INNOVATIVE APPLICATIONS AND RESEARCH METHODS IN AGRICULTURE, FORESTRY AND AQUACULTURE

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9 786258 513189

DOI:10.5281/zenodo.18049952

Innovative Applications and Research Methods in Agriculture, Forestry and Aquaculture

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Publisher
Platanus Publishing®

Editors in Chief
Prof. İsmet Daşdemir, Ph.D. & Assoc. Prof. Hikmet Yazıcı, Ph.D.

Cover & Interior Design
Platanus Publishing®

The First Edition
December, 2025

ISBN
978-625-8513-18-9

Platanus Publishing®
Address: Natoyolu Cad. Fahri Korutürk Mah. 157/B, 06480, Mamak, Ankara, Turkey.

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CONTENTS

CHAPTER 1

Fertilization and Plant Nutrition for Sustainable Lawn Areas

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1. INTRODUCTION

Lawns are the most important component of green areas. When we look at all the plant materials used in landscaping, we see that grass plants are the most commonly used. Considering the aesthetic and functional benefits provided by the use of grass plants, as well as their environmental benefits, this result is not surprising. Lawns, which perfectly cover the area they occupy, effectively prevent erosion with their dense and deep root systems, while their homogeneous appearance and attractive green colors enhance the beauty and therefore the economic value of the landscape. In addition to their aesthetic benefits, grass fields provide a natural, comfortable, safe, durable, and perfect playing surface for football, golf, tennis, and other sports fields (Beard, 1974; Salisbury, 1992; Acikgoz, 1994; Emmons, 2000; Henry et. al., 2002; Reynolds and Flint, 2009; Avcioglu, 2014).

In Türkiye, we often observe that turfgrass fields in landscaped areas are not properly established and maintained, and that the turfgrass cover eventually loses its uniformity and becomes bald under the pressure of weeds and diseases. However, if properly maintained, turfgrasses established using the right species and varieties of turfgrass can maintain their quality by creating a strong turfgrass cover for many years and provide the expected aesthetic and functional benefits. While maintenance of turfgrasses includes watering, mowing, fertilizing, rolling, sanding, preventing matting, and combating diseases, pests, and weeds, fertilization and plant nutrition, is the most important factor for plant growth, development, and adaptation to the environment (Acikgoz, 1994; Avcioglu, 2014; Sever Mutlu, 2017).

The substances added to the soil to increase the fertility of cultivated soils and to improve the quality and quantity of the crop are called fertilizers, and the process itself is called fertilization. Until the end of the 19th century, especially in Europe, large amounts of potassium and phosphorus, and later nitrogen, began to be used in agriculture. Observations and research have shown that nitrogen, phosphorus, potassium, sulfur, calcium, magnesium, silicon, and iron are extremely important for plant growth (Garling and Boehm, 2001; Browman, 2003; Barton and Colmer, 2006; Espinoza et. al. 2007).

Plant nutrients, or beneficial elements, are divided into two groups: "macro" and "micro" elements, depending on their amount within the plant or the amount required by the plant. The macronutrients, especially essential and beneficial for turfgrass, are nitrogen, phosphorus, potassium, sulfur, calcium, and magnesium, while the micronutrients are iron, zinc, silicon, copper, and manganese (Brohi and Aydeniz, 1991; Kacar and Katkat, 2023).

Organic materials derived from plant, animal, and human waste or residues, or artificially derived inorganic materials, can be used as plant nutrient sources for fertilization. Organic plant nutrients include farm manure, chicken manure, compost, green manure, liquid feces, sewage sludge, plant waste, industrial and food processing factory waste, grain straw, hay, and husks, aquatic plants, and seaweed. Fertilizers add organic matter to the soil, improve its physical properties, accelerate microbiological activity, regulate structure, increase aeration and water-holding capacity, supplement macronutrients, and increase phosphorus availability. Inorganic plant nutrients are materials that have been artificially produced and used for plant nutrition, particularly in the last century (Reicher and Throssell, 2000; Mangiafico and Guillard, 2006; Trenholm and Unruh, 2009; Landschoot, 2016; Zengin, 2022; Kacar and Katkat, 2023).

2. FERTILIZING LAWN AREAS

Grass plants require certain nutrients for healthy growth and development. The source of plant nutrients is the breakdown and dissolution of organic matter and minerals in the soil and the accumulation of applied commercial fertilizers. Fertilization is extremely important for creating high-quality and healthy lawns. However, answering the questions of when, at what dose, and how fertilizer should be applied is just as important as fertilization itself. Failure to fertilize with the most accurate answers to these questions can negatively impact the quality and development of grass plants and potentially harm the environment. Improper application techniques and excessive application doses, in particular, can cause fertilizer to leach into surface water, soil, and groundwater resources (Beard, 1972; Acikgoz, 1994; Avcioglu, 2014).

Grass plants have an ideal root structure for nutrient uptake. Nutrients are divided into two categories: "macro" nutrients, which plants require in large quantities, and "micro" nutrients, which plants require in smaller amounts. Nitrogen, phosphorus, and potassium are the essential macronutrients required by grass plants in large quantities. Among micronutrients, iron is the most frequently added element to turf fertilization programs. However, for turfgrass growing on athletic fields containing high amounts of sand, such as golf courses (especially on the greens) or football fields, and in soils with high pH levels (excessively alkaline), it may be advisable to add other micronutrients such as copper, zinc, manganese, and macronutrients such as magnesium to their fertilization programs (Brohi and Aydeniz, 1991; Browman, 2003; Barton and Colmer, 2006; Espinoza et. al. 2007; Kacar and Katkat, 2023).

The starting point for developing a suitable fertilization program for turfgrass is a soil analysis. This analysis should be performed during the preparation phase before establishing the turfgrass and repeated at least every three years thereafter. Analysis results provide important information, particularly regarding adjusting soil pH, determining the need for phosphorus, potassium, and other nutrients, and determining application rates. For optimal growth and development, the pH level of turfgrass should be maintained between 5.5 and 7, phosphorus between 21 and 40 ppm, and potassium between 100 and 200 ppm. Fertilization should be applied if phosphorus and potassium levels fall below these limits. The most commonly used macronutrient and micronutrient fertilizers for turfgrass are listed below (Brohi and Aydeniz, 1991; Reicher and Throssell, 2000; Mangiafico and Guillard, 2006; Trenholm and Unruh, 2009; Landschoot, 2016; Zengin, 2022; Kacar and Katkat, 2023).

2.1. Macro elements used in turfgrasses

2.1.1. Nitrogen (N) and Nitrogen Fertilizers

Because nitrogen primarily develops the green parts of plants, Anatolian farmers call it “branch fertilizer”. Because nitrogen is a crucial element in turfgrass, affecting shoot and root growth, shoot density and color, and resistance to diseases, pests, and other stress conditions, regular supplementation of the soil's nitrogen content is essential. Because it is the most needed nutrient and a key factor in growth and development, turfgrass fertilization programs are built around nitrogen fertilizers. Overuse, as well as deficiency, can have negative consequences. Excessive or untimely nitrogen applications lead to a grass cover with weak and shallow roots. Grasses with weak root systems are generally less tolerant to various stress conditions. Therefore, the amount used and the timing of application should be carefully adjusted. Typical symptoms of nitrogen deficiency include stunted shoot growth, decreased tillering, and a light yellowish green color in the turfgrass (Brohi and Aydeniz, 1991; Mangiafico and Guillard, 2006; Trenholm and Unruh, 2009; Landschoot, 2016; Zengin, 2022; Kacar and Katkat, 2023). There are two basic types of nitrogen fertilizer. Water-soluble (fast-release nitrogen) and water-insoluble (slow-release nitrogen, meaning it's taken up slowly by the plant) nitrogen fertilizers (Brohi and Aydeniz, 1991; Kacar and Katkat, 2023). Water-soluble nitrogen fertilizers provide nitrogen that can be quickly absorbed by the plant, and it's possible to see a response in grass within a few days. Ammonium sulfate (21% N), ammonium nitrate (33% N), and urea (46% N) are examples of water-soluble/fast-release fertilizers commonly used in green areas. These fertilizers persist in the soil for about three to four weeks, but heavy rainfall or excessive irrigation after application can easily leach out and wash away quickly. Slow-release fertilizers, on the other hand, require much more complex processes (soil temperature, microorganism activity, etc.) for their breakdown and uptake by the plant, resulting in a slower plant response. However, they provide the plant with the elements it needs in a balanced manner and persist in the soil for a considerable period (approximately 10 weeks or more). Urea formaldehyde (38% N) and sulfur-coated urea (32% N) are examples of slow-release fertilizers commonly used in lawns. Fast-release fertilizers are much cheaper and widely used, but if applied incorrectly, such as at high doses, they are highly likely to burn turfgrass. Slow-release fertilizers, on the other hand, have low water solubility rates, so they are not washed away from the soil, and even if applied incorrectly, they are very unlikely to burn turfgrass. It is possible to find lawn fertilizers containing both slow- and fast-release nitrogen forms on the market. These combine the advantages of both fertilizers and mitigate their disadvantages, offering the potential to be highly effective lawn fertilizers. Another nitrogen source that can be considered in the water-insoluble fertilizer group is natural organic fertilizers such as milorganite (6% N). These fertilizers contain low levels of nitrogen and have a low potential to burn turfgrass, but most require a certain temperature to demonstrate their effectiveness. Many factors, including the type of turf used and its intended use, the expected turf quality, environmental conditions, soil structure, and the extent of mowing residue collection, influence the total amount of nitrogen applied to turfgrasses (Beard, 1974; Brohi and Aydeniz, 1991; Salisbury, 1992; Acikgöz, 1994; Emmons, 2000; Reynolds and Flint, 2009; Avcioglu, 2014; Kacar and Katkat, 2023).

Turfgrass Type: Turfgrass species differ in their total nitrogen requirements due to differences in nitrogen use efficiency and growth rate. For example, turfgrasses such as *Zoysia japonica* Steud., *Buchloe dactyloides* (Nutt.) Columbus, and *Festuca ovina* L. perform significantly better under relatively low nitrogen application rates than turfgrasses such as *Poa pratensis* L. and *Lolium perenne* L (Beard, 1974; Acikgoz, 1994; Avcioglu, 2014).

The expected turfgrass quality and intended use: Turfgrasses such as football, golf, and tennis, which receive high traffic and therefore require intensive maintenance such as short and frequent mowing and increased irrigation, and where dense, lush, and high-quality turf are desired, require more nitrogen than a low-maintenance, less densely textured, and lower-quality home garden. For example, in the hot conditions such as Izmir and Antalya, a football field established with hybrid *Cynodon dactylon* (L.) Pers. (Bermuda grass) on a sandy-textured surface might require a total of 75 g m^{-2} of nitrogen over the course of a year. On the other hand, a home garden established with *Zoysia japonica* in the same location might require only 5-10 g m^{-2} of nitrogen annually. Similarly, in the cool conditions such as Tokat, Sakarya, and Ankara, a low-maintenance home garden established with *Festuca arundinacae* Schreb. might require 5 g m^{-2} of nitrogen annually. Furthermore, for a football field established with a turfgrass mixture containing *Poa pratensis* and *Lolium perenne* in the same location, the nitrogen rate could be increased to 35 g m^{-2} annually. The most important point to remember is that more nitrogen application means more mowing and watering (Beard, 1974; Acikgoz, 1994; Trenholm and Unruh, 2009; Avcioglu, 2014).

Vegetation Period: The longer the growing season, when turfgrasses are actively growing, the more nitrogen they need (Beard, 1974; Acikgoz, 1994; Avcioglu, 2014; Reicher and Throssell, 2000; Mangiafico and Guillard, 2006; Landschoot, 2016).

2.1.2. Phosphorus (P) and Phosphorus Fertilizers

Phosphorus is referred to as “fertility fertilizer” by Anatolian farmers because it increases productivity and fruiting. Phosphorus, which plays a key role in the adenosine triphosphate (ATP) system, which facilitates energy transfer within plant tissues, is essential for all growth and developmental components, particularly germination, new root and shoot cells, and energy supply. It is less sensitive to phosphorus, which is responsible for the nitrogen and cells of grass flowers. Because it plays a critical role in the germination, rooting, and tillering of grass plants, it is particularly important for newly established lawns to store phosphorus-containing fertilizers before transport and planting. It is found in commercial fertilizers as phosphate (P_2O_5) (Brohi and Aydeniz, 1991; Kacar and Katkat, 2023). The amount of phosphorus applied to lawns, which is supplemented by nitrogenous fertilizers, should always be calculated based on soil tests. Turfrasses respond positively to fertilization when phosphorus levels in the soil fall below 5 ppm. However, applying phosphorus in large amounts, even without a soil test, can cause excessive accumulation of this element in the soil. Excessive phosphorus accumulation in the soil can initially inhibit the uptake of other micronutrients, especially iron, by the roots, leading to perpetuating plant deficiencies. Since phosphorus is relatively immobile in the soil, the method of application is crucial for ensuring it reaches the roots. In lawn areas experiencing phosphorus deficiency at this temperature, fertilizer application should be made after soil aeration, ensuring phosphorus root formation. Ammonium phosphate, calcium- and sulfur-containing superphosphate, and triple superphosphate are commonly used phosphorus fertilizers in lawns. If a soil analysis is performed, the annual phosphorus application can be 1/4 of the total nitrogen amount (4N:1P) (Garling and Boehm, 2001; Browman, 2003; Barton and Colmer, 2006; Espinoza et. al. 2007).

2.1.3. Potassium (K) and Potassium Fertilizers

Potassium is referred to as "honey fertilizer" by Anatolian farmers because it improves the overall quality of plants. Potassium, abundant in young grass plants, is crucial for water uptake and balancing osmotic pressure. It is the most commonly used nutrient in turfgrass after nitrogen. It is crucial for increasing turfgrass' resistance to drought, high temperatures, diseases, and trampling. Potassium also increases turfgrass' tolerance to low temperatures. Therefore, it is common practice to increase winter hardiness by applying high-potassium fertilizers to turfgrasses in the fall in cool climates. Potassium fertilizers are formulated as potash (K20) (Brohi and Aydeniz, 1991; Kacar and Katkat, 2023). The total annual potassium application rate is determined based on soil test results. If a soil analysis is not available, potassium (2N:1K) can be applied at a rate of half the total nitrogen application rate. For example, a home garden with loamy soil and a 1000 m-2 lawn can receive a total of 20 kg of nitrogen, 10 kg of potassium, and 5 kg of phosphorus annually. If the growing medium is sandy, the amount of potassium applied can be increased to equal the amount of nitrogen, with an application of 20 kg (1N:1K). Similarly, if the goal is to increase the resistance of turfgrass to drought, trampling, and cold tolerance, or if turfgrass clippings are collected from the area rather than left on the surface, the amount of potassium applied can be increased. Potassium sulfate is a commonly used potassium fertilizer in turfgrass. Potassium, although not as much as nitrogen, can also be lost through leaching from the soil. Slow-release polymer-coated potassium fertilizers are also used in turfgrass (Beard, 1974; Brohi and Aydeniz, 1991; Kacar and Katkat, 2023).

2.1.4. Magnesium (Mg)

Magnesium, a key component of the chlorophyll molecule, is another macronutrient crucial for maintaining green color and growth in turfgrass. It also plays a role in phosphorus transport within the plant, positively impacting phosphorus utilization. However, it is not required in as high amounts as nitrogen, phosphorus, or potassium. Mg deficiency results in pale green grass and slows shoot growth. Severe deficiencies can cause complete yellowing of older leaves and necrosis. Magnesium deficiency is particularly prevalent on golf and football fields with high sand content. Deficiency symptoms begin to appear when the Mg content in the plant falls below 0.4%. Therefore, incorporating magnesium into the fertilization program, in addition to nitrogen, phosphorus, and potassium, is recommended, especially in sandy soils and high-traffic fields **(**Brohi and Aydeniz, 1991; Zengin, 2022; Kacar and Katkat, 2023**).**

2.2. Micronutrients Used in Turfgrass Areas

2.2.1. Iron (Fe)

Iron is essential for chlorophyll synthesis in turfgrass and is a key component of many enzymes. Therefore, the amount of iron available to the plant directly affects turfgrass color (Beard, 1974; Brohi and Aydeniz, 1991; Acikgoz, 1994; Avcioglu, 2014). Iron is the most common micronutrient deficiency in turfgrass. Iron chlorosis, characterized by a yellowish discoloration of leaves due to iron deficiency, is a common condition, particularly in soils with alkaline characteristics (soil pH 7.0 or higher), excessive moisture, and high phosphorus content. This condition is particularly common in *Poa pratensis*, *Cynodon dactylon*, and *Zoysia japonica*, while some species, such as *Festuca arundinaceae*, are more resistant to iron chlorosis. Iron in alkaline soils converts to complex forms that are not easily soluble, preventing plant roots from absorbing it. Iron application to turfgrass results in a rapid darker green hue. Iron deficiency chlorosis, seen in turfgrass, should not be confused with nitrogen deficiency. Chlorosis caused by iron deficiency results in randomly distributed patches of yellowing on grass surfaces, while nitrogen deficiency results in uniform yellowing across the entire area. Applying nitrogen to an area with iron deficiency can exacerbate the problem. Iron sulfate fertilizer or chelated iron can be used to correct iron chlorosis. Iron sulfate (20% Fe) is more commonly used to quickly correct iron deficiency. For this purpose, 0.6-0.9 grams of iron sulfate should be dissolved in water for every 1 m^{-2} of turf area, and the mixture should be applied immediately to the leaves. The turfgrass regains its green color within 24-48 hours of application, but the effects wear off after a few weeks (Beard, 1974; Brohi and Aydeniz, 1991; Acikgoz, 1994; Avcioglu, 2014; Zengin, 2022; Kacar and Katkat, 2023).

2.2.2. Zinc (Zn)

Zinc is a micronutrient that plays a role in chlorophyll synthesis and amino acid synthesis, as well as the synthesis of the growth hormone indoleacetic acid. Zinc prevents plant stunting, increases plant height and stem thickness, accelerates tillering and stem formation in grasses, and prevents leaf yellowing and shrinkage. While plants' zinc requirements are lower than those of other nutrients, if the soil is zinc deficient, yields will be low even if all other nutrients are sufficient. (Beard, 1974; Brohi and Aydeniz, 1991; Zengin, 2022; Kacar and Katkat, 2023).

Zinc deficiency affects both plant yield and human health. Therefore, the demand for zinc as a micronutrient in agriculture is increasing. (Hopkins, 2014). Although zinc deficiency affects many crops, turfgrass are more susceptible to this deficiency than others. It is estimated that half of the land used for turfgrass cultivation suffers from zinc deficiency, posing a critical threat to human health, especially children's health, in zinc-deficient regions. Because latent zinc deficiency can cause significant yield losses, soil testing is the best method for identifying zinc deficiency. Plant tissue analysis is a complementary method to visual inspection. For example, a graminea plant experiencing zinc deficiency develops white or yellow stripes parallel to the midrib on young leaves (Brohi and Aydeniz, 1991; Kacar and Katkat, 2023).

Zinc fertilization plays a crucial role in achieving adequate yields. Zinc deficiency in plants can be addressed with the right fertilizers. Plants typically require small amounts of zinc (20-100 ppm). Applying zinc fertilizers to the soil is the best method for correcting deficiencies. Foliar application is best. Zinc must be dissolved in water for the plant to absorb it. It's important to remember that chelated micronutrients remain soluble in the soil for a longer period, allowing the plant more time to absorb them. Zinc can be taken in the form of zinc chelate or zinc sulfate to address zinc deficiencies (Brohi and Aydeniz, 1991; Kacar and Katkat, 2023).

2.2.3. Sulfur (S)

Sulfur, which has effects such as green color, shoot growth and density, root growth, carbohydrate reserves, and reduced disease susceptibility, is also found in some amino acids, proteins, membranes, and coenzymes. Sulfur deficiency causes chlorosis (Brohi and Aydeniz, 1991; Zengin, 2022; Kacar and Katkat, 2023).

Over time, leaching of acidic substances from the soil and the use of acidifying fertilizers tend to gradually lower pH. The only way to monitor changes is through periodic soil testing. Alkaline soils (pH above 7.0) are unsuitable for turfgrass cultivation due to reduced nutrient availability. Alkaline soil problems can generally be addressed by applying elemental sulfur. The amount of elemental sulfur applied to established turfgrass should not exceed 2.5 kg ha. The optimal time for such applications is spring or fall, preferably in conjunction with seed planting. Sulfur is slow to react, so soil pH should be retested after 6-12 months to measure changes. Unfortunately, the addition of sulfur may not lower pH in all soils; Some soils are highly buffered and resist attempts to lower the pH (Zengin, 2022; Kacar and Katkat, 2023).

3. 3. Interpretation of Fertilizers

Fertilizer packaging generally includes the relative values of nutrients (N-P-K) and basic information about the source used. The three numbers in a series on the fertilizer represent the percentages of nitrogen, phosphorus (available P_2O_5), and soluble potassium (K_2O), respectively. For example, a fertilizer with a 25-10-15 ratio on the packaging label means it contains 25% nitrogen, 10% phosphorus (available P_2O_5), and 15% potassium (soluble K_2O). Furthermore, if the fertilizer contains other nutrients in addition to these three essential nutrients, such as iron and magnesium, their percentages are also indicated on the packaging. In addition to compound fertilizers containing two or more macronutrients, there are also fertilizers containing only a single nutrient. For example, urea fertilizer (46-0-0) contains only 46% nitrogen. In addition to these commonly used fertilizers, lawn fertilizers formulated for lawns are also sold. Most lawn fertilizers contain high levels of nitrogen and smaller amounts of phosphorus and potassium (e.g., 20-6-12), along with important micronutrients such as magnesium and iron. Furthermore, lawn fertilizers containing less nitrogen but higher levels of phosphorus and potassium (e.g., 5-20-20) have also been developed specifically for pre-seeding applications (Brohi and Aydeniz, 1991; Kacar and Katkat, 2023).

4. FERTILIZATION TIME AMOUNT AND TECHNIQUE

The conditions for effective fertilization are: a) applying the appropriate type of fertilizer, b) applying the amount of fertilizer required by the plant, c) applying the fertilizer properly, and d) applying the fertilizer at the appropriate time.

The optimal fertilization time is between the last frost date in spring and 15 days before the first frost date in autumn. During this period, the total annual fertilizer amount can be divided into monthly, fortnightly, or weekly periods. However, in areas where any sporting activity takes place year-round, fertilization can be done year-round with appropriate planning (Acikgoz, 1994; Avcioglu, 2014).

In Türkiye, the fertilizer amount can be 15-10-10 kg da^{-1} in regions with relatively low temperatures, low irrigation, and short vegetation periods, and in soils with high water retention capacity. In regions with relatively high temperatures, high irrigation, and sandy soils with low water retention capacity, the amount can be 75-30-30 kg da^{-1} or slightly more (Salman et. al. 2011). Generally, an N-P-K fertilizer amount of 25-15-15 kg da^{-1} is ideal. Fertilizer application techniques generally involve applying fertilizer manually in small areas, manually or with spreading machines in larger areas, or, where pressurized irrigation systems are available, with irrigation water (Yilmaz, 2003; 2011; Bilgili and Acikgoz, 2007; 2011).

For turfgrass plants to benefit most from the fertilizer, it is essential that they have a healthy root system and that the fertilizer be applied at the appropriate time, considering its impact on growth and development. Fertilizer application should be strictly avoided on lawns under drought stress and in lawns where average air temperatures are 35 °C or above (Beard, 1972; Acikgoz, 1994; Avcioglu, 2014).

Generally, the most suitable time for fertilizing is when turfgrass is actively growing. Cool-season turfgrasses such as *Festuca arundinaceae*, *Festuca rubra rubra* L., *Festuca rubra commutata* L., *Lolium perenne*, and *Poa pratensis* exhibit good growth and development in spring and autumn. Therefore, cool-season turfgrass species should be fertilized during these specified periods, and preferably, most of the annual fertilizer application should be shifted to the autumn months. For example, in a home garden established with *Lolium perenne* in the such as Tokat and Ankara climates, the annual nitrogen requirement of 15 g m^{-2} can be divided into three doses: May, September, and November. Nitrogen applied during May and September under optimum temperature conditions increases the number of shoots and encourages the formation of a healthy and strong root system. A second fertilization in the first half of November, when shoot development slows but the root system is still active, helps the plant accumulate carbohydrate reserves. It is also recommended to apply potassium (5 g m^{-2}) equal to the amount of nitrogen applied in November. This will make our grass plants more resistant to winter and will green up early in the spring. Fertilizing cool-season grass species in July and August should be avoided. This is because cool-season grass species exhibit optimal growth and development between 16-24°C and become stressed in the summer when temperatures average

26°C or higher. Applying nitrogenous fertilizers to cool-season grasses during this period, especially in the form of fast-release fertilizers, increases the incidence of fungal diseases, disrupts the shoot/root development balance, reduces the plant's stress tolerance, and significantly reduces quality. If nitrogen fertilizer is required during this period, it is preferable to use a low dose (2.5 g m^{-2}) and, if possible, a slow-release nitrogen fertilizer (Yilmaz, 2003; 2011; Bilgili and Acikgoz, 2007; 2011). The most suitable fertilization times for warm-season turfgrasses are late spring and summer. *Cynodon dactylon*, *Paspalum vaginatum* Sw. (Syn.: *Seashore paspalum*), *Stenotaphrum secundatum* (Walt.) Kuntze., *Buchloe dactyloides*, and *Zoysia japonica* are warm-season turfgrass species and are suitable for use on the Aegean and Mediterranean coastlines, which have Mediterranean climates. Nitrogen application in early spring is not recommended for warm-season turfgrass species, as it will encourage weed growth. Nitrogen fertilizers applied in late fall are also not recommended, as they will reduce their winter resistance. For example, if a total annual nitrogen application of 15 g m^{-2} is to be applied to a lawn planted with *Zoysia japonica* in Izmir and Antalya, this rate can be divided into three doses, preferably in May, June, and August (Salman et. al. 2011).

Fertilizers can be applied in two different ways: solid granular or liquid foliar fertilizers. In turfgrass, solid granular fertilizers should be broadcast and distributed over the area. For a uniform application, the total amount should be divided in half, with half applied in a north-south direction and the other half in an east-west direction. This minimizes the likelihood of unfertilized or over-fertilized areas. To minimize the risk of leaf burn, irrigation should be carried out immediately after fertilization to ensure the fertilizer is washed from the grass leaves into the soil (Beard, 1972; Brohi and Aydeniz, 1991; Acikgoz, 1994; Avcioglu, 2014; Zengin, 2022; Kacar and Katkat, 2023).

5. CONCLUSION AND RECOMMENDATIONS

Turfgrass managers understand that each growing season is different from the previous one. Fertilization practices need to be adjusted according to environmental changes. Certain conditions, such as extreme cold and rain or hot and dry weather, require different fertilization practices than normal conditions. Cool and wet conditions and nitrogen leaching, favorable for the growth of cool-season grasses, may require additional fertilization compared to normal growing conditions. Hot and dry conditions, unfavorable for the growth of cool-season grasses, generally require less fertilization than normal conditions.

Furthermore, differences in soil or light-shade conditions can determine different fertility regimes. Nitrogen applications are generally more frequent for grasses growing in sandy soils than for finer-textured or high-organic soils. Nitrogen leaching is more likely in sandy soils than in these other soil types. In shady conditions, the lower end of the fertility range should be used, as the grass's nitrogen needs are lower in shade than in full sunlight.

Mowing and irrigation practices should also be considered when developing a fertility program. When turfgrass clippings are returned to the lawn, one-fourth less actual mineral nutrients can be applied than when clippings are not returned. The shredding of grass clippings can provide additional minerals to the lawn. Heavily irrigated lawns often require supplemental fertilizer due to leaching and increased turfgrass growth. Fertilization of lawn areas should be planned after making changes to other management practices to maintain turf quality.

Developing a successful lawn fertilization program requires more than simply applying a high-nitrogen fertilizer in early April. This requires both technical knowledge and turf management experience. Developing a comprehensive fertilization program should consider the grass type and growth cycles, the lawn's use, the minerals needed for growth and development, soil fertility, fertilizer characteristics, environmental and management conditions, and the application schedule. Successful lawn managers, whether they realize it or not, must consider these factors holistically when deciding how, when, and how much to fertilize.

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CHAPTER 2

A Practical Solution to High Water Table; Mole Drainage

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1. INTRODUCTION

The most fundamental inputs in the domain of plant production are soil, water and sunlight. Soil constitutes the fundamental basis upon which plants are able to attain an upright position. It is evident that soil fulfils a dual function: it provides the ground on which plants can take root, and it supplies the water and plant nutrients necessary for their growth. Soil, the medium in which plants are cultivated, comprises three distinct phases: solid, liquid and gas. The solid phase, consisting of sand, silt, clay and solid organic waste, constitutes the majority of the soil. This solid phase is instrumental in enabling the plant to anchor itself to the soil and maintain an upright position. The residual liquid and gas phases constitute the void space in the soil. The liquid phase in this context is designated the soil solution, which supplies nutrients to the plant by means of dissolution. The gas phase is the air of the soil and plays a pivotal role in the aeration of plant roots. Within this void space, which is the sum of the liquid and gas phases, the liquid (water) and gas (air) must be present in certain proportions. Disruption of this ratio in favour of water or air has been known to result in a concomitant decrease in the soil's productivity potential. Plants must be able to easily meet both their water and air needs from the soil. In the event of a disruption to the water-air balance within the voids, the plant may experience a deficiency in either water or aeration. Should the volume of water within the voids of the plant's root zone exceed the desired level, the plant roots will be unable to achieve sufficient aeration, resulting in excessive water stress. In such instances, an excess water problem arises for the root zone. The problem of excess water accumulating in the land, especially in the root zone, and not draining away on its own can only be solved with drainage systems.

Drainage can therefore be defined as the process of extracting excess water from agricultural land, irrespective of its source, with a view to creating a well-aerated root zone and sufficiently dry topsoil for agricultural activities (Güngör et al., 2011). The established drainage system has been shown to remove excess water, thereby providing numerous other benefits, including salinity control, early soil warming, easier soil cultivation, increased yield, protection of existing structures, and mosquito control.

Drainage systems can be categorised into three distinct groups: surface, subsurface and mole drainage systems. A wide range of equipment is utilised in the installation of these systems, ranging from small hand tools to large machinery. While some machines only open the channel to provide drainage, in contrast, other machines have been engineered to lay pipes underground without causing any disruption to the soil. When deciding which type of drainage system to install, the characteristics of the land are the primary determinant. Subsequently, factors such as soil type, crop characteristics, and prevailing economic conditions influence the type of drainage selected. The extent and spread of the drainage problem also affect the required drainage volume, thereby influencing the type of drainage system to be installed.

In the context of small-scale agricultural operations, the development of simple and economical solutions is a frequently observed phenomenon. However, due to a lack of awareness regarding these economic solutions, farmers frequently opt to refrain from undertaking drainage work, which results in their agricultural land being left fallow. When there is a risk of a drainage problem, the installation of a drainage system should be considered under normal circumstances. As with the importance of investing in irrigation, the necessity of installing a drainage system for land that requires drainage is equally important.

In this study, general drainage systems are discussed and detailed information is given about mole drainage, which can be practically applied to groundwater conditions that restrict or make it impossible to grow crops, especially in small agricultural enterprises.

2. DRAINAGE SYSTEMS

2.1. Surface Drainage Systems

Surface drainage systems are systems installed on land to remove excess surface water such as excessive rainfall, excess irrigation water or surface runoff from sloping lands, usually through trapezoidal channels opened to the soil surface. Water collected in the field channels, called tertiary channels, established in the soil, is conveyed to the secondary channel at the end of the field and from there to the main channel or a discharge source. As demonstrated in Figure 1, surface drainage systems are of paramount importance in this context.

Figure 1. Surface drainage systems (Anonymous 2025, Anonymous 2025a)

The most significant advantage of surface drainage systems is that initial installation costs are low, as these systems are created simply by shaping the soil. Conversely, the primary disadvantage associated with this method is the division of the land into separate parcels, which hinders the unobstructed movement of motorised machinery between plots. In this system, since the channels are made of soil, there is constant grazing and sediment accumulation problems at the channel bottom. This situation inevitably leads to high maintenance costs. Since the side surfaces of the channels have a very low slope, the channels cause extensive land loss. Due to the aforementioned disadvantages, surface drainage systems are not typically favoured within agricultural fields; rather, they are more commonly utilised in secondary channels that possess collecting properties. However, it is common practice to install open surface drainage systems known as belt channels at the point where the sloping area transitions to flat land. The purpose of this installation is to protect the flat areas at the bottom of sloping and surface flow-prone lands from surface runoff.

2.2. Subsurface Drainage Systems

In instances where high groundwater levels do not recede naturally, the necessity arises to lower the groundwater to the desired depth below the root zone, thereby enabling the root zone to undergo the process of aeration. The most common method employed for this purpose is subsurface drainage. Subsurface drainage is achieved through the implementation of perforated drainage pipes, which are installed with a specific gradient, immediately below the desired depth at which the groundwater level is to be lowered. These pipes, buried underground, function by collecting excess water from the soil through their perforations and subsequently draining it away along the gradient. The water conveyed outside the field is discharged either into a secondary channel or another receiving environment like a river or a lake. Consequently, rising groundwater is maintained at the desired level underground. As demonstrated in Figure 2, a subsurface drainage system is employed (Anonymous 2025b).

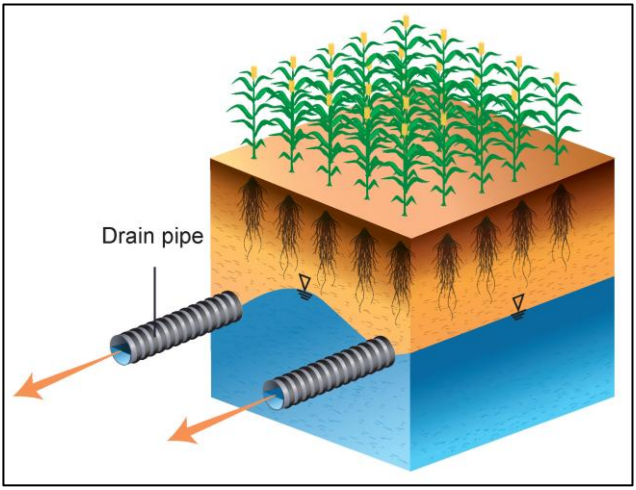

Figure 2. Subsurface drainage system

Subsurface drainage systems are the most prevalent type of system utilised in fields. Despite the initial high costs, this method is the most favoured due to numerous advantages, including the avoidance of land division and the low maintenance costs. Nevertheless, the substantial initial financial outlay required to participate in the scheme is likely to be a key factor deterring farmers with limited financial means from opting for the system. The installation of subsurface drainage systems in extensive areas is typically undertaken using machinery that is not owned by the farmers themselves. Some of these machines are only trench opening machines (trenchers). The drainage pipe is installed in an upward direction, commencing from the secondary channel that functions as the outlet of the trench. In instances where the pipe is covered with its own filter material, the installation of a drainage filter may not be necessary. However, the implementation of a gravel filter around the drainage pipe, which is to be installed in the trench, will ensure the system's longevity. The trench is then filled in, thus completing the system's installation. The trenchless machine is capable of burying the drainage pipe directly at the required depth, thus obviating the need to dig a trench in the soil. These machines utilise filter-covered pipes. The installation of drainage systems is a process that requires a significant investment of time and resources. However, the utilisation of trenchless is resulted in enhanced efficiency, thereby enabling the installation of drainage systems over larger areas to be completed in a shorter timeframe. As demonstrated, the implementation of subsurface drainage systems necessitates the utilisation of pipes, the procurement of costly machinery, and the potential requirement for filter material. Consequently, the initial investment cost for subsurface drainage increases, which may act as a deterrent for farmers considering this system.

2.3. Mole Drainage Systems

Another method employed to address high watertable levels is the implementation of mole drainage systems. The utilisation of mole drainage systems is associated with a number of advantages and disadvantages when contrasted with subsurface drainage systems. The selection of the most appropriate drainage system is determined subsequent to a thorough evaluation of the associated advantages and disadvantages.

It is evident that a significant proportion of farmers lack the requisite knowledge about drainage. In the contemporary era, a significant proportion of farmers are unable to cultivate half of their land due to issues related to drainage, while others continue to cultivate their land despite high watertable levels, thereby accepting a loss in yield. In light of the substantial financial burden imposed by agricultural inputs, it becomes evident that the production of low yields constitutes a considerable risk. For farmers with inadequate economic resources, the undertaking of investments in irrigation and drainage, and the installation of drainage systems on their land, has become unfeasible for a considerable number of farmers in the present day. Consequently, farmers either acquiesce to low yields or opt to leave the problematic parts of their land fallow. Therefore, mole drainage emerges as a pragmatic and cost-effective solution for farmers lacking the financial resources to install a conventional drainage system.

2.3.1. What is mole drainage?

The system created by the specialised equipment known as mole ploughs, which farmers attach to the rear of their tractors and manoeuvre through the soil to create artificial pipes that facilitate the drainage of water from the soil, is referred to as mole drainage. The implementation of mole drains by farmers does not necessitate the use of additional materials such as pipes or filter materials, nor does it require the execution of costly operations such as trench digging. This characteristic renders mole drainage a highly economical drainage method.

The mole equipment is mounted onto the tractor using a three-point hitch system (Figure 3). The method commences with the identification of the location at which the drainage water will be discharged. The discharge area under discussion is typically the secondary channel, located at the lower edge of the field. The installation of mole drains starts from the secondary channel where the water will be discharged and moves from bottom to top, almost parallel to the slope of the land. As soon as the mole tube is created, at least 50 cm long PVC pipe should be installed at the outlet. This configuration enables the water from the mole drain to be directed into the channel without compromising the integrity of the secondary channel slope. Moreover, the installation of an animal barrier at the extremity of the PVC pipe is recommended in order to prevent the ingress of animals into the mole drain.

The installation of mole drains is typically straightforward on slopes ranging from 0.1% to 0.6%. In the presence of high-velocity flow conditions at steeper slopes, the stability of the mole drain may be compromised. The optimal slope for effective drainage is 0.2%.

During the installation of the mole drainage system, it is imperative that the mole plough is pulled at a speed of 2-4 km/h to ensure optimal pipe formation. At higher velocities, the puller's expander causes the pipe walls to crack due to the exertion of tensile forces. This situation can result in a reduction of the service life of the mole drain, for instance, to a period of one or two years (Anonymous 2025c).

Figure 3. Mole plough and its attachment to the tractor (Anonymous 2025d)

The mole plough is lowered into the secondary channel and the mole blades are stuck into the channel edge, 40-60 cm below the field surface. The mole drain is installed from the end to the beginning, with the mole plough remaining in the soil. The installed drain comprises a cylindrical pipe that has been compacted into the soil. The implement used for tilling the soil, known as the ploughshare, creates a crack in the topsoil. However, these cracks also facilitate drainage, as illustrated in Figure 4. Mole drains can be installed starting from 80 meters in low-sloping lands and up to 200 meters in high-sloping lands. (Güngör et al., 2011).

