soils. Fennel (*Foeniculum vulgare*) can reach a height of 2-2.5 m and possesses upright, stiff branches and bright green, hairy, thread-like leaves that resemble those of dill (*Anethum graveolens*). The plant is distinguished by its yellow flowers, which bloom during the summer months. The fruits of the plant are characterised by their thick skins, elongated and rectangular-oval in shape, and reach maturity in October. The propagation of this species may be achieved through the utilisation of seeds, roots, or crowns (see Figure 4) (Rather et al., 2016; Badgujar et al., 2014; Diao et al., 2014; Noreen et al., 2023; Rafieian et al., 2023).



Figure 4 *Foeniculum vulgare* (a) stem (b) bulb (c) leaves and flowers (d) *F. vulgare* population (Noreen et al., 2023)

Fennel seeds have been found to contain varying amounts of carbohydrates, fats, proteins and fibre. In addition, the leaves are rich in minerals and vitamins, including calcium, potassium, sodium, phosphorus, iron, riboflavin, niacin and vitamin C. Furthermore, the fruits/seeds contain oil components such as oleic acid, linoleic acid, palmitic acid, and petrosilic acid in varying proportions, as well as phenolic compounds including rosmarinic acid, chlorogenic acid, and flavonoids such as quercetin and apigenin. Furthermore, the presence of volatile compounds in Fennel has been identified as the source of its distinctive aniseed aroma, a quality that has garnered significant attention in the culinary realm, where it is frequently employed as a flavouring agent in various food products. These include trans-anethol, the main component known for its medicinal effects, as well as etragol, limonene, fenchone, and  $\alpha$ -phellandrene. The primary medicinal effects of these compounds encompass antibacterial, antifungal, antiviral, anti-inflammatory, antimutagenic, and memory-enhancing properties (Rather et al., 2016; Badgujar et al., 2014; Diao et al., 2014; Noreen et al., 2023; Rafieian et al., 2023).

#### 1.1.5. Ginger (*Zingiber officinale Roscoe*)

*Zingiber officinale Roscoe* is a species belonging to the genus *Zingiber* of the *Zingiberaceae* family, which comprises 1,300 different species worldwide. It is a herbaceous perennial plant with aromatic rhizomes. Despite its provenance in India and the Pacific Islands, it is now cultivated across the tropical and subtropical regions of the world. The leading producers are India, China, and Nigeria. Ginger (*Zingiber officinale*) is a perennial plant that grows to a height of approximately 1 m. It has fibrous roots, green, usually lanceolate, hairless leaves, and yellow-green flowers (see Figure 5). Its rhizomes, which possess medicinal properties, are yellowish-brown in

colour and have a thick, aromatic scent. The optimal climatic conditions for its growth include warm and humid weather conditions and loamy soils, with altitudes of up to 1,500 metres above sea level being particularly conducive. The period from planting to harvest is of particular significance. The optimal planting period is typically in April or May, with harvesting concluding in winter (Bauza et al., 2019; Semwal et al., 2015; Zhang et al., 2021; Ali et al., 2008; Mao et al., 2019).



Figure 5 Parts of the ginger plant (Begum A., 2024)

The distinctive aroma of ginger is attributable to the presence of phenolic and terpene group compounds within its chemical structure. The phenolic compounds have been identified as the principal components responsible for its bioactivity, particularly its pungent taste when used as a spice or flavouring. The main phenolic compounds responsible for ginger's pungent taste are gingerol, shogaol, zingerone and paradol. While gingerols are recognised as the primary component responsible for the pungent taste, they undergo a transformation into shogaols at elevated temperatures due to alterations in their stable structure, thereby acquiring a spicy character. Conversely, sesquiterpenoids, diterpenoids and monoterpenoids belonging to the terpene group constitute the primary components of essential oil. The most prevalent components include  $\beta$ -bisabolene,  $\alpha$ -curcumin, zingiberene,

$\alpha$ -farnesene,  $\beta$ -sesquiphellandrene, camphene, cineole, eugenol, curcumen, citral, geraniol and terpinenol. It has been determined that these components, which form part of its structure, have been utilised for medicinal and culinary purposes, with a view to alleviating symptoms such as headaches, colds and nausea, a practice that has been in evidence since time immemorial (Bauza et al., 2019; Semwal et al., 2015; Zhang et al., 2020; Ali et al., 2008; Mao et al., 2019).

#### 1.1.6. Turmeric (*Curcuma longa*)

*Curcuma longa*, which belongs to the *Zingiberaceae* family, is a herbaceous and perennial plant that is widely cultivated in tropical countries, primarily in Asia, particularly in India, where it accounts for 78% of production and consumption. It is also cultivated in China. The turmeric plant (*Curcuma longa*), from which the rhizomes are harvested for use in both spice and medicine production, possesses a pleasant aroma and a bitter taste. It possesses spear-shaped, green shoots that can reach lengths of approximately 1 m and up to 12 leaves (see Figure 6). The plant in question produces yellow flowers, which are surrounded by purplish bracts. Turmeric (*Curcuma longa*) is a plant that is native to tropical and subtropical regions, but it is able to thrive in a wider range of conditions. It is able to grow in humid, sandy, nutrient-rich soils with sufficient annual rainfall, and it grows best at temperatures between 20°C and 30°C. The maturation process takes 7 to 9 months, and turmeric is harvested between January and April (Jyotirmayee and Mahalik, 2022; Fuloria et al., 2022; Iweala et al., 2023).



Figure 6 Certain parts of the turmeric plant b) rhizome c) dried powder form (Fuloria et al., 2022)

The colour of the substance ranges from yellow to brown, and it is this colouration that is responsible for the majority of its medicinal benefits. These benefits are derived from curcumin, which is the main component of the phenolic compound group in its structure. Furthermore, it has been

determined that the substance contains over 300 active components, which are classified into the following groups: polyphenols, terpenes, sterols and alkaloids. Research has determined that these bioactive compounds in its structure have antioxidant, hepatoprotective, anti-osteoarthritic, anti-inflammatory, anti-cancer, anti-arthritic, neuroprotective, anti-diabetic, anti-depressant, wound healing, memory enhancing and anti-ageing properties (Jyotirmayee and Mahalik, 2022; Fuloria et al., 2022; Iweala et al., 2023).

### 1.1.7. Sage (*Salvia officinalis* L.)

*Salvia officinalis* is the largest genus in the *Lamiaceae* family, with approximately 900 to 1,000 known species worldwide. It is a perennial aromatic shrub. Despite its global distribution, spanning Asia, Africa, America and Europe, the provenance of sage is firmly rooted in the Mediterranean region. The sage plant is characterised by a woody stem, long, serrated, lanceolate leaves that are greyish green in colour, and purple to bluish flowers (see Figure 7). Sage is a hardy plant that typically flourishes between the spring and summer months. It is capable of thriving in a range of environmental conditions, including high altitudes, low temperatures, and across a broad pH spectrum (Ghorbani and Esmaeilizadeh, 2017; Jakovljević et al., 2019; Akacha et al., 2024; Assaggaf et al., 2022).



Figure 7 The sage plant (Ertaş et al., 2023)

Sage is notable for its high content of phenolic compounds and terpenes, which endow it with significant medicinal and aromatic properties. A range of studies have identified the following main volatile compounds: borneol, caryophyllene, elemene, humulene,  $\alpha$ -thujone, camphor, 1,8-cineole, limonene and  $\beta$ -thujone. Furthermore, phenols such as caffeic acid, carnosic acid, carnosol, and rosmarinic acid, as well as flavonoids such as apigenin, luteolin, and quercetin, have been reported as prominent components in sage extract (Ghorbani and Esmaeilizadeh, 2017; Jakovljević et al., 2019; Akacha et al., 2024; Assaggaf et al., 2022).

### 1.1.8. Garlic (*Allium sativum* L.)

*Allium sativum* L. is a bulbous, perennial, herbaceous species belonging to the *Amaryllidaceae* family, which is known to comprise 750 genera and approximately 600 species. Its provenance is believed to be Central Asia, and contemporary countries with the highest production worldwide include China, India, the United States, Turkey, Korea, Egypt, and Spain. A study of the morphology of garlic reveals that the part consumed, responsible for the pungent aroma and flavour, is the bulb. This is encased in a white skin or membrane and contains 10 to 20 cloves (see Figure 8). The aboveground part of the plant is characterised by sparse stems and leaves that measure 2 to 3 centimetres in width. The utilisation of these bulbs as propagation material is a key aspect of the study. Garlic cultivation is a straightforward process, and in temperate climates, it can be planted throughout the year. The bulbs are harvested after they have reached full maturity (Ammarellou et al., 2022; Okoro et al., 2023; Shang et al., 2019; Batiha et al., 2020; Jikah et al., 2024).



Figure 8 *Allium sativum* underground onions (Magrys et al., 2021)

Garlic's flavour and aroma are derived from its aromatic properties, specifically from the phenolic compounds, organic sulphides, saponins, and polysaccharide group bioactive compounds that are present in its structure. These include, in particular, allicin—a sulphur-containing compound known as garlic's primary component—followed by diallyl sulphide, diallyl disulphide, diallyl trisulphide, ajoene, S-allylcysteine, and vinyl dithione. The primary phenolic compound present is identified as  $\beta$ -resorcylic acid (Ammarellou et al., 2022; Okoro et al., 2023; Shang et al., 2019; Batiha et al., 2020; Jikah et al., 2024).

### 1.1.9. Mint (*Mentha piperita* L.)

*Mentha piperita* L. is a perennial medicinal and aromatic plant belonging to the *Lamiaceae* family, which comprises approximately 7,000 species and 260 genera. Originating in Europe, this species is now cultivated extensively in temperate climate regions worldwide, particularly in America and Africa. In terms of morphology, the stem of a mint plant is typically square in form and branched, with a range of heights between 30 and 90 centimetres. The leaves are oblong-ovate in shape with serrated edges. The plant is distinguished by its flowers, which exhibit a colouration that can be described as purple or pinkish. As illustrated in Figure 9, the propagation of plants can be facilitated by the development of underground shoots. While mint can grow in a wide range of pH levels, it has been observed to thrive optimally in well-drained, humus-rich soils with a pH range between 6 and 7.5 (Singh et al., 2015; Mahendran and Rahman, 2020; Hudz et al., 2023; Gholamipourfard et al., 2021).



Figur 9 General appearance of *M. piperita* (Mahendran and Rahman, 2020)

The essential oils present in the leaves are the primary contributors to its medicinal properties. The predominant volatile components, namely menthol, menthone, menthofuran and menthyl acetate, account for approximately 90% of the total oil composition. Furthermore, a range of terpenoids, including  $\alpha$ -pinene,  $\beta$ -pinene, carvone and 1,8-cineole, have also been identified as components. Peppermint extract is characterised by a high flavonoid content, including rutin, quercetin, and naringenin, as well as phenolic acids such as caffeic and rosmarinic acid. Notably, peppermint extract is a rich source of ursolic acid, which belongs to the triterpenoid group (Singh et al., 2015; Mahendran and Rahman, 2020; Hudz et al., 2023; Gholamipourfard et al., 2021).