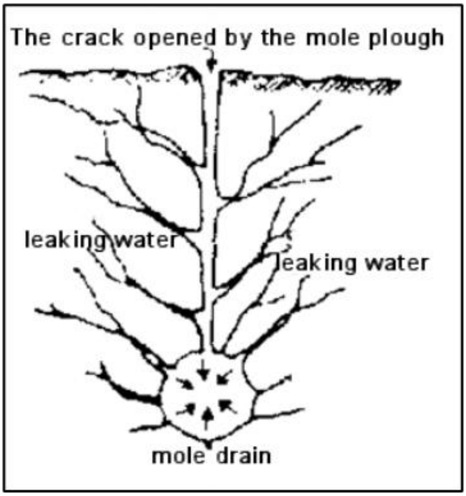

Figure 4. Cross-section of an installed mole drain and flow through cracks

A drain line then established from the secondary channel to the upper edge of the field, and the blades are extracted at the highest point of the field. Thus, the installation of the drains is completed. In the instance of a double-bladed mouldboard plough being utilised, two drain lines are installed in a single pass. It is important to note that the distance between two drains (typically 2.4 m) is generally maintained, and that the creation of new drains commences from the same distance in the subsequent pass. This ensures that the entire field parcel is covered with evenly spaced mole drains.

2.3.2. Advantages and disadvantages of mole drains

The utilisation of mole drains is associated with a number of advantages and disadvantages when contrasted with alternative drainage systems. The advantages of mole drains can be enumerated as follows.

1. The installation of mole drains can be executed at a remarkably minimal cost.
2. Farmers have the capacity to install mole drains autonomously, obviating the necessity for external service provision.
3. The subterranean location of mole drains ensures that they do not impact traffic on the land.
4. The reinstallation of mole drains on the same land does not necessitate any additional work.
5. It has been demonstrated that the performance of these drainage systems is superior when used in heavy soils in comparison to other drainage systems.

Nevertheless, the system is not without its drawbacks.

1. It is evident that the capacity of mole drains to be opened only superficially results in their provision of only partial drainage of the soil surface. Consequently, their suitability is confined to areas in which the cultivation of plants with shallow roots, such as pastures, is prevalent.
2. The system is not long-lasting due to the fact that no pipe-like material is placed underground; rather, the soil is shaped to form small pipes. Nevertheless, these can be readily re-established in the event of any impairment.
3. It is imperative that these systems, which are characterised by their shallowness and low yield, are opened at regular and frequent intervals.
4. It is important to note that these units are not compatible with light-textured soils. It is imperative that the soil has a high clay content (more than 35%).
5. The presence of aggregate structure is not evident in soils with elevated sodium content. The soil exhibits a single-particle structure. Consequently, the cohesion between soil particles is weak. This makes it difficult to install mole drains. The collapse of the small pipes formed under the soil may be early. Consequently, the implementation of mole drainage is not recommended for sodic soils.

In the context of installing a drainage system on land, the most challenging aspect for farmers is the financial aspect of the initial investment. In the context of suboptimal economic conditions, agricultural producers frequently eschew investment in drainage infrastructure. However, the installation of mole drains can be a highly cost-effective solution in areas where alternative methods are not feasible. For a farmer who possesses a tractor, the financial outlay is confined to the rental expense of the mole plough and the tractor's fuel requirements. The cost of renting a mole plough is negligible, given that large areas can be drained in a short time. In such a scenario, the financial outlay intrinsic to the system installation will be exclusively that of fuel expenses. In the context of favourable soil conditions, mole drains have been demonstrated longevity of up to 10 years. In the event of deterioration and damage to mole drains, the ease and cost-effectiveness of reinstallation renders them suitable for repeated implementation within the same field. Indeed, in circumstances where a mole drainage system has been compromised by damage to only a proportion of the drains, it is possible to install a new mole drainage system in close proximity to the existing one without the necessity of making any modifications to the existing drains.

3. CONCLUSION

Drainage problems are a potential concern in agricultural areas, with the potential causative factors including excessive rainfall, surface runoff or high watertable levels. Drainage problems, especially in the bottom lands consisting of flat areas at lower elevations, are an important factor limiting production in these areas. Farmers encountering such drainage issues are first considered the installation of subsurface or surface drainage systems as a potential solution. However, due to the high costs involved, they either abandon the idea of installing a drainage system or leave the problematic land out of agricultural use. Farmers lacking awareness of mole drainage systems do not contemplate them as a viable option. However, the majority of drainage issues can be readily addressed by the implementation of mole drainage systems.

Mole drainage is a pragmatic and cost-effective approach that has the capacity to resolve the majority of drainage-related issues. The method has been found to be particularly successful in heavy soils and in conditions where shallow-rooted plants are cultivated, and it is often considered the primary method.

It is important to note that, in certain cases, mole drainage may not be an effective solution to drainage problems of a serious nature. Nevertheless, it is a method that will support the solution of all kinds of drainage problems. The implementation of mole drainage systems has been demonstrated to facilitate drainage in areas where both subsurface and surface drainage systems are in operation.

Mole drainage systems serve a dual purpose; they are utilised not only to facilitate the removal of excess water during the growing season, but also to ensure that the land dries out in a timely manner prior to the commencement of planting activities. This facilitates earlier planting. In certain regions, the early planting of crops can create an opportunity for the cultivation of a second crop in the same season following the harvest of the first crop. This has been shown to result in increased profits from production.

In the event of contemplating the installation of alternative drainage methodologies, the implementation of mole drainage is demonstrably more practical. In cases of major drainage problems, even temporary installation of a mole drain may be considered if other drainage methods are not feasible. The installation of the mole drainage system is a relatively straightforward process, and it can be implemented expeditiously as a provisional solution.

The system's installation is so cost-effective that, even if it must be opened annually despite having a normal lifespan of 5-10 years, it will still be more economical than other methods. In order to enhance the system's efficiency, it is recommended that additional mole drains be opened in subsequent years. Drains opened later will not cause any damage to the previous system.

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CHAPTER 3

Reverse Osmosis Method in Reuse of Wastewater in Agriculture under the Impact of Climate Change

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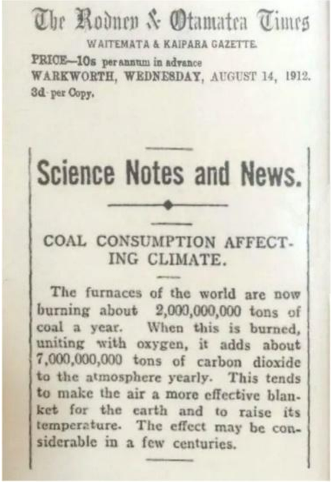
1. INTRODUCTION

With the first industrial revolution, which lasted from the 1760s to the 1830s, humanity began to meet a large portion of its energy needs by burning coal. Excessive coal consumption during this period led to the first awareness about the environment. Humankind first realized the existence of global warming in the early twentieth century. Since there were not many vehicles using petroleum-derived fuels in those years, the cause of this warming was attributed entirely to coal and its derivatives. The first warning about global warming was a 1912 New Zealand newspaper report that stated, "The furnaces of the world now burn about 2,000,000,000 tons of coal annually. This, when burned, combines with oxygen and releases into the atmosphere about 7,000,000,000 tons of carbon dioxide annually. This tends to make the air a more effective blanket for the earth and to raise its temperature. The effect may be considerable within a few centuries." (Figure 1) (Anonymous 2025).



The Rodney & Otamatea Times
WAITEMATA & KAIPARA GAZETTE
PRICE—10s per annum in advance
WARKWORTH, WEDNESDAY, AUGUST 14, 1912.
3d per Copy.

Science Notes and News.

COAL CONSUMPTION AFFECTING CLIMATE.

The furnaces of the world are now burning about 2,000,000,000 tons of coal a year. When this is burned, uniting with oxygen, it adds about 7,000,000,000 tons of carbon dioxide to the atmosphere yearly. This tends to make the air a more effective blanket for the earth and to raise its temperature. The effect may be considerable in a few centuries.

Figure 1. Newspaper clipping from 1912 about global warming (Anonymous 2025)

The main source of warming the Earth is the Sun. However, the Earth is warmed by the light of the Sun, not by its heat. Photons coming from the sun are first captured in the stratosphere, that is, the ozone layer, as they pass through the atmosphere. Photons passing through the atmosphere reach the earth's surface and are absorbed by objects and radiated into the environment as heat. Some gases in the atmosphere, like terrestrial objects, absorb these rays and convert them into heat. This results in a warming of the atmosphere.

Today, there are still scientists who claim that there is no global warming and that the temperature increase observed is oscillations within normal limits.

However, as a result of significant and clear statistical evidence, the number of people who think this way is decreasing day by day.

The most important of these gases, called greenhouse gases, which capture sunlight in the atmosphere and convert it into heat, are carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), ozone (O_3) and chlorofluorocarbon (CFC) gases. The amount of light energy coming to the world does not actually change. If these gases did not absorb light, the light would reach the earth anyway. In this case, it might seem counterintuitive that the Earth is warming. However, the function of gases in warming is to absorb light before it reaches the earth and warm the atmosphere. Because the light reaching the earth is absorbed by plants and used in photosynthesis, and no excessive heat is released into the environment. In other words, if the photons reaching the Earth are used in a process such as photosynthesis or are converted into another form of energy as in photovoltaic sources, then no heat is emitted into the environment. However, greenhouse gases only absorb light and radiate it into the environment as heat. Therefore, greenhouse gases make a significant contribution to the warming of the atmosphere. Greenhouse gases also trap infrared rays reflected from the earth into space, preventing them from escaping into space, thus creating a complete greenhouse effect on earth.

Most of the gases that cause warming in the atmosphere are carbon-derived gases. As is known, carbon is actually coal. It is black in color and is extremely effective in absorbing sunlight. For this reason, the disruption and increase in the carbon balance in the atmosphere directly triggers atmospheric warming.

The amount of greenhouse gases has increased by 40-50% since the industrial revolution (1750) (Anonymous 2025a). Due to this increase, the temperature of our planet has risen almost 1.5°C above what it should be. IPCC (The Intergovernmental Panel on Climate Change), an organization affiliated with the United Nations, is working to keep the Earth's temperature at +1.5 °C by 2030. If the current trend continues, it is estimated that the Earth's temperature will reach +4.5 °C in 2100.

2. CLIMATE CHANGE AND ITS EFFECTS

As a result of global warming, some changes have begun to be seen in climate, especially since the second half of the 20th century (Figure 2).

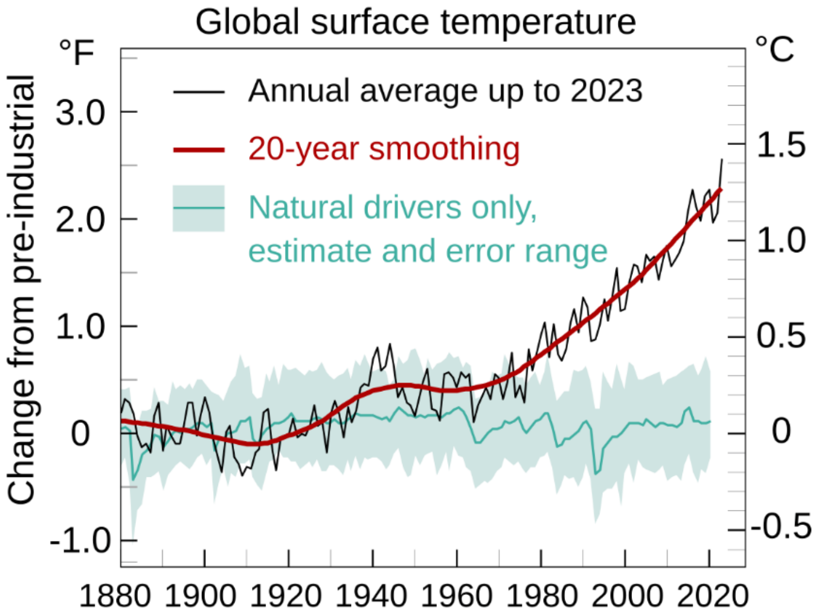


Figure 2. Changes in global surface temperatures (Anonymous 2025b)

Global surface temperatures have increased significantly over the last 50 years, increasing by +1.5°C compared to the natural value. This warming has numerous negative impacts on human life. These significant impacts can be examined under several headings.

1. The polar regions are the ones most affected by these negativities. Because the glaciers in the polar regions are melting rapidly and retreating by up to 250 meters per year. Polar bears, in particular, are facing the loss of their natural habitats, and hunting has become almost impossible for them to feed. Sea water levels are expected to rise due to melting glaciers.
2. Another effect is forest fires that cover very large areas and are very difficult to extinguish. Global warming is one of the primary causes of these fires. However, these forests are humanity's most important source of support against global warming. Massive forest fires not only mean that forests will be destroyed, but also that global warming will accelerate.
3. Another important effect is the deterioration of the distribution of water resources. This situation causes a decrease in water resources, a decrease in volume, and a significant decrease in flow regimes. Some streams and lakes are shrinking and drying up. This situation is especially important for regions that use these water resources for irrigation. Especially in latitudes like ours, where agriculture is dependent on irrigation, the decrease in water resources presents significant problems on a national scale. As a result of the restriction of irrigated agriculture due to water shortage, the supply of food products will also be

significantly affected. For these reasons, significant human migration is expected in societies that cannot be fed.

4. Ocean and sea waters have been gradually warming. This situation creates significant effects, ranging from the displacement of marine habitats to the extinction of some species.

Many countries and organizations around the world are trying to take measures to stop the increase in global warming so that the negative effects described above do not grow. Various agreements around the world are trying to prevent carbon emissions, increase forest cover, and prevent sources that release gases such as carbon and methane into the atmosphere.

3. DROUGHT PRECAUTIONS

We observe the biggest effects of global warming in our country as the decrease in water resources used for agricultural irrigation, imbalance in rainfall and floods. In this study, we will not discuss the precautions that can be taken against climate change, but instead, we will explain the precautions that can be taken against the drought effect that affects our country the most.

The most important effect of climate change observed in Türkiye is the decrease in reserves of water resources and unexpected floods. Türkiye is a country suffering from water scarcity and is getting closer to being a water-poor country day by day. The decrease in water resources has a deteriorating effect on social life and environmental factors. High temperatures, irregular rainfall and depletion of water resources will soon lead to desertification. Due to the decrease in water reserves, irrigation water supply poses a significant problem today. This problem causes serious conflicts between irrigation water providers and farmers in many agricultural areas. Some farmers open illegal wells and waste groundwater recklessly to meet the water shortage in agriculture. While some farmers are choosing to grow crops that consume as little water as possible, others are abandoning agriculture. Finding irrigation water in agriculture has become one of the most important problems of farmers. For this reason, regardless of its quality, it is seen that water of such low quality that it is not suitable for use in agriculture is used in agriculture.

Low quality water not only affects the plant and reduces production, but also causes significant damage to the soil that is almost impossible to reverse. Unfortunately, many farmers only realise this when they use poor quality water and begin to lose their crops and soil.

Measures to eliminate the effects of global warming are very long-term. It is currently not even possible to stop the increasing warming. Therefore, the most important measures to be taken for Türkiye should be;

1. In agriculture, practices that cause water waste for any reason should be prevented and serious sanctions should be imposed on farmers who waste water.
2. In order to use existing water resources optimally, it is urgently necessary to make plans that will maximize yields with the available water. In this context, the production of products with low water use efficiency should be restricted and the proportion of plants that consume relatively little water in the plant pattern should be increased. Farmers who have given up on production should be encouraged to return to agriculture and, if necessary, produce with deficit irrigation. It should not be forgotten that the decrease in production potential would create a famine environment in the country.
3. The use of groundwater should be kept under control and the importance of protecting groundwater reserves should be emphasized. In regions where production can be done with dry farming, dry farming should be recommended instead of fallow so that total production potential is not lost.
4. Alternative water resources should be found for agriculture. Necessary studies should be carried out to purify water that is not of suitable quality for irrigation in agriculture and make it usable in agriculture.

Although 74% of the total water used in our country is used for irrigation purposes, only 15% is allocated for domestic use. Despite this, it is very strange that efforts are made to save water in domestic use and that the same effort is not made to encourage water saving in agriculture. However, a 10% saving on water in the agricultural sector corresponds to a 50% saving in domestic use. In this case, the first target sector for water saving should of course be agriculture. An inventory of all wasted water resources should be made and those that can be used after treatment should be treated and reused.

4. REUSE OF WASTEWATER

Pollutants entering an ecosystem through wastewater can be classified as degradable, non-degradable (conservative), or bioaccumulative (persistent) (Arceivala, 1998). The three main methods used in the treatment of polluted water are physical, chemical and biological treatment processes. Physical treatment is based on the principle of filtering the retained substances in the polluted water by holding them in filters with various mesh sizes, from decimeter to nanometer, without using any support additives such as chemical or biological active substances. Chemical treatment is a treatment method in which chemical additives are used to transform the undesirable state in water into the desired state. It is also sometimes used to assist physical treatment or to speed up a process. Biological treatment is the method in which bioactive materials are used to eliminate contaminants in water. In fact, a biological treatment process is constantly taking place in nature. In this way, nature can clean itself. In biological treatment plants, the treatment process in nature is simulated and accelerated.

Some countries in the Middle East and the Arabian Peninsula, which have extremely limited water resources, are forced to purify wastewater and even purify seawater and use it for various purposes. For water-poor countries, water treatment and reuse is a necessary solution. Over the years, Turkey has come very close to the level of water-poor countries. Therefore, it is now mandatory to treat and reuse wastewater. Today, treatment processes are carried out in many sectors in the country. However, most of these treatments are carried out to improve the quality of water that already has a certain quality, according to the requirements of the sector. For example, city water is purified and used as drinking water. The purpose of this purification is to improve the quality of water that is already drinkable. In some industrial branches, the need for pure water is met by purifying potable water. However, today, the need for water has increased tremendously in many sectors, regardless of its quality. For this reason, it has become necessary to purify even the water that is too dirty to be used for any purpose and use it for some purposes.

When it comes to reusing treated water, agricultural or recreational irrigation are the first areas that come to mind. This is because the area with the highest water demand is irrigation water. In addition, it is possible to utilize treated water for different purposes such as use in industrial areas, vehicle washing, environmental cleaning, use in toilet flushing and feeding underground water resources.

5. REVERSE OSMOSIS METHOD

Until recently, water needs in areas experiencing water shortage were met by transferring water from areas where water was abundant. However, over the years, developments in treatment technologies and the decrease in treatment costs, coupled with the increase in transportation energy costs, have made water treatment more economical than water transfer. For this reason, the use of water treatment systems in many sectors and domestic uses is increasing day by day.

Although obtaining pure water is the first thing that comes to mind when water purification is mentioned, purification actually means removing unwanted substances from water. Therefore, making the unsuitable parameters in the water suitable for the sector in which the water will be used and making it available for use is an adequate procedure for purification. In fact, when you say dirty water, it means water that is not suitable for the purpose it is used for. In other words, the criterion that constitutes pollution in water actually depends on the purpose of use. A water source, may be considered dirty for washing your face, while the same water may be clean for washing a car. Water treatment is all processes that improves the quality of dirty water to make it appropriate for a specific purpose of use. The operations in these processes can sometimes be surprisingly easy. For example, a floodwater that is turbid and contains a high amount of sediment may be perceived as very dirty in appearance. However, it can be made usable for hundreds of purposes only through the filtration process. Therefore, for the efficient and sustainable use of water resources, regardless of the pollution level of water, it is possible to make suitable for some purposes after treatment. In fact, "**there is no dirty water that should not be treated; there is dirty water that is not economical to treat**".

The universe contains complex balances that cannot be explained by the laws of physics alone. If a large amount of salt is poured into just one corner of a freshwater pool, after waiting for a sufficient period of time, the entire pool will be filled with water of the same salinity. In other words, the salts are spread throughout the entire pool volume to create a balance in the water. If the same thing were done by dividing the pool into two parts with a semi-permeable membrane, the salts will not be able to pass to the other side. However, in order to establish the salinity balance, this time the fresh water will pass to dilute the salty side and thus different water loads will occur depending on the salinity difference on both sides of the pool. This phenomenon is called osmosis (Figure 3). Applying pressure to the saltwater section, allowing only water to pass through to the other side, reverses the osmosis process. That's why this phenomenon is called reverse osmosis (Figure 3).

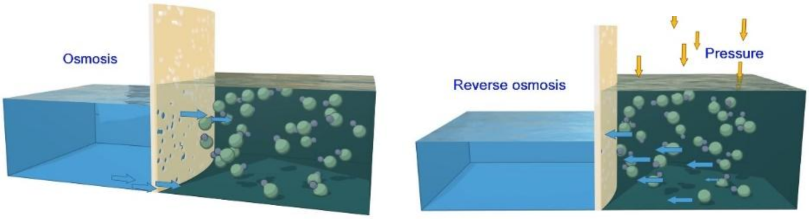


Figure 3. Occurrence of osmosis and reverse osmosis events (Anonymous 2025c)

Reverse osmosis is the process of forcing dirty water to pass through a special membrane under high pressure, thus retaining unwanted substances in the water in the membrane and allowing substances of a permitted size to pass to the other side. Since unwanted minerals, organic materials, pathogens such as viruses, bacteria and other undesirable substances in the water will be retained in the membrane, it is possible to provide water of the desired quality. Since reverse osmosis is a filtration method, it is an application form of physical treatment.

Since the reverse osmosis method does not use raw materials, it is much more economical than other purification technologies. The level of energy used is quite low. These systems, which can provide uninterrupted treatment, have a modular structure and are highly expandable, and can be installed in sizes ranging from very small to very large (Figure 4). It is a widely used method for producing water of the desired quality in many sectors such as pure water production, drinking water production, operational water production in factories, domestic water production in hotels, and pool water production. Since pure water can be produced using the reverse osmosis method, it can provide 100% purification from contaminants.

(Anonymous 2025d)

(Anonymous 2025e)

(Anonymous 2025f)

Figure 4. Reverse osmosis systems of different sizes

a. Use of Reverse Osmosis Method in Agriculture

Until a quarter of a century ago, when a well opened for irrigation turned out to be too salty to be used in agriculture, it would a complete disappointment and the well had to be closed despite all the expense and effort. At that time, treatment of salt water did not make sense for economic reasons. However, with developing technology, treatment costs have now decreased to reasonable levels. Today, due to the scarcity of water resources, people are looking for new water sources and are forced to purify and use water that is not suitable for agriculture. Therefore, reverse osmosis method is a method that can provide a way out for water scarcity, especially in agricultural areas. Two different sizes of reverse osmosis systems produced to provide irrigation water in agricultural areas are shown in Figure 5.

(Anonymous 2025g)

(Anonymous 2025h)

Figure 5. Reverse osmosis systems used in agricultural areas

Drainage water returning from irrigation in agricultural areas contains high levels of salt, fertilizer and pesticide residues. Since the removal of this water requires a drainage system, costly operations are required. However, it is possible to purify this water, which is considered waste, by reverse osmosis and reuse it in irrigation. Thus, both water source will be provided and wastewater disposal will be realized.

The biggest handicap of the reverse osmosis method is that during the treatment process, at least the same amount of water is released as waste. Although it may seem like there is a significant waste of water here, this loss is not an issue for agricultural areas. Because the more concentrated water released as waste after treatment is given back to the aquifer or water storage structure or taken back to the treatment unit, thus allowing reuse. This situation will of course cause deterioration in the quality of groundwater or water in the water storage structure over long periods. However, this effect will be seen after many years. The reverse osmosis method is completely harmless to the environment. It does not leave a polluting effect on nature, only takes clean water from dirty water and does not contribute to the total pollution load.

The positive effects of the reverse osmosis method on soil and plants can be listed as follows (Anonymous 2025i);

1. Water purified by reverse osmosis prevents salt accumulation in the soil, improving plant development and water uptake.
2. Since the reverse osmosis method does not use chemicals and heavy metals, the soil is protected and more natural and higher quality products are obtained.
3. Water purified by reverse osmosis allows plants to absorb water and nutrients more effectively, thus ensuring healthier plant development.
4. Water free from pollutants and pathogens increases the ability of plants to fight diseases and pests.

6.CONCLUSION

As can be seen, reverse osmosis method is one of the most important solutions for regions that do not have sufficient clean irrigation water but have sufficient water of a quality that is not suitable for agricultural irrigation. Compared to other methods of providing irrigation water, reverse osmosis is the only method that stands out in terms of both economy and applicability. Some precautions can also be suggested for special situations that may be encountered in the application of reverse osmosis method to obtain irrigation water. For example, if the irrigation water to be purified by reverse osmosis contains a large amount of sediment, then pre-filtering it will be beneficial for the healthy operation of the system. Since the reverse osmosis method is used to obtain water by passing it through very small openings, the oxygen balance of the water may be disrupted. Therefore, if drip irrigation system is used for irrigation, aeration of the water or increasing the oxygen content by adding hydrogen peroxide will be very beneficial. If sprinkler irrigation method is used for irrigation, there is no need to aerate the water. Water will already be aerated during the application of this method. In pressurized irrigation systems, there are problems such as calcification or clogging with sediment. When the reverse osmosis method is applied, the fact that irrigation systems will remain as clean as the day they were first installed and their service life will be greatly extended constitutes an important benefit of the method.

In order to facilitate the application of the reverse osmosis method, establishing two pools, one for dirty water and one for clean water, and collecting the incoming dirty water in the dirty pool and the purified clean water in the clean water pool, will provide great convenience in terms of management. Pool floors must be impermeable or membrane-covered.

Reverse osmosis is an economical treatment method that provides sufficient success in using water that is not suitable for direct use in irrigation in agricultural areas. Although the initial investment costs of reverse osmosis systems are somewhat high, they are systems that will recoup their initial investment costs in a short time due to advantages such as extending the life of the irrigation system, facilitating operation, and providing high efficiency. Reverse osmosis systems are the most important treatment method for irrigated agriculture in agricultural areas where water resources such as groundwater and drainage water are not sufficient for irrigation in terms of quality but are sufficient in quantity.

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CHAPTER 4

Biological, Environmental and Management Parameters Affecting Reproductive Efficiency in Medicinal Leech Aquaculture

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INTRODUCTION

Medicinal leeches have been an important component of traditional medicine throughout human history (Whitaker et al., 2004). Today, thanks to their bioactive secretions, they have become an indispensable “bio-therapeutic” agent in modern medicine, particularly in plastic and reconstructive surgery (Baskova et al., 2004; Sig et al., 2017). Hundreds of bioactive compounds such as hirudin, calin, and hyaluronidase found in the salivary glands of leeches provide anticoagulant, analgesic, and anti-inflammatory effects (Hildebrandt and Lemke, 2011; Bilden et al., 2025). However, the recognition of this therapeutic value has dramatically increased global demand (Elliott & Kutschera, 2011; Sawyer, 2013), and natural populations have come under serious threat due to conversion of wetlands to agriculture and overharvesting pressure (Saglam, 2018). Economically valuable species such as *Hirudo verbana* and *Hirudo medicinalis* have been placed under protection within the framework of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Williams et al., 2020; Liuzzo et al., 2025). Quotas on wild collection are insufficient to meet clinical demand, leeches are classified as “medical devices” (according to FDA and European Union medical device directives), and strict sterility requirements have made controlled farming mandatory (Rados, 2004; Abdualkader et al., 2013; Malek et al., 2019; Manav et al., 2019; Alaama et al., 2024).

This study comprehensively examines the effects of critical environmental, biological, and management parameters on reproductive physiology, factors that are essential for optimizing leech aquaculture under laboratory and farm conditions, in light of the current literature on medicinal leeches.

EFFECTS of ENVIRONMENTAL FACTORS on REPRODUCTION

Light

Light is one of the most fundamental environmental signals that regulates the biological clock, hormonal balance, and reproductive behaviors of leeches. A recent study conducted on *Hirudo verbana* revealed that manipulating the circadian rhythm (the natural day–night cycle) may create a stress adaptation that enhances reproductive efficiency. Compared to the natural daylight cycle (14 hours light/10 hours dark), leeches kept under 24 hours of continuous light or 24 hours of continuous darkness were found to produce a higher number of cocoons and offspring. It was determined that in broodstock leeches kept especially in the continuous-light group, the gestation period extended up to the 11th week and lasted an average of 6.75 weeks. In contrast, this period ended in the 8th week in the darkness and daylight groups, remaining between 5.33 and 5.41 weeks on average. Continuous light manipulation increased the number of cocoons per leech (50 cocoons in the continuous-light group, 45 cocoons in the continuous-dark group, and 40 cocoons in the natural daylight group) and raised the total offspring yield from 538 to 723. These data indicate that leeches respond to continuous light stress by increasing reproductive effort and that this method may be used to enhance productivity in breeding operations (Ceylan et al., 2024).

Temperature and Thermal Shock

Since leeches are ectothermic (cold-blooded) organisms, their metabolic rate, growth performance, and reproductive success depend directly on the ambient temperature. In a controlled experimental study (Ceylan et al., 2025a), it was determined that the optimum growth temperature for *Hirudo verbana* culture is 18.7 °C, and the highest proportion of healthy individuals is obtained around 19.7 °C. An increase in temperature to 25 and 30 °C leads to physiological impairments in leeches due to thermal stress. High temperatures increase regurgitation (vomiting of ingested blood), morphological deformities, and mortality rates; in leeches kept at 30 °C, growth nearly ceased, and the proportion of unhealthy individuals reached 26.67%.

In the aforementioned study, it was also reported that sudden temperature changes (thermal shock) during water exchanges have a decisive impact on leech health. Interestingly, leeches were found to tolerate cold thermal shock much better than heat shock. Water changes performed with water that was 15 °C colder than the current temperature (3.7 °C) triggered the immune system in leeches, reduced the deformity rate to 19.05%, and resulted in the highest proportion of healthy individuals (76.19%).

In the reproductive cycle, decreases in temperature serve as a critical signal that terminates reproductive activity; while this threshold value is 21.5 °C for *H. verbana*, the endemic species *Hirudo sulukii* can maintain reproductive activity even at much lower temperatures, such as 11.9 °C (Ceylan et al., 2021).

Cocoon Incubation Angle

The position of cocoons during the incubation (hatching) period determines hatching success by affecting gas exchange and embryonic development. When cocoons are incubated in a horizontal (0-degree) position, the decay rate is as high as 17.5%, whereas in the vertical (90-degree) position, this rate decreases to as low as 2.5%. The average number of offspring obtained from cocoons placed vertically or at an angle (45 degrees) (13.15 and 13.38, respectively) is numerically higher, although not statistically significant, compared to the horizontal position (12.13). The main reason for this difference is that the vertical position prevents the closure of the air pores formed by the opening of the opercula, which are located at the ends of the cocoon, and optimizes air circulation inside the cocoon. According to modeling, an angle of 78.73 degrees is recommended for optimum efficiency, and 60.63 degrees for maximum offspring number (Ceylan et al., 2023).

Environmental Material and Hygiene

In natural conditions, leeches deposit their cocoons into moist soil or moss. When peat, hydrogel, and shredded sponge were compared as cocoon-deposition materials under laboratory conditions, it was determined that the sponge environment is unsuitable because it causes irritation, high ammonia accumulation, and mortality in leeches. Although no statistical difference was found between peat and hydrogel in terms of cocoon and offspring yield (Peat: 3.13 cocoons/leech, Hydrogel: 2.80 cocoons/leech), the transparent structure of hydrogel provides operational advantages. It was evaluated that hydrogel may reduce pathogen risk and offer a more hygienic alternative, as it allows easy detection of cocoons and dead leeches and can be washed and sterilized (Uğural and Serezli, 2020).

Timing of Transfer to the Cocoon-Laying Environment

“Timing” is a critical factor in the reproductive physiology of leeches. Delays in transferring pregnant leeches from the water environment to the moist peat/soil environment after copulation were found to have fatal consequences. In *H. verbana* individuals that became pregnant but were not immediately placed in the reproductive environment and were kept in water; the mortality rate reached 15.6%, while in *H. medicinalis* it reached 14.3%. More importantly, it was determined that most of the cocoons deposited by leeches that were kept in water and transferred late were defective or contained dead offspring. This situation is explained by “necrotic intoxication,” which occurs because of reproductive material not being expelled from the leech’s body in time (Aminov et al., 2021).

EFFECTS of BIOLOGICAL FACTORS on REPRODUCTION

Age of Broodstock Leeches

The age of broodstock leeches shows an inversely proportional relationship with reproductive efficiency, and younger broodstock are more suitable for production. In a study conducted on *Hirudo verbana* (Ceylan, 2020), it was determined that 1-year-old broodstock exhibited higher reproductive performance compared to 2- and 3-year-old individuals. While 1-year-old broodstock produced an average of 4.30 cocoons/leech, this number decreased to 2.80 in 3-year-old broodstock. Reproductive quality also deteriorates with age; while the deformation rate in cocoons is 0% in young individuals, it rises to

28.57% in older ones, and the offspring mortality rate increases from 10% to 40%. These data indicate that reproductive physiology weakens in older broodstock.

Body Weight of Broodstock Leeches

There is a strong positive correlation between the body weight of leeches and their reproductive capacity. Heavier leeches produce a greater number of cocoons, and the cocoons they produce are also larger. This relationship between body weight of leeches and both cocoon number and cocoon size highlights the importance of selecting heavier individuals in production planning. Furthermore, although leeches lose up to 70% of their initial body weight during the reproductive process, they can still maintain reproductive activities, which demonstrates that the species' reproductive instinct outweighs its metabolic cost and that they exhibit a strong reproductive effort (Ceylan et al., 2015).

Physical Anomalies (Body Constrictions)

Physical deformities observed in the bodies of leeches are an important biological factor that directly affects reproductive ability. It has been determined that these anomalies almost completely eliminate the reproductive capacity of leeches. The cocoon-laying rates of leeches with such constrictions are significantly lower than those of healthy individuals (4.8 cocoons/leech in healthy *H. verbana*, compared to 0.45 cocoons/leech in individuals with constrictions). In addition, a large proportion of these leeches die by regurgitating the blood they have ingested before they can lay cocoons. It has been reported that this anomaly results from an immunological conflict between the blood ingested by the leech and its own tissues and is observed in approximately 5% of the population (Aminov et al., 2020).

Inter-species Differences and Life-history Strategies

Species belonging to the genus *Hirudo* have developed different reproductive strategies. *H. verbana* exhibits characteristics of a typical “r-strategist,” with high fecundity, small offspring size, and rapid growth potential. This strategy facilitates adaptation to variable and unstable environmental conditions. In contrast, *H. medicinalis* produces fewer cocoons and fewer offspring, but its offspring are larger in size and have higher survival rates; this indicates characteristics of a “K-strategist” (adapted to stable environmental conditions) (Petrauskienė et al., 2011).

The species *Hirudo sulukii*, which is endemic to Türkiye, reaches sexual maturity earlier and produces a higher number of cocoons compared to *H. verbana*. However, the deformation rate in *H. sulukii* cocoons (31.6%) is considerably higher than that in *H. verbana* (4.9%), and it exhibits higher mortality (13%) during the growth period (Ceylan et al., 2021).

EFFECTS of AQUACULTURE MANAGEMENT and FEEDING

Stocking Density

The density of broodstock leeches is one of the most important managerial parameters limiting reproductive success, due to competition for resources per unit area, waste accumulation, and stress factors. At low stocking densities (1-2 leeches per liter), the number of cocoons per leech is around 3.83, whereas when the density is increased to 16 leeches per liter, this number drops to 0.44. Overcrowded environments not only reduce numerical yield but also increase the deformation rate in cocoons up to 78%, trigger embryo mortality, and lead to maternal deaths by increasing stress-induced cannibalism. The recommended stocking density for optimal reproductive performance and leech welfare is 2 leeches per liter (Ceylan et al., 2019).

Feeding Source and Content

The source and biochemical composition of the blood used to feed leeches directly affect their growth rate and physiological health. In comparative studies, the highest growth rates were recorded in leeches fed with heparinized bovine blood (3.83% Specific Growth Rate - SGR) and natural goat blood (3.61% SGR). In contrast, sheep blood (1.87% SGR) and feeding with live frogs produced the lowest growth performance. The poor performance of sheep blood has been associated with its high cholesterol content (118 mg/dL), as high cholesterol is thought to negatively affect the digestive system microbiota and cause metabolic stress (Ceylan et al., 2025b).

When external glucose supplementation to the blood was examined, it was found that glucose positively affected growth up to a certain level (2500 mg/dL), but glucose levels above 750 mg/dL sharply reduced survival and pregnancy in leeches. This indicates that high glucose creates a toxic effect by causing osmotic stress or metabolic disorders. In addition, a strong negative correlation was found between high basophil levels in the blood and regurgitation cases in leeches. It is estimated that histamine and heparin-like substances secreted by basophils prevent coagulation of the blood inside the leech’s digestive system and increase digestive tolerance (Karataş et al., 2022; Ceylan et al., 2025b).

Host Health

The health status of the host from which the blood is obtained for feeding is of vital importance. In leeches fed with blood taken from sick animals or humans (varicose veins, inflammation, etc.), mortality rates increase 3-4 times compared to those fed with blood from healthy hosts. It is thought that the high leukocyte and immune cell levels in the blood of sick hosts collapse the immune defense in the leech's digestive system and lead to the leech's death. This situation also suggests that leech death after feeding could serve as an early diagnostic tool (express diagnostics) for physiological disorders in the host (Aminov, 2023).

Reproduction and Resting

Resting and feeding leeches before they enter successive reproductive cycles is of vital importance for sustainable production. In leeches that were put back into production after the first reproduction without being rested and fed, mortality increased to 4–6%, cocoon yield decreased, and high rates of deformation and mortality (10–15%) were observed in the offspring. In contrast, in groups rested for at least 2 months after reproduction and re-fed, mortality remained at 1–1.6%, and physiological condition improved rapidly. These findings show that reproduction imposes a significant metabolic burden on leeches and that a rehabilitation period is therefore essential (Aminov et al., 2022).

PATHOLOGICAL AND BEHAVIORAL PROBLEMS

Deformations

Morphological deformities observed in leeches that are kept under inappropriate environmental conditions or after feeding impede their medical use and reduce reproductive efficiency. In deformed cocoons, the incomplete formation of the outer spongy layer disrupts moisture regulation, leading to a dramatic decline in hatchling emergence. While the hatching failure rate in normal cocoons is 11%, this rate increases up to 70.8% in deformed cocoons (Ceylan et al., 2019). It is also thought that overfeeding causes micro-cracks on the intestinal surface of leeches, leading to tissue deterioration and triggering permanent morphological deformities (Ülger and Ayhan, 2025).

Cannibalism and vomiting

Cannibalism in leeches is a behavioral disorder that typically emerges under conditions of stress, inadequate feeding, or high temperatures. It has been observed that starved leeches attack their less mobile and satiated conspecifics in an attempt to suck blood. Vomiting (regurgitation), on the other hand, is an indicator of severe stress, toxicity, or digestive problems. High temperatures (25-30 °C) and unsuitable blood sources (blood with high ash or moisture content) increase the incidence of regurgitation. It has been reported that the vomited blood initially stimulates nearby leeches but quickly loses its attractiveness, and that this event degrades water quality, predisposing colonies to secondary infections and mass mortalities (Ceylan and Erbatur, 2012; Aminov, 2019; Ceylan et al., 2025b).