#### 1.1.10. Cumin (*Cuminum cyminum*)

Cumin (*Cuminum cyminum*) is a plant species that belongs to the *Apiaceae* family. It is generally considered to be an annual species and is characterised by its aromatic properties. The provenance of this plant is the Eastern Mediterranean and Middle East regions, from which it has now been cultivated in many temperate climate regions, primarily in India,

Iran, Turkey, and Mediterranean countries (Khan et al., 2016; Patel and Singh, 2019). The plant is typically 30-50 cm in height and possesses a slender, branched stem. The leaves of the plant are thin, lobed and devoid of hair. The flowers are diminutive in size, with a white or pink hue, and are arranged in umbrella-shaped clusters (see Figure 10). Cumin is predominantly propagated through seed, and optimal growth conditions include well-drained, slightly clayey, organic-rich soils with a pH range of 6-8 (Ali and Kumar, 2018).



Figure 10 Cumin herb and seed (Johri, 2011)

The most significant medicinal constituent of cumin is the volatile oil content found in its seeds. The chemical composition of the essential oil is dominated by cuminaldehyde, thymol, carvacrol,  $\gamma$ -terpinene and  $\beta$ -pinene, with the total volatile oil content ranging between 70-80%. Furthermore, the seeds have been found to contain significant concentrations of flavonoids, phenolic acids, and other biologically active substances (Zhao et al., 2020; Riaz and Khan, 2022). Cumin's antioxidant, antimicrobial, and digestive-facilitating properties are attributable to the chemical compounds it contains (Gupta and Verma, 2017).

#### 1.1.11. Partridge (*Satureja hortensis*)

The common name for *Satureja hortensis* is summer savory. This is an annual, herbaceous, aromatic plant belonging to the *Lamiaceae* family. It is also referred to as savory, savory herb, and savory plant. The *Satureja*

genus, which is native to southern Europe and the Mediterranean region, comprises approximately 50 different species worldwide, 16 of which are endemic to Turkey. The plant under discussion is capable of reaching a height of between 30 and 60 centimetres. It produces flowers that are purplish in colour and bloom during the summer months. The leaves of the plant are lanceolate to linear in shape, sessile, green to bronze in colour and hairy. These leaves are used as a spice. As illustrated in Figure 11, summer savory, which typically thrives in rocky environments, can be propagated through the use of seeds (Fierascu et al., 2018; Ejaz et al., 2023; Borojo et al., 2018; Körük and Gedik, 2024; Mašković et al., 2024).



Figure 11 The above-ground branched stem portion of *Satureja hortensis* L.  
(Hassanzadeh et al., 2016)

The plant is notable for its high concentration of biologically active components, particularly in its leaves. These are rich in minerals such as potassium, phosphorus, iron, and calcium, as well as vitamins A, C, and riboflavin. Furthermore, its extracts and essential oils have been found to contain phenolic compounds, tannins, flavonoids, steroids, rosmarinic acid, caffeic acid, mucilage, and volatile components. The bioactive components of the essential oil are carvacrol,  $\gamma$ -terpinene, thymol, and p-cymene. The bioactive components of the plant have been shown to possess medicinal properties, including antioxidant, anti-inflammatory, antimicrobial, and antispasmodic effects. The essential oil is employed in a variety of domains, including but not limited to the pharmaceutical industry, the cosmetics

industry, and the food industry (Fierascu et al., 2018; Ejaz et al., 2023; Borojo et al., 2018; Körük and Gedik, 2024; Mašković et al., 2024).

### 1.1.12. Carnation (*Syzygium aromaticum*)

*Syzygium aromaticum* L. is a perennial medicinal and aromatic plant that belongs to the *Myrtaceae* family. The species is believed to have originated in Indonesia, and is now widespread in tropical regions such as Madagascar, Sri Lanka, India and Tanzania. Carnation is a plant that can grow to a height of 10 to 20 metres and is categorised as an evergreen tree. The leaves exhibit a dark green hue, a lustrous appearance, and an oval or elliptical morphology. The plant is notable for its red and pink flowers, and its buds are hand-picked before harvest and used in spice production (Figure 12). Carnation (*Syzygium aromaticum*) is a plant that thrives in conditions of high humidity and temperature, as well as in well-drained, humus-rich, slightly acidic soils (Xue et al., 2022; Cetin, 2014).



Figure 12 The carnation plant and its buds

The essential oil extracted from the flower buds is the primary component that is utilised for its medicinal properties. The essential oil under scrutiny is predominantly composed of eugenol, a constituent that has been identified as the primary active ingredient responsible for the characteristic scent and

medicinal benefits of cloves. However, components from the terpenoid group, such as eugenyl acetate,  $\beta$ -caryophyllene,  $\alpha$ -humulene, and methyl salicylate, are also found in clove essential oil. Non-volatile components include tannins, flavonoids, sterols, and triterpenes. The phenolic compounds it contains have been shown to possess antibacterial, antifungal, antioxidant, and insecticidal properties. The essential oil is utilised in a variety of industries, most notably in the pharmaceuticals, food, and cosmetics sectors (Teixeira et al., 2013; Szente and Szejtli, 2004; Gonzalez et al., 2021).

### 1.1.13. Cinnamon (*Cinnamomum zeylanicum*)

*Cinnamomum zeylanicum*, belonging to the genus *Cinnamomum* of the *Laureaceae* family, which has approximately 250 species, and commonly known as true cinnamon or Ceylon cinnamon, originates from India, Sri Lanka and the tropical regions of Asia. Zeylanicum is a tree-like, evergreen species that can reach a height of approximately 12 metres. It can be distinguished from other species by its acute-shaped leaves, which turn from red to dark green, and its greenish flowers. (Figure 13) Although it can grow at elevations of 500 metres, it is generally more common at lower elevations. Its fruits ripen between May and August (Ranasinghe et al., 2013; Jayaprakasha and Rao, 2011; Behbahani et al., 2020; Unlu et al., 2010; Weerasekera et al., 2021).



(a)



(b)

Figure 13 Cinnamon a) green stem b) bark (Husain et al., 2018)

All parts of the plant, including the bark, leaves, fruit and roots, contain bioactive compounds, and are used both medicinally and as food or spice. The essential oils analysed in this study were cinnamaldehyde, eugenol, linalool, beta-caryophyllene, eucalyptus and benzaldehyde. It has been determined through rigorous analysis and experimentation that cinnamaldehyde and eugenol are the primary active components of cinnamon extract. Furthermore, cinnamaldehyde has been identified as the main component of the essential oil, which possesses antioxidant effects (Ranasinghe et al., 2013; Jayaprakasha and Rao, 2011; Behbahani et al., 2020; Unlu et al., 2010; Weerasekera et al., 2021).

#### 1.1.14. Maral Root (*Rhaponticum carthamoides*)

*Rhaponticum carthamoides* is a perennial plant that belongs to the Asteraceae family. It is commonly known by names such as maral root and Russian leuzea. The plant's provenance is the Altai and Sayan Mountains in southern Siberia, where it is endemic, growing at elevations ranging from 1,200 to 2,300 metres. The maral root is capable of attaining a length of approximately 150 centimetres. It possesses black, branched, vertical rhizomes and a smooth root structure with an elastic texture, which are the primary components employed in its extract (Figure 14) (Todorova et al., 2022; Kokoska and Janovska, 2009 Głażowska et al., 2018; Todorova et al., 2023).



Figure 14 The maral root with its flowers in bloom (Kokoska and Janovska, 2009)

The Maral root plant is characterised by a high concentration of phytoecdysteroids, which are its primary bioactive compounds and have been demonstrated to be beneficial to health. These compounds have been found to be present in both the generative and vegetative parts of the plant but are particularly abundant in the root section. The primary active compound is succeeded by phenolic and steroid groups. Research has indicated the presence of various constituents within the plant, including flavonoids, sesquiterpenes, monoterpenes, and glycosides. The essential oil, isolated from the root parts of the plant, contains bioactive components that are comprised of terpenoid group compounds. These active components are used in medical treatments for a variety of purposes, including the treatment of kidney and cardiovascular diseases, the alleviation of physical fatigue, the promotion of muscle development, the stimulation of protein synthesis, and the prevention of bacterial, antioxidant, and anticarcinogenic processes (Kokoska and Janovska, 2009; Głażowska et al., 2018; Todorova et al., 2023; Todorova et al., 2022).

### 1.1.15. Chicory (*Cichorium Intybus L.*)

*Cichorium intybus*, a species within the *Asteraceae* family, is found in Europe, Asia and Africa in its wild state. However, it is originally a plant that thrives in a Mediterranean climate, and is a perennial medicinal plant commonly found throughout Europe and Asia. The plant known as chicory can reach heights of between 20 and 150 centimetres. It possesses a green stem of considerable strength, green leaves, and flowers that are predominantly blue, but which can also be whitish in colour. These flowers open in conditions of bright sunlight and are rich in nectar and pollen. The leaves exhibit a green hue and can be classified into various shapes, including oval, lanceolate, or oblong, with a hairy texture (see Figure 15) (Janda et al., 2021; Mulabagal et al. 2009; Birsa and Sarbu, 2023; Abbas et al., 2015).



Figure 15 The open flowers and green stems of the chicory plant (Khan et al., 2020)

Notwithstanding the fact that the edible components are the flowers and buds, it is notable that all parts of chicory are abundant in bioactive compounds. The roots contain a number of chemical compounds, including sesquiterpenes, lactones, phenolic acids such as caffeic and cichoric acids, triterpenes, intybin glycoside, and inulin. The leaves contain vitamins A, B and C, minerals such as calcium, potassium, phosphorus, iron and zinc, and phenolic compounds that are medically significant. In addition

to the numerous health benefits of its components, the anti-inflammatory properties of its anthocyanin content are well-documented, and inulin has a variety of industrial applications, including in coffee and animal feed (Janda et al., 2021; Duda et al., 2024; Mulabagal et al. 2009; Birsa and Sarbu, 2023; Abbas et al., 2015)

### 1.1.16. *Echinacea* (*Echinacea purpurea* L.)

The *Asteraceae* family and *Echinacea purpurea* L., belonging to the *Echinacea* genus, which is known to comprise 9 to 13 species, is the most commonly used species for medicinal purposes. Despite its provenance in North America, the plant is classified as a flowering, herbaceous, perennial, and medicinal species. It is predominantly distributed across the central and south-eastern regions of the continent, extending to Europe. The plant's growth potential is evident in its ability to reach a height of approximately 60 to 150 centimetres, accompanied by a robust and well-developed root and stem structure. The leaves of the plant under scrutiny are of a linear-lanceolate configuration and display a green pigmentation. They are also characterised by the presence of stiff hairs. The flowers of this species bloom during the summer months and display a pinkish hue (see Figure 16) (Manayi et al., 2015; Nagy et al., 2022; Dosoky et al., 2023; Mohamed et al., 2023).

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Figure 16 The spear-shaped upper leaves and flowers of echinacea (Lim, 2014)

### 1.1.17. *Hypericum perforatum*

*Hypericum perforatum* L., a perennial plant in the *Hypericaceae* family, is highly prized for its medicinal properties. The *Hypericum* genus is comprised of approximately 400 species on a global scale, with around 70 species being present in Turkey. This species, which originated in Europe, is now widespread in temperate and tropical climate zones. *Hypericum* is herbaceous in form, with yellow flowers and hairy leaves (see Figure 17) (Ekren et al., 2010; Cakmak and Bayram, 2003).

The medicinal value of the plant is attributed to the flowers, which contain bioactive compounds. In addition to flavonoids and tannins, the main bioactive components are understood to be hypericin, sitosterol, essential oils, pseudohypericin, hyperforin, choline, and pectin. The bioactive components present in it have been shown to possess antidepressant, wound healing, antiseptic, antiviral, and sedative properties (Cakmak and Bayram, 2003; Burunkaya et al., 2021; Ekren et al., 2010).



Figure 17 *Hypericum perforatum L.* (Gritsenko, 2021)

### 1.1.18. Hot Pepper (*Capsicum annuum L.*)

*Capsicum annuum L.* is a member of the *Solanaceae* family and is a perennial, shrub-like plant that is native to North and South America and is extensively cultivated in Asia, Africa and the Mediterranean. The plant is referred to by a number of common names, including hot pepper, red pepper, bell pepper and jalapeño. Morphologically, *Capsicum* has been observed to attain a maximum length of approximately 1 m. The morphology of its leaves exhibits significant variation in shape, yet they are predominantly classified as oval-lanceolate or oblong-oval in form. The flowers of this plant range from purple to white in colour, and the fruits it produces can be red, green, yellow or orange. As illustrated in Figure 18, the optimal temperature range for seed germination is 25 to 30°C, while 18 to 30°C is required for fruit development. The optimal climatic conditions for the plant's growth are those that are humid and warm, with loamy soils that are well-drained, characterised by high water retention capacity and abundant organic matter content (Silva et al., 2013; Zhigila et al., 2014; Olatunji and Afolayan, 2018).

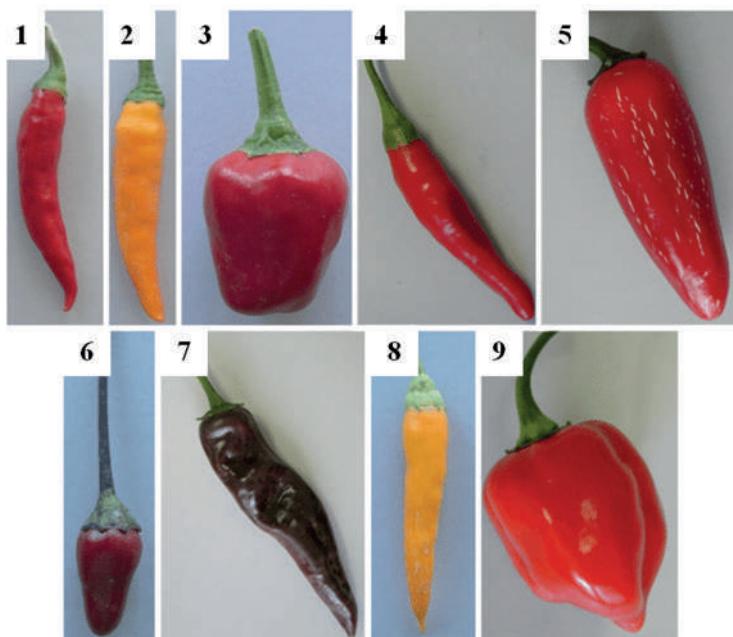


Figure 18 *Capsicum annuum* varieties 1) Black Cobra Pepper 2) Kilian Pepper 3) Greek Pepperoncini Pepper 4) Tabasco Pepper 5) Jalapeño Pepper 6) Black Prince Pepper 7) Chocho Pepper 8) Medusa Pepper 9) Red Habanero Pepper (Mňahončáková et al., 2021)

The *capsicum* genus of plants is characterised by a high nutritional content, including vitamins A, C and E, which have been demonstrated to play a significant role in human health. In addition to these vitamins, the genus contains minerals such as calcium, phosphorus and potassium, as well as carotenoids, which are known to be beneficial to human health. Furthermore, these fruits contain a plethora of bioactive compounds, including capsaicinoids, phenolic compounds, flavonoids, volatile oils and alkaloids. The medicinal effects of chilli are attributed to the active ingredient capsaicin, while its pungency is attributed to active ingredients in the alkaloid group. In a study, the main active component of two different *capsicum* varieties was determined to be betulin and the main volatile component was methyl benzoate (Silva et al., 2013). The bioactive compounds present in peppers have been shown to possess antioxidant, anti-inflammatory, and anticancer properties, among other health-promoting effects. These valuable compounds have led to the utilisation of peppers as a food, spice, and colouring agent. In addition, peppers have been employed in the treatment of various diseases, including cancer, stomach disorders, eye disorders, and

cholesterol (Silva et al., 2013; Zhigila et al., 2014; Olatunji and Afolayan, 2018; Sanati et al., 2018).

## 2. Conclusion

Medicinal and aromatic plants represent a promising group of natural resources that can be effectively utilised as functional feed additives in animal nutrition. Their rich phytochemical composition, including phenolic compounds, terpenes, alkaloids, and sulphur-containing metabolites, provides a wide spectrum of biological activities such as antimicrobial, antioxidant, and immunomodulatory effects. When incorporated into animal diets, these plants not only enhance feed efficiency and growth performance but also strengthen the immune system, improve animal welfare, and contribute to the production of high-quality, residue-free animal products. Furthermore, their use offers sustainable alternatives to synthetic additives and antibiotic growth promoters, aligning with the increasing demand for environmentally friendly and safe livestock production systems.

Despite these advantages, the utilisation of medicinal and aromatic plants as feed additives faces certain challenges. The variability in phytochemical composition among plant species, as well as differences in cultivation conditions, extraction methods, and processing, may significantly affect their efficacy. Moreover, the beneficial effects of phytochemicals are dose-dependent, and inappropriate inclusion levels may lead to adverse health consequences. Therefore, standardisation of plant-based feed additives, along with comprehensive evaluations of their safety and effectiveness, remains an essential research priority.

Future studies should focus on elucidating the precise mechanisms of action of plant-derived bioactive compounds, optimising dosage levels for different animal species, and developing innovative delivery systems to maximise their stability and bioavailability. Additionally, integrating medicinal and aromatic plants into precision feeding strategies, in combination with modern biotechnological tools such as metabolomics and microbiome analysis, may offer new opportunities to enhance their functional potential. Ultimately, a deeper understanding of these natural resources will facilitate their wider adoption as safe, effective, and sustainable functional feed additives in modern animal production systems.

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# Artificial Intelligence-Based Beehive Monitoring Systems in Modern Beekeeping 8

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## Abstract

Honeybees play a vital role in maintaining the balance of ecosystems and supporting human life. Their primary contribution is pollination, which enhances agricultural productivity and biodiversity. Additionally, they produce valuable natural products such as honey, royal jelly, and propolis, which have nutritional and medicinal benefits. In recent years, increasing threats to bee populations have emphasized the need for improved beekeeping practices that ensure both the conservation of bees and the efficiency of hive management. Traditional beekeeping methods, while effective, are often labor-intensive and time-consuming. In contrast, precision beekeeping also known as smart beekeeping integrates technology to monitor and manage hives more efficiently. Artificial intelligence (AI)-based hive monitoring systems have emerged as powerful tools in this field. These systems use sensors and AI algorithms to collect and analyze data on temperature, humidity, bee activity, sound, and other environmental factors. They enable early detection of diseases, swarming, or other threats, allowing beekeepers to take timely action. Over the past decade, many studies have explored the use of machine learning and deep learning techniques in these systems. This chapter aims to provide a comprehensive overview of recent advancements and emerging trends in AI-supported hive monitoring, offering valuable insights to guide future research and technological innovation in modern beekeeping practices.

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## 1. Introduction

Honeybees are among the important living groups that maintain balance in the ecosystem. These bees are primary pollinators among plant species. Moreover, honeybees hold a significant position economically, socially, and culturally because they contribute positively to food security, production, and pollination (Kayışoğlu & Türksoy, 2023). Honeybees naturally live in colonies. Bee colonies produce beneficial nutrients such as honey, propolis, royal jelly, beeswax, and pollen (Mutlu, Akbulut, Aydin, & Mutlu, 2023). Honey is highly beneficial for health because it contains valuable minerals like calcium and magnesium (Budak, 2023). Therefore, the demand for bees and bee products is increasing worldwide, leading to a rapidly growing business sector in this field (Çalışan, Balat, & Yavuz, 2022).

Beekeeping has always been one of the most important agricultural activities contributing to the economy. According to data from the Food and Agriculture Organization of the United Nations (FAO, 2024), our country ranks among the top in the world in honey production. Beekeeping is practiced commercially in our country, but also as a hobby to meet natural food needs. The beekeeping sector has important advantages such as being able to operate with small capital and generating income within one year. The amount of honey produced in beekeeping is not solely related to the number of colonies. For example, although Turkey ranks among the top countries in terms of bee colonies and honey production, it does not show the same success in honey yield per colony. The main reasons for this failure are unconscious production processes, environmental effects, and various diseases in the colonies (Kutlu & Kılıç, 2020). In this area, productivity can be increased by methods such as controlling knowledge, experience, and environmental conditions.

Bee species conservation can be done by classical or modern beekeeping methods. Classical methods require high labor and costs. Modern beekeeping methods aim to increase productivity and quality while reducing costs. Modern beekeeping methods also appear as precision beekeeping technology. Precision beekeeping involves the electronic monitoring and tracking of honeybees' roles and functions in nature, such as honey production and life cycles. The most commonly used methods in precision beekeeping are bee hive monitoring systems that include artificial intelligence-based machine learning and deep learning methods.

Hive monitoring systems and machine learning algorithms are becoming increasingly important for bee production and behavior. Hive monitoring systems consist of data collection and data analysis sections. Bee monitoring

and tracking systems are important for assessing colony health and increasing production efficiency. Sensors, cameras, and Global Positioning System (GPS) devices are used to collect data on bee behaviors. Machine learning algorithms are used to define bee behavior patterns and detect anomalies. For example, image processing algorithms can detect abnormalities on bees' bodies. Machine learning algorithms can be used to increase hive efficiency. Additionally, data analysis can be performed to determine the timing of nectar collection and the most productive areas. By using machine learning algorithms, relationships can be established between bee movement data inside the hive and colony health and production performance (Zacepins, Brusbardis, Meitalovs, & Stalidzans, 2015).

To ensure quality and efficient honey production, regular inspection of hives is necessary. Environmental factors such as temperature, humidity, wind, wild animal attacks, and parasites like varroa negatively affect hives and reduce yield. Regular monitoring of hives against these factors can be easily achieved using information technology-based devices. Data can be collected to evaluate some conditions occurring in hives and analyzed using machine learning methods. Images can be collected to detect diseases such as varroa. In this way, information about the most productive conditions for honey production can be obtained. With the development of Internet of Things (IoT) technology, the number of sensors is increasing, enabling more data collection and more accurate analyses.