CONCLUSION AND RECOMMENDATIONS

In light of current literature and recent findings, the critical success factors and recommendations for sustainable, economical, and high-yield medicinal leech farming are summarized below:

Broodstock Leech Selection

Broodstock leeches should consist of young, 1-year-old individuals that have not yet deteriorated and possess the highest possible body weight. Individuals with segmental constrictions on their bodies should be immediately removed from the production line due to their low reproductive capacity and high mortality risk.

Low stocking density

To minimize stress factors, cocoon deformities, and cannibalism, stocking density should be limited to a maximum of 2 leeches per liter. High density directly results in yield loss and breeder mortality.

Sensitive environmental optimization

Temperature: Water temperature must be precisely maintained within the optimal range of 18.7-19.7 °C for growth and health. Sudden temperature increases during water changes should be avoided; if necessary, cold-shock applications that support the immune system may be used. ***Light:*** Continuous light regimes (24 h) that manipulate circadian rhythm should be used as a strategic tool to extend gestation duration and increase cocoon and hatchling yields.

Incubation angle

To reduce cocoon losses from 17.5% to 2.5% and maximize gas exchange, cocoons should be incubated vertically or at a steep angle in moist peat, rather than horizontally.

Critical timing

As soon as gestation is detected in breeders, they must be immediately transferred from the aquatic environment to moist peat/soil. Delays in this transfer lead to necrotic intoxication and mass mortality.

Feeding regime

The use of heparinized bovine blood or natural goat blood is important for optimal growth and health parameters. Due to its high cholesterol content, sheep blood should be avoided, as should human blood due to pathogen risk and induced aggressiveness. If supplementation is required, glucose may be added to raise blood glucose levels to 750 mg/dL; exceeding this threshold may trigger mortality. Knowing the baseline glucose level of the blood beforehand is therefore beneficial.

Broodstock leech rehabilitation

Broodstock leeches must be rested and fed before being reintroduced into production; this is not optional but mandatory. When broodstock leeches are used without adequate recovery, mortality increases and offspring quality declines.

DECLARATION

During the preparation of this study, the author used Google Gemini Pro for translation and language refinement purposes. After using this tool, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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CHAPTER 5

The Botanical Properties of the Common Street and Park Trees in Kastamonu, Türkiye

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Introduction

Türkiye, located in the Northern Hemisphere between 36°–42° N latitudes and 26°–45° E longitudes, lies within the temperate climate zone. Türkiye, a predominantly mountainous country in Southwest Asia, spans approximately 783,000 km², with 97% of its territory in Asia and 3% in Europe. Its terrain ranges from sea level to 5,137 meters above sea level at Mount Ararat, with an average elevation of 1,130 meters (Ekim and Güner, 2000).

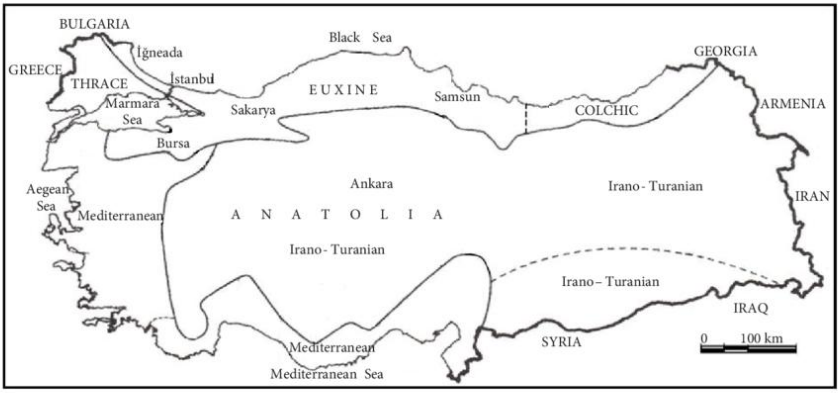

Figure 1. The phytogeographical regions of Türkiye (Davis, 1971; Kavgaci et al., 2016).

Significant climatic variations exist across different regions of Türkiye, particularly between Northern Anatolia, Southern Anatolia, the Western Mediterranean and Aegean coasts, and the inland region of Central Anatolia. These regional climate differences have exerted a strong influence on the distribution, composition, and diversity of the country's flora (Gür et al., 2024). Flora represents a key element of the landscape, significantly shaping both the visual and functional aspects of green spaces. Vegetation not only enhances landscape aesthetics but also provides vital ecosystem services, including productivity and environmental regulation. Trees and shrubs profoundly affect the surrounding environment and the living conditions of other organisms. As long-lived organisms, woody plants generally withstand short-term ecological fluctuations, although their ability to adapt depends on species-specific traits and environmental conditions. Compared to surrounding regions, Türkiye exhibits exceptionally high plant diversity. This richness is primarily attributed to the variety of habitats, diverse climatic conditions, differences in soil and geomorphological features, and, more broadly, the heterogeneity of its ecological characteristics. In addition to its geographic position, Turkey's location at the intersection of three distinct phytogeographical regions further contributes significantly to its floristic diversity (Avcı, 2014). The geographic location of Türkiye, overlapping three major global biodiversity hotspots (Mediterranean, Caucasus, and Irano-Anatolian), contributes to its exceptional plant diversity and the prevalence of endemic species (Davis, 1971) (Figure 1). In the Western Black

Sea region, average annual precipitation varies between 1,050 and 1,370 mm, rising to over 2,000 mm in the Eastern areas. The highest rainfall typically occurs during autumn and winter. Along the Western coast, the presence of a pronounced summer drought can limit the establishment and regeneration of Euxine forests, which are predominantly found in the inland and eastern parts of the region. Conversely, a coastal strip in the east supports vegetation more typical of Mediterranean ecosystems. Türkiye's vascular flora, encompassing both flowering plants and ferns, totals around 12,000 - 13,000 species, with an exceptionally high endemism rate of approximately 34% (Güner at al., 2012). This considerable proportion of endemic species underscores Türkiye's position as a global biodiversity hotspot, resulting from its unique geographic location at the intersection of Europe, Asia, and the Middle East, combined with its diverse topography and climatic conditions that support a wide variety of ecological niches (Özhatay et al., 2013).

Türkiye, hosting an extraordinary diversity of both herbaceous and woody plant species, ranks among the world's leading countries in terms of botanical richness. This remarkable floristic diversity has drawn significant scientific interest and has been the subject of numerous research investigations. Türkiye spans three major floristic regions—the Euro-Siberian, Mediterranean, and Irano-Turanian zones (Davis 1965–1968). The coexistence of these distinct biogeographical units underscores the country's exceptional botanical diversity and the high ecological heterogeneity of its flora.

Table 1. Distribution of Vascular Plant Species in Türkiye Across Floristic Regions

Floristic Region	**Approx. Number of Vascular Plant Species**	**Percentage of Endemic Species**	**Notable Characteristics / Dominant Vegetation**
Euro-Siberian	2,500 – 3,000	15–20%	Dense, humid forests; beech, fir, oak species; mesic habitats
Mediterranean	4,000 – 4,500	25–30%	Sclerophyllous shrubs and forests; olive, pine, maquis vegetation; drought-adapted species
Irano-Turanian	3,500 – 4,000	35–40%	Steppe and semi-arid vegetation; endemic herbs and shrubs; high continentality
Circumboreal / Transcaucasian Overlaps	500 – 1,000	10–15%	Alpine and subalpine flora; cold-tolerant species; mountain meadows and screes
Total (all regions)	~12,000 - 13,000	~34%	Türkiye as a whole is a major biodiversity hotspot with high endemism

In biodiversity, although forests play a key role in nature, urban afforestation is progressively viewed as a crucial approach for supporting biodiversity and strengthening ecosystem services that help alleviate the effects of climate change in urban areas (Resemini et al., 2025). Nature-based Solutions are strategies that protect, restore, and sustainably manage ecosystems to address social, economic, and environmental challenges while enhancing human well-being, ecosystem services, resilience, and biodiversity (UNEA, 2025). Urban green spaces, including parks and gardens, play a critical role in enhancing environmental quality and the well-being of city residents. Urban afforestation, the systematic planting of trees and shrubs in these areas, provides numerous ecological, social, and climatic benefits that are essential for sustainable urban development. In this study, the species of woody plants commonly used in the parks and along the streets of Kastamon were listed. The aim was to identify the composition, diversity, and prevalence of urban tree species in different types of green spaces, providing insights into current urban afforestation practices. Understanding the selection and distribution of these species is crucial for enhancing urban biodiversity, improving ecological services, and informing future urban planning and greening strategies in the region.

Materials and Methods

The study was carried out in the central area of Kastamonu, Türkiye. Kastamonu lies within Türkiye's Western Black Sea Region, extending between 41°21'–42°01' N latitudes and 32°17'–34°06' E longitudes. Bordered by the Black Sea to the north, the province encompasses a wide range of landforms, including expansive forested areas, pronounced valleys, and elevated plateaus. Covering roughly 13,064 km^2, of which nearly 67% is forested, Kastamonu ranks among the most heavily wooded provinces in Türkiye. The region is characterized by diverse landscapes, including mountain ranges, forested valleys, deep canyons, and fertile plains. Kastamonu has one of Türkiye's highest forest cover percentages, with over 65% of the province covered by forests dominated by beech, fir, pine, and oak species. The dense forest structure and varied topography contribute to its designation as one of the key biodiversity areas of the Black Sea hinterland. Kastamonu is located within the Euro-Siberian phytogeographical region.

Figure 2. Kastamonu is located in the Western Black Sea Region of Türkiye.

In this study, all trees growing along major streets and within public parks in the central districts of Kastamonu were systematically surveyed. Each individual tree was photographed in the field to document its general morphology, growth form, and condition. For accurate species identification, diagnostic plant materials—including fresh needles, leaves, cones, fruits, buds, and other descriptive vegetative and reproductive structures—were collected when available. These samples were examined using standard dendrological identification keys and regional floras. Species were recorded together with their locations, and ambiguous taxa were cross-checked with herbarium references and expert consultation to ensure taxonomic accuracy.

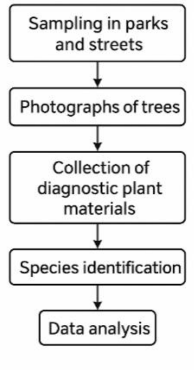

Figure 3. Field survey and data collection protocols for woody plant inventories in Kastamonu’s central parks and streets

Results

Assessing Regional Climate Change in Kastamonu: Evidence from the Last 30 Years

In this section annual mean values of temperature and precipitation data of Kastamonu over the last 30 years were given. Long-term climate data were obtained from the Turkish State Meteorological Service. Kastamonu region is characterized by a continental climate, marked by cold winters and relatively wet summers. According to data from the Turkish State Meteorological Service, the mean annual air temperature is 9.9 °C, with monthly averages ranging from −4.5 °C in January—the coldest month—to 28.2 °C in August, the warmest period of the year. Annual precipitation totals approximately 485.1 mm, exhibiting a distinct seasonal pattern. The highest monthly precipitation occurs in May (75.4 mm), while the lowest is recorded in February (27.1 mm). These climatic conditions play a significant role in shaping the ecological performance, distribution, and management requirements of woody plant species in the region.

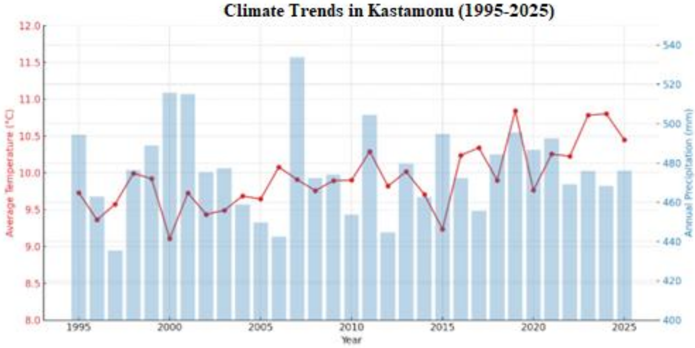

Figure 4. The average annual temperature and precipitation for Kastamonu Centre (1995–2025). Red line showing the average annual temperature (°C) and a gradual warming trend. Blue indicating the annual precipitation (mm) and variability but no clear long-term trend.

Over the past three decades, Kastamonu has experienced noticeable shifts in both temperature and precipitation patterns. The average annual air temperature shows a gradual increase from 9.2 °C in the mid-1990s to approximately 10.5 °C in the early 2020s. Winter temperatures have risen more sharply, with January averages increasing from −5 °C to around −2 °C, indicating milder winters. Summers have also warmed, with August temperatures climbing from 27 °C to 29 °C, increasing the risk of heat stress for vegetation. Annual precipitation has shown high interannual variability, fluctuating between 420 mm and 530 mm, but with no statistically significant long-term increase or decrease. Seasonal shifts are evident: spring (March–May) precipitation has decreased slightly, while autumn (September–November) precipitation shows a slight increase, potentially affecting soil moisture regimes and agriculture.

Urban Tree Species Commonly Used in the Streets and Parks of Kastamonu City Centre

Urban forests play a critical role in supporting ecological sustainability, enhancing air quality, and providing essential recreational and social benefits. However, the establishment, health, and long-term performance of woody plants in urban environments are largely constrained by climatic conditions—particularly temperature extremes and fluctuations in water availability. Kastamonu Province, situated in northern Türkiye, occupies a transitional climatic zone influenced by both the Black Sea and continental regimes. The region experiences mean annual temperatures of approximately 9.7 °C and an average annual precipitation of about 490.5 mm. Seasonal water deficits, most notably during the summer months, present significant challenges for the survival, growth, and maintenance of trees in urban green spaces.

Figure 5. A variety of native and ornamental tree species have been planted throughout the streets and parks of Kastamonu city centre to enhance urban greenery and ecological resilience.

A diverse assemblage of tree species is planted throughout the streets and parks of Kastamonu city centre, reflecting both the region's ecological conditions and long-standing urban landscaping practices. The urban canopy includes several species of *Tilia*—*Tilia cordata*, *T. tomentosa*, and T. *platyphyllos*—which are widely valued for their dense shade and ornamental qualities. Additional broadleaf species commonly used in the city's green spaces include *Fraxinus angustifolia*, *Platanus orientalis*, *Aesculus hippocastanum*, *Acer pseudoplatanus*, *Acer negundo*, *Robinia pseudoacacia*, *Betula pendula*, as well as moisture-loving willows such as *Salix babylonica* and *S. matsudana*. Coniferous taxa are also well represented, particularly ornamental and drought-tolerant species such as *Cedrus deodora*, *Cedrus atlantica*, *Cedrus libani*, *Cupressus sempervirens*, *Pinus sylvestris*, *Pinus nigra*, *Abies nordmanniana* subsp. *equi-trojani*, *Picea pungens*, and *Picea abies*. Collectively, these species contribute to the structural diversity, aesthetic value, and ecological functioning of Kastamonu's urban green spaces.

Table 2. Botanical Comparison of Tree Species in Kastamonu City Centre

Tree species	**Growth Form / Size**	**Leaf Characteristics**	**Reproductive Features**	**Bark & Crown Traits**	**Notable Botanical Features**
Tilia cordata	Medium–large deciduous	Small, cordate, serrated	Fragrant yellow flowers; nutlets with bracts	Dense rounded crown; grey bark	High nectar production; shade tolerant
Tilia tomentosa	Large deciduous	Silvery underside, tomentose	Aromatic flowers	Broad symmetrical crown	Drought-tolerant; reflective leaves
Tilia platyphyllos	Large deciduous	Broad heart-shaped leaves	Fragrant cymes	Deeply fissured bark	Moisture-loving; pollinator-friendly
Fraxinus angustifolia	Tall deciduous	Pinnate leaves	Winged samaras	Straight bole	Fast-growing; riparian species
Platanus orientalis	Massive deciduous	Large lobed leaves	Monoecious spherical heads	Exfoliating bark	Long-lived; pruning tolerant
Aesculus hippocastanum	Large deciduous	Palmately compound	Showy panicles; spiny capsules	Broad canopy	High ornamental value
Acer pseudoplatanus	Medium–large deciduous	Palmately lobed	Paired samaras	Grey flaking bark	Pollution- and wind-tolerant

Acer negundo	Medium deciduous	Pinnate leaves	Paired samaras	Green stems	Highly adaptable; dioecious
Robinia pseudoacacia	Medium deciduous	Pinnate	White racemes	Deeply furrowed	Nitrogen-fixing; durable wood
Betula pendula	Medium deciduous	Triangular, serrated	Catkins	White peeling bark	Cold-adapted
Salix babylonica	Medium deciduous	Narrow lanceolate	Catkins	Weeping branches	Moisture-dependent; rapid growth
Salix matsudana	Medium deciduous	Narrow leaves	Catkins	Upright to arching	More drought-tolerant than S. babylonica
Cedrus deodara	Large evergreen	Bluish-green needles	Barrel-shaped cones	Nodding branch tips	Ornamental Himalayan species
Cedrus atlantica	Large evergreen	Bluish clusters	Upright cones	Pyramidal crown	Drought-tolerant
Cedrus libani	Large evergreen	Dark green needles	Upright cones	Horizontal branches	Highly drought-resistant
Cupressus sempervirens	Tall evergreen	Scale-like	Small woody cones	Columnar form	Mediterranean species
Pinus sylvestris	Tall evergreen	Blue-green paired needles	Conical cones	Orange upper bark	Cold-tolerant
Pinus nigra	Large evergreen	Long rigid needles	Ovoid cones	Dark fissured bark	Drought- and heat-tolerant
Abies nordmanniana subsp. equi-trojani	Large evergreen	Glossy dark needles	Upright cones	Symmetrical crown	Shade tolerant; native
Picea pungens	Medium evergreen	Sharp blue-green needles	Pendant cones	Conical crown	Glaucous ornamental foliage
Picea abies	Large evergreen	Quadrangular needles	Long pendant cones	Drooping branchlets	Fast-growing; cool-moist habitat

Several tree species exhibit strong ecological suitability for the semi-continental climatic conditions of Kastamonu, particularly those capable of tolerating seasonal summer drought, pronounced temperature fluctuations, and common urban stressors. *Cedrus libani, Pinus nigra, Cupressus sempervirens, Robinia pseudoacacia, and Cedrus atlantica* demonstrate high levels of drought resistance, substantial tolerance to heat and air pollution, and the capacity to grow in nutrient-poor or compacted soils. These traits align closely with Kastamonu's warm, dry summers and cool winters, positioning these species as highly promising candidates for climate-resilient urban forestry strategies. In addition, species such as *Picea pungens* and *Acer negundo* perform reliably under local environmental conditions due to their broad ecological amplitude and capacity to withstand site disturbances.

A second group of species shows moderate suitability for the climatic regime of Kastamonu. Although these species grow satisfactorily under typical conditions, they tend to exhibit increased physiological stress during prolonged summer droughts. *Tilia tomentosa, Platanus orientalis, Acer pseudoplatanus, and Pinus sylvestris* generally tolerate urban habitats but benefit from supplemental irrigation during periods of moisture deficit. Similarly, *Abies nordmanniana* subsp. *equi-trojani, Fraxinus angustifolia,* and *Cedrus deodara* require relatively stable soil moisture to maintain optimal vigor and growth. While these species can be effectively integrated into parks, boulevards, or partially shaded areas, their long-term success will depend on careful site selection, soil preparation, and irrigation management.

Most widely used species exhibit only low to moderate suitability for Kastamonu's environmental conditions. *Tilia cordata, Tilia platyphyllos, Aesculus hippocastanum,* and *Betula pendula* typically perform well in cool, moist temperate climates but become increasingly vulnerable under extended drought or high summer temperatures. Their high moisture requirements and sensitivity to water stress often result in reduced growth rates, leaf scorch, premature senescence, or shortened life expectancy in exposed urban environments. These species may still be cultivated successfully in irrigated or well-maintained park settings; however, they are less appropriate for street landscapes or sites with limited soil moisture availability.

Species characterized by high water demand and low drought tolerance exhibit the lowest suitability for Kastamonu's climatic conditions. *Salix babylonica* and *Betula pendula* require consistently moist soils and are highly susceptible to root desiccation during the dry summer months. Their reliance on abundant soil moisture and sensitivity to hydrological fluctuations often leads to early decline or mortality when planted in typical urban soils. Consequently, their use should be restricted to riparian corridors, lake margins, or irrigated parks. In the context of projected climate change, the preferential selection of drought-resilient species is essential to ensure long-term stability and ecosystem service provision.

Overall, Kastamonu's transitional climate—characterized by moderately cold winters, warm and dry summers, and an average annual precipitation of approximately 490 mm—favors species with strong drought tolerance, resilience to thermal extremes, and the capacity to endure fluctuating moisture regimes. These findings underscore the necessity of incorporating climate-adaptive criteria into urban forestry planning and species selection. Prioritizing highly suitable species such as *Cedrus libani, Pinus nigra,* and *Cupressus sempervirens* will enhance the structural resilience, ecological functionality, and longevity of urban green spaces. Conversely, species with higher water requirements should be used more selectively and supported through appropriate soil management and irrigation practices. Such evidence-based approaches will become increasingly important as climate change intensifies summer droughts and amplifies thermal stress across the region.

Table 3. Commonly Used Tree Species in Kastamonu and Their Ecological Requirements

Tree species	Commonly used Sites	Soil Requirements	Water Requirements	Climate Tolerance
Tilia cordata	Streets and parks	Deep, moist, fertile loams	Moderate–high moisture	Tolerates cold; sensitive to drought
Tilia tomentosa	Streets and parks	Well-drained, moderately fertile soils	Moderate	Very drought-tolerant; heat-resistant
Tilia platyphyllos	Streets and parks	Moist, nutrient-rich soils	High moisture demand	Prefers cool, humid climates
Fraxinus angustifolia	Parks	Alluvial, moist soils	High	Tolerates periodic flooding; moderate drought tolerance
Platanus orientalis	Streets	Deep, moist, fertile soils	High	Tolerates urban pollution; moderate drought tolerance
Aesculus hippocastanum	Streets and parks	Moist, nutrient-rich,	High	Prefers cool climates;

		well-drained soils		sensitive to drought
Cedrus deodora	Parks	Well-drained, slightly acidic soils	Low–moderate	Prefers mild climates; sensitive to severe frost
Cedrus atlantica	Parks	Rocky, well-drained soils	Low	Drought-tolerant; tolerates heat
Cedrus libani	Streets and parks	Rocky, calcareous, well-drained soils	Low–moderate	Very drought- and heat-resistant
Acer pseudoplatanus	Streets and parks	Moist, fertile soils	Moderate	Prefers cool, moist climates; shade-tolerant
Acer negundo	Paks	Wide soil tolerance; disturbed sites	Moderate	Highly adaptable; tolerates drought and pollution
Cupressus sempervirens	Streets and parks	Well-drained, calcareous soils	Low	Very drought- and heat-tolerant
Pinus sylvestris	Parks	Sandy, well-drained, acidic soils	Low	Cold-tolerant; moderate drought tolerance
Pinus nigra	Parks	Rocky, well-drained soils	Low	Highly drought-tolerant; tolerates harsh climates
Abies nordmanniana subsp. *equi-trojani*	Parks	Moist, deep, fertile soils	Moderate–high	Prefers cool, humid climates; frost-tolerant
Picea pungens	Parks	Well-drained, moist soils	Moderate	Cold-tolerant; moderately

				drought-tolerant
Picea abies	Parks	Moist, fertile, well-drained soils	High	Prefers cool, humid climates; drought-sensitive
Robinia pseudoacacia	Streets and parks	Poor, dry, disturbed soils	Low–moderate	Very drought- and pollution-tolerant
Salix babylonica	Parks	Moist, deep soils near water	High	Prefers humid climates; drought-sensitive
Salix matsudana	Parks	Moist to wet soils	High	More drought-tolerant than *S. babylonica*
Betula pendula	Parks	Sandy, moist, well-drained soils	Moderate–high	Prefers cool climates; drought-sensitive

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CHAPTER 6

Commercial Decapod and Stomatopod Crustaceans in the Turkish Seas

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Introduction

Fishery resources of the world are determined as common property and open-access. Recently, decline in fishery resources was characterized by decreasing in total cathes and in some manners, the economic fall of the fishing industry was observe (Berkes, 1986). The demersal resources on the Turkish coast have been subjected to increasing exploitation since 1960. The rapid increase in trawling capacity has resulted in the decline of the resources and operations have become less profitable. Fishing continues to be a necessity for global food security for people worldwide (Golden et al., 2016; Hicks et al., 2019). The proliferation of fishing for decapod crustaceans can be attributed to a combination of natural abundance and high market value compared to other seafood. Decapod crustaceans, including but not limited to species such as crab, lobster, and shrimp, represent a significant component of fishing activities (Boenish et al., 2022).

Decapod and Stomatopod crustaceans play a noticeable role in commercial fisheries in the Mediterranean Ecosystem and their roles in marine environment are more or less known.

A recent paper by Bakır et al. (2024) includes a total of 288 species of decapod and stomatopod crustaceans reported from the Turkish coast. Brachyuran crabs are most diverse regarding the number of species. Dendrobranchiate shrimps contribute to less extent to the number of species. Decapod crustaceans with commercial importance are evaluated under three major groups such as shrimps (Penaeidae, Pandalidae), brachyuran crabs (Portunidae, Majidae), squat lobsters, and lobstres (Nephropidae, Palinuridae). Penaeid shrimps are mostly distributed in tropical and warm temperate waters, on the contrary, pandalids are boreal species in distribution. Crab species occurr all oceans and seas of the world. All decapod crustaceans are found at the depths from tidal to abyssal, but commercial fisheries are largely limited to the depths of less than 200 m (Caddy, 1989). Decapod and Stomatopod fisheries on Turkish coast may be clarified to be focused on several species such as pink shrimp, *Parapaneus longirostris* and Norway lobster, *Nephrops norvegicus* or other pink shrimps, *Aristeus antennatus* and *Aristeomorpha foliacea*.

Results

1. Decapod and Stomatopod Crustaceans in Turkish Seas

Up to date, a total of 288 (279 decapod and 9 stomatopod crustaceans) species were reported from the Turkish coast (Bakır et al., 2024). Of these 288 reported species, 25 are commercially important (Table 1). New records in last two years for decapod crustaceans for from Turkish coast are shrimps, *Farfantepenaeus subtilis* (Pérez Farfante, 1967) by Gokoglu and Ozvarol (2013), *Lysmata kempi* Chace, 1997 (Froglia and Deval, 2014), *Periclimenes aegylios* Grippa and d'Udekem D'Acoz, 1996 (Duris et al., 2013), the brachyuran crabs, *Actaea* *savignii* (H. Milne Edwards, 1834) (Karhan et al., 2013), *Monodaeus guinotae* Forest, 1976 (Gönülal et al., 2014), and the gost crab, *Gebiacantha talismani* (Bouvier, 1915) (Sezgin et al., 2016).

Despite of there are no comprehensive surveys carried out to cover all the Turkish coast as regards deceapod and stomatopod crustaceans of the Turkish shores, knowledge that is considered to be sufficient was achieved in last 50 years. This information was based relevant scientific works, mainly Demir, 1952; Holthuis, 1961; Geldiay, 1969; Geldiay and Kocatas, 1969; Kocatas, 1979; Kocatas, 1983; Müller, 1986; Katagan et al., 1988; Balkıs, 1994; Kocak et al., 2001; Kocatas and Katagan, 2003; Ates, 2003; Özcan, 2007; Bakır et al., 2014.

2.1. Pandalid shrimps

Several species of pandalid shrimps occur along the continental shelf and slope of the western Mediterranean Sea. Notwithstanding, and despite their widespread occurrence in demersal fisheries research samples, very few studies have focused on aspects of their biology and ecology in the Mediterranean Sea (Carbonell and Abello, 1998).

2. Commercial Species in Turkish Waters

Table 1. Decapod and Stomatopod crustaceans with importance commercially on Turkish coast. BS: Black Sea, TSS: Türk Boğazları Sistemi, AS: Aegean Sea, MS: Mediterranean Sea.

TAXA	BS	TSS	AS	MS
DECAPODA				
Aristeidae				
Aristaeomorpha foliacea (Risso,1827)	-	-	+	+
Aristeus antennatus (Risso, 1816)	-	-	+	+
Penaeidae				
Penaeus aztecus Ives, 1891	+	-	+	+
Penaeus merguiensis De Man, 1888	-	-	-	+
Penaeus semisulcatus De Haan, 1844	-	+	+	+
Marsupenaeus japonicus (Spence Bate, 1888)	-	+	-	+
Melicertus hathor (Burkenroad, 1959)	-	-	+	+
Metapenaeus affinis (H. Milne Edwards, 1837)	-	-	+	-
Metapenaeus stebbingi Nobili, 1904	-	-	-	+
Parapenaeus longirostris (Lucas, 1846)	-	+	+	+
Trachysalambria palaestinensis (Steinitz, 1932)	-	-	+	+
Pandalidae				
Pandalina profunda Holthuis, 1946	-	+	+	-
Plesionika edwardsii (Brandt, 1851)	-	-	+	+
Plesionika heterocarpus (A. Costa, 1871)	-	+	+	+
Plesionika martia (A. Milne-Edwards, 1883)	-	+	+	+
Plesionika narval (Fabricius, 1787)	-	+	+	+
Nephropidae				
Homarus gammarus (Linnaeus, 1758)	-	+	+	-
Nephrops norvegicus (Linnaeus, 1758)	-	+	+	-
Palinuridae				

Palinurus elephas (Fabricius, 1787)	-	+	+	+
Scyllaridae				
Scyllarides latus (Latreille, 1803)	-	-	+	+
Calappidae				
Calappa granulata (Linnaeus, 1758)	-	+	+	+
Portunidae				
Callinectes sapidus Rathbun, 1896	+	+	+	+
Portunus segnis (Forskål, 1775)		+	+	+
Majidae				
Maja squinado (Herbst, 1788)	+	+	+	+
Squillidae				
Squilla mantis (Linnaeus, 1758)	-	+	+	+

2.1.1. *Pandalina profunda* Holthuis, 1949

This species occurred within a depth range of 241 to 525 m. It was found in 14 samples, most of them collected between 300 and 500 m depth (table 1). The estimated values of abundance were very low and reached maximum averages of up to 5 individuals per hour in the western Alboran Sea (Carbonell and Abello, 1998).

2.1.2. *Plesionika narval* (Fabricius, 1787)

Plesionika narval (Fabricius, 1787) is a cosmopolitan species occurring from the surface down to 910 m of depth in a large variety of habitats including muddy, sand-muddy, rocky bottoms and submarine caves (Holthuis, 1987; Thessalou-Legaki, 1989). This species is distributed in the eastern Atlantic Ocean, the Mediterranean Sea, the Red Sea and Indo-West Pacific from Madagascar to French Polynesia (Sousa et al., 2014). This species occurred within a depth range of 66 to 364 m. It was present in only 13 samples, most of them collected between 200 and 400 m depth (table 1). The percentage occurrence reached 22% within the 300-400 m depth stratum (Carbonell and Abello, 1998).

Figure 1. *Plesionika narval* (Fabricius, 1787) (Photo by Özcan, 2015)

2.1.3. *Plesionika edwardsii* (Brandt, 1851)

Other pandalid shrimp, *Plesionika edwardsii* occurs within a depth range of 269 to 522 m. The highest percentage occurrence in the western Mediterranean Sea was found between 300 and 500 m. The highest densities are encountered between 200 and 400 m depth in Spanish waters (Carbonell and Abello, 1998).

Figure 2. *Plesionika edwardsii* (Brandt, 1851) (Photo by J. Poupin) Figure 3. *Aristeus antennatus* (Risso, 1816) (Photo by C. Taylor)

2.2. *Penaeid shrimps*

Penaeid shrimps have a considerable importantance in both fiheries and aquaculture production due to their economic value. They comprise dense populations in both estuarine and littoral ecosystems (Korun et al., 2013). 8 species (*Marsupenaeus japonicus* (Bate, 1888), *Metapenaeus monoceros* (Fabricius, 1798), *Penaeus semisulcatus* de Haan, 1844, *Melicertus hathor* (Burkenroad, 1959), *Metapenaeopsis aegyptia* Galil, 1990, *Metapenaeopsis moigensis consobrina* (Nobili, 1904), *Metapenaeus stebbingi* (Nobili, 1904), and *Trachysalambria curvirostris* (Steinitz, 1932)] of alien penaeid shrimps appear on the Mediterranean Sea coast of Turkey (Özcan et al., 2006). Only seven species of penaeid shrimps (*Marsupenaeus japonicus*, *Melicertus kerathurus*, *Metapenaeus monoceros*, *M. stebbingi*, *Penaeus semisulcatus*, *Parapenaeus longirostris* and *Trachypenaeus curvirostris*) were stated to be commercially important for Turkish fisheries. All of these are commonly caught by commercial trawlers in the neritic zone, especially at the depths between 5 and 30 m on the eastern Mediterranean coast of Turkey (Kumlu et al., 1999).

2.2.1. *Aristeus antennatus* (Risso, 1816)

Red shrimp, *Aristeus antennatus* (Risso, 1816), is the most important decapod in landed weights fished by the trawlers in the western Mediterranean Sea. Its price is usually one of the highest amongst crustaceans. Norway lobster, *Nephrops norvegicus* is the second crustacean in volume of catches after red shrimp (Carbonell et al., 1999). *A. antennatus* lives specifically on the muddy bottoms of fishery grounds at the depths between 200 and 2000 m in the western Mediterranean. This species is a target species because of its commercial value

(Garcia-Rodriguez and Esteban, 1999). *A. antennatus* was observed at an average depth of 626 m (8.0 kg in hectar) between 1992 and 1997 at the Baleric Islands (the southern Spain) (Carbonell et al., 1999). This species was previously reported in Greek waters of the Ionian Sea and the Aegean Sea by Koukouras and Kattoulas (1974) and Thessalou-Legaki (1994). Red shrimp, *Aristeus antennatus* (Risso, 1816) was reported at depths of between 550 m and 670 m by means of a trawl on the Marmaris coast (the Turkish Aegean Sea) (Özcan et al., 2009).

2.2.2. *Aristaeomorpha foliacea* (Risso, 1827)

The giant red shrimp, *Aristaeomorpha foliacea* (Risso, 1827) is a demersal, deep-sea species with one of the widest geographical ranges known. *A. foliacea* is a dominant species in Mediterranean Sea megafaunal assemblages, mainly occupying the middle slope between 450 and 600 m. It is sympatric with other species of the family Aristeidae, sharing similar morphology and ecology with *Aristeus antennatus* at mid-bathyal depths of the Mediterranean, and with *Aristeus varidens* and *Aristeus antillensis* in tropical and subtropical Atlantic waters. *A. foliacea* is an cosmopolitan species that is also widely distributed in the Mediterranean and was caught in different areas of the Mediterranean Sea (Baleric Basin, the southern Sicily, the western Italy, the southern Sardinia, Ionian Sea, the southern Cyprus, the southern Turkey) at the depths between 455 and 600 m by commercial bottom trawls (Cartes et al., 2014). The comparative importance of *A. foliacea* and *Aristeus antennatus* in deep-water fisheries alters for the west and east parts of the Mediterranean. Furthermore, knowledge limited is known as regards *A. antennatus* in the Mediterranean Ecosystem (Kapiris et al., 1998). Yet, *A. foliacea* was rarely reported in the Aegean Sea (Koukouras and Kattulas, 1974; Mytilineou and Politou, 1997; Özcan et al., 2009).

Figure 4. *Aristaeomorpha foliacea* (Risso, 1827) (Photo by Tin-Yam Chan) Figure 5. *Melicertus kerathurus* (Forskål, 1775) (Photo by Sokou, 2011)

2.2.3. *Melicertus kerathurus* (Forskål, 1775)

The prawn *Melicertus kerathurus* (Forskål, 1775) appears on muddy or muddy-sand bottoms of marine and brackish waters environments. *M. kerathurus* is found at the depth ranges from 0.5 to 90 m, but mainly up to 40 m. Its geographical distribution range is limited to the eastern Atlantic coasts from the northern Angola to the southern England, and the Mediterranean. *M. kerathurus* has a considerable importance for commercial fishery in the Mediterranean Ecosystem. Due to competition with other exotic shrimps (especially penaeids) penetrated to the eastern Mediterranean by Suez Canal, it almost disappeared on Israel, Syria, and Turkey coast. Native habitat of *M. kerathurus* was extinguished mainly by other penaid shrimp, *Marsupenaeus japonicus* (Kevrekidis and Thessalou-Legaki, 2011). Speciemens of *M. kerathurus* were monthly captured between May 2004 and April 2005 from İzmir Bay (Küçükdermenci and Lök, 2012).

2.2.4. *Metapenaeus affinis* (H. Milne Edwards, 1837)

Metapenaeus affinis (H. Milne Edwards, 1837) is distributed in the Indo-West Pacific, Indonesia, China, the Philippines, New Guinea, Arabian Sea. Its adults prefer sandy-muddy and muddy areas. *M. affinis* has a great commercial importance and it is cultured in the Philippines and it is commercially valuable in several Asian countries. This species is caught by trawlers and trammel nets. *M. affinis* is for the first time reported from İzmir Bay (the eastern Aegean Sea) for Turkish Seas (Aydın et al., 2009). Due to this species has a commercial importance continuously growing, it have been commonly hunted by fishermen in regions where it is found (Dinçer and Aydın, 2014).

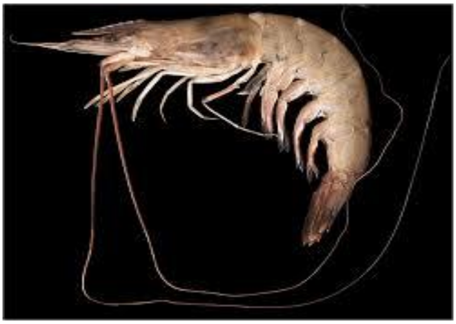

Figure 6. *Metapenaeus affinis* (H. Milne Edwards, 1837) (Photo by Aydın 2009) Figure 7. *Fenneropenaeus merguiensis* (de Man, 1888) (Photo from http://www.hk-fish.net).

2.2.5. *Fenneropenaeus merguiensis* (de Man, 1888)

Fenneropenaeus merguiensis has a wide distribution area includes the Indo-West Pacific Ocean, the Persian Gulf, the Arabian Sea, New Guinea, Australia, New Caledonia and Fiji. It prefers mostly sandy or muddy bottoms of the depths less than 55 m. *F. merguiensis* is commercially important in the Persian Gulf and Pakistan. This species is produced approximately 2 tons in the world. An adult female individual belongs to *Fenneropenaeus merguiensis* (De Man, 1888) was captured at the depths between 20 and 35m of İskenderun Bay (Turkish Mediterranean Sea) (Özcan et al., 2006).

2.2.6. *Parapenaeus longirostris* (Lucas, 1846)

The pink shrimp, *Parapenaeus longirostris* (Lucas, 1846) exists in the whole Mediterranean Sea and lives on sandy-muddy bottoms of the depths between 20 and 750 m (Kapiris, 2004*). P. longirostris* is commercially caught by bottom trawls on the Turkish coasts of Turkey. Duration allowed for catching on Turkey coast with bottom trawls is approximately 5 months within 6 miles of the coast of the Aegean Sea (Kaykac et al., 2009).

Figure 8. *Parapenaeus longirostris* (Lucas, 1846) (Photo by García Rodríguez) Figure 9. *Penaeus semisulcatus* De Haan, 1844 (Photo by Totti, 2022)

2.*2.7. Penaeus semisulcatus De Haan, 1844*

The first experimental culture of *Penaeus semisulcatus* was conducted in soil pond systems in Adana (eastern Mediterranean) in 1994. Between 1995 and 1998, it was cultivated in a semi-intensive system in Antalya (the western Mediterranean) (Türkmen, 2007). More than 85% of individuals shrimp caught belongs to *Penaeus semisulcatus* in Spring periods for the eastern Mediterranean shores of Turkey (Kumlu et al., 1999).

2.3. *Portunid crabs*

Among the brachyuran crabs, portunid crabs is one of the better documented taxonomic groups. Portunid crabs are economically valuable, and among them, two exotic species, the blue crab, *Callinectes sapidus* Rathbun, 1896, and *Portunus segnis* (Forskål, 1775), have established populations in Turkish waters. According to data from TÜİK, the total weight of *C. sapidus* captured off the Turkish coast between 2006 and 2012 was approximately 48 tons.

2.3.1. *Callinectes sapidus* (Rathbun, 1896)

Blue crab, *Callinectes sapidus* was previously reported from European Atlantic and north Sea coasts (Nehring et al. 2008), the Mediterranean (Gennaio et al., 2006), Nigeria's Atlantic coast (Ololade et al., 2008), the Pacific coast of Japan (Iwasaki 2006, Otani 2006).

C. sapidus was observed in eastuarine and marine environments of the geographic areas cited above (Williams, 1974). Blue crabs can tolerate the environmental variables with a wide range. They live in different habitats of hipersalin lagoons with the salinity from the freshwater to 117 ppt (Williams, 1984). Their tolarance to environmental temperature is too high (<3ºC to >35ºC) (Tankersely and Forward, 2007). Commercial blue crab fishing are made in the Mediterranean and many crabs captured are exported to many European and United States (Küçükgülmez et al., 2006). Exporting of oysters to European countries in 19th and 20th centuries has been a vector for penetrating of blue crab's juveniles to European waters. Major commercial blue crab's fishing is carried out on bay's coasts in the Atlantic United States and as of 2009, more than 70.000 tons of crabs have been caught (NMFS, 2009). *C. sapidus* was previously reported from the Turkish Aegean Sea (Øksnebjerg et al., 1997), the Sea of Marmara (Zaitsev & Mamaev 1997), the Black Sea (Zaitsev and Mamaev 1997, Micu and Abaza 2004).

Figure 10. *Portunus segnis* (Forskål, 1775) (Photo by Özcan, 2012) Figure 11. *Callinectes sapidus* (Rathbun, 1896) (Photo by Ateş)

3. Stomatopods

Despite the commercial fishing of several stomatopod species in the Mediterranean, their economic value is generally low due to their status as bycatch. The practice of stomatopod fishing in the Mediterranean Sea is centred on the capture of stomatopod species inhabiting shallow waters (depths exceeding 80-90 m) characterised by muddy or sandy bottoms (Ateş et al., 2015). However, significant stomatopod fishing occurs in the Mediterranean for *Squilla mantis* (Linnaeus, 1758). *S. mantis* constitutes a significant proportion of the marine species caught by trap fishing in the Adriatic (Ahyong et al., 2013).

3.1. *Squilla mantis* (Linnaeus, 1758)

Squilla mantis (L., 1758) is known to occur in high densities throughout the Mediterranean Sea, with a preference for habitats comprising fine sand and sandy-muddy seabeds. *S. mantis* is categorised as a semi-marine food source, and is commercially fished along the Mediterranean coasts of Italy, Spain, France, Egypt, and Israel (Ateş et al., 2015).

Figure 12. *Squilla mantis* (Linnaeus, 1758) (Photos by Sartore, 2025; Özcan, 2015)

4. Lobsters

Commercially significant lobster species that are suitable for human consumption are widely distributed throughout the Mediterranean Sea. These species, which are of significant importance in the marine ecosystem, include *Homarus gammarus*, *Nephrops norvegicus*, and *Palinurus elephas*. In areas of the Mediterranean where intensive fishing is prevalent, a decline has been observed in the population sizes of certain lobster species. This decline commenced prior to the 20th century, and with the advent of modern technologies, current stocks have diminished even further. Nevertheless, there was a marked increase in the catch of *P. elephas* in the Mediterranean during the 1960s, after which a decline occurred (Spanier, 2015).

4.1. *Palinurus elephas* (Fabricius, 1787)

The spiny lobster, *Palinurus elephas* is known to inhabit the western and central Mediterranean, extending to the Turkish coastline (Soldo et al., 2001). The economic importance of *P. elephas* fishing in the Mediterranean ecosystem is well-documented. Following the initial capture of *P. elephas* using bait traps, trammel nets have now become the preferred method of capture. The substitution of traps for trammel nets has resulted in an increase in the harvesting of *P. elephas* along the Mediterranean coast (Ateş et al., 2015).

Figure 13. *Palinurus elephas* (Fabricius, 1787) (Photo by T. Özcan). Figure 14. *Nephrops norvegicus* (Linnaeues, 1758) (Photo by T. Özcan).

4.2. *Nephrops norvegicus* (Linnaeus, 1758)

Nephrops norvegicus L. is considered a target species in the context of commercial fishing in the Mediterranean Sea at depths of approximately 400 m. The species is popular due to its high food quality, relatively sparse population, and high market value (Sarda, 1998). The highest biomass of *N. norvegicus* in the Saros Bay of the northeastern Aegean Sea was reported to be 433.22 (kg m-2) during the spring period (İşmen et al., 2013). According to data from TÜİK (2013), a total of 5.7 tons of *N. norwegicus* were captured in the Aegean Sea off the Turkish coast.

5. Importance of decapod crustaceans in diets of demersal fish

Decapod crustacean assemblages represent a significant fishery resource in the Mediterranean Sea. The fishing activities in question comprise trawling and deep-sea fishing (at depths greater than 150 m) conducted at depths below 50 m. Deep-water pink shrimp, *Parapenaeus longirostris*, deep-water red shrimp, *Aristenus antennatus*, and Norway lobster, *Nephrops norwegicus* are abundant in waters deeper than 150 m (Cartes et al., 2002). R. clavata is not consumed as human food. But, this species is caught as bycatch, particularly from the demersal beds of the Aegean Sea using trawl nets, and is exported to other European countries. The main food source for deep-sea fish species such as stingrays consists of decapod crustaceans (Daban et al., 2022). Previous studies conducted in the northeastern Aegean Sea have recorded that *Goneplax rhomboides*, a decapod crustacean, is predominant in the stomachs of *Raja clavata* individuals (Yığın and İşmen, 2010; Daban et al., 2022). In *R. clavata* individuals along the Anatolian coast of the Black Sea, the most abundant species (67%) is the ghost crab, *Upogenia pusilla* (Sağlam and Başçınar, 2008).

Figure 15. *Raja clavata* (Linnaeus, 1758) (Photo by Hillewaert, 2008)   Figure 16. *Goneplax rhomboides* (Photo by Paggiani, 2011)

Despite the considerable decline in the population of *R. clavata* in the Black Sea ecosystem, there has been a concomitant increase in the biomass of the portunid crab, *Liocarcinus depurator*, which is preyed upon by this species (Demirel et al., 2020).

Figure 17. *Liocarcinus depurator* (Linnaeus, 1758) (Photo by Paggiani, 2011)

Moreover, Pandalid, Penaeid, and Crangonid shrimps were identified in the stomachs of demersal fish species (*Etmopterus spinax*, *Galeus melastomus*, *Scyliorhinus canicula*, and *Squalus blainville*) inhabiting the deep waters (150-550 m) of Sığacık Bay (eastern Aegean Sea) (Bengil, 2019).

Remarks

The global rise of decapod crustacean fishing presents opportunities for poverty reduction, but achieving an optimal balance between economic and ecological integrity will be a necessary challenge. Although the fishing of decapod crustaceans is increasing rapidly worldwide, the costs and risks of these activities are not being given sufficient consideration. For over 60 years, researchers have been conducting studies to assess the global socioeconomic and ecological impacts of fishing activities. However, it is believed that reducing the impact of human activities on marine ecosystems would also benefit decapod fishing.

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CHAPTER 7

Alfalfa Pests

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Alfalfa (*Medicago sativa* L.) is recognised worldwide as the most important perennial leguminous forage crop due to its high yield potential, superior nutritional value, digestibility, and wide adaptability (Appiah et al., 2024). Because of these characteristics, it has earned the title of "queen of forage plants" in agricultural production systems (Radović et al., 2009). Alfalfa is notable not only for its contribution to animal production but also for its positive effects on ecosystem sustainability, soil fertility, the carbon cycle, and biodiversity. The recent surge in demand for animal products has significantly increased the need for alfalfa and other forage crops. Therefore, the development of high-yielding, high-quality, and environmentally stress-tolerant alfalfa varieties has become a critical issue for food and feed security. However, pests and diseases are among the most important biotic stress factors limiting yield and quality in alfalfa production. These factors not only negatively affect plant growth and biomass production but also cause toxic effects in animal nutrition and economic losses (Yang & Zhao, 2022). Therefore, the development of pest-resistant varieties is emerging as an environmentally friendly, low-cost, and sustainable control strategy (Zhang & Wang, 2025).

This section addresses the main pests of alfalfa, their biology, damage patterns, and economic importance, and evaluates integration-based control approaches (cultural, biological, and chemical methods) for each pest. The aim is to compile existing knowledge on pests and to propose sustainable solutions within the framework of integrated pest management.

1. Alfalfa stem weevil (*Hypera postica* Gyllenhal, 1813)

Alfalfa is one of the most widespread and highly nutritious forage crops worldwide. However, its production is threatened by various pests. Among these pests, the alfalfa weevil (*Hypera postica* Gyllenhal, 1813) is among the most economically important. *H. postica* causes serious leaf and shoot losses in the plant, particularly during the early growth stages, thereby reducing yield. A thorough understanding of the pest's biology and ecology is fundamental to determining the effectiveness of control strategies.

1.1. Morphological Characteristics

Adult individuals are 4-5.5 mm long and have a broad, dark-coloured stripe extending from the pronotum to the elytra on the dorsal side. The rostrum is short and thick, while the frons is approximately twice as narrow as the rostrum. The eggs are yellowish, approximately 0.5 mm long, and broadly oval.

When first hatched, larvae have a dark black head capsule and a dirty white body. In later stages of development (L2-L4), the body turns light green and white stripes become prominent on the dorsal side (Figure 1). The mature larva is approximately 8 mm long and has a white dorsal stripe on its greenish body.

Larval development comprises four stages (Pellissier et al., 2017; Koçakoğlu et al., 2024).

Figure 1. Larva of *Hypera postica* (Anonymous, 2025a)

Figure 2. Adult stage of *Hypera postica* (Anonymous, 2025b)

1.2. Biology and Life Cycle

Pest development is mainly dependent on temperature, with a development threshold of 8.9 °C (Whitworth et al., 2011). The insect overwinters in the egg and adult stages. Adults (Figure 2) are usually found in soil cracks, plant debris or the root zone (Manglitz & App, 1958). From mid-February onwards, when temperatures rise above 9 °C, adults become active; they feed on shoots and leaves, then lay 5-20 eggs on stems and shoot tips (Litsinger & Apple, 1973). A female can lay approximately 4200 eggs during her lifetime (Coles & Day, 1977).

The larvae develop in 2-4 weeks, depending on the temperature. Pupation occurs in mid-April, and the pupal stage lasts 10-14 days (Summers et al., 1981). The new generation of adults begins to appear in early May. Adults spend the summer months dormant underground or among plant debris, becoming active again in autumn to lay eggs and entering hibernation by November. *H. postica* produces one generation per year (Gözüaçık, 2022).

1.3. Damage Form and Economic Importance

Both adults and larvae cause damage, but the larvae cause the bulk of it. The first two larval stages (L1-L2) feed on the buds and shoot tips of the plant, while the subsequent stages (L3-L4) feed on leaf tissues. The larvae move downwards, destroying the shoot tips and leaf axils (Pons & Nuñez, 2020).

In dense populations, if the number of larvae exceeds 50 per square metre, only the vascular parts of the plant remain, and damage can reach up to 90% (Behdad, 1989; Degooyer et al., 1995). The period of highest damage is before the first harvest, so control during this period is of great importance (Altınok & Karakaya, 2002).

1.4. Biological Control

The alfalfa weevil has numerous natural enemies (predators).

- Larval parasitoids: *Bathyplectes curculionis* (Thomson, 1887) and *B. anurus* (Thomson, 1887) (Hymenoptera: Ichneumonidae) are the most common species. *B. curculionis* kills its host after spinning a cocoon and can suppress populations by more than 60% (Davis, 1974). Studies conducted in Turkiye have found parasitism rates of up to 22.2% in Mardin and 57.5% in Iğdır (Efil, 2018; Gözüaçık, 2019).
- Adult parasitoids: *Microctonus aethiopoides* (Figure 3) (Hymenoptera: Braconidae) (Flanders et al., 1994).
- Egg parasitoids: *Anaphes luna*, *A. fuscipennis* (Hymenoptera: Mymaridae) and *Fidiobia rugosifrons* (Hymenoptera: Platygastridae) (Ellis, 1973; Gözüaçık & Pricorp, 2017).
- Predators: *Coccinella septempunctata* (Figure 4), Chrysopidae, Nabidae and spider species (Ouayogode & Davis, 1981).
- Entomopathogenic fungi: *Beauveria bassiana* (Bals.-Criv.) Vuill. (1912), *B. pseudobassiana*, *Zoophthora phytonomi* and *Erynia* phytonomi (Yücel et al., 2015, 2018).

Figure 3. *Microctonus aethiopoides* (Anonymous, 2025c)

Figure 4. *Coccinella septempunctata* (Anonymous, 2025d)

1.5. Cultural and Chemical Control

Cultural measures should be prioritised in pest management. Early mowing is one of the most effective methods for reducing larval populations (Summers, 1998). Raking and grazing after mowing reduce the population by crushing or eating the eggs (Senst & Berberet, 1980). Pre- and post-harvest irrigation slows larval development. Burning with fire or crushing with a roller during the dormant period also damages eggs.

In chemical control, the economic damage threshold is typically set at 1.5-3 larvae per stem or 20 larvae (Evans, 1989). Ireç et al. (2021) reported that spraying when the plant height was 5–10 cm increased green forage yield by 37%.

However, uncontrolled and frequent pesticide applications destroy the pest's natural enemies, creating an imbalance in the ecosystem.

1.6. Integrated Pest Management Approach

The most effective strategy for controlling *H. postica* is an integrated approach. Monitoring the pest's biology, protecting natural enemy populations, spraying at the economic damage threshold, and implementing alternative cultural practices together provide sustainable control.

H. postica is one of the most important pests causing yield losses in alfalfa production in Turkey and worldwide. The fact that the pest's life cycle is closely linked to climate and temperature makes timing critical in control efforts. Instead of relying solely on chemical control, integrated control programmes incorporating biological, cultural, and mechanical methods ensure environmental sustainability and minimise economic losses.

2. Alfalfa Leaf Beetle (*Gonioctena fornicata* Brüggeman, 1873)

The Chrysomelidae family, known as leaf beetles, is one of the most prominent families in the Coleoptera order, with approximately 50,000 species, over 2,000 genera, and 19 subfamilies (Reid, 2005; Güven et al., 2024). Members of this family include pest species that cause significant economic losses in agricultural production worldwide. Among these species, the alfalfa leaf beetle (*Gonioctena fornicata* (Brüggemann, 1873)) causes severe damage to plants belonging to the Fabaceae family, particularly alfalfa, in Europe and Asia, including Turkey.

2.1. Morphological Characteristics

Adult individuals are oval-shaped, 5-7 mm long, and brownish-red in colour. Their elytra have dark spots (Figure 5). The eggs are oval and yellow. The larvae are blackish in colour, have a soft body structure, and usually feed on the underside of leaves (Koçakoğlu et al., 2024).

Figure 5. *Gonioctena fornicata* (Anonymous 2025e)

2.2. Biology and Life Cycle

G. fornicata usually produces one generation per year (Grigorov, 1976). The pest spends the winter in the adult stage in the soil, plant debris or root zone. It becomes active with the rise in spring temperatures, feeding on shoots and leaves to gather energy, and then lays its eggs in clusters on the leaf surface. The larvae that emerge from the eggs quickly destroy leaf tissues, leading to a decrease in the plant's photosynthetic activity and, in general, a loss of biomass.

2.3. Damage Pattern and Economic Importance

Both the larvae and adults of the pest feed on the plant's leaves, shoots, buds, and flowers. As a result of this feeding, the leaf tissue is destroyed, the plant turns grey, and stunted growth is observed. Particularly in dense populations, losses of up to 60% of vegetative mass and 100% of seed yield can occur (Grigorov, 1976).

Observations in Turkey indicate that the pest multiplies rapidly under suitable conditions and causes the most damage during the pre-first-mowing period. Therefore, pest population density remains lower in areas where early and frequent mowing is practised.

2.4. Biological Control

Within the scope of biological control, it has been reported that entomopathogenic fungi play an important role among the natural enemies of *G. fornicata* populations. *Beauveria* species, in particular, contribute to suppressing population density by causing natural infections in many leaf beetles (Coleoptera: Chrysomelidae) (Zimmermann, 2007). Furthermore, it has been reported that entomopathogenic fungi are a significant factor in suppressing the dynamics of harmful insect populations in natural ecosystems. It has been determined that they can achieve significant infection rates in natural populations, particularly within the Coleoptera order (Augustyniuk-Kram & Kram, 2012; Wakil et al., 2014).

2.5. Cultural and Chemical Control Methods

Cultural control measures such as early mowing, frequent mowing, and temporarily covering the field with water damage the pest's egg and larval stages. Hand-picking larvae and adults may be effective in small areas (Grigorov, 1976).

The pest reaches high population densities in spring and summer, causing severe damage to alfalfa leaves. Chemical methods are considered a complementary control option in pest management, particularly when population density exceeds the economic damage threshold (Baysal et al., 2019).

G. fornicata is one of the most important leaf pests threatening alfalfa production in Turkey. It causes significant yield losses, especially when it forms dense populations during spring and summer. Before resorting to chemical control, it is recommended to apply environmentally friendly methods such as early mowing, grazing, mechanical collection, and biological control. Monitoring the pest's biology and climate-related population dynamics is critical for the effectiveness of integrated pest management programmes.

3. Ligus (*Lygus* sp.)

Alfalfa is particularly important among forage crops due to its high nutritional value and perennial growth habit. However, especially during seed production, pests can significantly reduce plant yield and seed quality. Among these pests, *Lygus* species (Hemiptera: Miridae), commonly known as "ligus bugs" are among the most harmful species in alfalfa seed production (Orak & Gökkaya, 2014).

3.1. Morphological Characteristics

Adult *Lygus* species are small and oval-bodied. Their average length ranges from 3 to 6 mm. A triangular or "V" shaped pattern is usually seen on their backs (Figure 6). Their body colours can vary from light green to brown. These insects are quite mobile and can easily move between plants.

Figure 6. *Lygus* sp. (Anonymous, 2025d)

3.2. Biology and Life Cycle

Lygus species (Hemiptera: Miridae) are pests that undergo incomplete metamorphosis. Their development consists of an egg stage, a nymph stage, and an adult stage. Females lay their eggs within plant tissue, ensuring rapid embryonic development. Nymphs progress through five successive stages, while adults are 4-6 mm long and highly mobile and capable of flight. Species typically overwinter as adults and can produce multiple generations per season under favourable temperature conditions. The sensitivity of their development periods to temperature can cause the population to reach economically damaging levels within a short period (Capinera, 2001).

3.3. Damage Form and Economic Importance

Adult females usually lay their eggs on plant tissue near fresh shoots. *Lygus* bugs have a sucking mouthpart. They cause damage by sucking the plant sap and secreting toxic substances, particularly during budding and flowering periods. This results in discolouration, drying, and shedding of flowers. *Lygus* bugs damage not only flowers and buds but also immature seeds. Seeds that have been sucked on lose their germination ability, leading to significant declines in seed yield and quality (Orak & Gökkaya, 2014).

Lygus species cause significant economic losses, particularly in alfalfa fields where seed production occurs. If dense populations develop during the flowering period, seed set decreases and germination rates fall. Therefore, effective monitoring and control of this pest group is critical for obtaining high-quality seeds.

3.4. Biological Control

Among the most effective natural enemies of *Lygus* species are *Peristenus* species in the family Braconidae. These parasitoids can significantly reduce population density by achieving high parasitism rates in nymphs (Day, 1996). In the predator fauna, predators such as *Nabis* spp., *Geocoris* spp. and *Orius* spp. Feed on nymphs and adults, thereby strengthening biological control (Wheeler, 2001).

3.5. Cultural and Chemical Control Methods

Cultural measures should be prioritised for controlling *Lygus* species.

- Early tillage in seed production areas can prevent pest infestation.
- Clearing weeds from the field eliminates alternative hosts for the pest.
- Removing plant debris after harvesting reduces the population.
- Chemical control may also be applied in areas with high pest density if the population exceeds the economic damage threshold. However, careful timing is crucial, as spraying during the flowering period may adversely affect pollinator (bee) activity.

Chemical control of *Lygus* species is typically used when population density exceeds the economic damage threshold, and targeting nymphs is especially effective. Insecticide groups such as organophosphates (malathion), pyrethroids (lambda-cyhalothrin, bifenthrin) and neonicotinoids (imidacloprid, thiamethoxam) are most commonly used. The ability of some *Lygus* species to rapidly develop resistance to certain insecticide groups makes chemical control a short-term solution. Therefore, it is recommended to rotate the use of active ingredients with different modes of action, reduce host plants through field margin weed management, and time applications to coincide with peak nymph abundance.

3.6. Integrated Pest Management Approach

Integrated pest management is a broad approach that considers the pest's biology, its relationships with host plants, and its population dynamics. An effective integrated pest management programme requires integrating early population monitoring, decision-making based on economic injury thresholds, cultural controls, biological controls, and selective chemical applications when necessary. Cultural measures include managing alternative host plants (especially weeds) in the field, removing plant debris after harvest, and reducing shelter areas between plants. In biological control, supporting nymph parasites (e.g., *Peristenus* spp.) and predators (e.g., *Nabis, Geocoris,* and *Orius*) is an important part of integrated pest management (Day, 1996; Wheeler, 2001). Chemical applications should only be used when the population density exceeds the economic damage threshold, and they should primarily be used during the nymph stage. To prevent resistance from developing, it is recommended to rotate insecticides with different modes of action. This integrated approach reduces environmental impacts and ensures sustainability in the long-term management of *Lygus* populations.

Lygus species are among the most significant pests affecting alfalfa seed production by hindering flower and seed development. Sucking damage prevents seed filling and maturation, resulting in yield loss and reduced germination capacity. Close monitoring of pest biology and the adoption of integrated control methods, supported by appropriate cultivation and management practices, are of great importance for sustainable seed production.

4. Alfalfa seed weevil (*Bruchophagus roddi* Gussakovskiy, 1933)

One of the most important pests in alfalfa production, the alfalfa seed weevil (*Bruchophagus roddi* Gussakovskiy, 1933), causes severe economic losses, particularly in seed production areas.

4.1. Morphological Characteristics

Adult individuals are small, shiny black in colour and exhibit typical Hymenoptera morphology (Figure 7). Female individuals have an organ called an ovipositor, which resembles a needle and is used to lay eggs inside the seed. This structure allows females to oviposit by piercing the still-unhardened seed tissue.

Figure 7. *Bruchophagus roddi* (Anonymous, 2025e)

4.2. Biology and Life Cycle

Females lay their eggs inside the seed coat using their ovipositor before the seeds have fully hardened. The larvae that emerge from the eggs feed on the inside of the seed, consuming the internal tissue completely and leaving only an empty shell behind. Therefore, the pest completely prevents the seed from germinating. Larval development and pupation occur entirely within the seed. Depending on climatic conditions, the pest may produce one or two generations per year.

4.3. Damage Form and Economic Importance

B. roddi causes damage to alfalfa seeds in two ways:

- Complete consumption of the seed's internal tissue by larvae developing within the seed,
- Adult feeding, during which the seed coat is eaten, leaving only the shell.

As a result of these two forms of damage, the seeds lose their germination ability, and their seed quality is impaired. It has been reported that under favourable conditions, damage can reach up to 80%. The pest particularly prefers seeds that have not yet hardened and causes intense damage during this period.

4.4. Biological Control

The use of natural enemies is also considered an effective method. Various parasitoid Hymenoptera species that can be effective against *B. roddi* (Gussakovskiy, 1933) during its egg and larval stages can naturally suppress the pest population.

4.5. Cultural and Chemical Control Methods

Cultural control is one of the most basic and environmentally friendly methods for managing *Bruchophagus* species. Deep ploughing applications bury pupae in the ground, causing them to die, which significantly reduces the number of adults that will emerge the following year. Using fallow land, you can stop the host plant from finding a host plant and interrupt its life cycle. Early cutting was done to delay flowering, and it keeps the period when females lay eggs from overlapping with the period when the plant is growing well, which limits population growth. Flood irrigation physically destroys seeds near the soil surface and seed capsules containing harmful larvae or pupae. These cultural practices, when used together, are a big help in reducing the population density of harmful organisms.

Chemical control is recommended if the population density exceeds the economic damage threshold. However, in seed production, the timing of spraying must be carefully determined, taking into account the risk of chemical residues.

Alfalfa seed weevil is a pest that significantly reduces germination rates and seed yield, particularly in areas where seed production takes place. It causes damage to the plant's generative organs during both the adult and larval stages. To prevent damage, an integrated control approach should be adopted; cultural methods such as deep ploughing, fallow, irrigation, and cultivation timing should be applied in conjunction with biological and, when necessary, chemical control.

4.6. Integrated Pest Management Approach

Integrated management of *B. roddi* includes using a variety of methods, such as adjusting planting and harvesting times to match the pest's flight period, deep fall ploughing, avoiding planting seeds after harvest, and protecting natural enemies. Thoroughly cleaning seeds prevents the spread of infestation. Chemical control should not be used only when bees are active and necessary, such as during the flight period.

5. Aphids

Aphids are a group of important pests frequently encountered in production and capable of reaching the economic damage threshold. Although many aphid species cause economic damage to alfalfa, the most common and important ones are as follows: (Orak & Gökkaya, 2014; Jabbour et al., 2016)

Spotted alfalfa aphid (*Therioaphis maculata*)

Blue alfalfa aphid (*Acyrthosiphon kondoi* Shinji, 1938)

Pea aphid (*Acyrthosiphon pisum* Harris, 1776)

Alfalfa aphid (*Macrosiphum creelii*)

Spotted alfalfa aphid (*Therioaphis maculata*)

It is considered the most damaging species on alfalfa. Individuals that feed by sucking on the plant leave toxic substances with their saliva. These substances cause symptoms such as injury, yellowing and drying of young seedlings in the tissues they feed on.

Damaged plants show stunted growth, leaf spotting, or leaf shrivelling. Since they can produce many generations in a season, even low populations at the outset can quickly become a dense infestation and require control measures (Orak & Gökkaya, 2014).

Blue Alfalfa Leaf Aphid (*Acyrthosiphon kondoi* Shinji, 1938)

It typically shows high density in spring months and on the growing tips of plants. They feed on young shoots and buds, secreting a sticky, honeydew-like substance. This secretion creates conditions conducive to mould growth on the plant surface. Blue aphids leave toxic secretions on the plant tissues they contact, causing slow growth and leaf deformation.

Pea Aphid (*Acyrthosiphon pisum* Harris, 1776)

Although its primary host is the pea plant, it can also cause damage to the growing points and leaves of the alfalfa plant in particular. It is not as harmful as the spotted aphid; however, in dense populations, mould development, stunted growth and yield loss may occur (Orak & Gökkaya, 2014).

Alfalfa Leaf Aphid (*Macrosiphum creelii*)

It has a similar biology to the pea leaf aphid. Especially in dense populations, it feeds on plant leaves and shoots, reducing photosynthetic capacity and thus lowering biomass production. As population density increases, sticky substance accumulation on the plant surface and indirect fungal development also increase.

5.1. Biological Control

One of the most effective and environmentally friendly methods for controlling aphids is biological control. Many beneficial organisms naturally suppress aphid populations. These include ladybirds (Coccinellidae), mites, parasitoid wasps (Aphidiidae), and various predatory insect larvae. These natural enemies feed on aphids at all stages, preventing population densities from exceeding the economic damage threshold. Ladybirds, in particular, have a high predation capacity in both their adult and larval stages. An adult ladybird can consume an average of 50 aphids per day, which reduces colony density in a short period of time. Therefore, adopting practices that maintain biological balance in agricultural ecosystems is highly effective for natural control of aphids (Hodek et al., 2012).

5.2 Cultural and Chemical Control Methods

Another way to reduce aphid damage is through cultural practices such as using resistant alfalfa varieties and early mowing. Avoiding excessive nitrogen fertilisation also limits aphid proliferation.

Insecticide applications may be necessary in dense populations. However, these applications should be carefully implemented within the framework of integrated pest management principles, as they can have adverse effects on pollinators and natural enemies.

Aphids cause severe economic damage to alfalfa production, both directly and indirectly, by rapidly forming dense populations. The spotted alfalfa aphid (*T. maculata*) is particularly prominent as the most harmful species. To ensure adequate control, it is recommended to protect biological control agents, take cultural measures, and only use chemical applications when necessary.

5.3. Integrated Pest Management Approach

The integrated control approach requires a holistic perspective that considers the pest's biology, population dynamics, and its relationship with the ecosystem. The first step in integrated pest management strategies is making decisions based on early population monitoring and the economic damage threshold. To control aphids, cultural practices include reducing the density of host plants, controlling weeds, using balanced fertilisers, and avoiding excessive nitrogen, which encourages aphid growth. Biological control is the most important part of the integrated pest management approach. To protect these beneficial organisms, the unnecessary use of broad-spectrum insecticides should be avoided, and chemical control should only be implemented when population density exceeds economic thresholds. Choosing selective preparations and doing the work when sunburn and bee activity are low both help the environment and keep natural enemies alive. This multifaceted approach is recognised as the most effective method for managing aphids in a sustainable and environmentally friendly manner.

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CHAPTER 8

The Botanical Properties of the Common Street and Park Trees in Kastamonu, Türkiye

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Introduction

Türkiye, located in the Northern Hemisphere between 36°–42° N latitudes and 26°–45° E longitudes, lies within the temperate climate zone. Türkiye, a predominantly mountainous country in Southwest Asia, spans approximately 783,000 km², with 97% of its territory in Asia and 3% in Europe. Its terrain ranges from sea level to 5,137 meters above sea level at Mount Ararat, with an average elevation of 1,130 meters (Ekim and Güner, 2000).

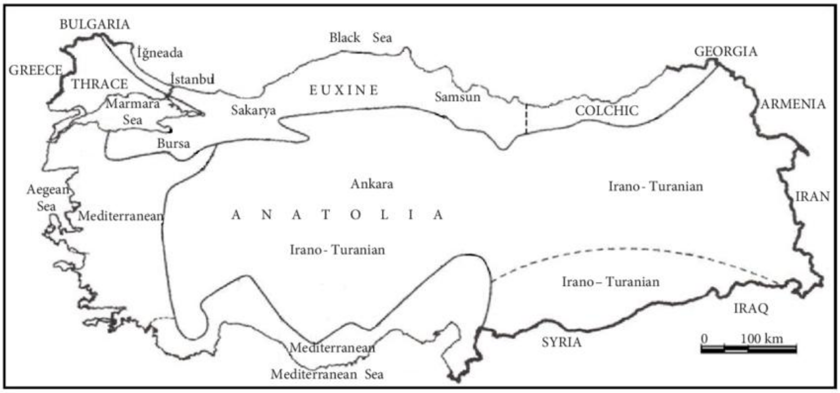

Figure 1. The phytogeographical regions of Türkiye (Davis, 1971; Kavgaci et al., 2016).

Significant climatic variations exist across different regions of Türkiye, particularly between Northern Anatolia, Southern Anatolia, the Western Mediterranean and Aegean coasts, and the inland region of Central Anatolia. These regional climate differences have exerted a strong influence on the distribution, composition, and diversity of the country's flora (Gür et al., 2024). Flora represents a key element of the landscape, significantly shaping both the visual and functional aspects of green spaces. Vegetation not only enhances landscape aesthetics but also provides vital ecosystem services, including productivity and environmental regulation. Trees and shrubs profoundly affect the surrounding environment and the living conditions of other organisms. As long-lived organisms, woody plants generally withstand short-term ecological fluctuations, although their ability to adapt depends on species-specific traits and environmental conditions. Compared to surrounding regions, Türkiye exhibits exceptionally high plant diversity. This richness is primarily attributed to the variety of habitats, diverse climatic conditions, differences in soil and geomorphological features, and, more broadly, the heterogeneity of its ecological characteristics. In addition to its geographic position, Turkey's location at the intersection of three distinct phytogeographical regions further contributes significantly to its floristic diversity (Avcı, 2014). The geographic location of Türkiye, overlapping three major global biodiversity hotspots (Mediterranean, Caucasus, and Irano-Anatolian), contributes to its exceptional plant diversity and the prevalence of endemic species (Davis, 1971) (Figure 1). In the Western Black

Sea region, average annual precipitation varies between 1,050 and 1,370 mm, rising to over 2,000 mm in the Eastern areas. The highest rainfall typically occurs during autumn and winter. Along the Western coast, the presence of a pronounced summer drought can limit the establishment and regeneration of Euxine forests, which are predominantly found in the inland and eastern parts of the region. Conversely, a coastal strip in the east supports vegetation more typical of Mediterranean ecosystems. Türkiye's vascular flora, encompassing both flowering plants and ferns, totals around 12,000 - 13,000 species, with an exceptionally high endemism rate of approximately 34% (Güner at al., 2012). This considerable proportion of endemic species underscores Türkiye's position as a global biodiversity hotspot, resulting from its unique geographic location at the intersection of Europe, Asia, and the Middle East, combined with its diverse topography and climatic conditions that support a wide variety of ecological niches (Özhatay et al., 2013).

Türkiye, hosting an extraordinary diversity of both herbaceous and woody plant species, ranks among the world's leading countries in terms of botanical richness. This remarkable floristic diversity has drawn significant scientific interest and has been the subject of numerous research investigations. Türkiye spans three major floristic regions—the Euro-Siberian, Mediterranean, and Irano-Turanian zones (Davis 1965–1968). The coexistence of these distinct biogeographical units underscores the country's exceptional botanical diversity and the high ecological heterogeneity of its flora.

Table 1. Distribution of Vascular Plant Species in Türkiye Across Floristic Regions

Floristic Region	**Approx. Number of Vascular Plant Species**	**Percentage of Endemic Species**	**Notable Characteristics / Dominant Vegetation**
Euro-Siberian	2,500 – 3,000	15–20%	Dense, humid forests; beech, fir, oak species; mesic habitats
Mediterranean	4,000 – 4,500	25–30%	Sclerophyllous shrubs and forests; olive, pine, maquis vegetation; drought-adapted species
Irano-Turanian	3,500 – 4,000	35–40%	Steppe and semi-arid vegetation; endemic herbs and shrubs; high continentality
Circumboreal / Transcaucasian Overlaps	500 – 1,000	10–15%	Alpine and subalpine flora; cold-tolerant species; mountain meadows and screes
Total (all regions)	~12,000 - 13,000	~34%	Türkiye as a whole is a major biodiversity hotspot with high endemism

In biodiversity, although forests play a key role in nature, urban afforestation is progressively viewed as a crucial approach for supporting biodiversity and strengthening ecosystem services that help alleviate the effects of climate change in urban areas (Resemini et al., 2025). Nature-based Solutions are strategies that protect, restore, and sustainably manage ecosystems to address social, economic, and environmental challenges while enhancing human well-being, ecosystem services, resilience, and biodiversity (UNEA, 2025). Urban green spaces, including parks and gardens, play a critical role in enhancing environmental quality and the well-being of city residents. Urban afforestation, the systematic planting of trees and shrubs in these areas, provides numerous ecological, social, and climatic benefits that are essential for sustainable urban development. In this study, the species of woody plants commonly used in the parks and along the streets of Kastamon were listed. The aim was to identify the composition, diversity, and prevalence of urban tree species in different types of green spaces, providing insights into current urban afforestation practices. Understanding the selection and distribution of these species is crucial for enhancing urban biodiversity, improving ecological services, and informing future urban planning and greening strategies in the region.

Materials and Methods

The study was carried out in the central area of Kastamonu, Türkiye. Kastamonu lies within Türkiye's Western Black Sea Region, extending between 41°21'–42°01' N latitudes and 32°17'–34°06' E longitudes. Bordered by the Black Sea to the north, the province encompasses a wide range of landforms, including expansive forested areas, pronounced valleys, and elevated plateaus. Covering roughly 13,064 km^2, of which nearly 67% is forested, Kastamonu ranks among the most heavily wooded provinces in Türkiye. The region is characterized by diverse landscapes, including mountain ranges, forested valleys, deep canyons, and fertile plains. Kastamonu has one of Türkiye's highest forest cover percentages, with over 65% of the province covered by forests dominated by beech, fir, pine, and oak species. The dense forest structure and varied topography contribute to its designation as one of the key biodiversity areas of the Black Sea hinterland. Kastamonu is located within the Euro-Siberian phytogeographical region.

Figure 2. Kastamonu is located in the Western Black Sea Region of Türkiye.

In this study, all trees growing along major streets and within public parks in the central districts of Kastamonu were systematically surveyed. Each individual tree was photographed in the field to document its general morphology, growth form, and condition. For accurate species identification, diagnostic plant materials—including fresh needles, leaves, cones, fruits, buds, and other descriptive vegetative and reproductive structures—were collected when available. These samples were examined using standard dendrological identification keys and regional floras. Species were recorded together with their locations, and ambiguous taxa were cross-checked with herbarium references and expert consultation to ensure taxonomic accuracy.