Bees produce sounds at different frequency ranges depending on various activities (Hadjur, Ammar, & Lefevre, 2022). Classification studies can be performed using machine learning methods based on these sound frequencies. The number of bees or the status of the hive can be monitored by images taken from around or the entrance of the hive. AI-based applications are important in beekeeping, allowing the examination of many characteristics affecting the basic structure of the hive such as the queen bee, nectar level, feeding, and swarming (Güler; Zacepins et al., 2015). Bee colonies face environmental factors, parasites, and problems like Colony Collapse Disorder (CCD) that negatively affect their survival. Important threats to bees include CCD, varroa parasite, habitat degradation, and genetic species factors (Dimitrijević & Zogović, 2022). These problems in bees cause reduced production in the food chain, negatively impacting global economies. Today, beekeepers develop various methods such as bee hive monitoring to combat negative situations like hive theft, colony health problems (CCD, parasites), and weather conditions (Meikle & Holst, 2015). To accurately determine the parasite load of bee colonies, it is statistically necessary to test at least 300 bee individuals (Lee, Moon, Burkness, Hutchison, & Spivak,

2010). Manual performance of these processes is prone to error and time-consuming. Technology-based applications can perform these processes in a shorter time.

Nowadays, problems that were previously difficult to solve, such as classification and pattern recognition, are solved more easily. Time and economic savings in analyses are also highlighted. Artificial intelligence applications provide significant savings in this regard. Classification applications are used in tasks such as counting bees, determining pollen status, detecting pests and diseases like varroa, and providing suitable environmental conditions for bees.

Artificial intelligence includes many subfields. Machine learning is a subfield of artificial intelligence, and deep learning is a subfield of machine learning. Machine learning automates the model creation process by enabling systems to learn from training data to solve tasks in computer science. It is also described as imparting learning ability to computers. There is no single best algorithm to solve a problem. The algorithm's performance is affected positively or negatively by many features such as problem type, number, and variable ratio (Mahesh, 2020). Large amounts of input data are not necessarily required to perform analysis with machine learning. Data are labeled by experts according to their class. Preprocessing such as rotation, scaling, and filtering may be necessary to handle missing or inconsistent data. Data augmentation techniques increase data quantity and improve learning outcomes, thereby enhancing model robustness. Adequate knowledge about observed data is essential.

Deep learning consists of neural networks with many layers and parameters (Shinde & Shah, 2018). It is a concept within machine learning based on Artificial Neural Networks (ANN) methods (Janiesch, Zschech, & Heinrich, 2021). In many studies, deep learning approaches produce better results than traditional machine learning methods. Its greatest advantage is good performance with high-dimensional data. The biggest disadvantage of deep learning is the high computational requirement. Today, improvements in Graphics Processing Unit (GPU) performance largely eliminate this disadvantage.

Traditional machine learning approaches are less universal and more complex than Convolutional Neural Network (CNN)-based architectures, which are a type of deep learning. In traditional methods, object detection or classification is divided into individual stages. The first stage is computer vision, and the second is the machine learning algorithm. CNN is a special type of supervised machine learning algorithm. Its use increased in computer

vision after its performance in the 2012 ImageNet competition (Bilik et al., 2024). It can easily classify objects without requiring preprocessing. It allows the use of pre-trained networks for general problems.

One of the important fields enabling the increased use of AI-based applications in the beekeeping sector is IoT technology. IoT technology is defined as the exchange of data between objects over the internet. It enables machine-to-machine communication (M2M) (Zikria, Ali, Afzal, & Kim, 2021). In beekeeping, IoT technology allows data collection from hives such as temperature, humidity, and images through sensors, enabling AI-supported analysis or real-time control systems.

Nowadays, modern beekeeping applications have become an important area for monitoring hive conditions automatically, thereby increasing production and yield. However, beekeepers often lack sufficient knowledge about automatic hive monitoring systems. This section aims to inform beekeepers about modern beekeeping and discuss studies in this field. Examples are provided regarding the use of IoT sensors in monitoring bee behavior and how data collected from hives via these sensors are analyzed using AI-based algorithms.

## 2. Literature Review

Precision beekeeping is an advanced agricultural field that optimizes hive productivity using digital infrastructure. At both hive and colony levels, it aims to maximize efficiency while minimizing resource consumption. The digitalization of the beekeeping sector began with the development of sensors in the field of IoT and the collection of data. In studies related to beekeeping, the most commonly used data come from sensors related to image (Tashakkori, Hamza, & Crawford, 2021; Wachowicz, Pytlik, Małysiak-Mrozek, Tokarz, & Mrozek, 2022), sound (Nolasco & Benetos, 2018), and temperature (Mahamud et al., 2019; Zacepins, Kviesis, Pecka, & Osadcuks, 2017). These are valuable because bees within the hive produce different sound frequencies depending on conditions such as the absence of a queen, swarming, or emergence of a new queen (Truong et al., 2023). In addition, high temperatures and humidity inside hives adversely affect honey production and yield. Therefore, determining optimal environmental conditions based on this data is essential (Wardhani, Hidayat, & Jhoswanda, 2020).

According to the literature, a study on smart bee health monitoring systems for improving honey production emphasized the importance of maintaining hives in good condition for bee activity (Yusof, Billah, Kadir,

Ali, & Ahmad, 2019). Stingless bees are very sensitive to environmental changes. In the study, hives were monitored using sensors measuring temperature, humidity, carbon dioxide ( $\text{CO}_2$ ) levels, and hive weight. These were tracked in real-time via IoT technology.

According to a study in the literature, significant challenges have been identified in automatically detecting the status and health of bee colonies within the scope of precision beekeeping (Robles-Guerrero, Saucedo-Anaya, Guerrero-Mendez, Gómez-Jiménez, & Navarro-Solís, 2023). The study aimed to reduce power consumption in automated monitoring systems, extend battery life, and monitor colony stress to prevent yield losses. Since many hives are located in remote areas with limited access to electricity, solar energy is needed. The choice of power model positively affected battery life. Machine learning methods including Logistic Regression (LR), Support Vector Machines (SVM), Random Forest (RF), K-Nearest Neighbors (KNN), and Neural Networks (NN) were evaluated for computational requirements and execution performance. Model performance was assessed using confusion matrix, accuracy, precision, recall, F1 score, and ROC curve. Acoustic samples were collected from five carniolan bee colonies using an Raspberry Pi 2-based omnidirectional monitoring system powered by a 10 Ah power bank and a 10 W solar panel. Data were recorded every 10 minutes in 30-second intervals. For feature extraction, the classical Mel-Frequency Cepstral Coefficients (MFCC) method was used. 70% of the 504 samples were used for training and 30% 216 samples for testing. SVM and NN achieved the best performance with faster classification.

In a study for detecting varroa mite infestations, image analysis and machine learning techniques were applied to visuals captured at hive entrances (Schurischuster, Remeseiro, Radeva, & Kampel, 2018). A homogeneous background was established and image patches containing individual bees were extracted. Raw images were converted to RGB format, and processing used brightness information separated from color using CIELab and HSV color spaces. Each frame of video footage 1920 x 1080 pixels, 30 fps was extracted and analyzed. From 12 videos, 1300 image patches were obtained. Due to the small number of varroa samples, the dataset was balanced. 80% of data 163 samples was used for training, 20% 43 samples for testing. Classification results using Naive Bayes, SVM, and RF were evaluated for accuracy and F1 score, with RF performing best.

In a study explained that varroa mites were initially seen in Eastern honeybees (*Apis cerana*) and later spread to Western honeybees (*Apis mellifera*), posing a significant threat to their health (Picek, Novozamsky,

Frydrychova, Zitova, & Mach, 2022). In Western bees, varroa reproduces in worker brood. The study used 400 images from 100 hives taken with a mobile phone. If the varroa size was less than 12x12 pixels, detection was not possible. A shallow CNN architecture called VarroaNet achieved 96% and 93% accuracy in fall and winter, respectively. The model was evaluated using sensitivity and specificity metrics. Advantages of the proposed model include low cost, real-time detection, and sufficient data provision on infestation.

In a study proposed a system for monitoring varroa infestation levels using deep learning with video images (Bjerge et al., 2019). The model achieved an F1 score of 97% for counting bees and 91% for counting mites using a CNN architecture with fixed-size images of varroa mites. Figure 1 shows images of varroa and other objects from the study.

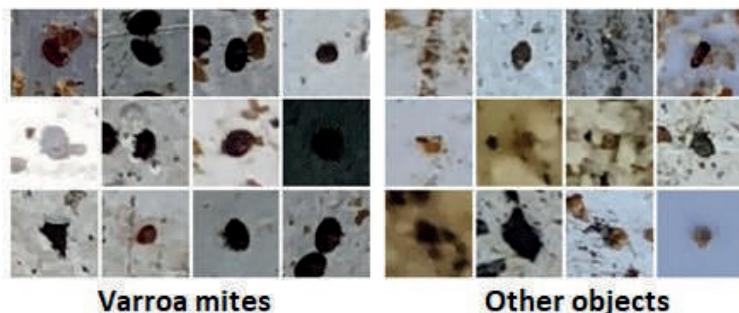


Figure 1. Visualization of varroa and other objects (Bjerge et al., 2019)

AI and IoT applications in beekeeping generally involve data collection through devices such as sensors or cameras. These data are automatically analyzed using machine learning and deep learning methods.

### 3. Artificial Intelligence-Based Applications and Internet of Things Technology in Beekeeping

Honeybees play a critical role in ecosystems, especially through pollination. Bee health is evaluated at the colony level, not individually. In recent years, the investigation of colony losses in *Apis mellifera* has become an important agricultural issue. Correct practices in beekeeping positively affect colony health. Beekeepers play a key role in colony management, especially when faced with threats. Physical or chemical interventions can help prevent dangers. Conditions affecting bee health and survival are shown in Figure 2.

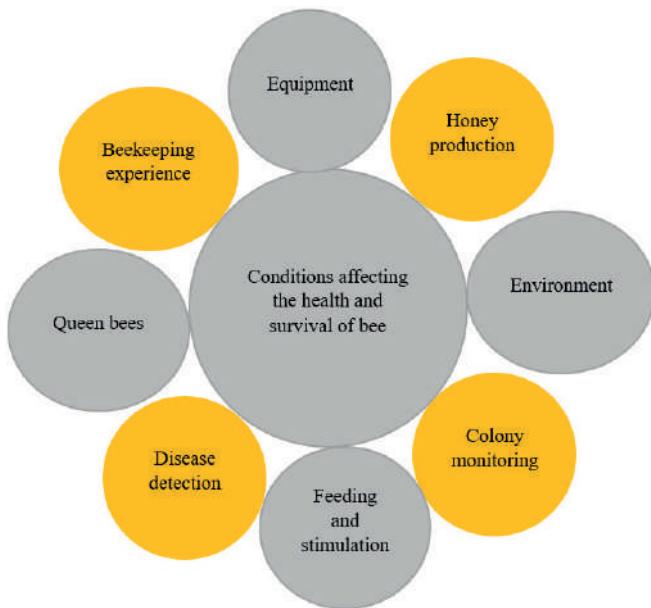


Figure 2. Bee health and survival conditions (El Agrebi et al., 2021)

Simultaneous monitoring of hives was previously costly and difficult. Today, technologies like IoT provide remote access to hive data. Real-time data access has brought significant convenience. Automatic data transfer has also greatly facilitated analysis. When sufficient data is not available, data augmentation with machine learning and deep learning methods can enhance analysis.

Analyzing honeybee images is increasingly important. Human eyes may fail to distinguish parasites on bees. Deep learning methods, especially CNNs, are effective for image-based analyses.

In beekeeping, classification methods commonly include RF (Albayrak, Çeven, & Bayır, 2021), SVM (Rahman, Lee, Venkatesan, Lim, & Shin, 2022), Decision Trees (DT) (Çukur & Çukur, 2022), KNN (Rafael Braga, G Gomes, M Freitas, & A Cazier, 2020), and LR. Deep learning methods include CNN (Braga, Madureira, Scotti, Piuri, & Abraham, 2021), Deep Neural Networks (DNN) (Zgank, 2021), and Feedforward Neural Networks (FNN) (Dimitrijević & Zogović, 2022).

### 3.1. Evaluation of Classification Results in Artificial Intelligence-Based Applications

Analyses conducted using machine learning and deep learning methods are generally evaluated based on the ROC curve or the confusion matrix (Lasko, Bhagwat, Zou, & Ohno-Machado, 2005; Sokolova & Lapalme, 2009). The confusion matrix consists of four different outcomes (Handelman et al., 2019):

- TP (True Positive): The number of correctly classified and actually correct values,
- TN (True Negative): The number of incorrectly classified and actually incorrect values,
- FP (False Positive): The number of correctly classified but actually incorrect values,
- FN (False Negative): The number of incorrectly classified but actually correct values.

In beekeeping-related classification studies, metrics such as accuracy, precision, recall, and F1 score are commonly used for analysis.

### 3.2. Machine Learning and Deep Learning Studies in Beekeeping

Deep learning is essentially formed by increasing the number of layers in ANN models, which are a type of machine learning method. In the field of precision beekeeping, image data has been increasingly utilized in recent years. Deep learning methods, which are based on machine learning, offer higher performance in this area. Example classification results from machine learning and deep learning-based approaches in precision beekeeping are presented in Table 1.

**Table 1. Machine learning- and deep learning-based approaches in beekeeping**

Reference	Implementation	Method	Performance (%)
(Kaur, Ardekani, Sharifzadeh, & Varastehpour, 2022)	Varroa detection	CLAHE + DCGAN + CNN	F1: 99.9
(Bjerge et al., 2019)	Bee counting Varroa detection	CNN	F1: 97.0 F1: 91.0
(Schurischuster & Kampel, 2020)	Varroa detection	DeepLabv3	F1: 95.0
(Yoo, Siddiqua, Liu, Ahmed, & Hossain, 2023)	Bee counting Varroa detection Pollen recognition	BeeNet	Acc: 92.45 Acc: 94.50 Acc: 99.18
(Ngo, Rustia, Yang, & Lin, 2021)	Pollen recognition	YOLOv3-tiny	Pre: 91.0 Rec: 99.0 F1: 94.0
(Berkaya, Gunal, & Gunal, 2021)	Varroa detection	Pre-trained DNN	Acc: 93.22 Pre: 94.99 Rec: 97.24 F1: 95.34

In deep learning, the success of classification results largely depends on the quantity and quality of the available data. In cases where the dataset is limited, the outcomes often tend to be ambiguous or suboptimal. However, by applying data augmentation techniques to expand and diversify the training data prior to classification, significant improvements in accuracy and model performance can be achieved.

#### **4. Artificial Intelligence in Beekeeping: Evaluation and Future Vision**

In the field of precision beekeeping, studies are conducted on tracking pollen-carrying bees, obtaining additional information about subspecies, distinguishing bee species, and analyzing their health status (infected or healthy). These studies are generally conducted using electronic systems mounted on the hives. This paper reviewed methods (deep learning and machine learning) and targeted applications (pollen recognition, varroa detection, bee traffic monitoring) used in automatic hive monitoring systems. Furthermore, articles published in the last 10 years on this topic were analyzed.

The analysis revealed that deep learning-based approaches are increasingly preferred due to their deeper architectures and better performance on large

datasets. Therefore, the usage of deep learning has significantly surpassed that of traditional machine learning methods. Based on the reviewed studies, it is anticipated that the use of AI-based hive monitoring systems in precision beekeeping will continue to grow, particularly due to advantages in speed and cost.

## 5. Conclusion

Honeybees are affected by adverse conditions such as colony collapse disorder, varroa mites (parasites), and unfavorable environmental factors. Early detection of problems within beehives is essential to protect bee populations and ensure stable production levels. Identifying hive issues manually is often time-consuming and costly. Precision beekeeping systems offer advantages in terms of cost and labor, making beekeeping more efficient.

AI-supported systems used in precision beekeeping can rapidly detect unfavorable conditions and potential problems within beehives. However, such smart monitoring systems are not yet widely adopted due to factors like lack of training and limited access to technology. This section aims to contribute to the proliferation of AI-supported hive monitoring systems. It is expected that in the future, the adoption of AI-based applications in beekeeping will become more widespread. As a result of this expansion, more informed production practices will emerge, leading to increased yield and quality per hive.

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# Knowledge and Awareness Levels of Agricultural Faculty Students Regarding Organic Agricultural Products 8

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## Abstract

People are turning to more eco-friendly options as the usage of chemical items rises and harms the environment. People are more interested in organic farming now since it protects the environment and encourages biodiversity. The organic product industry in Turkey is growing very quickly since more people are getting educated and have more money to spend. This study aims to determine the organic food consumption habits of students within the agricultural faculty. In March 2024, an online survey was performed with 341 students using the basic random sample method. Students were judged based on the amount of money they made each month, including scholarships and student loans. The results show that most students correctly what organic products are and what their logotype looks like. Also, 63.4% of students said that they get organic food. The most popular organic foods are fresh fruits, vegetables, and nuts. Red meat and fowl are less popular. Increased health knowledge, trust in inspections, and easy access to sales sites are the main factors that affect people's decisions to buy organic food. Students' purchasing habits for organic food and the reasons they buy it stay the same no matter how much money they make each month. As a result, it has been determined that students in agricultural faculties, who are seen as future experts in the field of agriculture, have a significant awareness and tendency towards organic products. Contrary to popular beliefs, wealth level does not greatly influence agriculture faculty students, indicating that knowledge and awareness are more essential in purchasing behavior. It is essential to put awareness-raising programs first and develop plans that include cheaper and more compactly packed items to make it easier for people on a budget, like students, to buy things.

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## **1. Introduction**

The negative impacts on the environment and the health risks of using a lot of chemical fertilizers and pesticides are making people prefer safer and more sustainable farming methods (Doğan & Karpuzcu, 2019; Eryılmaz et al., 2019). Global environmental problems and the rise of healthy eating have greatly increased the demand for organic agricultural products. Organic farming is a way of producing food that doesn't use chemicals at any stage of the process (ÖzTÜRK, 2012). Organic farming is a way to keep the land fertile over time while also protecting biodiversity. This approach uses biological control methods to get rid of pests and organic fertilization methods to feed plants (Çetiner, 2005). This strategy helps protect the current ecology and makes the environment stronger for future generations.

Like in other parts of the world, the market for organic goods in Turkey is growing quickly. The improvement of customers' education and buying power is a big part of this growth. Modern consumers consider not just the price and quality of goods, but also how the way they are made affects the environment and their social duty (Turhan et al., 2017).

Consumer trends include how people choose, buy, and use things to meet their needs and wants (Kılıç & Göksel, 2004). Studies show that socioeconomic factors have a big role in what buyers choose when they buy organic items. Income level, educational achievement, and lifestyle significantly affect the purchasing behavior of organic products (Gündüz & Kaya, 2007; Kekeç & Seçer, 2021; Şahin & Yercan, 2022). Rising domestic demand is leading university students to demonstrate heightened interest in organic food consumption, thereby promoting higher awareness of organic farming techniques (Demirbaş & Yılmaz, 2021). Understanding consumers' evaluations of organic agricultural products, the factors that encourage purchases, the reasons for avoiding organic products, and the knowledge and awareness of agricultural faculty students on this subject is essential for grasping the perspectives of future agricultural professionals.

The main goal of this study is to find out if students at Ankara University's (AU) Faculty of Agriculture eat organic foods differently depending on how much money they make each month. The study aims to ascertain whether organic food purchasing behaviors, determinants influencing organic food acquisitions, motivations for refraining from organic food, and critical evaluations of organic food vary according to monthly income levels.

## 2. Material and Methods

The main data for the study come from students at Ankara University's Faculty of Agriculture who were enrolled in the 2023-2024 academic year. There are 3001 students enrolled in the Faculty of Agriculture for the 2023-2024 school year. To determine the ideal sample size for effectively reflecting the consumer population, 341 surveys were collected using a simple random sampling method, as outlined in Equation 1 (Oğuz & Karakayacı, 2017).

$$n = (N \cdot p \cdot q) / [(N - 1) \cdot (D^2) + p \cdot q] \quad \text{Equation 1}$$

In the equation,  $n$  is the sample size,  $N$  is the population size,  $D^2 = (d/t)$ ,  $q = 0.5 - p = 0.5$ ,  $d$  is the maximum error allowed by the researcher (0.05), and  $t$  is the confidence interval (1.96).

The survey was conducted electronically in March 2024. Studies show that wealthy people are the most likely to buy organic products (Tosyalı, 2023; Turhan et al., 2019). The study divided students into three groups depending on their monthly income, which included scholarships and loans: low-income group (3999 ₺ and below/171 individuals), middle-income group (4000 ₺ to 5999 ₺ /112 individuals), and high-income group (6000 ₺ and above/58 individuals). The collected data was examined by creating cross-tables. The students' comprehension of organic food, their purchasing patterns about organic agricultural products, and their perspectives were revealed.

## 3. Findings and Discussion

There is a link between students' economic status and their demographic traits and household behaviors. More than half of the students (51.1%) have a poor income. Their average monthly income, which includes scholarships and loans, is 4291 ₺. The income level affects the residential status and the people who do the grocery shopping in a home. Most of the students that live in dorms are from low and middle-income families. This means that students are responsible for getting their own meals. Students with higher incomes live in student housing. Because of this, housemates and the group as a whole go grocery shopping more often. Table 1 shows the student' socio-economic traits.

**Table 1. Socio-economic characteristics of students**

		Income Level			
		Low	Middle	High	Overall
Gender (%)	Female	64.9	65.2	62.1	64.6
	Male	35.1	34.8	37.9	35.4
Residence status (%)	Dormitory	57.3	42.9	8.6	44.5
	Student house	11.1	32.1	67.2	27.5
	Family/Relative	31.6	25.0	24.1	28.1
Monthly income including scholarship-loan (avg.- ₺)		2753.5	4588.4	8224.1	4291.2
Class (%)	1st year	19.3	6.3	3.4	12.3
	2nd year	25.1	36.6	25.9	28.9
	3rd year	38.0	35.7	37.9	37.1
	4th year	17.5	21.4	32.8	21.6
Person Doing Grocery Shopping in Household	Me	52.0	43.8	15.5	43.0
	A family member	16.4	14.3	17.2	15.8
	Roommate/dormmate	4.1	12.5	22.4	10.2
	All together	27.5	29.5	44.8	31.0
Total	frequency	171	112	58	341
	%	50.1	32.9	17.0	100.0

The study conducted in Istanbul indicated that consumers see organic food as a natural product (Özkan, 2019). Consumers sometimes mix up the terms “natural food” and “organic food.” To test this comprehension among students, we included a number of questions. The survey showed that 83.3% of students knew the difference between the two conceptions, and the students had a very good (55.7% + 20.2%) comprehension of organic products (Table 2). Hamilton and Hekmat (2018) found that how much people know about organic food affects how much they eat, while Çam and Karakaya (2018) found that 73.4% of consumers know a lot about organic products and Aral and Cufadar (2024) found that 29.4% of university students know a lot about organic products, with 63.2% having some knowledge.