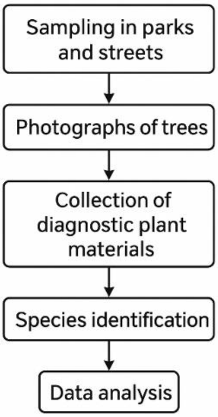

Figure 3. Field survey and data collection protocols for woody plant inventories in Kastamonu's central parks and streets

Results

Assessing Regional Climate Change in Kastamonu: Evidence from the Last 30 Years

In this section annual mean values of temperature and precipitation data of Kastamonu over the last 30 years were given. Long-term climate data were obtained from the Turkish State Meteorological Service. Kastamonu region is characterized by a continental climate, marked by cold winters and relatively wet summers. According to data from the Turkish State Meteorological Service, the mean annual air temperature is 9.9 °C, with monthly averages ranging from −4.5 °C in January—the coldest month—to 28.2 °C in August, the warmest period of the year. Annual precipitation totals approximately 485.1 mm, exhibiting a distinct seasonal pattern. The highest monthly precipitation occurs in May (75.4 mm), while the lowest is recorded in February (27.1 mm). These climatic conditions play a significant role in shaping the ecological performance, distribution, and management requirements of woody plant species in the region.

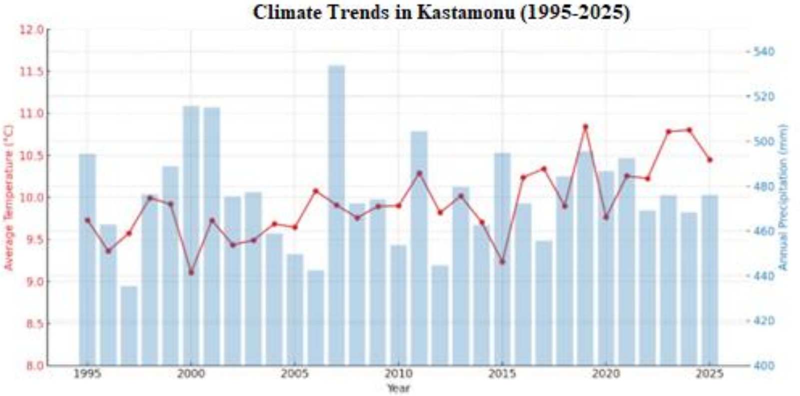

Figure 4. The average annual temperature and precipitation for Kastamonu Centre (1995–2025). Red line showing the average annual temperature (°C) and a gradual warming trend. Blue indicating the annual precipitation (mm) and variability but no clear long-term trend.

Over the past three decades, Kastamonu has experienced noticeable shifts in both temperature and precipitation patterns. The average annual air temperature shows a gradual increase from 9.2 °C in the mid-1990s to approximately 10.5 °C in the early 2020s. Winter temperatures have risen more sharply, with January averages increasing from −5 °C to around −2 °C, indicating milder winters. Summers have also warmed, with August temperatures climbing from 27 °C to 29 °C, increasing the risk of heat stress for vegetation. Annual precipitation has shown high interannual variability, fluctuating between 420 mm and 530 mm, but with no statistically significant long-term increase or decrease. Seasonal shifts are evident: spring (March–May) precipitation has decreased slightly, while autumn (September–November) precipitation shows a slight increase, potentially affecting soil moisture regimes and agriculture.

Urban Tree Species Commonly Used in the Streets and Parks of Kastamonu City Centre

Urban forests play a critical role in supporting ecological sustainability, enhancing air quality, and providing essential recreational and social benefits. However, the establishment, health, and long-term performance of woody plants in urban environments are largely constrained by climatic conditions—particularly temperature extremes and fluctuations in water availability. Kastamonu Province, situated in northern Türkiye, occupies a transitional climatic zone influenced by both the Black Sea and continental regimes. The region experiences mean annual temperatures of approximately 9.7 °C and an average annual precipitation of about 490.5 mm. Seasonal water deficits, most notably during the summer months, present significant challenges for the survival, growth, and maintenance of trees in urban green spaces.

Figure 5. A variety of native and ornamental tree species have been planted throughout the streets and parks of Kastamonu city centre to enhance urban greenery and ecological resilience.

A diverse assemblage of tree species is planted throughout the streets and parks of Kastamonu city centre, reflecting both the region's ecological conditions and long-standing urban landscaping practices. The urban canopy includes several species of *Tilia*—*Tilia cordata*, *T. tomentosa*, and T. *platyphyllos*—which are widely valued for their dense shade and ornamental qualities. Additional broadleaf species commonly used in the city's green spaces include *Fraxinus angustifolia*, *Platanus orientalis*, *Aesculus hippocastanum*, *Acer pseudoplatanus*, *Acer negundo*, *Robinia pseudoacacia*, *Betula pendula*, as well as moisture-loving willows such as *Salix babylonica* and *S. matsudana*. Coniferous taxa are also well represented, particularly ornamental and drought-tolerant species such as *Cedrus deodora*, *Cedrus atlantica*, *Cedrus libani*, *Cupressus sempervirens*, *Pinus sylvestris*, *Pinus nigra*, *Abies nordmanniana* subsp. *equi-trojani*, *Picea pungens*, and *Picea abies*. Collectively, these species contribute to the structural diversity, aesthetic value, and ecological functioning of Kastamonu's urban green spaces.

Table 2. Botanical Comparison of Tree Species in Kastamonu City Centre

Tree species	**Growth Form / Size**	**Leaf Characteristics**	**Reproductive Features**	**Bark & Crown Traits**	**Notable Botanical Features**
Tilia cordata	Medium–large deciduous	Small, cordate, serrated	Fragrant yellow flowers; nutlets with bracts	Dense rounded crown; grey bark	High nectar production; shade tolerant
Tilia tomentosa	Large deciduous	Silvery underside, tomentose	Aromatic flowers	Broad symmetrical crown	Drought-tolerant; reflective leaves
Tilia platyphyllos	Large deciduous	Broad heart-shaped leaves	Fragrant cymes	Deeply fissured bark	Moisture-loving; pollinator-friendly
Fraxinus angustifolia	Tall deciduous	Pinnate leaves	Winged samaras	Straight bole	Fast-growing; riparian species
Platanus orientalis	Massive deciduous	Large lobed leaves	Monoecious spherical heads	Exfoliating bark	Long-lived; pruning tolerant
Aesculus hippocastanum	Large deciduous	Palmately compound	Showy panicles; spiny capsules	Broad canopy	High ornamental value
Acer pseudoplatanus	Medium–large deciduous	Palmately lobed	Paired samaras	Grey flaking bark	Pollution- and wind-tolerant

Acer negundo	Medium deciduous	Pinnate leaves	Paired samaras	Green stems	Highly adaptable; dioecious
Robinia pseudoacacia	Medium deciduous	Pinnate	White racemes	Deeply furrowed	Nitrogen-fixing; durable wood
Betula pendula	Medium deciduous	Triangular, serrated	Catkins	White peeling bark	Cold-adapted
Salix babylonica	Medium deciduous	Narrow lanceolate	Catkins	Weeping branches	Moisture-dependent; rapid growth
Salix matsudana	Medium deciduous	Narrow leaves	Catkins	Upright to arching	More drought-tolerant than S. babylonica
Cedrus deodara	Large evergreen	Bluish-green needles	Barrel-shaped cones	Nodding branch tips	Ornamental Himalayan species
Cedrus atlantica	Large evergreen	Bluish clusters	Upright cones	Pyramidal crown	Drought-tolerant
Cedrus libani	Large evergreen	Dark green needles	Upright cones	Horizontal branches	Highly drought-resistant
Cupressus sempervirens	Tall evergreen	Scale-like	Small woody cones	Columnar form	Mediterranean species
Pinus sylvestris	Tall evergreen	Blue-green paired needles	Conical cones	Orange upper bark	Cold-tolerant
Pinus nigra	Large evergreen	Long rigid needles	Ovoid cones	Dark fissured bark	Drought- and heat-tolerant
Abies nordmanniana subsp. equi-trojani	Large evergreen	Glossy dark needles	Upright cones	Symmetrical crown	Shade tolerant; native
Picea pungens	Medium evergreen	Sharp blue-green needles	Pendant cones	Conical crown	Glaucous ornamental foliage
Picea abies	Large evergreen	Quadrangular needles	Long pendant cones	Drooping branchlets	Fast-growing; cool-moist habitat

Several tree species exhibit strong ecological suitability for the semi-continental climatic conditions of Kastamonu, particularly those capable of tolerating seasonal summer drought, pronounced temperature fluctuations, and common urban stressors. *Cedrus libani, Pinus nigra, Cupressus sempervirens, Robinia pseudoacacia, and Cedrus atlantica* demonstrate high levels of drought resistance, substantial tolerance to heat and air pollution, and the capacity to grow in nutrient-poor or compacted soils. These traits align closely with Kastamonu's warm, dry summers and cool winters, positioning these species as highly promising candidates for climate-resilient urban forestry strategies. In addition, species such as *Picea pungens* and *Acer negundo* perform reliably under local environmental conditions due to their broad ecological amplitude and capacity to withstand site disturbances.

A second group of species shows moderate suitability for the climatic regime of Kastamonu. Although these species grow satisfactorily under typical conditions, they tend to exhibit increased physiological stress during prolonged summer droughts. *Tilia tomentosa, Platanus orientalis, Acer pseudoplatanus, and Pinus sylvestris* generally tolerate urban habitats but benefit from supplemental irrigation during periods of moisture deficit. Similarly, *Abies nordmanniana* subsp. *equi-trojani, Fraxinus angustifolia,* and *Cedrus deodara* require relatively stable soil moisture to maintain optimal vigor and growth. While these species can be effectively integrated into parks, boulevards, or partially shaded areas, their long-term success will depend on careful site selection, soil preparation, and irrigation management.

Most widely used species exhibit only low to moderate suitability for Kastamonu's environmental conditions. *Tilia cordata, Tilia platyphyllos, Aesculus hippocastanum,* and *Betula pendula* typically perform well in cool, moist temperate climates but become increasingly vulnerable under extended drought or high summer temperatures. Their high moisture requirements and sensitivity to water stress often result in reduced growth rates, leaf scorch, premature senescence, or shortened life expectancy in exposed urban environments. These species may still be cultivated successfully in irrigated or well-maintained park settings; however, they are less appropriate for street landscapes or sites with limited soil moisture availability.

Species characterized by high water demand and low drought tolerance exhibit the lowest suitability for Kastamonu's climatic conditions. *Salix babylonica* and *Betula pendula* require consistently moist soils and are highly susceptible to root desiccation during the dry summer months. Their reliance on abundant soil moisture and sensitivity to hydrological fluctuations often leads to early decline or mortality when planted in typical urban soils. Consequently, their use should be restricted to riparian corridors, lake margins, or irrigated parks. In the context of projected climate change, the preferential selection of drought-resilient species is essential to ensure long-term stability and ecosystem service provision.

Overall, Kastamonu's transitional climate—characterized by moderately cold winters, warm and dry summers, and an average annual precipitation of approximately 490 mm—favors species with strong drought tolerance, resilience to thermal extremes, and the capacity to endure fluctuating moisture regimes. These findings underscore the necessity of incorporating climate-adaptive criteria into urban forestry planning and species selection. Prioritizing highly suitable species such as *Cedrus libani, Pinus nigra,* and *Cupressus sempervirens* will enhance the structural resilience, ecological functionality, and longevity of urban green spaces. Conversely, species with higher water requirements should be used more selectively and supported through appropriate soil management and irrigation practices. Such evidence-based approaches will become increasingly important as climate change intensifies summer droughts and amplifies thermal stress across the region.

Table 3. Commonly Used Tree Species in Kastamonu and Their Ecological Requirements

Tree species	Commonly used Sites	Soil Requirements	Water Requirements	Climate Tolerance
Tilia cordata	Streets and parks	Deep, moist, fertile loams	Moderate–high moisture	Tolerates cold; sensitive to drought
Tilia tomentosa	Streets and parks	Well-drained, moderately fertile soils	Moderate	Very drought-tolerant; heat-resistant
Tilia platyphyllos	Streets and parks	Moist, nutrient-rich soils	High moisture demand	Prefers cool, humid climates
Fraxinus angustifolia	Parks	Alluvial, moist soils	High	Tolerates periodic flooding; moderate drought tolerance
Platanus orientalis	Streets	Deep, moist, fertile soils	High	Tolerates urban pollution; moderate drought tolerance
Aesculus hippocastanum	Streets and parks	Moist, nutrient-rich,	High	Prefers cool climates;

		well-drained soils		sensitive to drought
Cedrus deodora	Parks	Well-drained, slightly acidic soils	Low–moderate	Prefers mild climates; sensitive to severe frost
Cedrus atlantica	Parks	Rocky, well-drained soils	Low	Drought-tolerant; tolerates heat
Cedrus libani	Streets and parks	Rocky, calcareous, well-drained soils	Low–moderate	Very drought- and heat-resistant
Acer pseudoplatanus	Streets and parks	Moist, fertile soils	Moderate	Prefers cool, moist climates; shade-tolerant
Acer negundo	Paks	Wide soil tolerance; disturbed sites	Moderate	Highly adaptable; tolerates drought and pollution
Cupressus sempervirens	Streets and parks	Well-drained, calcareous soils	Low	Very drought- and heat-tolerant
Pinus sylvestris	Parks	Sandy, well-drained, acidic soils	Low	Cold-tolerant; moderate drought tolerance
Pinus nigra	Parks	Rocky, well-drained soils	Low	Highly drought-tolerant; tolerates harsh climates
Abies nordmanniana subsp. *equi-trojani*	Parks	Moist, deep, fertile soils	Moderate–high	Prefers cool, humid climates; frost-tolerant
Picea pungens	Parks	Well-drained, moist soils	Moderate	Cold-tolerant; moderately

				drought-tolerant
Picea abies	Parks	Moist, fertile, well-drained soils	High	Prefers cool, humid climates; drought-sensitive
Robinia pseudoacacia	Streets and parks	Poor, dry, disturbed soils	Low–moderate	Very drought- and pollution-tolerant
Salix babylonica	Parks	Moist, deep soils near water	High	Prefers humid climates; drought-sensitive
Salix matsudana	Parks	Moist to wet soils	High	More drought-tolerant than *S. babylonica*
Betula pendula	Parks	Sandy, moist, well-drained soils	Moderate–high	Prefers cool climates; drought-sensitive

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CHAPTER 9

Natural Behavioral Characteristics in Chicken Society

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1. INTRODUCTION

The investigation of specific behavioral characteristics in chickens (*Gallus gallus domesticus*) is of great importance in both the scientific and practical domains of animal husbandry. Chickens are among the most widely domesticated and studied animals, and yet their behavioral complexity is often underestimated. Their behaviors—whether related to reproduction, care of young, roosting, nesting, aggression, or feeding—are the product of a long evolutionary process and reflect their adaptation to both natural and human-altered environments (Collias & Collias, 1996).

Understanding the behavioral repertoire of chickens is not only essential for advancing scientific knowledge in ethology and animal cognition but is also a cornerstone of effective and ethical poultry management. Each of the fundamental behaviors that chickens display has direct implications for animal welfare, flock health, and production efficiency (Appleby et al., 1992; Mench & Keeling, 2001). For instance, behaviors such as nest selection, social interaction, and foraging are not merely habits, but are critical indicators of chickens' physical and psychological well-being. Disruption or suppression of these natural behaviors can result in elevated stress, reduced productivity, and compromised health (Savory, 1995). Moreover, the study of chicken behavior offers unique opportunities to understand the dynamics of animal societies, the mechanisms of learning and socialization, and the capacity of animals to adapt to different environments. These insights are invaluable not only for improving animal welfare standards but also for designing housing systems, enrichment strategies, and management practices that align with the species' natural needs (Appleby et al., 1992; Olsson & Keeling, 2000). In modern agriculture, where economic and ethical considerations increasingly intersect, the ability to interpret and support species-specific behaviors is a prerequisite for sustainable and humane farming systems.

Behavioral observations also serve as practical tools for monitoring flock health and welfare, since changes in normal activity patterns often serve as early warning signs of disease, social stress, or environmental inadequacies (Savory, 1995; Johnsen et al., 1998). In this sense, behavioral science is not only an academic pursuit but a day-to-day necessity for anyone responsible for the care and management of poultry. Finally, the study of chicken behavior compels us to reconsider our responsibilities toward domesticated animals. By recognizing the intrinsic value and needs of chickens as sentient beings with rich behavioral lives, we are better equipped to create systems that respect their welfare while meeting human needs for food production (Fraser, 2008). The continued exploration and appreciation of chicken behavior thus stands as a foundation for progress in both science and animal ethics, underscoring the essential relationship between knowledge, welfare, and responsible stewardship.

2. NATURAL BEHAVIORAL CHARACTERISTICS

Chickens (*Gallus gallus domesticus*) naturally possess a polygamous mating system, which is one of the defining social and reproductive characteristics of the species. In natural and semi-natural environments, a single dominant rooster typically oversees a group of 10 to 15 hens, forming a loose but discernible social and reproductive unit (Collias, 1987; Guhl, 1953). This polygynous structure promotes both genetic diversity and social stability within the flock, and is thought to be an evolutionary adaptation that balances the reproductive interests of both sexes. In commercial and controlled breeding systems, the male-to-female ratio is carefully managed to maximize both fertility rates and social harmony. Studies and industry guidelines generally recommend a ratio of one rooster to nine or ten hens (1:9–1:10) to achieve high rates of egg fertilization while minimizing disruptive competition and aggression among males (Bell & Weaver, 2002; Etches, 1996). However, for optimal egg fertility—especially in intensive breeding operations aimed at maximizing chick production—reducing the number of hens per rooster (e.g., 1:6 or 1:7) may further increase the likelihood of each hen being mated frequently, thus ensuring a consistent and fresh supply of sperm (Etches, 1996; Brillard, 2004).

Despite the dominant role of the rooster in flock structure and reproduction, not all hens in a group will necessarily be mated equally. Roosters display selective mating behaviors, often preferring certain hens over others and investing more time in courtship and copulation with their chosen individuals. This selectivity is influenced by factors such as hen health, reproductive status, plumage condition, and even social hierarchy within the female group (Collias, 1987). Conversely, some hens may be largely ignored, receiving little or no attention from the rooster. Such differential mating patterns reflect the action of both male and female choice, contributing to sexual selection and the maintenance of genetic diversity within the flock (Bateson, 1982).

A unique feature of avian reproductive biology is the hen's ability to store viable sperm in specialized crypts in the infundibulum portion of the oviduct, known as "sperm storage tubules." After a successful copulation, hens can retain and utilize sperm for fertilization over an average period of 10 days, though this can vary depending on age, breed, and individual differences (Brillard, 2004; Etches, 1996). This adaptation reduces the necessity for frequent mating, as a single copulation can result in a sequence of fertilized eggs over several days. Thus, intermittent mating is sufficient for maintaining high fertility rates, even in flocks with relatively low rooster-to-hen ratios.

The mating process in chickens is characterized by a complex sequence of courtship behaviors, which serve both to attract females and to signal the male's fitness and readiness for mating. Courtship typically begins with the rooster's active pursuit of the hen's attention. One common display is "tidbitting," where the rooster finds and offers a desirable food item, such as an insect or seed, to the hen while making specific vocalizations and head movements (Collias, 1987). This behavior not only attracts the hen but also demonstrates the rooster’s foraging ability and social attentiveness. Roosters also perform visual displays, such as "wing dragging" or "wing pulling," in which they lower and extend one wing while circling the hen in a ritualized manner. These displays signal dominance, vigor, and genetic quality, and are often combined with soft vocalizations and subtle movements. The hen’s response to these courtship behaviors determines the outcome: a receptive hen will crouch and lower her head, signaling her willingness to mate, while an unreceptive hen may attempt to evade the rooster’s advances. If the hen attempts to escape, the rooster may intensify his display, adopting the so-called "turkey pose"—stretching his neck, fluffing his feathers, spreading his wings, and fanning his tail as he chases the hen. Upon catching the hen, mating proceeds with the hen assuming a crouched position, while the rooster mounts her from behind, balancing himself by grasping the hen’s neck feathers and wings with his beak and feet. Copulation culminates with the simultaneous eversion of both birds’ cloacas, allowing sperm transfer into the hen’s oviduct (Etches, 1996; Brillard, 2004). After mating, the rooster often crows or briefly struts away to resume other social or protective behaviors, while the hen typically shakes her feathers and returns to her normal activities. This seemingly routine act is, in reality, the outcome of complex social interactions, sexual selection, and evolutionary pressures that govern reproductive success in chickens (Bateson, 1982). The combination of polygamous mating, sperm storage, and elaborate courtship rituals ensures both high fertility rates and the maintenance of genetic diversity in chicken populations. Furthermore, these processes are directly influenced by social hierarchy, individual health, and environmental conditions, all of which must be considered in both natural and commercial flock management (Bell & Weaver, 2002; Brillard, 2004).

Hens exhibit complex, instinct-driven behaviors when selecting a nesting site for egg laying—behaviors that are the result of millions of years of evolution and are critical for both individual welfare and reproductive success. In both natural and managed environments, nest site selection is not random; it is a decision-making process involving environmental assessment, social negotiation, and personal experience (Cooper & Appleby, 1996). In their natural habitats, wild and feral hens generally seek out nesting sites that are secluded, quiet, and partially shaded, often slightly elevated above ground level and lined with soft, clean materials. Such sites provide protection against predators and adverse weather, while also reducing the risk of parasitism and egg predation (Collias & Collias, 1967). The instinctive drive for safety and comfort leads hens to invest significant time and energy into searching for a location that meets these criteria. In commercial or backyard settings, the absence of suitable nesting sites may result in increased stress, vocalizations, and undesirable behaviors such as floor laying (laying eggs outside of nests), which can compromise both welfare and production (Appleby et al., 1992).

To accommodate the biological needs of hens and minimize floor laying, it is essential that nest boxes in managed systems are designed to be comfortable, dark, clean, and lined with high-quality, dust-free bedding. Research has shown that hens strongly prefer nests that provide a sense of enclosure and security; for example, adding curtains or partially covering nest entrances can significantly increase nest use and reduce floor eggs (Cooper & Appleby, 1996). The strategic placement of nests—such as aligning them along side walls or raising them slightly above floor level—can also enhance their attractiveness. Random or poorly planned nest placement may confuse hens or increase competition, resulting in higher rates of floor laying or aggression (Appleby et al., 1992). Hens are creatures of habit and often repeatedly use sites where they previously felt safe and successful. This site fidelity is reinforced by both positive past experiences and social learning, as younger or lower-ranking hens may observe and mimic the nesting choices of older, more experienced individuals. However, competition for preferred nests can be fierce, especially in flocks with pronounced social hierarchies. Dominant or older hens usually gain priority access to the best nests, while subordinate hens may be forced to use less desirable locations or wait for a preferred one to become available (Cooper & Appleby, 1996). In some cases, group nesting occurs, where multiple hens lay in the same nest simultaneously. While this can be peaceful, overcrowding can also increase the risk of egg breakage, stress, and aggression.

Scientific studies consistently demonstrate that providing individual nest boxes, rather than communal group nests, yields better outcomes in terms of welfare and productivity. Individual nests reduce stress and competition, lower the incidence of floor eggs, and promote more hygienic conditions for egg collection (Appleby et al., 1992). The optimal ratio is generally considered to be one nest per 4–5 hens, but this can vary based on flock size, breed, and management practices (Cooper & Appleby, 1996). The pre-laying behavior of hens is particularly elaborate and informative. As the time for laying approaches, hens often display restlessness, increased vocalization ("pre-laying cackling"), and a heightened tendency to explore or pace around the coop. This search for a suitable site is driven by both instinct and individual temperament; some hens may spend considerable time inspecting and preparing nests, while others may quickly choose a familiar spot. In flocks with high levels of aggression or crowding, subordinate hens may go to great lengths to find isolated or less competitive nests, sometimes even attempting to escape the enclosure to find a suitable site. Stressed or anxious hens are more likely to lay eggs outside of designated nests, further emphasizing the importance of environmental design and social management. Once a nest is selected, hens perform a series of preparatory behaviors: they may scratch at the bedding to create a shallow depression, rearrange materials to adjust softness and comfort, and circle within the nest to ensure safety. This “nest building” is followed by a crouched posture and egg laying, after which hens may remain in the nest for some time before rejoining the flock (Cooper & Appleby, 1996). Environmental and management factors thus have a profound influence on nesting behavior. By aligning nest box design, placement, and maintenance with hens’ natural preferences and behaviors, producers can enhance both animal welfare and production efficiency. Proper nest management not only reduces undesirable behaviors such as floor laying and egg pecking, but also facilitates cleaner, more efficient egg collection, directly impacting the quality and safety of the final product (Appleby et al., 1992). In summary, the nesting behavior of hens is a sophisticated, adaptive process shaped by instinct, experience, and environmental context. Recognizing and accommodating these innate preferences through thoughtful management and design is fundamental to ensuring both the well-being of the animals and the success of commercial and backyard egg production.

Chick development before and immediately after hatching is shaped by a series of intricate behavioral and physiological processes, many of which are essential for survival and effective integration into the social structure of the flock. One of the earliest and most critical milestones is the development of auditory capability. Starting around the 15th day of incubation, chicken embryos (*Gallus gallus domesticus*) begin to detect and respond to sounds in their environment (Vince, 1966; Gottlieb, 1971). The most common and consistent auditory stimuli are the clucking, cooing, and vocalizations made by the brooding hen. Through repeated exposure, embryos not only become habituated to these maternal sounds but also begin to recognize and distinguish their mother’s voice from the sounds of other hens or environmental noise (Collias & Collias, 1996).

The ability to recognize and respond to the broody hen’s voice upon hatching is a key adaptive trait. Experimental research demonstrates that chicks can discriminate between their broody hen's calls and those of unfamiliar hens almost immediately after emergence (Vince, 1969; Gottlieb, 1971; Bolhuis, 1991). This early vocal communication, which includes the soft peeping sounds chicks make inside the egg, plays a vital role in synchronizing hatching times among siblings and initiating the first social bonds both with the broody hen and among the brood (Rogers, 1995; Collias & Collias, 1996). The development of this auditory bond is so fundamental that hens who are deaf or unable to perceive the sounds made by their eggs during incubation often fail to recognize their chicks after hatching. In such cases, the absence of auditory-mediated bonding can lead to neglect, aggression, or even lethal pecking toward the chicks (Bolhuis, 1991). As hatching approaches, the broody hen exhibits a distinctive change in behavior. Broody hen becomes exceptionally vigilant and quiet, minimizing movement and external disturbance to facilitate a calm environment for the emerging chicks (Rogers, 1995). Unlike some species, the hen does not physically assist her chicks in breaking out of the shell; the process is entirely driven by the chicks themselves, which is crucial for proper muscular and respiratory development. Once all chicks have hatched, the hen immediately shifts to a highly protective role, enveloping her offspring under her wings to provide warmth, shelter, and protection from potential threats (Collias & Collias, 1996; Rogers, 1995). This period marks the onset of an intense mother-offspring bond, reinforced by constant vocalizations and physical contact.

The first 24–36 hours post-hatching are a period of heightened sensitivity for chicks—a critical window for the process known as "imprinting." Imprinting is a rapid, irreversible form of learning in which the chick forms a strong attachment to the first moving object or animate being it encounters, most commonly the mother hen (Lorenz, 1935; Bolhuis, 1991). This process is not solely visual; it is strengthened by the combined effects of auditory, tactile, and olfactory cues. The chicks' tendency to imprint ensures that they follow their mother, learn appropriate behaviors, and avoid potential dangers. Notably, chicks hatched in the absence of a mother will imprint on artificial objects, such as moving boxes or flashing lights, demonstrating the innate nature of this adaptive behavior (Bolhuis, 1991; Rogers, 1995). Imprinting also plays a crucial role in sibling recognition and social cohesion within the brood. The continuous exchange of vocalizations and physical proximity among siblings fosters strong intra-brood bonds, facilitating coordinated movement, group foraging, and collective responses to threats in the days and weeks following hatching (Gottlieb, 1971; Collias & Collias, 1996). This early socialization lays the groundwork for the more complex flocking and social behaviors that emerge as the chicks grow.

The broody hen faces significant challenges in managing a brood of 10–15 chicks, each at varying stages of readiness to leave the nest. Chicks do not leave the nest simultaneously; this staggered emergence allows the mother to more effectively monitor, guide, and protect her offspring, reducing the risk of predation or accidental separation (Wood-Gush, 1971; Collias & Collias, 1996). Chicks display a powerful instinct to track moving objects, most intensely during the first week of life. During this period, chicks will follow the mother's movements closely, learning essential skills such as foraging, predator avoidance, and social interactions (Lorenz, 1935). The strength of this following response diminishes after about eight days; chicks that have not imprinted on a mother by this time are less likely to form strong attachments to newly introduced adult hens or objects, emphasizing the temporal sensitivity of this developmental window (Bolhuis, 1991).

These processes—auditory recognition, imprinting, and early social learning—are fundamental to both individual chick survival and the cohesion of the flock as a whole. They are shaped by an interplay of innate instincts and environmental interactions, ensuring not only the continuity of the species but also the establishment of stable, well-functioning social groups (Gottlieb, 1971; Collias & Collias, 1996). For poultry producers, an understanding of these mechanisms is crucial for optimizing animal welfare, as interventions that disrupt maternal bonding or early chick development can have lasting negative effects on behavior, stress resilience, and productivity. In summary, the broody hen-chick relationship in chickens is founded on a complex suite of sensory, cognitive, and social processes that begin before hatching and rapidly develop in the first days of life. These mechanisms are not only fascinating from an ethological perspective but also of practical importance in poultry management and welfare.

Nest selection is a highly nuanced and instinct-driven process in domestic hens (*Gallus gallus domesticus*), shaped by both environmental and social factors. In natural or free-range conditions, hens typically seek out sites for egg-laying that are quiet, semi-shaded, slightly elevated above ground level, and lined with soft, clean nesting material (Collias & Collias, 1967). Such locations offer security from predators, environmental stressors, and provide a sense of seclusion that is critical for the hen's sense of safety during oviposition. Scientific observations have shown that hens often develop strong site fidelity, repeatedly selecting nest locations where they have previously experienced successful and undisturbed egg-laying (Cooper & Appleby, 1996). This behavior is reinforced by positive past experiences, social learning, and the need for security. When several hens show interest in the same nest, social hierarchy becomes decisive; dominant or older hens are usually given priority access, while subordinate individuals may have to wait or use less desirable sites (Appleby et al., 1992; Cooper & Appleby, 1996). Interestingly, when resources are limited, or nest boxes are not optimally distributed, it is not uncommon to find multiple hens sharing a single nest peacefully, especially if they are socially compatible. Such observations have prompted some producers to install communal or group nests rather than traditional individual boxes. However, scientific research consistently demonstrates that individual nest boxes are superior to group nests in promoting welfare: they reduce stress, minimize floor-laying, and result in better egg hygiene and collection efficiency (Appleby et al., 1992).

Nest boxes in commercial facilities are typically arranged along sidewalls or placed centrally on bedding, often in two or three tiers to maximize space. The quality and type of nesting material—dust-free straw, wood shavings, or synthetic mats—along with factors such as nest darkness, ventilation, and ergonomic entry design all significantly influence nest choice and use (Appleby et al., 1992). Improperly sited or unattractive nest boxes, on the other hand, increase the incidence of floor-laying, which not only diminishes egg quality but also raises hygiene and welfare concerns. Behavioral patterns preceding egg-laying are particularly fascinating. As the time for oviposition approaches, hens often become restless, vocalize (pre-laying cackling), and roam the coop in search of an appropriate nest. The intensity and duration of this pre-laying behavior can vary not only by breed but also by individual temperament. In flocks with a high proportion of aggressive or dominant individuals, subordinate hens may seek more isolated or less contested nests, sometimes even attempting to escape the coop to find a suitable site (Cooper & Appleby, 1996). Stress or social pressure may also lead to excessive pacing or repeated attempts to access preferred nest sites, occasionally resulting in competitive interactions or even injury.

Once a nest is chosen, the hen typically engages in nest-building behavior: she scratches the nesting material to form a shallow depression, adjusts the bedding for softness and comfort, and circles the nest several times to inspect her surroundings. This behavior is reminiscent of wild ancestors preparing a secure place for egg-laying (Collias & Collias, 1967). The final act of oviposition is performed in a crouched posture, and after laying, the hen may linger to recover before rejoining the flock. Providing adequate and strategically placed, comfortable nest boxes is essential for both welfare and productivity. The recommended ratio is generally one nest per four or five hens, but this may vary according to flock size, breed, and housing type (Appleby et al., 1992). Well-designed nests help reduce floor-laying, decrease stress and competition, and facilitate hygienic egg collection. In conclusion, nest selection in chickens is a multifaceted process, deeply rooted in natural instincts, shaped by environmental conditions, and influenced by social dynamics. Satisfying these preferences through science-based management not only improves animal welfare but also enhances egg quality and production efficiency. Understanding and accommodating the behavioral needs of hens regarding nesting is thus a fundamental aspect of modern poultry husbandry.

Before laying an egg, hens typically engage in an elaborate process of nest selection and inspection. Rather than choosing a location at random, most hens will visit and examine multiple nesting sites within the coop or available environment. This exploratory behavior allows them to assess the suitability of each option in terms of safety, comfort, seclusion, and substrate quality (Cooper & Appleby, 1996). It is well documented that many hens exhibit strong site fidelity, often returning to the same nest where they previously laid their last egg, especially if that experience was undisturbed and successful (Appleby et al., 1992). However, in some individuals, particularly those that are indecisive or exposed to social stress, the nest selection process can be prolonged lasting up to several hours in extreme cases (Cooper & Appleby, 1996). These hens may repeatedly alternate between nests, entering and leaving boxes, vocalizing, and exhibiting signs of restlessness. This behavior is thought to be influenced by a combination of individual temperament, previous nest experiences, social hierarchy, and environmental factors such as nest availability and competition.

Once a hen has made her decision, it typically performs a sequence of preparatory behaviors. Hen will scratch and rearrange the nesting material, often deepening the center of the substrate to create a shallow, comfortable depression for egg-laying. This nest-building behavior is an instinctive act shared with their wild ancestors, serving to both cushion the egg and reduce the risk of breakage or predation. Immediately before settling to lay, hens will frequently turn in circles within the nest, much like a dog preparing a spot to lie down. This circling behavior is believed to further assess the environment for safety and to optimize the comfort and structure of the nesting site (Appleby et al., 1992; Cooper & Appleby, 1996). Finally, the hen adopts a slightly crouched, semi-seated posture and proceeds to lay her egg, often remaining in the nest for a short period afterward to recover and ensure the safety of the egg. These detailed pre-laying and nest selection behaviors highlight the complexity of instinct-driven routines in domestic hens and underscore the importance of providing appropriate nest environments that accommodate these natural preferences. Such accommodations not only support animal welfare but also improve productivity and egg quality in managed flocks.

After oviposition, hens display a wide range of individual behaviors that reflect both physiological and evolutionary influences. Some hens, appearing physically exhausted or simply experiencing a sense of relief, remain quietly seated in the nest for a period of time. They may rest motionless, close their eyes, or preen gently, seemingly recovering from the egg-laying process (Appleby et al., 1992). In contrast, other hens immediately become highly active, leaping from the nest and vocalizing loudly emitting the well-known “cackling” or “cackling call” that is characteristic of laying hens. This post-laying vocalization has been the focus of considerable scientific interest. Researchers propose that the cackling call serves not only as a form of self-advertisement but also as a social or communicative signal with deeper evolutionary roots (Collias & Collias, 1967; Collias & Joos, 1953). The behavior is remarkably consistent across domestic breeds and is also observed in wild ancestors, such as the red junglefowl, suggesting it has adaptive significance. Ethologists have hypothesized that the loud post-laying call originally evolved as a “reassembly” or “reunion” signal—helping the hen relocate and rejoin her flock after the vulnerable and solitary act of laying an egg, which often takes place in concealed or isolated locations (Collias & Collias, 1967; Appleby et al., 1992).

In the context of domesticated chickens, this vocalization may also serve as an anti-predator strategy: by moving away from the nest and vocalizing elsewhere, the hen may help distract potential predators from the location of her eggs (Collias & Joos, 1953). The “cackling call” thus has both social and survival value. There is also evidence that the rooster is particularly sensitive and responsive to the hen’s post-laying call. When hearing this vocalization, roosters often respond by calling loudly in return and, in some cases, by moving rapidly toward the hen’s location. This behavior is interpreted as a means of flock cohesion—if a hen becomes separated from the group during nesting, the rooster’s response may help guide her back to the safety of the flock (Collias & Collias, 1967). Some researchers have described this interaction as a form of “flock gathering call,” emphasizing its function in maintaining group unity and communication (Collias & Joos, 1953). The diversity of post-laying behaviors in hens therefore reflects both individual temperament and deep-seated evolutionary adaptations. These behaviors—particularly the distinctive vocalizations—are of great interest to researchers studying animal communication, social behavior, and the evolutionary origins of domestic species.

During the incubation period, chick embryos gradually develop functional auditory organs, typically acquiring the ability to perceive sounds from approximately day 14 or 15 of incubation. At this stage, the cochlea and related auditory structures mature enough to process environmental sounds (Saunders, 1985). The most prominent sounds in the environment of the developing embryo are the vocalizations produced by the broody hen. These maternal calls, which include clucking and specific communication sounds, are repeatedly heard by the embryos before hatching. This prenatal exposure enables the chicks to become familiar with their mother's vocalizations, so much so that newly hatched chicks can immediately recognize and preferentially respond to the maternal voice among other sounds. This early recognition significantly facilitates the rapid formation of a social bond between the hen and her chicks after hatching (Gottlieb, 1971; Vince, 1966). In addition to perceiving external sounds, late-stage embryos themselves produce faint peeping or clicking vocalizations. These calls serve as communicative signals both to the mother hen and to sibling embryos. The hen responds to these embryonic vocalizations, which helps synchronize the hatching process and strengthens the social relationship between the mother and her offspring. The mutual exchange of vocal signals is thus crucial for establishing a robust social bond immediately after hatching. Research has demonstrated that auditory communication during incubation is essential for the development of appropriate maternal behavior. Hens that are deaf or unable to hear embryonic vocalizations often fail to form normal social bonds with their chicks. In some cases, such hens have been observed to peck at or even kill their own chicks, likely due to the absence of the prenatal auditory signals that normally facilitate the establishment of maternal recognition and care (Collias & Joos, 1953; Vince, 1975). These findings underscore the importance of prenatal auditory experience for both the chick's recognition of the mother and the mother's attachment to her offspring.

When a broody hen senses that her eggs are about to hatch, she becomes noticeably calm and remains quietly on the nest, minimizing any disturbance to the hatching process (Collias & Collias, 1996). During this period, the hen adopts a remarkably passive role—she does not attempt to assist the chicks in breaking out of their eggshells, nor does she interfere with their efforts; rather, she patiently waits until all chicks have successfully emerged (Rogers, 1995). There is no evidence in the scientific literature to suggest that hens ever hinder the hatching of their chicks; on the contrary, the broody hen's non-interventionist behavior is considered essential for proper development and successful hatching (Bateson, 1966). Once the fragile chicks have freed themselves from their shells, the broody hen becomes extremely attentive and cautious. She seldom moves from her place in order to avoid accidentally injuring the vulnerable hatchlings and actively defends the nest against intruding animals, thus ensuring a safe environment for her offspring (Collias & Collias, 1996; Rogers, 1995). Immediately after hatching, the mother instinctively gathers her chicks under her wings, providing necessary warmth and shelter. This period of intense brooding is critical, as chicks are highly susceptible to environmental influences, dehydration, and hypothermia during the first 36 hours of life (Bateson, 1966; Horn, 2004). During this sensitive phase, the chicks display an extraordinary responsiveness to moving objects or beings in their vicinity and rapidly form attachments—a process known as imprinting (Lorenz, 1937; Bateson, 1966; Horn, 2004). Imprinting is a rapid learning process by which a young animal establishes a strong and persistent attachment to the first moving object it perceives, which is usually its mother. These initial impressions are exceptionally persistent, shaping social behavior and recognition throughout life (Horn, 2004).

Interestingly, imprinting is not limited to the biological mother. Classic experiments have shown that chicks reared in artificial incubators, without exposure to a real hen, will reliably imprint on artificial stimuli such as moving cardboard boxes or flashing lights, following them as if they were their mother (Bolhuis, 1991; Horn, 2004; Lorenz, 1937). This demonstrates that certain behavioral patterns and responsiveness to specific stimuli are instinctive, and that imprinting is triggered by movement and other salient features, rather than species-specific cues (Bateson, 1966; Horn, 2004). However, the window for imprinting is not indefinite. Notably, chicks remain receptive to imprinting only up to about the eighth day of life. After this sensitive period, chicks that have not formed an attachment to a maternal figure will no longer readily accept a new hen as a social guide or follow her (Bateson, 1966; Bolhuis, 1991). This time-limited plasticity underscores the evolutionary importance of rapid and stable social bond formation in precocial birds.

Shortly after hatching and imprinting, chicks begin to venture out of the nest and explore their immediate surroundings, although their departure is typically staggered, rather than simultaneous (Collias & Collias, 1996). This staggered emergence allows the mother hen to maintain oversight and coordination, guiding her chicks in foraging for food and providing protection from potential dangers (Rogers, 1995). A broody hen is typically responsible for 10 to 15 chicks, and effective maternal care depends on her ability to monitor, gather, and manage her brood (Collias & Collias, 1996).

The initial communication between the hen and the still-unhatched chicks, which includes vocalizations from both sides, sets the stage for social bonding even before hatching (Gottlieb, 1971). After hatching, the visual and behavioral cues from the mother and siblings further reinforce the essential connections that ensure the cohesion and survival of the brood during the crucial first weeks of life (Horn, 2004; Bolhuis, 1991). These behavioral adaptations, rooted in both instinct and environmental responsiveness, illustrate the complex interplay between inherited predispositions and experiential learning in the early social development of domestic chickens. Such mechanisms ensure not only the survival of the individual chick but also the cohesion and coordinated functioning of the entire brood (Bateson, 1966; Horn, 2004; Rogers, 1995).

The first week of life represents a critical period of behavioral flexibility for both newly hatched chicks and broody hens in terms of recognition and acceptance (Bateson, 1966; Horn, 2004). Although chicks rapidly learn to recognize the vocalizations and appearance of their broody hen within the first hours and days after hatching—primarily through the process of imprinting—this attachment is not entirely fixed during the early post-hatch period (Lorenz, 1937; Bateson, 1966). Classic imprinting studies have demonstrated that, under certain circumstances, especially within the first week, chicks can adapt to a new maternal figure, even if this is not their biological mother (Bolhuis, 1991; Horn, 2004). This capacity for re-imprinting or social reassignment confers significant evolutionary advantages in both natural and managed settings, such as when fostering is necessary due to maternal loss or to manage large broods (Bateson, 1966; Rogers, 1995). For successful adoption, it is generally beneficial if the new hen closely resembles the original broody hen in appearance, particularly in plumage color and pattern. Chicks display a marked preference for maternal figures that match the visual features of their initial attachment object, as established in both field and laboratory research (Horn, 2004; Bolhuis, 1991). Nevertheless, a competent broody hen often exhibits remarkable acceptance and flexibility, readily adopting chicks that differ in appearance or origin, including those hatched from eggs of other hens or from artificial incubators (Rogers, 1995). This maternal flexibility is attributed to the hen’s strong instinct to brood and protect young, which can override discrimination based on external cues such as chick appearance (Collias & Collias, 1996).

The timing of the introduction is also crucial for fostering success. The optimal period for integrating chicks and a new hen is at dusk or during the night. Darkness reduces stress and aggression, facilitating a calm environment in which the hen and chicks can become accustomed to each other's presence (Bolhuis, 1991). Overnight, the physical proximity and the reduction of visual stimuli provide an opportunity for olfactory and auditory cues to facilitate mutual recognition and acceptance (Rogers, 1995). By the following morning, the likelihood of successful adoption is greatly increased, and the hen is more likely to treat the introduced chicks as her own, brooding and protecting them alongside her original brood (Bateson, 1966). This behavioral plasticity during the sensitive period of the first week is strongly supported by research on imprinting, social learning, and maternal care in precocial birds (Horn, 2004; Bateson, 1966; Bolhuis, 1991). It highlights the adaptive value of both chick and hen responsiveness to social and environmental cues, ensuring the survival and well-being of the brood even in changing or challenging circumstances (Rogers, 1995).During their first exploratory walks outside the nest, newly hatched chicks remain close to their mother, rarely venturing far from her protective presence (Collias & Collias, 1996). If a chick becomes separated from the hen and its siblings, it emits a loud, plaintive call known as the "distress" or "separation" call (Evans, Evans, & Marler, 1993). This is a highly effective vocalization, frequently heard in both natural and captive settings, as chicks are unable to visually identify their mother beyond a distance of approximately 10 meters (McBride & Foenander, 1962; Collias & Joos, 1953). In response to the distress call, the broody hen produces a series of distinctive "cluck-cluck" assembly calls, which serve as auditory beacons to guide the lost chick back to the group (Collias & Joos, 1953). Maintaining the cohesion of her brood is essential for the survival of the chicks, since it allows the mother hen to protect them from various dangers and to coordinate their activities. As a leader, the hen displays extraordinary vigilance and aggressive defensive behaviors. She confronts threats with an impressive and intimidating posture—head held high, feathers fluffed, and a piercing gaze—which often deters even much larger predators such as cats and dogs (Collias & Collias, 1996). Hens are particularly sensitive to aerial threats; upon detecting the silhouette of a raptor or other predatory bird in flight, the hen emits a sharp, specific alarm call (Evans et al., 1993). In response, chicks either run to her side, seek immediate cover, or freeze in place, remaining perfectly still to avoid detection (Evans et al., 1993). Remarkably, these responses are not learned through experience but are innate, instinctive behaviors. Both the hen's recognition of aerial predator silhouettes and the chicks' reactions to alarm calls are hardwired from birth (Horn, 2004). Experiments with artificially incubated chicks, reared in the absence of any maternal figure, have shown that playback of the hen's alarm call will trigger immediate hiding or freezing behavior, while the "cluck-cluck" assembly call prompts them to resume activity or emerge from hiding (Evans et al., 1993; Horn, 2004). This demonstrates that the neural circuits for processing these specific social and survival cues are present at hatching and do not require prior learning or exposure. Once the danger has passed, the hen produces a series of reassuring "cluck-cluck" calls, signaling to her chicks that it is safe to resume normal activity (Collias & Joos, 1953). This sophisticated system of vocal communication and the corresponding innate behavioral responses ensure the chicks' safety and cohesion. It highlights the evolutionary importance of instinctive signaling and social learning in precocial birds such as domestic chickens (Horn, 2004).

While chickens exhibit many innate, instinctive behaviors, their lives are not governed solely by instinct. Like all animals, chickens possess remarkable learning abilities, which are especially important for essential tasks such as foraging and food selection (Rogers, 1995). Newly hatched chicks display a strong, innate pecking drive, which compels them to investigate and peck at small moving objects such as insects (spiders, flies, ants) or objects resembling a grain of wheat (Collias & Collias, 1996). This predisposed behavior ensures that chicks are naturally attracted to potential food items in their environment. However, the ability to distinguish between edible and inedible items is not fully developed at birth and must be refined through social learning, primarily from the mother hen. The process of food learning in chicks is a clear example of social transmission. The mother hen plays a crucial role by demonstrating appropriate pecking and consumption of various food items in front of her chicks. She often accentuates these demonstrations with characteristic food calls—distinctive vocalizations that attract chicks' attention and encourage them to approach and investigate the food (Rendell et al., 2011). Through repeated exposure and observation, chicks learn not only to peck at and ingest appropriate food items but also to distinguish between palatable and unpalatable substances (Rogers, 1995). This learning is reinforced by positive associations with the vocal cues and the observed behavior of the mother, leading to more effective and efficient foraging as the chicks mature (Collias & Collias, 1996). Moreover, the capacity for social learning in chickens extends beyond food selection. Experimental studies have shown that chicks are capable of complex forms of learning, including observational learning, imprinting, and even rudimentary forms of problem-solving (Rogers, 1995). Such abilities, combined with instinctive predispositions, support the survival and adaptation of chickens in dynamic and sometimes challenging environments.

Chickens, like many birds, dedicate considerable time to self-maintenance behaviors, particularly in the periods between foraging, egg-laying, and other activities. One of the most essential of these behaviors is preening, in which the hen uses her pointed beak with great dexterity to grasp and realign individual feathers, remove debris, and distribute oils produced by the uropygial (preen) gland located at the base of the tail. Preening is not only crucial for maintaining the structural integrity and waterproofing of the feathers, but also for ensuring efficient insulation and flight, as well as facilitating social signaling through feather condition. In addition to preening, chickens engage in what is perhaps the most distinctive form of body care: dust bathing. This behavior is typically observed during the early afternoon hours, especially when birds have opportunities for undisturbed rest and environmental conditions are suitable. For dust bathing, chickens settle into loose, dry soil or sand—sometimes on a sparse litter substrate—and begin to vigorously scratch, peck, and flick substrate onto their bodies. Birds often recline to one side, stretching out their wings and legs, and energetically wriggle or dig deeper into the substrate. These actions ensure that dust and fine particles are distributed through the feathers and onto the skin (Duncan, 1998). The dust and loose particles serve several important functions. Mechanically, they absorb excess oils and help dislodge and remove ectoparasites such as mites and lice, as well as shed skin and feather debris (Van Liere & Bokma, 1987). Scientific studies have shown that the absence of opportunities for natural dust bathing can lead to feather deterioration, increased parasite loads, and even abnormal repetitive behaviors in chickens, underscoring the importance of this activity for both health and welfare (Olsson & Keeling, 2005). Dust bathing is usually concluded with vigorous shaking and ruffling of the entire body, which helps to remove loose dust and any remaining debris. This behavior is not only instinctive but is considered a strong behavioral need in chickens (Duncan, 1998). Even in winter, when natural dry soil may be unavailable, it is important to provide chickens with suitable substrates for dust bathing to maintain their health and well-being. Overall, self-maintenance activities such as preening, and dust bathing play a vital role in the hygiene, comfort, and physical health of domestic chickens. These behaviors are deeply rooted in avian biology and should be accommodated in both backyard and commercial settings to promote optimal welfare.

Domestic chickens (Gallus gallus domesticus) exhibit feeding preferences that are influenced more by tactile and physical characteristics of food than by taste. While birds possess taste buds and can detect basic flavors, research indicates that chickens primarily use tactile cues—such as the texture, size, shape, hardness, and surface structure of feed particles—to recognize and select their food (Gentle, 1985; Savory, 1999). This reliance on touch is particularly important given their natural feeding behavior, which involves rapid pecking and swallowing. Chickens prefer feed items that are easy to grasp and consume, favoring those that fit the "peck and swallow" strategy. They tend to avoid feeds that are difficult or time-consuming to break down, as their feeding efficiency and energy intake are optimized by quickly ingesting suitable food particles (Savory, 1980; Forbes & Shariatmadari, 1994). For example, coarsely ground or whole grains are generally preferred over finely ground mash feeds because they are more satisfying and easier for chickens to consume in larger, discrete bites. When presented with a variety of grains, wheat is typically the most preferred, followed by corn (maize), barley, rye, and oats (Forbes & Shariatmadari, 1994). The structure and size of grains significantly affect how quickly chickens can reach satiety. Finely ground feeds require longer to be consumed to achieve fullness, which can lead to decreased feeding efficiency and lower preference (Savory, 1980; Forbes & Shariatmadari, 1994). Chickens' natural selection for larger, intact grains helps explain their preference for whole foods over processed or powdered rations. In terms of green feeds, chickens show a strong preference for tender, succulent, and smooth-leaved plants, such as rape (Brassica napus), wild chicory, young cabbage leaves, Swiss chard, and beet greens (Savory, 1999). They readily consume fleshy and juicy leaves but avoid tough, hairy, or mature plants, likely due to the ease of ingestion and palatability. This selectivity is closely linked to the tactile properties of the plants, as chickens are less likely to consume materials that are fibrous or difficult to break down with their beaks. While the notion that "if chickens were the size of humans, few other species would survive" is anecdotal, it reflects the remarkable efficiency and voracity of chickens when exploiting available food resources. Their rapid "peck and swallow" feeding style allows them to consume large quantities of food quickly, demonstrating the ecological impact and competitive strength of this species. Overall, the feeding behavior of domestic chickens highlights the dominance of tactile cues over taste in food selection, the evolutionary adaptation to maximize feeding efficiency, and the importance of food structure in dietary preference and intake.

In chickens, the early stages of social behavior and hierarchy establishment begin as early as two weeks of age. At this stage, chicks start to engage in playful strength-testing behaviors, which serve as precursors to more serious social interactions later in life (Guhl, 1953). These activities include running towards each other with outstretched wings, leaping onto one another, and engaging in mock chases. Such playful encounters are accompanied by vigilant postures, as the chicks watch their surroundings and suddenly dart towards their next playmate. Within a few days of these initial games, more directed social behaviors emerge, such as pecking at the faces of peers. When one chick pecks another, the recipient often retreats, displaying clear submission. These early acts represent the first signs of social competition and the establishment of dominance relationships (Guhl, 1953; McBride & Foenander, 1962). Known as the "pecking order," this system of social ranking is a well-studied phenomenon in poultry and serves to minimize serious conflict within the group. As chicks approach sexual maturity, the nature of these interactions becomes more intense and consequential. During this period, social classes within the flock are established through a series of aggressive encounters and fights. These contests can be fierce and, at times, bloody, but rarely result in death (Guhl, 1953; McBride & Foenander, 1962). The resolution of these battles leads to the clear identification of dominant and subordinate individuals, and each bird learns its precise position within the flock's hierarchy. Once the social hierarchy is established, the group generally experiences stability and reduced aggression. The knowledge of one's social rank allows most disputes to be settled through displays or minor confrontations, rather than actual fighting (Rushen, 1982). Only under unusual circumstances—such as the introduction of new individuals, changes in group composition, or environmental stress—do serious fights recur. The formation of social hierarchies is essential for the cohesion and functioning of chicken flocks. It regulates access to resources such as food, water, and mates, and provides a framework for predictable social interactions, reducing the incidence of injurious aggression and promoting group stability (Guhl, 1953). These social dynamics are not only observed in domestic chickens, but also in their wild relatives and other bird species, highlighting the evolutionary importance of dominance hierarchies in avian social systems.

Immediately after hatching, chicks exhibit little to no fear and display high levels of curiosity and approach behavior toward novel stimuli (Jones, 1987a; Vallortigara et al., 2001). However, within a few days, early signs of fear and withdrawal become apparent, particularly in response to unfamiliar sounds or sudden movements. These innate fear responses serve as important survival mechanisms but can also create risks in managed environments. For example, when startled, chicks may crowd together in a corner, leading to smothering and injury or even suffocation (Blokhuis & Arkes, 1984). To mitigate this danger, poultry producers often use brooder guards or round enclosures to eliminate sharp corners and prevent chicks from piling up. Adult chickens also display heightened sensitivity and suspicion toward unfamiliar situations, objects, or living beings, a trait that persists throughout life (Jones, 1986; Jones, 1996). Sudden, novel auditory or visual stimuli can trigger panic or extreme excitement, sometimes resembling the instinctive fear experienced when confronted with natural predators such as foxes or raptors (Peitz & Pertz, 2007). Domestic chickens, even when habituated to common farm animals like cats, dogs, or rabbits, may react intensely to unusual objects—such as a child entering the coop with a toy bear—demonstrating their predisposition for neophobia, or fear of the new and unfamiliar (Jones, 1987a; Jones, 1996). This tendency is particularly pronounced toward moving or fuzzy objects, which can be perceived as potential threats. The neurobiological basis for such fear responses in chickens is rooted in their comparatively large but less-developed brains, which limit their learning capacity and adaptability (Peitz & Pertz, 2007). As a result, much of their behavioral repertoire, including fear reactions, is inherited rather than acquired through experience (Jones, 1987a).

Fear is a key factor in the welfare and productivity of poultry. Researchers describe fear as a state of alarm and unease experienced in the face of danger, which triggers a cascade of psychophysiological responses involving the brain and neuroendocrine systems (Jones, 1987b; Boissy, 1995). The severity of the fear response depends on the animal's sensory perception, prior experience, hormonal status, and the intensity or novelty of the eliciting stimulus (Jones, 1986; Boissy, 1995). Mild fear can enhance an animal's ability to adapt and cope with environmental challenges, but severe or chronic fear is maladaptive, leading to behavioral disturbances, reduced welfare, and diminished productivity (Jones, 1996; Elrom, 2001). In practical terms, intense fear can provoke escape, freezing, or defensive aggression in chickens—responses that are adaptive in the wild but can cause significant problems under intensive production systems. Chronic exposure to fear-inducing stimuli or environments not only disrupts daily activity and social behavior but also contributes to economic losses through reduced growth, lower egg production, and increased mortality (Elrom, 2001; Jones, 1996). For these reasons, minimizing fear and stress is a major concern in modern poultry husbandry, and understanding the biological and environmental factors that affect fear responses is essential for improving both animal welfare and farm productivity.

Feather pecking and cannibalism represent major challenges in poultry production, particularly in commercial laying hen systems. What often begins as seemingly harmless exploratory pecking can escalate into severe feather loss, skin injuries, and, in extreme cases, cannibalism and death (Savory, 1995; Blokhuis & Arkes, 1984). This complex behavioral disorder typically starts with one bird, often out of curiosity, boredom, or frustration, approaching and plucking the protruding feathers of another, most frequently from the neck, tail, or vent area. Initially, the feather-pecker may examine and manipulate the feathers for extended periods. If the pecked bird resists or tries to escape, the behavior can quickly turn aggressive, resulting in feather removal and skin trauma (Savory, 1995). As feather pecking progresses, the behavior can become self-reinforcing and spread rapidly through the flock. The exposed, sometimes bleeding skin attracts further pecking from other hens, as chickens are instinctively drawn to the color red and moving tissues (Hughes & Gentle, 1995). In severe cases, cannibalism can occur, with birds attacking and consuming the flesh or internal organs of injured flockmates, particularly when the vent area or intestines become exposed (Bilcik & Keeling, 2000).The development of feather pecking and cannibalism is multifactorial, involving genetic, environmental, nutritional, and social factors (Rodenburg et al., 2013). Nutritional deficiencies—especially of protein, essential amino acids, minerals (such as calcium), and fiber—have been implicated in the development and escalation of feather pecking (Leeson & Summers, 2005; Savory, 1995). Additionally, feed form plays a significant role: finely ground or pelleted diets that are consumed quickly leave birds with excess free time and unfulfilled foraging motivation, increasing the risk of abnormal pecking (Blokhuis & Arkes, 1984; Hartcher et al., 2015). Environmental and management factors are also critical. High stocking densities, lack of foraging and dustbathing opportunities, poor air quality, inadequate enrichment, and insufficient space all contribute to boredom and frustration, key drivers of feather pecking (Rodenburg et al., 2013; Lambton et al., 2010). Flocks lacking a rooster may also experience increased social instability, as roosters often play a stabilizing role in group dynamics.

Preventing and managing feather pecking requires a multifaceted approach. Ensuring a balanced diet that meets all nutritional requirements—including appropriate levels of protein, fiber, minerals, and vitamins—is essential (Leeson & Summers, 2005). Providing environmental enrichment, such as scattering grains in the litter for foraging, supplying pecking stones, and ensuring access to dustbathing and scratching areas, helps satisfy the birds' behavioral needs and reduce boredom (Rodenburg et al., 2013; Hartcher et al., 2015). Stocking density should be minimized, and housing should be designed to avoid overcrowding and provide sufficient space for movement and exploration. Clean, well-ventilated environments, as well as outdoor runs or covered scratching areas, are particularly important during periods of inclement weather when birds cannot go outside. Flock size should not exceed the capacity of the housing system, and keeping a few birds less than maximum capacity improves overall welfare. Early intervention is critical. Birds exhibiting feather pecking should be removed promptly for isolation and rehabilitation with mineral-rich diets. Severely injured birds should be treated, disinfected, and protected from further attacks until fully healed. Topical deterrents, such as oily or bitter-tasting substances, can be applied to wounds, but their effects are generally short-lived and require frequent reapplication (Hughes & Gentle, 1995).Beak trimming, the removal of a portion of the upper and lower beak, is sometimes used as a last resort to minimize damage from pecking (Gentle, 2011). While effective at reducing severe injury, it is controversial from an animal welfare perspective, as it can cause acute and chronic pain and impair normal behaviors such as preening and foraging, particularly if the beak is excessively shortened (Gentle, 2011; Blokhuis & Arkes, 1984). Foraging chickens, as opposed to those fed only fine mash, are especially affected by loss of tactile sensitivity. Temporary plastic beak guards have also been tried, but adaptation can be difficult for the birds. Ultimately, attention to flock management, nutrition, environmental enrichment, and early detection are the most effective long-term strategies for controlling feather pecking. Immediate culling of injured birds should be avoided, as this may leave only aggressive individuals in the flock. Rehabilitation, protection, and, if necessary, separation of victims until recovery remain best practices.

3. CONCLUSION

The natural behavioral characteristics of chickens are the result of a complex interplay between evolutionary adaptations, environmental influences, and social dynamics. The detailed exploration of these behaviors—ranging from reproductive strategies and parental care to foraging, nesting, self-maintenance, and social interactions—highlights the remarkable sophistication and flexibility inherent in chicken society. Far from being mere agricultural commodities, chickens exhibit a breadth of instinctive and learned behaviors that reflect both their wild ancestry and their capacity to thrive in diverse, often human-modified environments.

Understanding and supporting the expression of these natural behaviors is not only essential for advancing scientific knowledge but is also foundational to ethical and effective poultry management. The ability of chickens to form intricate social hierarchies, engage in cooperative and competitive interactions, and exhibit complex learning and adaptive responses underscores their status as sentient beings with significant cognitive and emotional capacities. The processes of imprinting, social learning, and the establishment of stable social structures are not only crucial for individual welfare and flock cohesion but also serve as key determinants of overall productivity and health within both backyard and commercial production systems.

Modern poultry husbandry increasingly recognizes that the suppression or neglect of these natural behaviors can have profound negative consequences, including elevated stress levels, increased aggression, and a higher incidence of abnormal behaviors such as feather pecking and cannibalism. Conversely, management practices that prioritize environmental enrichment, adequate space, social stability, and opportunities for natural behaviors contribute not only to improved welfare but also to enhanced productivity, disease resistance, and product quality. The integration of behavioral science into poultry production thus represents a convergence of ethical responsibility and practical necessity, aligning animal welfare with the goals of sustainable and efficient agriculture.

Furthermore, growing consumer awareness of animal welfare and ethical food production places additional impetus on producers and policymakers to design systems that respect and foster the behavioral needs of chickens. This shift is not merely a response to market demands, but a reflection of an evolving societal understanding of the intrinsic value of animals as sentient individuals. By fostering environments in which chickens can express their full behavioral repertoire—whether it is through opportunities for dust bathing, nesting, social interaction, or exploration—producers can ensure not only the well-being of their flocks but also contribute to the broader movement toward humane and sustainable food systems.

In conclusion, the study and appreciation of natural behavioral characteristics in chickens bridge the gap between scientific inquiry, ethical stewardship, and practical management. By centering animal welfare in production practices and recognizing the complex behavioral lives of chickens, we take meaningful steps toward a future in which agricultural systems are both productive and humane. The ongoing challenge and opportunity lie in translating this understanding into everyday practice, ensuring that the remarkable behaviors and needs of chickens are respected and supported in all settings.

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CHAPTER 10

Characters and Social Interactions in Chicken Society

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1. INTRODUCTION

Plant breeding and animal domestication are not merely agricultural practices but cornerstone developments that have had wide-reaching implications, catalyzing transformative changes in human societies and their relationship with the natural world. Their study offers invaluable insights into the co-evolution of humans and domesticated species, as well as the ongoing challenges and opportunities presented by contemporary agricultural and environmental concerns. The domestication and breeding of animals have played a fundamental role in the development of human civilization. Throughout history, humans have either directly exploited a wide variety of animal species or utilized the opportunities these animals provided to meet their basic needs, such as food, clothing, transportation, hunting, and protection (Zeder, 2012). Domestication not only brought animals under human control but also led to the transition of human societies to settled life, the beginning of agricultural production, and the complexity of social organization (Larson & Fuller, 2014).

The wolf, known as the first domesticated animal species, evolved into the dog (*Canis lupus familiaris*) and became humanity's oldest companion. Dog domestication constitutes one of the oldest examples of the relationship between humans and animals and is thought to predate other domesticated animal species (Perri, 2016). Throughout history, dogs have played important roles not only in hunting and guarding, but also in providing social and emotional support. The bond formed between animals and humans is considered a dynamic, mutually beneficial relationship. In this context, a close link has been demonstrated between the health and well-being of animals and the psychological and physiological well-being of humans (Beetz et al., 2012). Dogs, cats, other domesticated animals, and farm animals have long been viewed as companions by their caregivers, treated with love and care. Emotional relationships with pets, in particular, reduce people's stress levels, prevent social isolation, and improve their overall quality of life.

From a scientific perspective, sound animal husbandry is not limited to meeting animals' physical needs; it also involves understanding their behavior and responding appropriately. Observing animal behavior allows for the assessment of many important parameters, such as stress, disease, welfare, and adaptation to the environment (Grandin, 2014). This can improve animals' living conditions, increase their productivity, and enhance the quality of human-animal relationships.

Chickens (*Gallus gallus domesticus*) are one of the most widely raised domestic animals in the world and hold a significant place in global food systems for both egg and meat production. In recent years, with increasing public awareness of animal welfare, accurate human understanding of chicken behavior has become increasingly important. Accurate interpretation of chicken behavior not only improves animal welfare but also plays a critical role in production efficiency, disease prevention, sustainability, and ethical production. Human understanding of chicken behavior is essential for monitoring animal welfare. Chickens cannot directly verbally express hunger, thirst, stress, fear, or pain. However, they exhibit various behavioral changes in these situations. For example, feather plucking, excessive inactivity, aggression, cannibalism, abnormal vocalizations, or a tendency to flee can be indicators of welfare disorders or environmental stressors (Rodenburg et al., 2013). Early detection of these behaviors prevents unnecessary animal suffering and reduces potential economic losses for producers. Understanding chicken behavior is an important tool for improving production systems. Behavioral problems are frequently encountered in systems where chickens' natural behavioral needs are not considered, such as those raised in cramped cages or in unenriched environments (Bracke & Hopster, 2006). Encouraging natural behaviors such as free roaming, digging, pecking, and social interaction both enhances the psychological well-being of the animals and contributes to their overall health. Indeed, meeting the behavioral needs of animals helps strengthen their immune systems, increase disease resistance, and reduce existing stress levels (Jones & Dawkins, 2010). Furthermore, understanding chicken behavior is important for human-animal interactions. Scientific research shows that positive interactions with animals reduce their stress levels and increase their productivity. In particular, farm workers and caretakers who observe chicken behavior and accurately assess the health and welfare of the animals provide the opportunity for early intervention (Hemsworth & Coleman, 2011). Furthermore, negative human behaviors, such as suddenly handling, chasing, or disturbing animals with loud noises, can increase chronic stress and lead to behavioral disorders in chickens.

Consumer expectations for products today are no longer limited to product quality and price. In line with increasing social awareness and ethical sensitivities, consumers want to be assured that the animal products they purchase are produced ethically and with respect for animal welfare. Especially in recent years, with the awareness-raising efforts of social media, NGOs, and international organizations on animal welfare, consumer sensitivity to this issue has increased significantly. This has brought pressure on producers for transparency and accountability. In animal welfare-sensitive production systems, environmental regulations are implemented that allow chickens to exhibit their natural behaviors. For example, free-range areas, meeting basic behavioral needs such as digging, soil bathing, pecking, and social interaction contribute to chickens' psychological and physiological health. Consumers want to see labels such as "free range," "organic," "label rouge," "traditional label rouge," and "high welfare standards" on products raised in such systems and are inclined to purchase these products at higher prices. Indeed, research shows that products derived from production systems where animals can exhibit their natural behaviors gain wider public acceptance and increase their market value (Fraser, 2008). This trend is encouraging producers and farm owners to move away from traditional cage systems and toward alternative systems with higher animal welfare standards. Animal welfare-sensitive production is considered not only an ethical necessity but also an economic opportunity. Thanks to this transformation driven by consumer demand, producers gain both social acceptance and the opportunity to increase their market share. Consequently, encouraging systems where chickens can exhibit their natural behaviors not only has positive consequences for animal welfare but also contributes to the widespread adoption of sustainable and ethical food production. Therefore, an accurate and comprehensive understanding of chicken behavior is crucial for ensuring animal welfare and developing ethical and sustainable production systems.

2. CHARACTERS IN A CHICKEN FLOCK

A chick is the name given to the hatched offspring of the domestic chicken species *Gallus gallus domesticus*, which are not yet fully developed and are still in the growth phase. Chicks differ biologically and behaviorally from adult chickens. During the first stages after hatching, they are quite sensitive and require appropriate environmental conditions and care practices for survival. The development and age of chicks are determined by both biological processes and the objectives of production systems. In the scientific literature, the chicken period generally covers the period from hatching to 6–8 weeks of age (North & Bell, 1990; Bell & Weaver, 2002). During this period, chicks undergo rapid growth and undergo fundamental developmental changes such as feathering, the acquisition of thermoregulation (body temperature control), and the maturation of the digestive system. After six weeks of age, chicks become, in most physical and physiological terms, considered young birds. However, depending on different criteria such as breed, rearing purpose, and production system, this age limit can be extended up to 8 weeks (Scanes, 2015).

In backyard poultry farming, the role and behavior of chicks can be more natural and diverse compared to commercial production systems. Backyard-raised chicks are generally raised by a broody hen and have the opportunity to observe natural parental behaviors. The broody hen guides the chicks, encouraging them to find food, dig, and protect themselves from danger. During this period, chicks socialize, explore their environment, and exhibit natural behaviors typical of their species, such as pecking and scratching. Furthermore, backyard-raised chicks generally grow under less stress, and their immune systems are naturally strengthened. Providing clean water, appropriate feed, adequate temperature, and a sheltered area are key priorities for the healthy development of chicks in this environment. In commercial poultry farming, raising chicks requires a different approach.

In commercial production, particularly in broiler and layer lines, chicks are generally raised in large groups and under controlled environmental conditions. Hatched chicks are initially kept in hygienic coops with high temperatures and low airflow, and fed with modern feeding systems (Leeson & Summers, 2005). In commercial broiler production, chicks are rapidly reared until 5-6 weeks of age and then sent to slaughter, while in layer lines, chicks are raised until approximately 16-18 weeks of age, after which they transition to egg-laying. In commercial systems, environmental factors such as temperature, humidity, ventilation, and lighting are meticulously controlled to ensure healthy and rapid chick development. Furthermore, vaccination programs, biosecurity measures, and regular health checks are implemented to prevent disease in commercial production (Bell & Weaver, 2002). In both production systems, care for chicks during their first weeks is crucial. Improper practices during this period can lead to chick mortality, developmental disorders, and later economic losses. With proper care, chicks grow healthily, reach adulthood, and contribute to their expected production potential.

Backyard farming is considered advantageous from an ethical and welfare perspective because it allows the animals to exhibit their natural behaviors, while commercial production prioritizes high yields and economic sustainability. In conclusion, chicks are the cornerstone of chicken farming, both in backyard and commercial production. The first 6-8 weeks of their life are a critical period for them to reach their health, welfare, and productive potential. Understanding and optimally meeting the biological and behavioral needs of chicks is essential for successful poultry farming in both small-scale and large-scale production systems.

In chicken species, growth and development are divided into a series of biological stages. Among these, the "pullet" and "cockerel" stages are critical stages during which young chickens are named according to their sex and undergo significant physiological changes. "Pullet" refers to the juvenile stage, when a female hen hatches but has not yet begun laying eggs. "Cockerel" refers to a young individual of the same age range but is male. These two terms are crucial for the proper management of animals based on their age, development, and production objectives, particularly in commercial and scientific poultry farming (Scanes, 2015; Bell & Weaver, 2002). The pullet and cockerel stages generally begin after the brooding period ends. Chicks are referred to by this name until approximately 6-8 weeks of age, after which time females are called pullets and males are called cockerels. The pullet stage typically lasts from 6-8 weeks of age to approximately 16-20 weeks of age. The cockerel period similarly covers the same age range (North & Bell, 1990; Leeson & Summers, 2005). However, these time intervals vary depending on the chicken breed. Generally, sexual maturity occurs early (16-18 weeks) in light breeds (Leghorn, Bantam Cochin, Thüringen, Serema), while it can extend up to 40 weeks in the Brahma breed. Furthermore, within the same breed, the age of sexual maturity can vary between males and females. For example, Rhode Island Red pullets begin laying eggs at 21-22 weeks, while fertile eggs from cockerels can only be obtained after 30 weeks.

The pullet and cockerel periods are a period during which animals experience rapid growth and development, enter puberty, and sex-related secondary characteristics begin to emerge. In pullets (young female chickens), body weight increases, skeletal structure develops, feather shedding is complete, and reproductive organs begin to mature. Egg-laying generally begins around 18-20 weeks of age (Bell & Weaver, 2002). During this period, appropriate feeding, lighting, and environmental conditions play a decisive role in future egg production. Overfeeding or underfeeding can lead to decreased productivity or health problems later in life (Leeson & Summers, 2005). During the cockerel period, male chicks continue to grow rapidly and develop sex-specific secondary characteristics (comb and wattles growth, changes in plumage, and the onset of singing behavior). Testes begin to mature during this period, and they reach sexual maturity at approximately 16-20 weeks of age. Cockerels are the males to be selected for future breeding in breeding flocks. Appropriate feeding and environmental conditions during this period are crucial for future reproductive success and health (Scanes, 2015).

Behavioral developments are also observed during this period. Pullets and cockerels begin to establish social hierarchies within the flock, pecking orders become clear, and adult social behaviors emerge. In cockerels dominance behaviors and increased vocalization are observed in the following weeks. In commercial production, these periods are critical stages in shaping the animals' future production potential. The healthy and appropriate development of pullets is crucial for egg-laying productivity. Similarly, the healthy growth and development of cockerels allocated for breeding directly impacts reproductive success. Vaccination programs, biosecurity practices, and flock management must be implemented meticulously during this period (Bell & Weaver, 2002).

In chicken (*Gallus gallus domesticus*) societies, the roles individuals assume throughout their life cycles vary according to both their biological sex and age. In this context, the terms "hen" and "rooster" are used to describe adult female and male individuals within the chicken society. These two concepts represent not only individual developmental stages but also the cornerstones of social structure, reproductive strategies, and production efficiency within the flock. A hen is a female chicken that has reached sexual maturity and is capable of laying eggs. Female chickens typically begin laying their first eggs when they reach 18-20 weeks of age, at which point they are called hens (Bell & Weaver, 2002; Leeson & Summers, 2005). In addition to egg production within a chicken flock, hens can also exhibit maternal behaviors such as incubation and brood care in natural settings. Especially in backyard or free-range flocks, natural maternal behaviors (such as incubating their own eggs, protecting and feeding their chicks) are quite prominent. In contrast, in modern commercial egg production, high egg production is achieved by using lines with suppressed brooding instincts, and maternal behaviors are generally not observed (Bell & Weaver, 2002).

Hens also play a role in the formation of the social structure within the flock, interacting with other females and roosters; they are active participants in maintaining the flock's hierarchy, foraging, and detecting danger. A rooster is a male chicken that has reached sexual maturity and is capable of reproduction. Similarly, male chickens reach sexual maturity at approximately 16-20 weeks of age, after which they are called roosters (Scanes, 2015; North & Bell, 1990). The primary biological function of roosters is to mate with females (hens) to contribute to reproduction. However, roosters' functions are not limited to producing fertilized eggs; they also play a central role in maintaining the social order and security of the flock. Roosters typically emerge as the most dominant male in the flock and occupy the top of the social hierarchy. They demonstrate dominance in their interactions with other roosters and females, establishing authority over the flock through secondary sexual characteristics such as vocalization, feather ruff, and crest and wattles. Roosters help maintain flock cohesion and serve as warning mechanisms against external threats. In natural habitats or free-ranging flocks, they both warn and direct the flock with loud vocalizations when danger is detected. They also signal to females when food is available, ensuring the sharing of food resources. Roosters' behaviors in maintaining and maintaining social order reduce stress within the flock and enhance individual welfare. When multiple roosters are present, dominance contests between them are often determined by physical combat and vocalization. This social competition contributes to increased genetic diversity and facilitates natural selection processes (Scanes, 2015). Before reaching the hen and rooster stages, chickens undergo specific developmental stages: chick stage (0-6/8 weeks), pullet/cockerel stage (6/8-16/20 weeks), and then adult stage (hen/rooster). Sexual maturity begins with the first egg in females and with the development of functional testes in males. This process is determined not only by genetic factors but also by external factors such as nutrition, environmental conditions, lighting, and flock size (Leeson & Summers, 2005; Scanes, 2015). In both natural and industrial poultry farming, the management of hens and roosters is crucial. A sufficient number of healthy roosters increases the rate of fertilized eggs and hatching success, while healthy hens ensure optimal egg and chick production. Particularly in breeding flocks, the rooster:hen ratio (usually 1:8-10) is a critical parameter for fertility and production success (Bell & Weaver, 2002). Furthermore, the social structure and behavioral balance within the flock directly impact the stress, immune status, and ultimately productivity of the animals. In systems where hens and roosters coexist, the display of natural behaviors is also considered important for animal welfare (Fraser, 2008).