*Table 2. Organic product awareness status (%)*

		Income Level			
		Low	Middle	High	Overall
Knowledge of the distinction between organic food and natural products	Same	11.1	4.5	5.2	7.9
	Different	79.5	88.4	84.5	83.3
	I don't know	9.4	7.1	10.3	8.8
Level of knowledge about organic products	I don't know	12.3	8.0	6.9	10.0
	I've only heard of it	15.8	13.4	10.3	14.1
	I know partially	52.0	55.4	65.5	55.7
	I have sufficient knowledge	19.9	23.2	17.2	20.2
Knowledge of organic product definition	Incorrect definitions	36.3	25.0	31.0	31.9
	Correct definition	63.7	75.0	69.0	68.1
Knowledge of organic product logo	Incorrect logos	33.3	24.1	20.7	28.1
	Correct logo	66.7	75.9	79.3	71.9

Studies show that consumers mix up different logos because they don't have enough information and don't trust the products (Diaz et al., 2012). The study provided numerous definitions and logos, indicating that a substantial majority of students correctly identified the concept of organic products (68.1%) and the organic product logo (71.9%) (Table 2). A similar survey found that 54.7% of students recognized the organic farming emblem (Akgül et al., 2020).

*Table 3. Importance level given to judgments about organic agricultural products (mean)\**

		Income Level			
		Low	Middle	High	Overall
Being fresh and tasty		4.4	4.6	4.7	4.5
Being natural and healthy		3.4	4.1	4.4	3.8
Being safe and certified		3.5	4.1	4.4	3.8
Not containing artificial chemicals		3.3	4.0	4.3	3.7
Being produced under environmentally friendly conditions		3.2	3.8	4.2	3.6
Being a food product unaffected by pollution		3.2	3.9	4.4	3.6
Being a product grown in natural conditions using traditional methods		3.0	3.8	4.3	3.5

\* Data was collected based on importance level from 1 (very low importance) to 5 (very high importance).

Most students think that organic food is fresh and tasty. Next comes natural, healthy, safe, and certified food that doesn't have any artificial ingredients and is made in ways that don't harm the environment (Table 3). A survey conducted in Turkey revealed that students regard organic foods as safe, advantageous for human health, and effective in diminishing pesticide usage in agriculture (Akgül et al., 2020). Ayaşan et al. (2021) emphasized that health safety, naturalness, and ecological concerns are of utmost importance. In contrast, Aral and Cufadar (2024) highlighted health benefits, nutritional value, safety, and environmental friendliness as the principal justifications for the choice for organic products.

*Table 4. Organic agricultural product purchasing behaviors (%)*

		Income Level			
		Low	Middle	High	Overall
Organic agricultural product purchasing status	Yes	53.2	70.5	79.3	63.4
	No	19.9	13.4	12.1	16.4
	No, but I would like to	26.9	16.1	8.6	20.2
Attention to logo on organic agricultural products	Yes	49.6	47.1	50.3	49.1
	No	50.4	52.9	49.7	50.9

More over half of the students (63.4%) said they bought organic food. Sarica et al. (2023) discovered that 85.7% of students consumed organic food in their studies. 49.1% of students who buy organic farm products think about the emblem when they decide what to buy (Table 4). A similar study showed that 16.7% of students think about the logo when they buy organic products (Ayaşan et al., 2021). This issue stems from the differences in the academic disciplines of the individuals participating in the sampling.

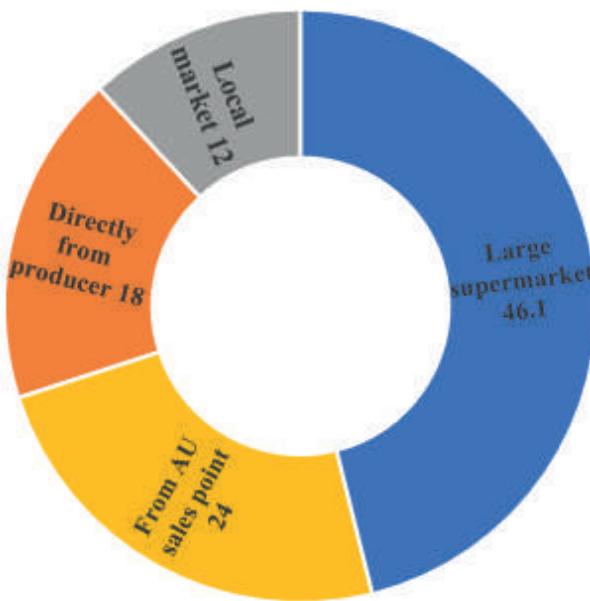


Figure 1. Place of purchase for organic agricultural products (%)

The accessibility of organic food positively impacts its consumption (Karabaş & Gürler, 2012; Singh & Verma, 2017). The current study done in Ankara province demonstrated that access to organic products is commonly available (81.0%). Students obtain products from prominent marketplaces, the sales platforms of Ankara University, directly from manufacturers, and from local markets, as illustrated in Figure 1. Ayaşan et al. (2021) found that most organic items come from villages (57.7%) and supermarkets (28.2%). Merdan (2018) found that they come from a variety of online platforms (26%), organic product retail stores (17%), and directly from producers (17%).

People who don't buy organic agricultural products (16.4%) say they don't because they are too expensive, there aren't enough organic options, or they don't trust organic agricultural products. Akgül et al. (2020) stated in their study that students are doubtful about the execution of organic food inspections and believe that such goods are not stored under hygienic conditions post-harvest.

**Table 5. Most frequently purchased organic agricultural products (%)<sup>\*</sup>**

General Ranking	Product	Income Level			
		Low	Middle	High	Overall
1	Fresh vegetables/fruits	32.7	49.1	72.4	44.7
2	Dried fruits/nuts	32.2	47.3	58.6	41.5
3	Milk and dairy products (milk, butter)	26.9	38.4	39.7	32.7
4	Grains/legumes	15.8	30.4	29.3	22.8
5	Red meat and poultry	15.2	22.3	25.9	19.3

*\* Totals exceed 100% as consumers made multiple choices..*

An examination of the organic agricultural bought by students indicated no variation in the leading three products among general and socioeconomic categories. After looking at everyone's likinges as a whole, it was clear that fresh fruits and vegetables were the most popular goods, followed by dried fruits and nuts. People typically think of these items as organic farming products. Red meat and poultry were chosen as the least popular items, which is also shown by the fact that groups didn't like them very much (Table 5). Research found that students predominantly consumed organic fresh fruits and vegetables at a rate of 54.6% (Ayaşan et al., 2021), while their intake of organic animal products included milk and dairy items (35.9%) and red or white meat (31.1%) (Aral & Cufadar, 2024). A study conducted in Diyarbakır revealed that customers preferred organic fresh fruits and vegetables (43%), milk and dairy products (23%), and meat (18%) (İnci et al., 2017).

Table 6. Reasons for purchasing organic agricultural products (%)<sup>\*</sup>

		Income Level			
		Low	Middle	High	Overall
Increased health awareness	1	1.3	2.9	1.9	2.0
	2	4.7	1.9	1.9	3.3
	3	94.0	95.2	96.2	94.7
	$\bar{x} \pm ss$	4.6±0.6	4.6±0.8	4.8±0.7	4.6±0.7
Knowing that inspections are carried out correctly	1	9.3	8.7	1.9	7.9
	2	7.3	2.9	1.9	4.9
	3	83.4	88.4	96.2	87.2
	$\bar{x} \pm ss$	4.1±0.9	4.4±0.9	4.7±0.7	4.3±0.9
Well-known brands producing organic food	1	27.3	14.6	3.8	18.9
	2	12.0	3.9	3.7	7.8
	3	60.7	81.5	92.5	73.3
	$\bar{x} \pm ss$	3.7±1.3	4.2±1.1	4.6±0.8	4.0±1.2
Proximity of sales location to home	1	22.7	11.6	5.6	15.7
	2	4.0	2.9	7.4	4.2
	3	73.3	85.5	87.0	80.1
	$\bar{x} \pm ss$	3.9±1.3	4.3±1.1	4.6±0.9	4.1±1.2
Increased promotion and advertising	1	36.0	24.3	5.6	26.8
	2	4.0	3.9	1.9	3.6
	3	60.0	71.8	92.5	69.6
	$\bar{x} \pm ss$	3.5±1.3	3.9±1.3	4.6±0.9	3.9±1.3
Tasting at markets, fairs, etc.	1	25.3	16.5	11.2	19.9
	2	6.0	9.7	3.7	6.8
	3	68.7	73.8	85.1	73.3
	$\bar{x} \pm ss$	3.7±1.2	4.0±1.2	4.4±1.1	3.9±1.2

\* Data was collected on a Likert scale from 1 (strongly disagree) to 5 (strongly agree).

“ $\bar{x}$ ” represents the mean, “ $ss$ ” represents the standard deviation.

Table 6 shows how pupils feel about getting organic agricultural products. Increased health knowledge (94.7%), confidence in correct inspections (87.2%), and ease of access to sales places (80.1%) are all thought to be important factors that affect the buying of organic products. Additionally, it has been demonstrated that improving marketing and advertising, together with offering sampling, leads to greater awareness of organic agricultural products and significantly impacts purchasing decisions. Güner and Ulusoy (2024) found a positive link between how students think about their health and how much organic food they eat. They concluded that a higher health perception greatly increases the likelihood of eating organic food.

#### **4. Conclusion and Recommendations**

As a result of their studies, students in the agricultural faculty know a lot about and comprehend organic farming. This level of awareness lessens the consequences of differences in income and other socioeconomic factors. It has been determined that knowledge and attitude exert greater influence than income. The heightened awareness among agriculture faculty students emphasizes the importance of education. To spread and develop knowledge throughout society, it is important to include topics like organic farming and food safety in school curricula. Also, non-governmental organizations and public institutions should start programs to teach consumers about things to raise awareness in society.

The majority of students are purchasing organic food. Instead than seeing organic food as a luxury, students look at it as a health benefit. Students from low-income try to get organic food. Across all income levels, the most popular organic foods are fresh fruits and vegetables, while the least popular are red and white meat. This situation is happening because agricultural faculty students eat in similar ways. To attract more people to eat organic food, especially students who are on a tight budget, need to come up with cheaper, smaller products and marketing strategies that focus on students.

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# The Use of Medicinal and Aromatic Plants in Livestock Production and Their Phytochemical Effects 8

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## Abstract

The use of medicinal and aromatic plants in animal husbandry and veterinary practice is increasingly recognised for their phytochemical richness and as sustainable alternatives to synthetic antibiotics. This review examines their roles as functional feed additives; stimulating appetite, promoting growth, combating bacterial, viral, and parasitic infections, and enhancing meat quality and aroma. In addition to these applied benefits, the active compounds in these plants modulate gene expression and transcriptomic pathways, thereby influencing metabolism, immunity, and muscle development. Growing concerns about antibiotic residues in animal products further underscore their significance. By integrating medicinal and aromatic plants into livestock feeding strategies, both productivity and welfare may be improved. This review highlights key chemical constituents, their molecular mechanisms, and implications for livestock production systems, proposing that such integration offers a promising, health-conscious, and environmentally relevant approach to modern animal production.