3. SOCIAL LIFE IN A CHICKEN FLOCK

The social life of domestic chickens is complex and well-organized, supporting their well-being both individually and at the group level. Understanding flock organization and social hierarchy in chickens is crucial for observing their natural behaviors and for creating healthier and more peaceful flocks in modern chicken farming. Domestic chickens feel much safer and more at peace when they are in a community. This is because chickens are biologically and behaviorally gregarious animals and require community membership to survive in nature and meet their basic needs, such as feeding and reproduction. A flock protects individuals from predators and other environmental hazards, while also providing a sense of social security and belonging. However, reaping the benefits of this community comes at a cost for the chickens. Each individual must adhere to certain social rules and hierarchies within the flock, deferring to superiors when necessary. A clear and definite hierarchical structure within the flock is essential for a well-functioning community. This social structure is shaped by a hierarchy, also known as the "pecking order," in which each hen or hen within the flock has a specific position. This hierarchical structure is typically formed through social struggles and fighting as chickens transition from juvenile to adult. Young chickens compete with each other to determine their place within the flock, and through these struggles, each individual's position within the flock is determined. This social status generally remains relatively unchanged after reaching adulthood, and the hen maintains this position throughout her life. Such hierarchical structures not only reduce inter-individual conflict but also facilitate order and resource sharing within the flock. Establishing social order reduces chickens' stress levels and ensures the sustainability of the advantages of group living. Ultimately, the complex social behaviors chickens exhibit while living in groups enable them to live healthy and balanced lives in accordance with their nature (Peitz and Pertz, 2007).

Red Jungle Fowl (*Gallus gallus*), the ancestors of chickens, live in small groups in the wild. These groups typically consist of a rooster, several females, and their young. In nature, this form of communal living has evolutionary advantages: flocks protect individuals from predators, facilitate food acquisition, and help reduce stress through social interactions. Chickens have largely retained this behavioral legacy since domestication. In chickens, a flock not only signifies coexistence but also the existence of a specific social order and division of labor. Social bonds and interactions among individuals within a flock are quite intense. Activities such as feeding, drinking, resting, roosting, and soil bathing are generally carried out in groups. Social interactions between chickens reduce stress within the group and allow individuals to learn from each other. The most obvious aspect of social order in chicken flocks is their hierarchical structure. This system, called the "pecking order," determines the social status of the chickens. Pecking order was first described in 1921 by Norwegian zoologist Thorleif Schjelderup-Ebbe. Each individual in the flock holds a specific social position relative to others, and this position is reflected in their interactions. Pecking order determines power relations and access rights within the flock. Higher-ranking hens (alpha individuals) have priority access to feed, water, and the best perches. Lower-ranking hens, however, often have to wait, and sometimes even submit, to access resources. The hierarchical structure within a flock is often formed through constant minor conflicts, threatening behavior, and physical struggles. These struggles become particularly pronounced when new individuals join the flock or when young hens mature. Pecking order is often determined through struggles that occur during juvenile development. Hens demonstrate their dominance through behaviors such as head-bobbing, wing-expanding, feather-ruffling, lunging, and direct pecking. Status within a flock is linked to the physical strength, age, experience, and aggressiveness of individuals. Once established, social status generally remains stable, and hens adhere to this order, often without engaging in serious fighting. However, when a new hen joins the flock, an individual becomes weakened due to illness or weakness, or a leader dies, the hierarchical structure is reshaped, and short-term conflicts can increase. Therefore, one of the most obvious sources of social stress in chickens is changes in group dynamics. Chickens use a variety of communication methods to maintain social hierarchies and regulate relationships within their groups. Examples include pecking, feather fluttering, wing flapping, vocalizations (vocal warnings), specific postures, and threatening stares. Furthermore, advanced social behaviors such as empathy, social learning, and imitation are also observed among chickens.

Social structure and hierarchy are critical for both welfare and production in commercial chicken production. Overcrowding, inappropriate environmental conditions, and excessive flock size can lead to abnormally aggressive behavior, injuries, and stress-related health problems among chickens. Therefore, considering social relationships and hierarchical structure among individuals in flock management is essential for both animal welfare and productivity. It is known that some chicken breeds (e.g., Sussex, Rhode Island Red, etc.) are more docile, while others (e.g., Aseel, Plymouth Rock) are more aggressive. This suggests that behavioral traits are related to both genetics and environmental factors. Environmental improvement efforts have been conducted to specifically prevent aggressive behavior. Examples include red-colored equipment, low lighting intensity, and calming wavelengths. In recent studies, some herbal extracts with antidepressant and sedative properties are added to chicken feed and drinking water. Some behavioral traits have also been used as selection criteria in poultry breeding. Some behavioral traits have disappeared or become more active due to their association with productivity. For example, brooding behavior is negatively genetically linked to the prolactin gene, which is associated with egg production, and has disappeared in chickens genetically improved for egg production. Conversely, the frequency of observed behaviors such as laying and feeding has increased in genotypes genetically improved for meat production.

4. ROLES OF CHARACTERS IN CHICKEN SOCIETY

The early weeks of life in chickens represent the most dynamic and formative period for social behavior and flock organization. Scientific research shows that chicken chicks (*Gallus gallus domesticus*) display remarkable levels of activity, playfulness, and social engagement during the initial 10–12 weeks post-hatch (Estevez, 2002). This period is characterized by frequent interactions, exploratory movements, and diverse playful behaviors, all of which serve as essential foundations for the development of social skills and the establishment of flock cohesion. Play behavior is particularly salient in young chicks and serves multiple developmental functions. One of the most commonly observed forms of play involves one chick picking up a small object or piece of litter in its beak and running, prompting several other chicks to chase after it. This "chase and retrieve" sequence not only stimulates physical activity but also acts as an early vehicle for social communication, competition, and learning about group dynamics (Jones & Faure, 1981). Through such playful interactions, chicks begin to recognize individual flock members, practice communication signals, and learn the basic rules of social engagement. Another frequently observed behavior is the sudden spreading of wings and running, often used by chicks to startle or displace their peers. These displays are not merely expressions of energy; they are early manifestations of the social hierarchy, or "pecking order," that will become more pronounced as the birds approach adolescence. Through playful skirmishes and mock confrontations, chicks assess each other's strength, speed, and assertiveness, laying the groundwork for future positions within the flock's social structure. These early playful competitions play a crucial role in both physical and psychological development. Physically, they help chicks enhance their coordination, agility, and reflexes. Socially, these activities foster the emergence of social bonds, improve conflict resolution skills, and reduce the likelihood of severe aggression later in life by allowing individuals to learn non-injurious ways to resolve disputes (Estevez, 2002; Fraser, 2008). Chicks learn to test boundaries, negotiate status, and develop the courage and resilience required for adult flock life. However, as chicks approach the transitional phase into adolescence—generally around 12 to 16 weeks of age—these innocent and playful behaviors often give way to more serious and sometimes violent dominance struggles. This period is marked by a sharp increase in competition as individuals strive to establish and stabilize their social status within the group (Guhl, 1953). Dominance interactions become especially pronounced among males, where fierce contests for rank can be observed. Physical aggression in these confrontations may involve pecking, wing flapping, feather plucking, and even chasing, which can occasionally escalate to injuries if competition is intense or resources are scarce (Fraser, 2008). The establishment of a stable pecking order through these dominance struggles is a fundamental feature of chicken social organization, as it helps minimize future conflict and allows for more predictable and harmonious group dynamics.

Upon reaching true sexual maturity—typically between 18 and 24 weeks of age for domestic chickens (*Gallus gallus domesticus*)—the previously intense rivalries and status competitions within the flock subside significantly. This transition marks the establishment of a stable social hierarchy, commonly referred to as the "pecking order," a term first introduced by Schjelderup-Ebbe in the early 20th century and supported by subsequent ethological studies (Schjelderup-Ebbe, 1922; Guhl, 1953). In this structured social system, each member of the flock recognizes their own status relative to others. The pecking order is characterized by a linear ranking, where dominant individuals consistently assert priority access to resources such as food, water, mates, and preferred roosting sites, while subordinate birds yield, thus reducing the need for continued aggressive encounters (Fraser, 2008). This hierarchical arrangement offers significant adaptive value for flock-living species. Once the pecking order is established, unnecessary conflicts and energy expenditure are minimized. Each hen, in understanding her rank, avoids direct challenges with higher-ranking individuals, maintaining respectful social distances and yielding when necessary. Such social awareness is routinely demonstrated through subtle behaviors—such as yielding space, lowering the head, or avoiding eye contact—rather than through overt aggression (Estevez, 2002). This "social distance" within the flock exemplifies the respect and status-consciousness that hens maintain toward one another, and it is essential for flock cohesion and individual well-being. However, even with a well-established hierarchy, chicken flocks are not entirely free from social tension. Competition, jealousy, and envy persist, particularly around access to limited or high-value resources. Common triggers for conflict include disputes over preferred or dominant mates during the breeding season, competition for the best feeding or watering spots, and access to the most comfortable or secure perching and nesting sites (Mench & Keeling, 2001). Alpha hens—those at the top of the social hierarchy—usually gain first access to these resources, reinforcing their dominant status. Subordinate hens, on the other hand, must either wait, use less favorable resources, or develop alternative strategies to meet their needs (Guhl, 1953; Fraser, 2008). Occasionally, lower-ranking hens may challenge the existing social order in an attempt to ascend the hierarchy. Such challenges typically provoke a display of dominance by higher-ranking individuals, manifesting as wing-flapping, feather-ruffling, chasing, and direct pecking. These confrontations, though often brief, serve to reaffirm the current social structure. In most cases, the more dominant or socially adept hen prevails, and the pecking order remains largely intact. Such episodic competitions, while seemingly disruptive, are integral to the maintenance of order and peace within the flock by clarifying boundaries and reinforcing social roles. The stability provided by a clear hierarchy is critical for both individual and collective welfare. It reduces the incidence of chronic stress, mitigates the risks of severe injury, and optimizes the allocation of resources within the group (Estevez, 2002; Fraser, 2008). Social hierarchy enables individuals to understand their roles and boundaries, facilitating smoother interactions and reducing the frequency and severity of aggressive encounters. This, in turn, promotes not only peace and security among flock members but also enhances overall productivity and flock health. In commercial and backyard poultry systems alike, disruption of the pecking order—such as by the sudden addition or removal of birds—can result in increased aggression, injury, and reduced productivity, underscoring the biological and practical importance of stable social structures (Mench & Keeling, 2001). In summary, social life and hierarchical organization are fundamental to the well-being, order, and efficiency of chicken flocks. The pecking order provides a framework for social interactions, minimizes conflict, and ensures a more equitable, predictable distribution of resources. Its existence is a testament to the complex social intelligence and adaptive strategies of chickens, contributing significantly to their evolutionary and practical success as one of the world’s most widespread domesticated animals.

In chicken society, an individual's hierarchical position within the flock is determined by the complex interaction of numerous physical and behavioral factors. One of the most important factors that allows a chicken to occupy a high position in the flock is its confident and courageous stance. Its tendency to be aggressive, its fighting spirit, and its endurance in the face of adversity also play a decisive role in achieving this status. Furthermore, physical appearance is another important factor influencing social standing; characteristics such as a sleek body structure, shiny and vibrant feathers, and a large and healthy comb increase attention and influence within the flock. Furthermore, age is a crucial factor in shaping the hierarchy. Older chickens generally occupy a higher position than younger, more inexperienced ones. This is related to the advantage older individuals have in maintaining their previously achieved social status against younger individuals. However, as physical strength and health decline with age, their status can be challenged by younger, more vigorous individuals. As long as flock size remains constant, the established social order is generally maintained for a long time. However, because chickens have limited social memory, an individual removed from the flock for any reason (such as illness, care, competition, or rearing in a different coop) can often lose its former status upon return. Other individuals in the flock often fail to recognize and re-evaluate newly added individuals who have been absent for extended periods. This leads to the new or returning individual engaging in social competition with other hens to reassess their status, often forcing them to climb the social ladder again.

The social hierarchy—or "pecking order"—within a chicken flock is not a static construct, but rather a fluid and dynamic structure that can be perturbed by any change in group composition. The addition or removal of even a single individual can disrupt the established social equilibrium, triggering a process of renegotiation in which the hierarchical order is reestablished (Guhl, 1953; Mench & Keeling, 2001). This phenomenon is rooted in the fact that chickens, like many social animals, rely on repeated interactions and recognition of conspecifics to maintain a stable social order (Fraser, 2008).

Introducing a new hen into an established flock—or reintroducing an individual after a prolonged absence—requires careful management to minimize the risk of severe aggression and social stress. The sudden presence of an unfamiliar or "forgotten" bird is commonly met with hostility, as resident flock members perceive the newcomer as a potential threat to their established ranks and access to resources (Estevez, 2002). Aggressive behaviors such as pecking, chasing, feather pulling, and even group attacks can occur, potentially resulting in injury or chronic stress for the new individual. To mitigate these negative outcomes, poultry keepers and researchers have developed several practical strategies. One widely recommended method is to introduce the new or returning hen at dusk or after nightfall, when the flock is already roosting and their activity is minimal (Mench & Keeling, 2001). By quietly placing the bird in the coop during this period of reduced arousal, the flock's ability to immediately identify and challenge the newcomer is diminished. In the morning, when all birds wake up together, the presence of the new individual is less likely to provoke a sudden, collective attack. While this approach can reduce initial aggression, it does not completely eliminate the need for the newcomer to "earn" its place within the social hierarchy. The process of acceptance into the flock, or regaining a former status after absence, is typically gradual. The new individual must participate in a series of social interactions, often involving displays of submission or brief confrontations, to establish its place within the pecking order (Estevez, 2002). Factors such as age, size, physical health, previous social experience, and even feather coloration can influence an individual's ability to ascend the hierarchy (Guhl, 1953; Fraser, 2008). For example, older or more robust hens may be more successful in asserting dominance, whereas younger or less assertive individuals may remain subordinate.

It is also important to note that social status in chickens is not fixed for life; it may shift in response to ongoing changes within the flock. Events such as the removal of a dominant hen, the addition of multiple new birds, or significant changes in resource availability can all lead to further renegotiation of the social order (Mench & Keeling, 2001). These dynamics underscore the need for careful, informed management in backyard and commercial poultry systems alike. Failure to account for the fluidity of social hierarchy can result in chronic stress, increased injuries, reduced productivity, and compromised animal welfare. Therefore, understanding and considering social dynamics in flock management is essential. Providing sufficient space, environmental enrichment, and resources can help reduce the severity of dominance-related aggression and facilitate smoother integration of new individuals (Fraser, 2008; Estevez, 2002). Ultimately, respectful and welfare-oriented management practices not only enhance the well-being of individual chickens but also contribute to the stability and productivity of the entire flock.

One of the most prominent and functionally essential elements of social hierarchy in chicken flocks is undoubtedly the rooster. The rooster's status as flock leader arises from a combination of physical strength, social skills, experience, and assertive behavior. This leadership is not a mere artifact of physical dominance; it reflects the rooster's capacity to mediate conflict, provide collective security, and promote social cohesion within the group (Guhl, 1953; Schjelderup-Ebbe, 1922). Typically, a rooster's rise to leadership within the flock is the result of a series of competitive interactions among male individuals—sometimes involving elaborate displays, vocalizations (crowings), and, when necessary, direct physical confrontations. Through these interactions, the rooster demonstrates his dominance by asserting himself in disputes, defending resources, and showing courage in the face of potential threats. Once a rooster has established his position at the top of the social hierarchy, his leadership is generally recognized by all members of the flock, and outright challenges become rare, especially if the rooster remains in good condition and continues to assert his authority (Guhl, 1953).

The responsibilities of flock leadership, however, are considerable. Beyond being the primary breeding individual, the rooster acts as a social coordinator, helping to manage the complex relationships among hens. Research shows that roosters often intervene in disputes among hens, using their physical presence, vocalizations, and sometimes gentle pecking or herding behaviors to defuse tensions and restore order (Collias, 1943). This mediation is particularly evident during competition for resources such as food, water, nesting or roosting sites, where the rooster's presence can prevent escalation of aggressive interactions among hens. Additionally, one of the rooster's most critical roles is that of a sentinel and protector. Roosters are highly vigilant and respond quickly to potential dangers, issuing loud alarm calls and adopting conspicuous postures to alert and rally the flock. When a predator is detected, the rooster often positions himself between the threat and the hens, sometimes even launching direct attacks to defend the flock (Collias, 1943). The presence of a rooster has been documented to reduce fear and stress levels among hens, leading to calmer social dynamics and higher productivity in the flock (Mench & Keeling, 2001). The presence or absence of a rooster has substantial effects on social order and harmony within the group. Flocks with a resident rooster generally exhibit fewer severe fights and less chronic tension among hens, whereas all-female (hen-only) groups tend to experience more frequent and intense competition, particularly around critical resources. In such hen-only flocks, dominant hens may adopt some "male-typical" behaviors and increase their aggression to fill the leadership vacuum (Fraser, 2008; Mench & Keeling, 2001). The rooster's leadership is also closely linked to reproductive behavior and success. Hens in the presence of a dominant, experienced rooster tend to be more relaxed and productive, showing higher rates of egg laying and more synchronized reproductive cycles (Jones & Faure, 1981). The rooster's courtship behavior—such as tidbitting (offering food), dance displays, and vocalizations—not only stimulates mating but also reinforces his social authority and the flock's cohesion.

In flocks with multiple roosters, a dominance hierarchy among the males usually develops. The oldest or most experienced rooster often retains the top position, while younger males remain subordinate, even if they surpass the dominant rooster in physical strength as they mature. This phenomenon demonstrates that experience and established social relationships can outweigh mere physical prowess in determining leadership; social memory and past achievements are highly valued within chicken societies (Guhl, 1953). However, the rooster's leadership is not always uncontested, especially in large flocks or those with particularly assertive hens. Occasionally, dominant hens may challenge the rooster's authority, attempting to exclude him from prime resources or undermining his role as mediator. In these cases, the rooster must remain vigilant, balancing his interactions to maintain both his dominance and group harmony. The complexity of these social dynamics highlights that leadership in chickens is not a simple function of sex or strength, but a sophisticated interplay of individual personality, social experience, and the unique makeup of the group (Mench & Keeling, 2001). In conclusion, the rooster's role within the flock extends far beyond being a breeding partner or symbol of dominance. He is a linchpin for social order, conflict management, and group security. The rooster's presence stabilizes the flock, mitigates conflict, and enhances reproductive success, but his leadership must constantly adapt to the shifting dynamics and personalities within the group. Effective flock management and welfare practices must recognize the nuanced, essential contributions of the rooster to the maintenance of peace, productivity, and social structure in chicken communities.

5. CONCLUSION

The social organization, behavioral repertoire, and welfare considerations of the domestic chicken reveal a remarkable degree of complexity and adaptability within a species that has been a cornerstone of human agriculture for millennia. As we have explored, the domestication and selective breeding of chickens have contributed not only to advances in food security but also to profound transformations in human societal structures, economic systems, and ethical frameworks. Understanding the behavioral ecology of chickens including their early life stages, the establishment and maintenance of social hierarchies, and the intricate interactions between individuals provides critical insights that extend far beyond the poultry house.

The chicken flock is a dynamic social entity, shaped by the interplay of innate biological drives, environmental influences, and individual experiences. From the earliest days post-hatch, chicks are engaged in a process of social learning and skill acquisition that lays the groundwork for lifelong adaptation and group cohesion. The development of the pecking order—a linear and often stable hierarchy—serves as a fundamental mechanism for minimizing conflict, regulating access to resources, and promoting group stability. Within this framework, the roles assumed by hens, roosters, pullets, and cockerels are not static; rather, they evolve in response to changing internal and external conditions, reflecting the fluidity of social dynamics in animal societies.

In both traditional and modern production systems, the well-being of chickens is inextricably linked to the degree to which their behavioral needs are understood and met. Allowing chickens to engage in natural behaviors such as foraging, dust bathing, social interaction, and play is not merely a matter of ethical necessity but also a determinant of physiological health and productivity. Conversely, environments that constrain these behaviors or induce chronic stress can precipitate a range of welfare problems including increased aggression, feather pecking, and reduced resistance to disease that have direct economic and ethical implications.

The role of human stewardship in shaping the quality of life for chickens cannot be overstated. Attentive management that takes into account the social structure, environmental enrichment, and psychological well-being of the flock translates into tangible benefits for both the animals and the producers. Moreover, growing consumer awareness and demand for ethically produced animal products are driving a shift toward farming practices that prioritize animal welfare and sustainability. This transformation is not only reshaping the marketplace but also fostering a deeper societal appreciation for the sentience and social sophistication of animals long regarded simply as commodities.

Ultimately, a comprehensive and empathetic understanding of chicken behavior and sociality enriches our scientific knowledge, informs best practices in animal husbandry, and challenges us to reconsider our responsibilities within the human-animal relationship. As research continues to elucidate the cognitive and emotional capacities of chickens, the imperative to provide environments that respect their natural instincts and promote their well-being becomes ever clearer. Thus, the study of chicken society is not an isolated endeavor but a vital component of the broader pursuit of ethical, sustainable, and humane food production systems for the future.

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CHAPTER 11

Principles of Anesthesia in Fish

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1. INTRODUCTION

Fish possess physiological specializations for aquatic life and, in many species, may rapidly succumb to asphyxia shortly after removal from water despite abundant atmospheric oxygen (Sedgwick, 1986). Considering the unique physiological adaptations of fish, prudent care is necessary when conducting any procedure with them. Immobilizing fish prior to even routine tasks is often imperative due to their inherent resistance during capture and handling, which directly affects their physiology and behavior (Iversen et al., 2013). Accordingly, anesthesia protocols are routinely applied in fish across diverse settings, ranging from standard husbandry and therapeutic management to imaging-based diagnostics, surgical interventions, and controlled experimental work (Sedgwick, 1986; Weber et al., 2009). In these contexts, calming aquatic animals and reducing their metabolic rates and stress levels are essential for aquaculture, veterinary care, and scientific research focused on aquatic life and fish populations (Anderson et al., 1997; Coyle et al., 2004; Cooper, 2011; Sneddon, 2012; Iversen et al., 2013; Küçük et al., 2016; Chatigny et al., 2018). Anesthesia and sedation reduce stress, pain, and the potential for self-inflicted injury during various procedures, including handling, diagnostics, and surgeries (Brown, 1988; Weber et al., 2009; Paruğ, 2012; Sladky and Clarke, 2016), and routine aquaculture operations such as sorting (Chatain and Corrao, 1992; Koumoundouros et al., 1997; Basaran et al., 2007), transportation (Cao et al., 2021), vaccination, grading, tagging, and morphometric measurements (Gilderhus and Marking, 1987; Brown, 1988; Munday and Wilson, 1997; Small, 2004; Bircan-Yildirim et al., 2010; Alagöz et al., 2021), blood sampling, and gamete collection via gonadal biopsy, stripping, or milt expression (Mattson and Riple, 1989; Küçük and Çoban, 2016; Basaran, Şen, et al., 2007).

1.1. Fish anesthesia: purpose and importance

Fish anesthesia is primarily applied to ensure humane handling and to minimize procedure-related stress and injury risks in both aquaculture and research settings. By attenuating acute stress responses and facilitating controlled manipulation, anesthesia improves animal welfare and also enhances the reliability of physiological and behavioral measurements obtained during sampling, diagnostics, or surgery (Brown, 1988; Weber et al., 2009; Sneddon, 2012; Sladky and Clarke, 2016).

1.2. Key definitions

In practical terms, sedation refers to a state of reduced activity and responsiveness that allows handling with minimal struggle, whereas anesthesia implies a deeper depression of the central nervous system, typically characterized by loss of equilibrium, diminished reflexes, and the inability to respond appropriately to stimuli. Analgesia, in contrast, denotes the reduction of pain perception and nociceptive processing; importantly, immobilization alone should not be assumed to guarantee analgesia, and the intended depth of anesthesia should be matched to the invasiveness of the procedure (Neiffer and Stamper, 2009; Sneddon, 2012; Chatigny et al., 2018). In this chapter, the term "deep anesthesia" is used to denote the stage of anesthesia sufficient for clearly invasive or surgical procedures, corresponding to what is often referred to as "surgical anesthesia" in clinical classifications. In the following sections, the progression from light sedation to deep anesthesia and subsequent recovery will be described using practical staging criteria to guide procedure-specific applications.

2. HISTORICAL OVERVIEW

The history of fish anesthesia extends back to early practices in which plant-derived extracts and other naturally occurring agents—some with ichthyotoxic or narcotizing effects—were introduced into water bodies to immobilize fish for capture. In different regions, rotenone-containing plants (e.g., *Derris spp*.) and other plant-derived infusions were traditionally used to immobilize fish and facilitate harvesting (Sedgwick, 1986). In the Nicobar Islands of India, seeds of Barringtonia asiatica were used for their ichthyotoxic effects in shallow tidewaters; following the tsunami of December 26, 2004, this practice markedly declined (Ravikumar et al., 2015). More broadly, many plant species have been used worldwide for fish capture (Dartay and Ateşşahin, 2010). Plant-derived toxins such as rotenone and croton oil have also been applied for pest control in tropical fishponds, but many such extracts are not selective and may exhibit considerable toxicity to a wide range of organisms, including vertebrates, invertebrates, and microorganisms (Baird, 1994). In contrast, extracts from some ichthyotoxic plants, such as spurge, may resemble anesthetic effects when consciously and appropriately applied at moderate concentrations (Ross and Ross, 2008; Alagöz et al., 2021).

The development of fish anesthesia has largely paralleled progress in human and veterinary anesthesia, with adaptations required by the aquatic environment. The transition from ether and chloroform in the 19th century to more refined and specific protocols today reflects broader advances in anesthesiology (Juvin and Desmonts, 2000; Paruğ, 2012; Robinson and Toledo, 2012). Approaches originally developed for human medicine, including inhalational and intravenous anesthesia, were adapted for use in fish by employing agents that can be delivered via immersion in water (Sedgwick, 1986; Paruğ, 2012).

In the early 1900s, some biologists investigated behavioral responses of aquatic animals by dissolving atmospheric gases (oxygen, nitrogen, carbon dioxide) and chemicals such as ammonia, nitrite, nitrate, and chlorine in water and exposing animals to these conditions (Shelford and Allee, 1913). Clayberg (1917) examined the effects of chloroform and ether on orange-spotted sunfish (*Lepomis humilis*) and described the behavioral responses observed during exposure; these agents were widely used historically but are also associated with notable toxicity concerns (Kazuń and Siwicki, 2012; Gupta, 2019). By the 1930s, ether had begun to be used experimentally for sedating rainbow trout (*Oncorhynchus mykiss*), and anesthetics subsequently became increasingly important in fish research and aquaculture (Summerfelt and Smith, 1990; Walsh and Pease, 2002). Over time, as interactions between fish physiology and anesthetic agents became better understood, safer and less toxic options were introduced (Fig. 1). Against this historical background, modern fish anesthesia can be summarized in terms of how agents enter the body, how they depress neural function, and how practical methods and staging are applied to achieve safe induction and recovery. Accordingly, contemporary fish anesthesia increasingly emphasizes welfare-oriented practice, safety margins, and the standardization of induction–maintenance–recovery procedures. For safe and consistent applications, it is essential to summarize the basic physiological principles, core mechanisms of action, and stress-related responses.

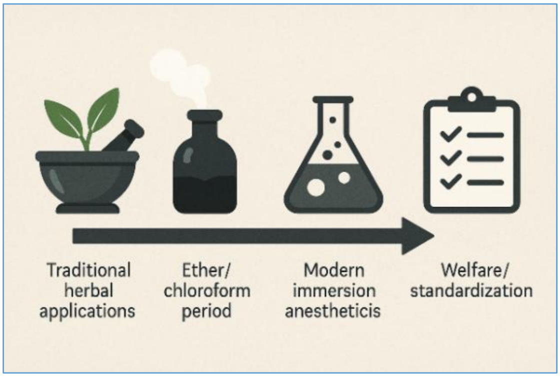

Figure 1. Historical development of fish anesthesia

3. BASIC PRINCIPLES: PHYSIOLOGY, MECHANISMS, AND STRESS

3.1. Uptake via gills and system-level distribution

In aquatic environments, anesthesia is most commonly achieved using pharmacological agents, whereas non-pharmacological methods may be applied in specific contexts. Depending on the agent and protocol, administration is typically by immersion and, less commonly, by injection. In most practical fish anesthesia protocols, agents are delivered via the surrounding water and absorbed primarily across the gill epithelium, which provides a large exchange surface and a short diffusion distance. Accordingly, uptake is closely coupled to ventilation and perfusion dynamics, and waterborne exposure functions as the aquatic analogue of inhalational anesthesia in terrestrial animals (Paruğ, 2012). Conceptually, the efficiency of waterborne uptake across the gills depends on the matching of branchial ventilation (water flow) and perfusion (blood flow), often described as the ventilation–perfusion (V/Q) relationship. Marked mismatches—

whether limited water flow relative to blood flow or limited blood flow relative to water flow—can constrain transfer across the lamellar surface and thereby reduce branchial transfer efficiency (i.e., increase physiological dead space); in immersion anesthesia, such conditions are expected to slow induction and increase variability in uptake kinetics, whereas more balanced conditions tend to support more rapid and predictable induction (Burggren, 1978). Although limited absorption may also occur across the skin and other epithelia, the gills represent the dominant route for systemic entry under immersion conditions (Neiffer and Stamper, 2009; Kazuń and Siwicki, 2012). Following entry into the circulation, anesthetic distribution is determined by circulatory transport and tissue perfusion, such that well-perfused tissues reach effective concentrations earlier and thereby affect the timing of central nervous system effects (Fig. 2). However, it should be noted that the induction, depth, and duration of anesthesia are shaped not only by ventilation–perfusion relationships but also by physicochemical, environmental, and biological determinants that influence effective exposure and tissue uptake, including water temperature, dissolved oxygen, pH, and salinity, as well as species-specific traits, ontogenetic stage (e.g., larval, juvenile, adult), and feeding status (gastrointestinal fullness) (Sylvester, 1975; Berka, 1986; Welker et al., 2007; Topic Popovic et al., 2012; Ballıkaya, 2016; Lee et al., 2023).

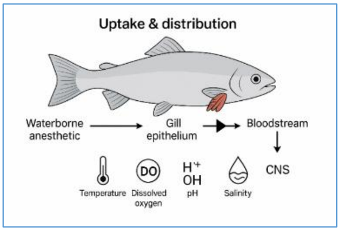

Figure 2. Representative schematic of waterborne anesthetic uptake and CNS distribution in fish

3.2. General mechanisms of action (CNS depression)

By reducing neuronal excitability and disrupting effective signal transmission within the central nervous system (CNS), anesthetic agents induce a progressive depression of neural function in fish. Mechanistically, this depression reflects compound-dependent actions on neuronal membranes, ion channels (Hedrick and Winmill, 2003), and synaptic pathways; depending on the agent, inhibitory transmission may be enhanced and/or excitatory transmission may be reduced, thereby decreasing network activity and responsiveness. For some commonly used agents, a prominent cellular component is the inhibition of voltage-gated Na^+ channels, which reduces action potential initiation and conduction and can attenuate afferent sensory input, including nociceptive signal transmission, contributing to loss of responsiveness at deeper planes (Velíšek et al., 2007; Ramlochansingh et al., 2014). This results in a loss of sensory function, equilibrium, movement, consciousness, and reflex action (Ucar and Atamanalp, 2010; Lozic-Djuric, 2015; Hao et al., 2020). In this manner, depending on the depth of anesthesia, it is possible to handle the fish easily during sedation, and all types of applications can be performed safely and gently during deep anesthesia (Weber et al., 2009; Sladky and Clarke, 2016; Brønstad, 2022). In practical terms, recovery largely depends on the decline of effective anesthetic concentrations at the target tissues, which is facilitated by adequate ventilation, circulation, and appropriate recovery-water conditions (Neiffer and Stamper, 2009).

3.3. Stress physiology in fish (HPI axis) and modulation by anesthesia

Anesthetics are instrumental in mitigating stress in aquaculture and research settings by supporting calmer handling and, when appropriately selected and applied, may attenuate neuroendocrine and metabolic components of the stress response (Martins et al., 2012). In teleost fish, acute stress is commonly framed through activation of the hypothalamic–pituitary–interrenal (HPI) axis, in which hypothalamic corticotropin-releasing hormone (CRH) stimulates pituitary adrenocorticotropic hormone (ACTH) release, which in turn activates interrenal steroidogenic cells to increase cortisol synthesis (Barton, 2002; Martínez-Porchas and Martínez-Córdova, 2009; Saccol et al., 2018; Sadoul and Geffroy, 2019) (Fig. 3). Cortisol is therefore a central endocrine mediator of the stress response, coordinating energy metabolism (e.g., gluconeogenesis/glycolysis) and contributing to ion–water balance; accordingly, elevations in plasma cortisol are frequently accompanied by increases in circulating glucose and lactate, and metabolic hormones such as insulin may also be evaluated depending on study objectives (Wendelaar Bonga, 1997; Pankhurst, 2011; Burt et al., 2013).

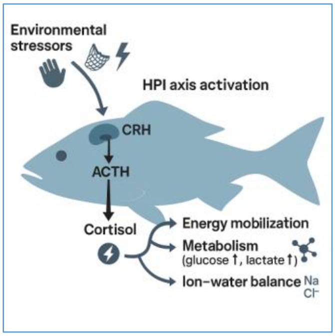

Figure 3. Schematic representation of the hypothalamic–pituitary–interrenal (HPI) axis in teleost fish.

Environmental and handling-related stressors activate hypothalamic corticotropin-releasing hormone (CRH) release, which stimulates pituitary adrenocorticotropic hormone (ACTH) secretion and subsequently promotes cortisol synthesis in interrenal tissue. Cortisol acts as a central endocrine mediator coordinating metabolic responses, ion–water balance, and energy mobilization during acute stress.

Beyond their direct pharmacological actions, anesthetic agents functionally modulate the stress response by reducing behavioral and sensory reactivity to environmental stimuli (Fig. 4). From light sedation onward, diminished perception of external cues, reduced locomotor activity, and a progressive slowing of metabolic processes constrain the amplification of HPI axis activation that would otherwise occur during handling or environmental disturbance (Barton, 2002; Martins et al., 2012; Sadoul and Geffroy, 2019).

Accordingly, researchers frequently benchmark anesthetic performance (including new organic alternatives) using circulating indicators such as plasma cortisol and associated metabolic markers (e.g., glucose and lactate), and—where relevant—metabolic hormones such as insulin, to compare stress load and physiological stability under standardized procedures (Small, 2004; Paruğ, 2012; Alagöz et al., 2021).

In this context, stress-related biomarkers are measured not only to characterize exposure to environmental or handling-related stressors, but also to evaluate and compare the capacity of different anesthetic agents and concentrations to limit stress escalation, or to assess the suitability of novel anesthetic alternatives under controlled experimental conditions (Small, 2004; Alagöz et al., 2021).

Prolonged or repeated stress can suppress immune function, slow growth, and impair reproduction, highlighting why minimizing handling-related stress is a core welfare and management objective (Barton, 2002; Ellis et al., 2012). Proper selection, dosage, and administration of anesthetics ensure effectiveness and safety, emphasizing the importance of species-specific considerations (Martins et al., 2012). Overall, understanding the endocrinological and metabolic components of the stress response in fish is essential for interpreting anesthetic effects and for managing stress in both natural and artificial aquatic environments.

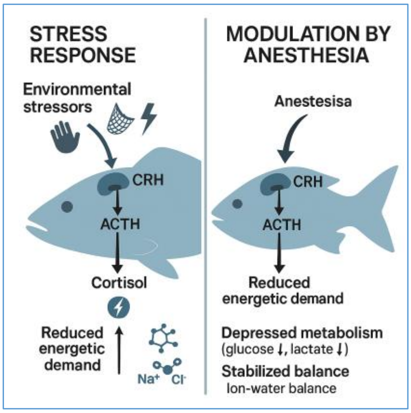

Figure 4. Conceptual illustration of the stress response in fish and its modulation by anesthesia.

(Left) Under unanesthetized conditions, environmental stimuli activate the HPI axis, leading to elevated cortisol secretion and increased metabolic demand. (Right) During anesthesia or sedation, reduced sensory input and central nervous system depression attenuate neuroendocrine activation, resulting in lower energetic demand, depressed metabolic activity, and greater physiological stability. The magnitude of modulation may vary depending on the anesthetic agent, dose, and exposure conditions.

4. PRACTICAL ANESTHESIA IN FISH: METHODS AND STAGING

4.1. Anesthesia methods: immersion, injection, and physical techniques

In routine aquaculture and research practice, fish are most frequently anesthetized by dissolving an agent in water and exposing the animals by immersion. Injection techniques and non-pharmacological approaches are used more selectively, typically in large individuals, prolonged procedures, or specific field situations. Regardless of the method, the objective is to achieve a controllable and reversible depression of central nervous system activity with minimal handling stress and an acceptable safety margin for both fish and operators (Coyle et al., 2004; Ross and Ross, 2008; Neiffer and Stamper, 2009; Paruğ, 2012).

In fish practice, the same agents are often used across a spectrum ranging from light sedation to full anesthesia: 'sedation' generally denotes partial depression of spontaneous activity without loss of responsiveness, whereas 'anesthesia' implies deeper central nervous system depression sufficient to prevent reactions to noxious or invasive stimuli. Within this general framework, immersion serves as the primary modality, whereas injection and non-pharmacological methods occupy more specialized roles in situations where waterborne delivery is impractical or specific operational constraints apply.

4.1.1. Immersion method

Immersion method relies on dissolving an anesthetic agent in the surrounding water, from which it is absorbed primarily across the gills and distributed systemically as outlined in Section 3.1. In practical use, fish are exposed within a defined treatment volume whose anesthetic concentration is known or can be closely approximated, either by transferring them into a pre-prepared bath or by adding the agent to a tank where the fish are already present, provided that mixing is sufficiently rapid to avoid pronounced local gradients. Induction proceeds as the agent is taken up from the water until the intended depth of sedation or anesthesia, as defined in Section 1.2, is reached, after which the animals are moved to clean, well-aerated water for recovery.

Relative to injectable techniques, immersion provides a waterborne route of delivery that can be applied simultaneously to groups of fish with minimal direct physical restraint, while allowing adjustment of overall exposure through changes in nominal concentration, exposure time or both. However, uptake and recovery remain sensitive to species-specific differences in gill area and ventilation, as well as to environmental factors such as temperature and water chemistry, so that protocols developed for one species or system often require empirical verification and, where necessary, modification before broader application (Bruecker and Graham, 1993; Ross and Ross, 2008).

4.1.2. Injection method

Injection techniques are used less frequently than immersion, but can be advantageous in large fish, individually handled high-value animals, or situations where waterborne delivery is impractical or undesirable, such as compromised gill function, very small working volumes, or combined procedures conducted under controlled laboratory conditions. Anesthetic agents are administered at a calculated dose per unit body mass, most commonly by intramuscular or intracoelomic injection; intravenous administration is rare in routine practice and is generally restricted to experimental applications requiring specialized expertise.

The principal advantage of injection methods lies in the precise control of administered dose, largely independent of water chemistry and environmental dilution, and with minimal generation of anesthetic residues in large water volumes. However, these techniques require firm physical restraint, accurate species- and size-specific dosing, and skilled personnel. Inappropriate injection site selection or technique may result in local tissue damage, variable systemic distribution or prolonged recovery. Because the administered dose cannot be adjusted once delivered, careful calculation, awareness of species-specific pharmacological characteristics and close observation of anesthetic depth are critical throughout the procedure (Ross and Ross, 2008; Neiffer and Stamper, 2009).