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## 1. Introduction

The valuable bioactive compounds of secondary metabolites also find applications in the livestock sector. The rapid growth of the global population continuously increases the demand for animal products, which directs the livestock industry towards innovations focused on productivity and sustainability. However, ensuring sustainability in production and maintaining animal health have become challenging, particularly due to the prolonged and uncontrolled use of antibiotics, which causes various issues (Gadde et al. 2017). In particular, the European Union's ban on antibiotics as feed additives in 2006 necessitated the exploration of natural and safe alternatives in the sector (Castanon, 2007).

In this context, medicinal and aromatic plants have gained prominence; due to their antibacterial, antioxidant, antiviral, and digestive-regulatory properties, they have been increasingly used to support animal health and enhance production performance (Windisch et al. 2008). The effects of medicinal and aromatic plants on important parameters such as meat quality, growth, development, and gene expression are being increasingly investigated across different animal species.

In ruminants, particularly cattle, sheep, and goats, the use of medicinal and aromatic plants supports feed efficiency through enhanced digestive functions, optimizes growth rates, and strengthens immune responses (Hashemipour et al. 2013a). In large animals with sensitive digestive systems, such as horses, these plants are particularly important for supporting digestion and immunity. Variations in metabolic characteristics and nutritional requirements among different breeds create diversity in the dosage and composition of these herbal additives (Srinivasan, 2016). Additionally, biological responses can vary across breeds. In poultry, especially chickens, medicinal and aromatic plants have been reported to positively affect growth performance, feed conversion ratio, and limb and claw health. Their effects may vary among different production-oriented breeds, such as broilers and laying hens (Brenes and Roura, 2010). The effects of bioactive compounds are evident in the development of muscle and adipose tissue, which are directly related to growth performance and meat quality. Medicinal and aromatic plants improve microbial balance in the digestive system, enhance nutrient absorption, and support gut health (Hashemipour et al. 2013b; Liu et al. 2020). This not only increases feed efficiency but also strengthens immune system functions (Windisch et al., 2008). Furthermore, certain plant compounds exert regulatory effects on the endocrine system, influencing the expression of growth-related hormones

and genes (e.g., IGF-1, mTOR, MyoD), resulting in positive effects on muscle and adipose tissue development (Liu et al. 2020).

These biological effects allow medicinal and aromatic plants to be considered in animal production systems not only as performance enhancers but also as natural health-supporting agents (Maqsood et al. 2014; Hashemi and Davoodi, 2012).

This review examines their roles as functional feed additives—stimulating appetite, promoting growth, combating bacterial, viral, and parasitic infections, and enhancing meat quality and aroma. In addition to these applied benefits, the active compounds in these plants modulate gene expression and transcriptomic pathways, thereby influencing metabolism, immunity, and muscle development.

## **2. Use of Medicinal and Aromatic Plants in Animal Feeding: Impacts on Physiology, Health and Performance**

The use of medicinal and aromatic plants (MAPs) in animal feeding is not limited to enhancing nutritional value but also exerts multifaceted effects on overall physiology and health. Particularly, these plant-based components, when included in diets, influence numerous biological processes in animals, ranging from feed utilization efficiency via digestive system regulation to modulation of hormonal balance and gene expression (Hashemi and Davoodi, 2012; Windisch et al., 2008; Greathead, 2003). The following sections provide a detailed evaluation of these effects in a holistic approach.

### **2.1. Effects on the Regulation of the Digestive System and Feed Utilization**

The healthy and efficient functioning of the digestive system in animals is among the key factors directly influencing growth. Volatile compounds such as carvacrol, thymol, and cinnamaldehyde, present in medicinal and aromatic plants (MAPs), enhance the activity of digestive enzymes, thereby improving the digestion of proteins and carbohydrates (Windisch et al., 2008). The ability of plant compounds to modulate microbial balance is critical for maximizing feed utilization (Ribeiro-Santos et al., 2015). The efficacy of these natural feed additives varies depending on animal species, breed, and diet composition, highlighting the importance of optimizing application doses (Wang et al., 2020). A meta-analysis in cattle by Benchaar et al. (2008) review evaluates the potential of plant-derived essential oils (EOs) as natural alternatives to antibiotics in response to growing concerns over antibiotic use in livestock production. Essential oils contain secondary

metabolites such as thymol, carvacrol, eugenol, and cinnamaldehyde, which possess antimicrobial properties and can modulate rumen microbial activity, thereby improving protein and energy utilization. In vitro studies have shown that EOs can reduce amino acid deamination and ammonia formation in the rumen, and certain components may also inhibit methane production. However, long-term in vivo studies indicate that rumen microbes may adapt to EOs, leading to diminished effects over time. The ability of EOs to alter rumen fermentation patterns in a manner similar to ionophore antibiotics (e.g., decreasing the acetate-to-propionate ratio) is considered beneficial for feed efficiency. Moreover, EOs have demonstrated strong bactericidal activity against foodborne pathogens such as *E. coli* O157:H7 and *Salmonella* spp. Nonetheless, identifying optimal doses that enhance rumen function without inhibiting overall fermentation and ensuring sustained efficacy remain major challenges. In conclusion, essential oils are promising natural feed additives that may enhance feed efficiency, reduce nitrogen and methane emissions, and help control pathogenic bacteria in ruminant production. Further in vivo research is required to clarify dose-response relationships, microbial adaptation mechanisms, and the long-term impacts of EO supplementation. Some in vitro studies suggest that aromatic plant essential oils (including fennel) may enhance rumen microbial protein synthesis or improve digestibility, though quantitative effects and consistent in vivo validation remain limited (Zhao et al. 2020). These plants are also significant for environmental sustainability due to their methane-reducing potential. Tannin-containing extracts can reduce ruminal protein degradation and thereby increase the flow of undegraded (bypass) protein to the small intestine; several meta-analyses and trials report improved nitrogen utilization with low-to-moderate tannin levels, although the magnitude of effects varies by tannin type, dose, and animal/breed (Jayanegara et al. 2019). Dietary supplementation with ginger (*Zingiber officinale*) has been shown to improve growth performance and feed efficiency in livestock and poultry. In broiler chickens, Hashemipour et al. (2013c) reported improved body weight gain and feed conversion ratio, whereas studies in small ruminants such as Awassi sheep have shown variable or modest effects depending on dose, duration, and diet composition. The study investigated the effects of a 10% aqueous garlic solution on parasitic infestation, live weight, and hematological parameters in Black Bengal goats. Garlic supplementation significantly reduced fecal egg counts while improving live weight gain and blood parameters (TEC, Hb, PCV). The findings suggest that garlic can serve as a natural antiparasitic and health-promoting feed additive for goats (Hasan et al., 2015). Similarly, cumin (*Cuminum cyminum*) and fennel

essential oils in Suffolk sheep increased body weight gain by 10% and total protein synthesis by 7%, while also improving liver function (Khan et al., 2021a). Thyme and clove (*Syzygium aromaticum*) essential oils in sheep diets increased growth rate by 9% on average and enhanced digestive enzyme activities by 15% (Al-Mamun and Islam, 2021).

In a study evaluated the effects of fennel and ginger supplementation on nutrient digestibility and milk production in 15 early-lactation Egyptian buffaloes. Both supplements did not influence feed intake but significantly improved the digestibility of dry matter, organic matter, crude protein, and fiber. Fennel and ginger increased milk yield and energy-corrected milk, as well as milk fat concentration and overall milk energy output. The supplements did not adversely affect blood biochemical parameters and reduced somatic cell count, indicating improved udder health. Overall, daily supplementation with 75 g of fennel or ginger enhanced milk productivity and quality, with fennel demonstrating a greater improvement in the nutritive value of milk (Fahim et al., 2022). In Niger, supplementation of laurel and fennel oils in Sahel goats increased growth rate by 11% and feed utilization by 8% (Oke et al., 2021). Studies in Alpine and Saanen goats demonstrated that ginger and cumin essential oils optimized rumen fermentation, increasing feed utilization by 6–9% (Hasan et al., 2024). According to Zakeri et al. (2014), supplementation of fresh garlic at levels of 30–70 g/kg on a dry-matter basis for 14 days may reduce milk fat content in goats by influencing acetate production in the rumen. Indeed, several researchers (Kongmun et al., 2011, Kholif et al., 2012) have shown that garlic derivatives exert an inhibitory effect on acetate formation. Similarly, administering 2 mL/day of garlic oil to lactating goats for three months has been reported to provide comparable beneficial effects (Kholif et al., 2012). In addition, supplementation of garlic at 20 g/kg (DM) for approximately five months in sheep (El-Shereef, 2019) led to an increase in conjugated linoleic acid (CLA) and omega-3 fatty acids—nutrients of particular importance for human health—in milk (Cavaliere et al., 2018; Trinchese et al., 2019). Likewise, Yang and He (2016) reported that adding 5 g/day of garlic oil to dairy cow diets for three weeks elevated the cis-9, trans-11 CLA content of milk; this fatty acid possesses anti-inflammatory, anti-diabetic, and anti-tumor properties (Tudisco et al., 2019; Thanh et al., 2021). Overall, the use of different forms of garlic across studies has contributed to considerable variation in outcomes. Standardization of garlic products would therefore be beneficial for determining the most appropriate dose and formulation aimed at improving ruminant milk quality. In poultry, research on the Ross 308 broiler strain, widely used in commercial meat production, has shown

that oregano and thyme essential oils improve feed conversion by 7–10% and enhance gut microbiota (Hashemipour et al. 2013a). In Bovans White chickens, rosemary (*Rosmarinus officinalis*) essential oil reduced feed intake while improving feed efficiency, altered yolk fatty acid composition, and modulated gut microflora (Kılıç, 2022).

## 2.2. Effects on the Immune System

The immune system is critical for sustaining growth. The antimicrobial and anti-inflammatory properties of medicinal and aromatic plants (MAPs) help reduce the risk of infections, thereby supporting animal health. Numerous studies have demonstrated that these plants, through their antioxidant and immunomodulatory effects, enhance immune function and mitigate oxidative stress. Research across various animal species—including cattle, sheep, goats, poultry, and horses—indicates that MAPs contribute both to health maintenance and productivity improvement.

In cattle, particularly beef breeds, essential oils derived from plants such as thyme (*Origanum vulgare*) and clove (*Syzygium aromaticum*) have been reported to enhance immune function, increase total antioxidant capacity, and balance liver enzyme activities (Michiels et al. 2010; Hashemipour et al. 2013a). Tassou et al. (2007) reported that dietary sage supplementation in sheep increased serum immunoglobulin G (IgG) levels by 15%.

In poultry, diets containing essential oil blends have been shown to reduce inflammatory cytokines by 20%, resulting in an 8–10% improvement in growth performance. Numerous studies in broiler chickens indicate that supplementation with thyme oil, garlic (*Allium sativum*) extract, ginger (*Zingiber officinale*), and cinnamon (*Cinnamomum zeylanicum*) enhances immune cell activity, lowers disease incidence, and limits oxidative damage (Brenes and Roura, 2010; Gadde et al. 2017). In species with more sensitive digestive systems, such as horses, the use of plants like Echinacea purpurea, fennel (*Foeniculum vulgare*), and thyme is recommended to support immune function. Their use has been associated with lower infection rates and more balanced blood parameters, particularly in performance horses (Srinivasan, 2016).

## 2.3. Effects on the Hormonal Regulation and Gene Expression

In recent years, medicinal and aromatic plants (MAPs) have been shown to exert effects in animal organisms not only at the macroscopic or biochemical levels but also at the molecular level. These molecular effects are particularly elucidated through gene expression and transcriptomic analyses.

By modulating metabolic pathways, components of the immune system, and muscle development at the genetic level, MAPs have the potential to enhance both product yield and animal welfare. Focusing on the epigenetic and transcriptomic effects of specific MAP compounds on the animal genome is of great importance for sustainable animal production and functional feed strategies (Luo et al. 2020; Wang et al. 2020).

Plants such as thyme (*Thymus vulgaris*), sage (*Salvia officinalis*), rosemary (*Rosmarinus officinalis*), peppermint (*Mentha piperita*), summer savory (*Satureja hortensis*), and ginger (*Zingiber officinale*) can positively influence gene expression in animals due to their antioxidant, anti-inflammatory, and immunomodulatory properties.

Similarly, the use of summer savory led to positive changes in anti-inflammatory gene expression and modulated immune responses (Jahanian and Ashnagar, 2015). Broiler chickens supplemented with thyme exhibited significant upregulation of antioxidant defense genes, including superoxide dismutase (SOD1), catalase (CAT), and glutathione peroxidase (GPX), demonstrating the efficacy of thyme compounds in combating oxidative stress (Khan et al. 2021b). In broilers supplemented with ginger, the expression of pro-inflammatory genes TNF- $\alpha$  and IL-1 $\beta$  decreased, while antioxidant gene expression increased (Ali et al. 2020). In pigs, Wang et al. (2020) demonstrated that plant extracts, including capsicum oleoresin, garlic, and turmeric, modulated the expression of immune-related genes in alveolar macrophages under PRRSV infection, thereby influencing the infection response. This suggests that MAPs can trigger protective mechanisms against infectious diseases at the genetic level. In sheep supplemented with sage, regulatory effects on inflammation-related cytokines (IL-6, TNF- $\alpha$ , IFN- $\gamma$ ) were reported, providing positive modulation of the immune system (Morales-Martínez et al. 2020). In cattle, rosemary extract increased the expression of PPAR $\gamma$  and FABP4, genes related to energy and lipid metabolism, contributing to improved meat quality (Wang et al. 2020). Similarly, gene expression analyses in Wagyu and Holstein cattle have shown significant differences in PPAR $\gamma$  and FABP4 expression levels, genes involved in lipid metabolism, suggesting that these genetic variations are associated with breed-specific differences in meat quality (Wang et al. 2020).

MAPs have also been reported to regulate the release of growth-related hormones and gene expression. Compounds such as capsaicin were found to increase growth hormone (GH) and insulin-like growth factor-1 (IGF-1) levels by 12–15%, supporting protein synthesis (Jin et al. 2016). Additionally, their anti-inflammatory effects promote the activation of genes associated

with muscle tissue repair and development. Experiments in broilers showed that essential oil blends increased the expression of genes related to muscle development by 10–12% (Cross et al. 2007). In a study examining the effects of dietary fennel essential oil (FO) on growth performance, antioxidant status, inflammatory responses, and liver histopathology in heat-stressed broiler chickens. FO supplementation, particularly at 3 g/kg, improved body weight gain and feed conversion ratio compared with the positive control and paracetamol groups. It also enhanced antioxidant enzyme activities, reduced MDA and pro-inflammatory cytokines, and downregulated hepatic IL1- $\beta$  and TGF- $\beta$  expression. Overall, 3 g/kg FO effectively mitigated the negative impacts of heat stress and improved growth and physiological responses in broilers (Amer et al., 2025).

#### 2.4. Effects on Muscle and Adipose Tissue

Carcass quality, one of the key indicators of growth performance, is directly associated with the development of muscle and adipose tissues. Phenolic compounds present in medicinal and aromatic plants (MAPs) can modulate lipid metabolism, leading to favorable changes in fatty acid profiles. Specifically, studies have shown that MAPs can increase the proportions of omega-3 and polyunsaturated fatty acids; thereby enhancing both the nutritional value and palatability of meat. In addition, these compounds help preserve muscle protein structure, reducing tissue hardness and consequently improving meat quality and consumer satisfaction (Patra and Yu, 2014).

Studies in cattle breeds such as Holstein and Angus have demonstrated that the use of MAPs positively affects fatty acid profiles and antioxidant capacity (Michiels et al. 2008). In sheep, rosemary extract supplementation was reported to improve meat tenderness and flavor characteristics (Tassou et al. 2007). Rosemary (*Rosmarinus officinalis*) is well known for its antioxidant and antimicrobial properties. In cattle, rosemary extract supplementation was found to enhance the expression of genes related to energy and lipid metabolism, contributing to improved meat quality (Wang et al. 2020).

The study investigated the effects of thyme oil, acetic acid, and their combination (each at 2%) on the quality and shelf life of raw beef stored at 4 °C. Treatments with either thyme oil or acetic acid individually reduced pH, TVBN, TBARS, and aerobic plate counts compared with the untreated control, thereby slowing chemical and microbial spoilage. The combined treatment exhibited the strongest preservative effect, maintaining acceptable sensory attributes and microbial quality up to 15 days of storage. Across all treated groups, lipid oxidation and nitrogenous compound formation were

substantially lower than in the control samples. Overall, the findings indicate that thyme oil and acetic acid—especially when applied together—can serve as effective natural preservatives to extend the shelf life and improve the quality of raw beef meat (El Asuoty et al., 2023).

In poultry, especially broilers, essential oils from thyme (*Thymus vulgaris*) and summer savory (*Origanum vulgare*) have been shown to enhance meat quality. These essential oils positively influenced pH, color, and water-holding capacity, reduced lipid peroxidation, and improved microbial stability. Sensory panelists described meat from essential oil-supplemented groups as “fresh,” “clean,” and “pleasantly herbal,” whereas control group meat exhibited slight rancidity. While the fatty acid composition, particularly unsaturated fatty acids, increased, no significant change in total fat content was observed. Zaazaa et al., (2022) reported that the effects of thyme and oregano essential oils, individually or in combination, on broiler growth performance, health parameters, and the prevalence of growth-related breast muscle abnormalities. Oregano oil supplementation resulted in the greatest improvements in body weight gain and feed conversion efficiency, while thyme oil also produced moderate performance benefits. However, the inclusion of either essential oil, alone or combined, markedly increased the incidence of white striping and wooden breast myopathies. No significant effects were observed on immune responses, as Newcastle disease antibody titers and interferon- $\gamma$  levels remained comparable to the control group. Overall, although essential oils enhanced growth performance, their association with increased muscle abnormalities warrants careful consideration in broiler production systems.

Consumer acceptance of animal products is largely influenced by sensory attributes, especially meat aroma and flavor quality. The inclusion of MAPs in animal diets affects not only the physical and chemical properties of meat but also its aromatic profile and overall sensory quality. Meat sensory quality, determined by color, texture, water-holding capacity, aroma, and taste, is closely linked to animal nutrition. In this context, phenolic compounds, flavonoids, terpenoids, and essential oils in MAPs can directly influence the aromatic and sensory characteristics of meat (Mandal et al., 2021; Maqsood et al., 2014). Sheep and goat meat can face limitations in consumer acceptance due to their characteristic “gamey” aroma. To mitigate these undesirable aromatic traits, dietary supplementation with MAPs has been investigated. In goats, supplementation with thyme and fennel essential oils altered the aromatic compounds in meat, increasing the levels of more pleasant odor-active compounds such as linalool and  $\alpha$ -terpineol. These changes were reported to suppress undesirable animal odors (Mandal et al. 2021). In a

sensory evaluation of sheep meat, the addition of garlic and thyme extracts to diets resulted in meat that was reported to be more tender, juicy, and aromatic. Taste panelists particularly noted that meat from this group had a “fresh and mildly herbal” flavor (Özkan et al. 2020). Hashemipour et al. (2013b) reported that the use of thyme oil in broiler diets reduced malondialdehyde (MDA) levels by 25%, leading to a 5–7% improvement in meat quality and growth rate.

## 2.5. Effects on Animal Health

Animal health and welfare are among the primary priorities in modern livestock production, with the rise of antibiotic resistance being one of the most significant challenges in these fields. The prolonged and uncontrolled use of antibiotics as growth promoters has facilitated the development of antimicrobial-resistant pathogens, posing a substantial threat to both animal and public health (Mathew et al. 2007). Consequently, many countries, particularly within the European Union, have banned such practices, leading to increased interest in and demand for alternative, natural feed additives (Hashemi and Davoodi, 2011). In this context, medicinal and aromatic plants (MAPs) have emerged as natural products with diverse pharmacological effects due to their bioactive constituents, including phenolic compounds, flavonoids, alkaloids, tannins, and essential oils. These compounds support the immune system of animals through their antiviral, antifungal, antibacterial, and antioxidant activities, thereby contributing to the control of infectious diseases and providing protection against pathogenic microorganisms (Burt, 2004; Windisch et al. 2008).

The effects of garlic extract on animal health were investigated by Hashemipour et al. (2013), focusing particularly on beef cattle. In this study, a total of 30 Holstein and Angus cattle were used, and garlic extract supplementation was found to enhance the activity of immune cells and strengthen resistance against infections. These results indicate that garlic extract acts as an immune modulator in cattle, helping to reduce the risk of infections.

Sage (*Salvia officinalis*), known for its antiviral and antibacterial properties, was shown by Tassou et al. (2007) to increase serum immunoglobulin G (IgG) levels in sheep, thereby enhancing immune function.

### 3. Conclusion

The use and research of medicinal and aromatic plants (MAPs) in animal husbandry and health are increasing day by day. With the advancement of modern feeding techniques, alternative feeding programs have emerged. Consequently, in recent years, MAPs have gained attention in animal nutrition, contributing significantly to enhancing the taste and flavor of animal products and addressing various nutritional challenges. By exhibiting prebiotic effects, MAPs promote the proliferation of beneficial bacteria and regulate microbial balance, thereby enhancing the activity of digestive enzymes. This contributes to improved gut health, increased feed utilization efficiency, and enhanced growth performance. Due to their bioactive constituents, these plants influence physiological systems in animals and exert multiple effects on these systems. When used as feed additives or incorporated into rations, they demonstrate antioxidant, antifungal, antimicrobial, immunomodulatory, and digestive-regulatory properties, serving both supportive and therapeutic purposes in livestock production. Such effects are particularly valuable in animal production systems aiming to reduce antibiotic use. Certain plant-derived compounds possess the potential to modulate the activity of specific genes at the molecular level, producing significant impacts on muscle development, metabolic processes, and stress responses. Additionally, they can play a regulatory role in the endocrine system, helping to balance hormone levels. To maximize the economic and health-related benefits of MAPs in animal production, careful consideration must be given to the plant species, dosage, duration of administration, and the animal species involved. It is also important to note that high doses of some of these plants may exert toxic effects on animals.

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# **Integrated Perspectives in Agriculture and Biology: Theory and Practice**

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