4.1.3. Physical and other non-pharmacological methods

Several non-pharmacological approaches are used in specific circumstances to induce profound sedation or short-term immobilization. These methods act primarily by altering physiological conditions rather than by direct receptor-mediated pharmacological effects, and their suitability for "true" anesthesia is debated (Ross and Ross, 2008).

Although supplemental oxygen does not induce anesthesia or immobilization on its own, oxygen enrichment is frequently used as a supportive measure during physical and other non-pharmacological applications to maintain adequate dissolved oxygen levels and to reduce hypoxia-related stress during short-term immobilization (Neiffer and Stamper, 2009).

Chilling and hypothermia are commonly applied in certain aquaculture operations, particularly for cold-tolerant species. Rapid cooling of the water slows metabolism and reduces spontaneous activity, which can facilitate brief handling. However, hypothermia alone may not provide adequate depression of central nervous system function for painful or invasive procedures and may itself act as a stressor if applied too rapidly or beyond the species' thermal tolerance. Consequently, hypothermia is best regarded as an adjunct or a method for very short, minimally invasive manipulations (Ross and Ross, 2008; Neiffer and Stamper, 2009).

Carbon dioxide narcosis is achieved by enriching water with CO_2, either by bubbling gas directly or by adding acidifying agents that increase dissolved CO_2. At suitable concentrations it can immobilize fish and is considered safe for human operators, with no restrictions arising from chemical residues. However, CO_2 often provides only partial and relatively slow immobilization, and repeated exposures may increase mortality. It does not produce true analgesia and therefore has a relatively shallow effect compared with standard pharmacological anesthetics, with characteristic physiological responses that include marked respiratory acidosis but little change in blood oxygen tension. As a result, CO_2 is best regarded as a stunning or short-term immobilization tool rather than a full anesthetic, and its independent use is increasingly confined to specific food-fish operations where very brief procedures and rapid post-treatment clearance are prioritized (Pirhonen and Schreck, 2003).

Electroanesthesia employs controlled electric fields to induce transient immobilization and loss of responsiveness. Properly configured, electrical stunning can produce very rapid induction and recovery, leaves no chemical residues, and can be integrated with capture or harvesting operations (Nguyen et al., 2019). However, it requires specialized equipment and careful control of voltage, current, exposure time, and electrode placement to avoid traumatic injuries such as vertebral fractures or hemorrhage. In addition to field strength and exposure duration, electrode polarity and spatial orientation influence current pathways through the body and are therefore critical determinants of both immobilization efficiency and the risk of neuromuscular or skeletal injury. Species-, size-, and water-conductivity–specific settings are essential, and personnel must be trained to minimize risks to both fish and operators (Ross and Ross, 2008; Neiffer and Stamper, 2009).

The principal methods currently used to induce anesthesia or transient immobilization in fish, together with their routes of action, typical applications and major welfare concerns, are summarized in Table 1.

Table 1. Overview of principal methods used for anesthesia or transient immobilization in fish

Method	Route / mechanism	Typical applications	Key advantages	Main limitations and welfare	References
Immersion (pharmacological)	Anesthetic dissolved or emulsified in water; uptake primarily across the gills.	Routine handling, grading, vaccination, deformity sorting, transport (sedation), and invasive procedures (deep anesthesia) across a wide range of species and sizes.	Fine control of anesthetic depth via concentration–time combinations; minimal physical restraint; suitable for group applications.	Sensitive to temperature, salinity and water chemistry; requires careful mixing and aeration; withdrawal times required for food fish.	Sedgwick, 1986; Keene et al., 1998; Coyle et al., 2004; Ross and Ross, 2008
Injection (pharmacological)	Agent administered at a calculated dose (mg kg^{-1}), usually intramuscular or intracoelomic.	Individual large or high-value fish; experiments requiring precise dosing; cases where immersion is impractical.	Precise dose independent of water quality; no residues in large water volumes.	Requires skilled personnel and physical restraint; dosing cannot be modified once administered.	Bruecker and Graham, 1993; Ross and Ross, 2008; Neiffer and Stamper, 2009
Hypothermia (chilling)	Rapid reduction of water temperature slows metabolism and spontaneous activity.	Very short, minimally invasive procedures in cold-tolerant species; adjunct to pharmacological anesthesia.	Simple implementation; absence of chemical residues.	Often insufficient for painful procedures; may act as a stressor if applied too rapidly or excessively.	CCAC, 2005; Ross and Ross, 2008
CO_2 narcosis	Elevation of dissolved CO_2 causing	Stunning and very brief procedures in	Rapid induction; no persistent	Pronounced aversive reactions	Pirhonen and Schreck,

Method	Route / mechanism	Typical applications	Key advantages	Main limitations and welfare	References
	respiratory acidosis and altered chemoreceptor activity.	selected food-fish operations.	chemical residues.	possible; limited analgesia; restricted use as sole anesthetic.	2003; CCAC, 2005
Electroanesthesia / electrical stunning	Application of controlled electric fields to induce transient immobilization.	Capture, stunning and slaughter operations; some field-based procedures.	Very rapid induction and recovery; integration with harvesting operations; no chemical residues.	Requires specialized equipment; risk of physical injury if misapplied.	CCAC, 2005; Ross and Ross, 2008; Neiffer and Stamper, 2009, Nguyen et al., 2019

4.2. Sedation and deep anesthesia according to procedure type

In fish practice, sedation and deep anesthesia represent different operational targets rather than distinct groups of agents. The same compounds are usually applied at lower concentrations and/or shorter exposures to obtain light sedation, and at higher effective doses or longer exposures to achieve deep anesthesia (Summerfelt and Smith, 1990; Coyle et al., 2004; Ross and Ross, 2008; Paruğ, 2012). Sedation is characterized by reduced spontaneous activity, calmer swimming and decreased responsiveness to non-painful stimuli, whereas deep anesthesia implies loss of equilibrium and protective reflexes together with an absence of purposeful reactions to noxious or invasive manipulations (Ross and Ross, 2008; Neiffer and Stamper, 2009; Navarro et al., 2016).

In routine aquaculture operations, light sedation is often sufficient and preferable. Mild depression of activity decreases struggling and scale loss during capture, grading, vaccination, external examination and short procedures such as deformity sorting, while preserving effective ventilation and allowing fish to maintain posture in the water column (Summerfelt and Smith, 1990; Coyle et al., 2004). Lower concentrations are also widely used during the transfer of cultured stocks, wild-caught fish or broodstock between facilities or from the field to the laboratory, where the aim is to stabilize behavior over many hours without loss of equilibrium (Ballıkaya, 2016; Cao et al., 2021; Lazrag, 2024). In particular, the use of anesthetics at sedative concentrations is crucial during live transport of fish, because the partial depression of activity and metabolism reduces oxygen consumption and waste excretion, helping to maintain water quality in limited water volumes and improving survival during transit (Ballıkaya, 2016; Lazrag, 2024). In such live-transport situations, any progression toward loss of equilibrium generally indicates that the sedative dose or exposure duration is greater than required for the task.

Deep anesthesia, by contrast, is mandatory whenever painful or clearly invasive procedures are undertaken. Surgical implantation of tags or transmitters, cannulation of vessels or ducts, internal biopsies, extensive fin or gill tissue sampling, and delicate ophthalmic or cranial procedures all require abolition of voluntary movements and reflex responses to avoid tissue damage and to meet accepted welfare standards (Sedgwick, 1986; Ross and Ross, 2008). For such interventions, the practical objective is to induce a stable surgical level rapidly, maintain it only for as long as the procedure demands, and then allow smooth recovery with minimal physiological disturbance.

The boundary between "adequate sedation" and "insufficient anesthesia" is therefore defined as much by the procedure as by the fish's behavior. Prolonged exposure at intermediate immersion concentrations can lead to substantial physiological exhaustion and delayed mortality without ever producing a reliable surgical level, especially under stressful environmental conditions (Small, 2004; Martínez-Porchas and Martínez-Córdova, 2009). For immersion techniques, species- and temperature-specific protocols usually specify practical combinations of concentration and exposure time that produce either light sedation for transport and routine handling, or deep anesthesia for invasive work, within predictable induction and recovery periods (CCAC, 2005; Lazrag, 2024). Similar principles apply to injectable agents, although the lack of control over exposure once the dose (mg kg^{-1}) has been administered makes accurate dose calculation and close monitoring even more critical (Ross and Ross, 2008; Neiffer and Stamper, 2009).

Non-pharmacological approaches such as hypothermia, CO_2 narcosis and electroanesthesia are most often used to provide short-term immobilization or profound sedation during capture, stunning or slaughter, rather than as stand-alone techniques for deep anesthesia (Pirhonen and Schreck, 2003; Coyle et al., 2004; Ross and Ross, 2008). Although they can induce rapid loss of equilibrium, their ability to provide true analgesia is limited or uncertain, and they may evoke pronounced aversive or cardiorespiratory responses if not carefully controlled (Neiffer and Stamper, 2009). As a result, these methods are generally reserved for very brief manipulations or are combined with pharmacological agents when more prolonged or painful procedures are anticipated.

In summary, choosing between sedation and deep anesthesia is an operational decision that must take into account the nature and duration of the procedure, species- and size-related sensitivity, environmental conditions and welfare considerations.

4.3. Stages of induction and recovery in fish anesthesia

The transition from normal behavior to deep anesthesia and back to full recovery (Fig. 5) represents a continuous physiological process, in which central nervous system activity, neuromuscular control, and cardiorespiratory function change in a graded and progressive manner, rather than through discrete and rigidly defined stages (Saccol et al., 2018). For practical purposes, this continuum has traditionally been divided into a series of stages that correspond to reproducible behavioral and reflex landmarks during induction and recovery, providing a common language with which researchers and practitioners can describe and compare anesthetic depth.

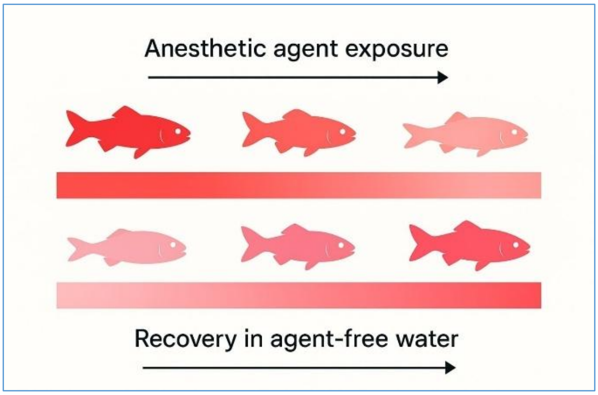

Figure 5. Conceptual diagram of the bidirectional continuum of anesthetic depth in fish, from normal activity to deep anesthesia and back to recovery in agent-free water.

Classical descriptions of fish anesthesia often use six- or seven-stage schemes to distinguish normal behavior, light sedation, loss of equilibrium, deep anesthesia, and the mirrored phases of recovery. These detailed systems provide a comprehensive conceptual framework, for interpreting anesthetic progression, but many applied studies in aquaculture and experimental biology only use a subset of stages that are directly relevant for the procedure at hand and the precision required by the study design (Keene et al., 1998; Zahl et al., 2012; Paruğ, 2012).

Although the staging tables provide a useful generalized framework, they are ultimately derived from the specific behavioral patterns observed in particular species and experimental settings. Accordingly, individual fish species and different anesthetic agents may show distinct behavioral sequences or omit some of the described stages altogether (Ballıkaya, 2016), and the schemes in Tables 2–3 should therefore be interpreted as flexible operational guides rather than rigid, universally applicable profiles. Where feasible, species- and agent-specific pilot trials can therefore be useful to refine these generalized schemes, validate the selected criteria, and to define operational stages that more accurately capture the behavioral trajectory under the environmental and procedural conditions of a given study.

Table 2. Stages of anesthesia in fish (modified from Keene et al., 1998; Zahl et al., 2012)

Stage	Behavioral characteristics in fish
A0	Normal swimming activity; fish keeps an upright orientation in the water; rapid response to visual and tactile stimuli.
A1	Slight reduction in swimming speed; reactions to external stimuli are slower but clearly present; opercular movements regular.
A2	Marked sedation; slow and sometimes uncoordinated swimming; reduced reaction to handling; opercular rate decreased compared with A0.
A3	Active swimming stops; fish mostly remains stationary in the water or near the bottom, but can still keep an upright orientation for short periods; only weak response to strong tactile stimuli.
A4	Loss of equilibrium; fish lies on its side or back; spontaneous movements absent; opercular movements slow but regular.
A5	Deep anesthesia; no response to firm tactile or pin-prick stimuli; opercular movements very slow and shallow.
A6	Medullary collapse; opercular movements cease and the fish is moribund (this stage should be avoided in routine procedures).

Table 3. Stages of recovery in fish (modified from Keene et al., 1998)

Stage	Behavioral characteristics in fish
R1	Opercular movements become visible or increase in amplitude; fish remains motionless on the bottom or lying on its side.
R2	Occasional fin movements and weak attempts to right the body axis; fish is still unable to maintain equilibrium for more than a few seconds.
R3	Continuous fin movements and short bouts of swimming; fish keeps an upright orientation most of the time but shows uncoordinated trajectories and delayed
R4	Coordinated, sustained swimming with normal equilibrium; rapid avoidance or fright responses reappear.
R5	Behavior indistinguishable from the pre-anesthetic condition; normal swimming pattern and full responsiveness to handling and environmental stimuli.

In practice, the full multi-stage framework is frequently condensed into simplified 2-, 3- or 4-stage variants. Two-stage systems usually distinguish only between an adequately anesthetized state and full recovery, focusing on clearly defined operational thresholds. Three-stage schemes typically separate light sedation, deep anesthesia suitable for invasive procedures, and the recovered state. Four-stage systems tend to treat loss of equilibrium as an explicit intermediate step between sedation and deep anesthesia, and to mirror this transition during recovery. Such adaptations emphasize easily recognized end points—loss of equilibrium, attainment of deep anesthesia and restoration of coordinated swimming—while collapsing intermediate transitions into broader categories (Keene et al., 1998; Mylonas et al., 2005; Zahl et al., 2012). An example of an operational three-stage induction–recovery scheme, suitable for routine handling and minor procedures, is summarized in Table 4.

Table 4. Stages of anesthesia and recovery in fish (modified from Iwama et al., 1989)

Stage of anesthesia	**Behavioral description in fish**
A1	Onset of loss of equilibrium. The fish begins to roll or lean to one side, with progressive impairment of its ability to maintain a stable orientation. Opercular movements remain clearly visible.
A2	Marked reduction of voluntary body movements. Apart from opercular activity, the body and fins are largely motionless; the fish usually lies on the bottom or remains immobile in the water column.
A3	Deep anesthesia state with complete immobilization. Opercular movements cease or become barely perceptible, and the fish shows no reaction to handling or strong tactile stimulation.
Stage of recovery	
R1	The body remains immobile, but opercular movements reappear or increase in amplitude. No sustained attempts at swimming are observed.
R2	Regular opercular movements are established and general body and fin movements resume. The fish may perform short, uncoordinated swimming bouts but cannot yet maintain stable equilibrium.
R3	Full recovery of equilibrium. The fish maintains a normal upright orientation and exhibits a typical swimming pattern with appropriate responses to environmental and handling stimuli.

Despite these differences in granularity, most staging systems share the same underlying structure. During induction, fish progress from normal behavior to a sedated state with reduced spontaneous activity and diminished responsiveness, then to loss of equilibrium, and finally to deep anesthesia in which purposeful reactions to noxious or invasive stimuli are abolished. Recovery follows the reverse sequence: initial return of opercular movements and reflexes, partial restoration of equilibrium with unstable swimming, and ultimately a return to sustained upright swimming, normal ventilation and appropriate behavioral responses.

4.4. Technical implementation of anesthesia: preparation, water quality and safety

Safe and reproducible use of anesthesia in fish depends not only on selecting an appropriate method and target depth, but also on how the solution is prepared, introduced and managed in the working system. In immersion techniques in particular, uneven mixing, inadequate aeration or unrecognized changes in water quality can convert an otherwise suitable protocol into a source of avoidable stress, toxicity or delayed mortality (Coyle et al., 2004; Ross and Ross, 2008; Paruğ, 2012).

For immersion method, stock solutions should be prepared in a controlled manner, using accurately weighed quantities of the active compound and volumetric equipment to achieve the intended nominal concentration. When water solubility is limited, the anesthetic is first dissolved in a small volume of an appropriate solvent such as ethanol or acetone, typically at approximately equal volumes of anesthetic and solvent (v/v), and then mixed with a small amount of water in a closed container and shaken vigorously to obtain a stable stock emulsion. For agents that are known to acidify the water, a suitable buffer may be added to maintain pH within the species' tolerance range; in determining the nominal concentration in the treatment tank, only the amount of active substance, and not the solvent volume, is taken into account (Coyle et al., 2004; Paruğ, 2012). The solution is then introduced into a vigorously mixed region of the system, away from the fish whenever possible, so that homogeneous distribution is achieved before substantial contact occurs. This gradual, well-mixed addition helps to avoid pouring highly concentrated anesthetic directly onto the animals and reduces the risk of local overdose.

Throughout induction and maintenance, basic water-quality variables require close supervision. The water used for anesthesia and recovery should be clean, of low turbidity and free from excessive suspended solids or organic debris, so as not to compromise gill function or interact unpredictably with the anesthetic. Although ventilation is depressed under anesthesia, dissolved oxygen should be maintained within the species-appropriate range; accordingly, vigorous aeration or oxygen supplementation is required, especially under high stocking densities during immersion procedures (Coyle et al., 2004; Ross and Ross, 2008). In addition to meeting oxygen demand, such aeration and associated water movement are essential for maintaining effective circulation of the anesthetic solution: if mixing is poor, particularly when poorly soluble agents are used, droplets or emulsified particles may accumulate at the surface or on the bottom depending on their density, leading to heterogeneous exposure in which some fish are scarcely affected while others experience local overdose.

Temperature should be held stable and within the normal tolerance of the species, although in some transport protocols a modest reduction within the tolerated range is used deliberately to reduce metabolic rate and oxygen demand. Salinity and general water chemistry influence both ionization state and partitioning of many agents and should therefore be considered when transferring protocols between systems or facilities (Ross and Ross, 2008; Neiffer and Stamper, 2009). Following use in food fish, spent anesthetic solutions and any solvent residues must be disposed of in accordance with local environmental regulations, and established withdrawal periods must be respected before slaughter or harvest (Coyle et al., 2004).

Comparable attention to detail is required for injectable and non-pharmacological techniques. For injectable agents, accurate estimation of body mass, correct calculation of dose (mg kg^{-1}), appropriate selection of injection site and needle size, and avoidance of intraluminal or intraperitoneal contamination are essential to minimize variability in exposure and procedure-related trauma (Ross and Ross, 2008; Neiffer and Stamper, 2009). In electrical and CO_2-based approaches, operator control over voltage, current, exposure time, gas delivery and water conductivity must be sufficient to prevent vertebral injury, severe cardiorespiratory disturbance or pronounced aversive reactions, even when the primary objective is stunning or slaughter rather than prolonged anesthesia (Ross and Ross, 2008; Neiffer and Stamper, 2009).

Operator safety also forms a critical component of good anesthesia practice. Many commonly used agents are irritant or sensitizing to the skin, eyes and mucous membranes, and some possess recognized human health risks when inhaled or absorbed chronically (Ross and Ross, 2008; Neiffer and Stamper, 2009). Concentrated formulations and stock solutions should therefore be handled with gloves and eye protection, in well-ventilated areas or under fume extraction, and spills should be cleaned promptly using appropriate absorbent materials. Careful labelling, secure storage away from food and personal items, and clear documentation of concentrations and preparation steps reduce the risk of dosing errors and unintended exposure. Well-defined emergency arrangements further support both operator safety and animal welfare.

5. PHARMACOLOGICAL AGENTS USED IN FISH ANESTHESIA

5.1. Criteria for selecting an anesthetic agent

In fish practice, the suitability of an anesthetic is determined less by its identity than by how well it meets a set of practical and biological criteria under specific operational conditions. An effective fish anesthetic can be characterized by the following core properties:

- Wide safety margin

The anesthetic should allow reliable induction without a narrow boundary between the desired depth and overdose, particularly under group treatment, variable environmental conditions, or limited individual monitoring (Marking and Meyer, 1985; Coyle et al., 2004).

- Predictable and sufficiently rapid induction and recovery

An effective fish anesthetic should permit attainment of the required depth within a practical and reproducible induction period and allow timely recovery following termination of exposure. In immersion-based studies, benchmark time frames are commonly employed when defining effective and reliable concentration–time combinations, with deep anesthesia typically targeted within approximately 3 minutes and full recovery expected within about 5 minutes after transfer to clean water (Keene et al., 1998; Coyle et al., 2004; Mylonas et al., 2005; Ross and Ross, 2008).

- Controllable anesthetic depth

The agent should permit reliable positioning along the sedation–anesthesia continuum in accordance with procedural requirements, rather than producing abrupt or poorly defined states (Coyle et al., 2004; Ross and Ross, 2008).

- Limited disruption of physiological homeostasis

Effective anesthetics should avoid excessive disturbance of respiration, circulation, acid–base balance, or endocrine stress responses that could compromise post-procedural tolerance (Marking and Meyer, 1985; Ross and Ross, 2008).

- Acceptable residue and withdrawal profile

In food fish, anesthetic use must be compatible with regulatory frameworks, either through negligible residue persistence or clearly defined and feasible withdrawal periods (Keene et al., 1998; Coyle et al., 2004; CCAC, 2005).

- Operational practicality and operator safety

The agent should be compatible with the intended method of delivery, scale of application, and available infrastructure, while also exhibiting low volatility, limited toxicity to humans, and handling characteristics that ensure operator safety during repeated or large-scale use (Keene et al., 1998; Coyle et al., 2004; CCAC, 2005).

5.2. Overview of major pharmacological anesthetics used in fish

In this section, a principle-based perspective is adopted, rather than an approach based on exhaustive or prescriptive dose–duration datasets, in order to reflect the broad biological and environmental variability inherent to fish anesthesia. This approach is grounded in the observation that dose and exposure times reported in the literature often display substantial variability as a result of multiple biological, environmental, and observation-dependent factors, making them better interpreted as context-dependent outcomes rather than as fixed or directly transferable benchmarks. In this context, individual anesthetic agents are discussed in terms of their general effectiveness and behavioral profiles, recognizing that optimal concentrations and responses are strongly influenced by species, developmental stage, physiological condition, environmental context, and the behavioral and reflex-based criteria used by researchers to assess progression through induction and recovery. Accordingly, the remainder of this section provides a concise overview of the major pharmacological agents used in fish anesthesia, taking into account their general chemical characteristics, typical patterns of anesthetic action, and principal practical advantages and limitations.

Tricaine methanesulfonate (MS-222) is a synthetic ester-type local anesthetic that has become one of the most widely used agents for fish since its introduction in the late 1960s (Ortuño et al., 2002; CCAC, 2005; Coyle et al., 2004). Chemically it is a sulfonated derivative of ethyl 3-aminobenzoate, supplied as a white crystalline powder that is highly soluble in both fresh and sea water, allowing the preparation of concentrated stock solutions and facilitating accurate dosing (Coyle et al., 2004; Ross and Ross, 2008). The sulfonate moiety renders working solutions acidic and may irritate gills and mucosal surfaces; therefore MS-222 baths are commonly buffered with sodium bicarbonate or similar agents to maintain water pH near neutrality and reduce local adverse effects (Coyle et al., 2004; Gourdon, 2007; Ross and Ross, 2008). MS-222 generally provides rapid induction and relatively prompt recovery over a wide range of species, and is considered to have a favourable safety margin when used within recommended concentration–time ranges and under appropriate environmental conditions (Coyle et al., 2004; CCAC, 2005; Ross and Ross, 2008). Nonetheless, both exposure and subsequent handling can elicit pronounced neuroendocrine and metabolic stress responses, including increases in plasma cortisol and lactate and alterations in respiratory and acid–base status (Molinero and Gonzalez, 1995; Coyle et al., 2004; Ross and Ross, 2008). The compound is eliminated mainly via the urine and tissue levels decline to near zero within approximately 24 h (Coyle et al., 2004; CCAC, 2005).

Benzocaine is a local ester-type anesthetic that is structurally related to tricaine but much less soluble in water. It is supplied either as a white crystalline powder with an aqueous solubility on the order of 0.4 g L^{-1} or as a free-base preparation that must first be dissolved in ethanol or acetone to produce a concentrated stock solution (typically 100–200 g L^{-1}) before dilution into the anesthetic bath (Gilderhus, 1991; Ortuño et al., 2002; Coyle et al., 2004; CCAC, 2005). In contrast to MS-222, benzocaine solutions are essentially pH-neutral at working concentrations, so unbuffered baths are less prone to induce initial hyperactivity or acute irritation of the gills and mucosal surfaces (Coyle et al., 2004). Functionally, benzocaine blocks voltage-gated Na^+ channels and, despite being classed as a local anesthetic in mammals, produces systemic anesthesia when administered by immersion in fish (Ortuño et al., 2002). Effective doses are broadly similar to those of MS-222, and the agent is generally regarded as having a satisfactory safety margin under appropriate environmental conditions (Coyle et al., 2004; CCAC, 2005). Nevertheless, elevated water temperature and prolonged exposures (> 15 min) are associated with increased risk of adverse effects, and recovery can be noticeably prolonged in large, fatty individuals or gravid females (Coyle et al., 2004; CCAC, 2005). Like MS-222, benzocaine can provoke hypoxia, hypercapnia, hyperglycemia and increased blood lactate when combined with handling stress (Ortuño et al., 2002).

2-Phenoxyethanol is an aromatic glycol ether used as a liquid immersion anesthetic and sedative in fish. It is a colorless to slightly straw-colored oily fluid with moderate solubility in water but complete miscibility with ethanol, does not appreciably alter the pH of fresh or sea water under typical aquaculture conditions, and has bactericidal and fungicidal properties that are advantageous during surgical procedures (Coyle et al., 2004; CCAC, 2005; Başaran et al., 2007; Neiffer and Stamper, 2009). From a practical standpoint, 2-phenoxyethanol is relatively inexpensive, can produce effects ranging from light sedation to deep anesthesia depending on the concentration–time combination, and is generally regarded as having a wide safety margin in many species, although studies in salmonids indicate that effective concentrations may approach toxic levels, narrowing the therapeutic index and necessitating careful monitoring during prolonged exposure (Sehdev et al., 1963; Takashima et al., 1983; Ortuño et al., 2002; Coyle et al., 2004; Ross and Ross, 2008). Because the undiluted compound can irritate skin, eyes and mucosal membranes and has been associated with hepatic and renal toxicity in mammalian toxicology data, appropriate protective measures are required during handling (Summerfelt and Smith, 1990; CCAC, 2005; Neiffer and Stamper, 2009).

Quinaldine is a synthetic quinoline derivative that has long been used as an immersion anesthetic, particularly in marine and tropical fish. In its base form it is a yellowish, oily liquid with very limited water solubility and therefore must be pre-dissolved in ethanol or acetone before being diluted into the anesthetic bath (Coyle et al., 2004; CCAC, 2005). Working solutions are acidic and are commonly buffered with sodium bicarbonate to maintain pH within a physiologically acceptable range and to reduce irritation of the gills and mucosal surfaces (Coyle et al., 2004). Although quinaldine is an effective anesthetic, it has an unpleasant odor and is classified as an irritant with potential carcinogenic properties, which constrains its desirability in some contexts (Coyle et al., 2004; CCAC, 2005; Ross and Ross, 2008). Nevertheless, its relatively low cost has supported widespread use in the collection of tropical aquarium fish and in bait and sport fisheries (Coyle et al., 2004). Induction with quinaldine typically occurs within about 1–4 min and is often accompanied by mild, transient muscle contractions; recovery is usually fairly rapid once fish are transferred to clean water (Coyle et al., 2004; CCAC, 2005). Reported effective concentrations vary considerably among species, with moderate doses being sufficient to induce loss of equilibrium and workable anesthesia in some cyprinids and grass carp, whereas much higher levels may be required to immobilize tilapias and other warm-water taxa (Gilderhus and Marking, 1987; Coyle et al., 2004). In general, quinaldine does not reliably produce the deep, fully relaxed surgical plane required for procedures that demand complete abolition of involuntary reflexes, and higher doses or prolonged exposures increase the risk of toxic effects (Gilderhus and Marking, 1987; Coyle et al., 2004; CCAC, 2005). Its potency tends to increase in hard and warm waters, so both species identity and water chemistry must be considered when defining safe and effective concentration–time combinations (Coyle et al., 2004).

Quinaldine sulfate is a related compound supplied as a pale yellow, crystalline powder that is readily soluble in water (on the order of 1 g L^{-1}), which simplifies preparation of immersion baths compared with the parent base (Coyle et al., 2004; CCAC, 2005). Although more expensive than quinaldine or MS-222, it has become a standard tool for marine biologists collecting fish from tidal flats and coral reefs because it typically induces anesthesia rapidly, recovery is usually short, and much of the drug is excreted without extensive biotransformation (Ortuño et al., 2002; Coyle et al., 2004). However, as with quinaldine, incomplete suppression of involuntary muscle activity has been reported, and several authors have questioned its suitability as a sole agent for surgery, precise tagging procedures or aquaculture operations especially at the relatively high doses sometimes used in practice (Gilderhus and Marking, 1987; Yanar and Kumlu, 2001; Coyle et al., 2004; CCAC, 2005).

Metomidate and **etomidate** are imidazole-based, non-barbiturate hypnotic agents originally developed for use in human and veterinary anesthesia and subsequently explored in fish. Both compounds are notable for producing effective anesthesia without the pronounced elevation of plasma cortisol that accompanies many immersion anesthetics, an effect attributable to suppression of steroidogenic pathways involved in cortisol synthesis rather than to true absence of physiological stress (Ross and Ross, 2008; Iversen et al., 2013). Metomidate is a water-soluble powder that typically produces smooth and rapid induction, minimal initial hyperactivity, and relatively fast recovery compared with MS-222 across a range of marine and freshwater species (Matson and Riple, 1989; Coyle et al., 2004; Ross and Ross, 2008). These properties have led to its preferential use in certain taxa, including cod and salmonids, particularly where rapid recovery is operationally desirable. Despite these apparent advantages, both metomidate and etomidate have important limitations. The cortisol-suppressive effect, once viewed as an indicator of stress-free anesthesia, is now recognized as a pharmacological blockade of endocrine stress responses rather than an absence of stress perception, raising concerns about interference with normal physiological regulation (Ross and Ross, 2008; Iversen et al., 2013). In addition, species- and life-stage-specific variability is pronounced: while metomidate has been reported as safe and effective in larger juvenile and adult salmonids and several marine fishes, inconsistent anesthesia and elevated mortality have been observed in larval stages of some species, including goldfish and red drum (Coyle et al., 2004; Ross and Ross, 2008). Occasional muscle twitching has also been reported, which may complicate procedures requiring immobility, such as blood sampling. Collectively, these features indicate that metomidate and etomidate can be valuable tools in selected contexts, but their use requires careful consideration of species, developmental stage, and experimental objectives rather than routine substitution for more conventional immersion anesthetics.

Clove oil is a pale yellow to brown aromatic essential oil distilled from the leaves, flower buds and stems of the clove tree *Syzygium aromaticum*. Its major active constituent is the phenolic compound eugenol, typically comprising around 70–95% of the oil, together with iso-eugenol and a range of other phenylpropanoid and terpenoid components that contribute to its characteristic odour and flavour (Soto and Burhanuddin, 1995; Coyle et al., 2004; Ross and Ross, 2008; Neiffer and Stamper, 2009). Owing to its very limited water solubility, clove oil is usually pre-dissolved in ethanol to prepare a concentrated stock solution, which can then be dispersed homogeneously into the anesthetic bath immediately before use (Keene et al., 1998; Coyle et al., 2004; Hoskonen and Pirhonen, 2004). When appropriately dosed it can produce stable anesthesia in a broad range of freshwater and marine fishes, with a wide separation between effective and lethal exposures and the possibility of achieving both light sedation and deep anesthesia within the same compound (Keene et al., 1998; Coyle et al., 2004; Roubach et al., 2005; Velíšek et al., 2005). Induction is generally smooth, whereas recovery is characteristically slower than with tricaine, reflecting respiratory depression and the lipophilic nature of the drug (Anderson et al., 1997; Keene et al., 1998; Coyle et al., 2004; CCAC, 2005). Comparative studies indicate that clove oil often elicits lower or more transient increases in plasma cortisol and lactate than MS-222 or 2-phenoxyethanol, and it has not been associated with marked immunodepression, suggesting a relatively mild acute stress profile in many species (Small, 2003; Wagner et al., 2003; King et al., 2005; Lazrag, 2024). Despite its long-standing use as a topical analgesic in human medicine, there is currently no direct evidence that clove oil provides true analgesia in fish, and it is best regarded primarily as a hypnotic–anesthetic agent (Keene et al., 1998; Sladky et al., 2001). From a practical standpoint, clove oil is straightforward to handle, has an agreeable odor, and—although high-purity laboratory preparations can be relatively expensive per unit mass—its high potency means that only small volumes are required to achieve effective anesthesia, making it a cost-efficient alternative to more conventional immersion anesthetics in many applications (Coyle et al., 2004; Ross and Ross, 2008; Lazrag, 2024). Iso-eugenol-based commercial formulations such as Aqui-S, which contain defined ratios of eugenol and iso-eugenol, represent refined analogues of clove-oil (Ross and Ross, 2008; Zahl et al., 2012).

Beyond the widely used immersion anesthetics discussed above, a number of other pharmacological agents have been evaluated in fish, but their application remains comparatively restricted. Injectable formulations explored primarily in experimental and physiological studies include steroid anesthetic mixtures such as alphaxolone–alphadolone (Saffan), barbiturates such as sodium pentobarbitone, non-barbiturate hypnotics such as propanidid, potent opioid–

tranquilizer combinations (e.g. etorphine–acetylpromazine), sedatives such as xylazine, and local anesthetics including lignocaine and procaine, most of which are adapted from human or veterinary medicine rather than developed specifically for aquatic use (Ross and Ross, 2008).

Among the injectable agents, **ketamine** has attracted particular interest as a dissociative anesthetic with both anesthetic and analgesic properties. In fish, it can produce rapid induction and intermediate-duration anesthesia after intramuscular or intravascular administration, but recovery is often prolonged and may be characterized by marked hyperactivity and ataxic swimming, and its safety margin tends to narrow at higher temperatures or doses. For these reasons, ketamine is generally reserved for specific research applications—sometimes in combination with adjunct sedatives—rather than being employed as a routine anesthetic in aquaculture or fisheries practice (Bruecker and Graham, 1993; Ross and Ross, 2008).

Oral delivery of sedatives and anesthetics, for example via diazepam-medicated feeds, has also been investigated as a relatively low-handling option, but slow and variable uptake, difficulties in controlling individual dose, and delayed onset of effect have so far limited this route to experimental use rather than widespread practical adoption (Ross and Ross, 2008).

5.3. Emerging plant-derived anesthetics and experimental alternatives in fish

In addition to the established pharmacological anesthetics outlined above, there is a growing body of research exploring plant-derived compounds and other experimental agents as potential alternatives for use in fish anesthesia. These efforts are largely motivated by the search for anesthetics that combine effective immobilization with reduced physiological stress, rapid and predictable recovery, minimal adverse effects on metabolism and endocrine function, and improved environmental and operator safety. In particular, the demonstrated utility of clove oil as an organic, immersion-based anesthetic has stimulated interest in identifying other botanically derived substances with comparable or improved profiles, even though most candidates remain at an exploratory stage and have not progressed to routine or commercial application. Accordingly, these lines of work build directly on the extensive experience accumulated with conventional anesthetics, applying similarly detailed physiological and stress-related endpoints to determine whether novel plant-derived compounds can match or surpass established agents in efficacy while reducing toxicological and welfare-related costs.

Recent work on plant-derived anesthetic candidates illustrates how diverse botanical sources are being screened not only for their ability to induce reliable anesthesia but also for their effects on physiological stress markers and longer-term welfare outcomes. For example, an aqueous macerate of spurge (*Euphorbia* *rigida*) was shown to produce operationally usable anesthesia in rainbow trout (*Oncorhynchus mykiss*), with dose-dependent induction and recovery times and plasma cortisol responses that compared favorably to 2-phenoxyethanol, supporting its potential as an organic immersion anesthetic in salmonids (Alagöz et al., 2021). In marine aquaculture, oregano (*Origanum sp.*) and eucalyptus (*Eucalyptus sp.*) essential oils have been evaluated as immersion anesthetics in European sea bass (*Dicentrarchus labrax*) and meagre (*Argyrosomus regius*), with oregano oil in particular showing induction and recovery profiles comparable or superior to clove oil and no mortality at optimized doses (Bodur et al., 2018). Temperature-dependent efficacy has also been demonstrated for rosewood (*Aniba rosaeodora*) essential oil in goldfish (*Carassius auratus*), where comparisons with 2-phenoxyethanol at 14 and 20 °C showed that appropriate dosing can achieve effective anesthesia across a range of thermal conditions while maintaining acceptable recovery profiles (Şahin and Kızak, 2023). Complementing these acute-handling scenarios, essential oil from *Myrcia sylvatica* has been investigated in silver catfish (*Rhamdia quelen*) during simulated transport, where sedation with this oil attenuated neuroendocrine activation, oxidative and nitrosative stress, and metabolic and osmoregulatory disturbances, suggesting a role for some plant-derived agents not only as anesthetics but also as modulators of transport-related stress syndromes (Saccol et al., 2018).

Some studies also illustrate that not all plant-derived preparations meet basic safety criteria for routine use. Essential oils from the Brazilian natives *Hesperozygis ringens* and *Ocotea acutifolia* produced effective anesthesia in silver catfish (*Rhamdia quelen*) without behavioral side effects, whereas two chemotypes of *Lippia sidoides* oil, although clearly anesthetic, were associated with marked mucus loss and mortality, leading the authors to recommend only *H. ringens* and *O. acutifolia* for anesthetic applications in this species (Silva et al., 2013).

Taken together, experimental studies on plant-derived anesthetics indicate that, when carefully formulated and applied, some preparations can provide anesthetic depth and recovery dynamics broadly comparable to conventional agents and, in certain contexts, may attenuate acute stress responses or transport-related disturbances. Conversely, reports of essential oils that, despite clear anesthetic activity, cause pronounced mucus loss, high mortality or other marked adverse effects at operational doses underline that botanical origin alone does not guarantee a wide safety margin or superiority over established drugs. Across this broader body of work, responses appear strongly dependent on species, life stage, temperature, formulation and exposure protocol, and robust data on standardization, chronic toxicity, residue kinetics and sensory effects on fish flesh remain limited. In this light, the examples summarized above should be viewed as illustrative rather than exhaustive, and most plant-based anesthetic candidates are best regarded at present as promising but still experimental tools and context-dependent complements to established agents rather than direct replacements.

CONCLUSION

Fish anesthesia represents a central component of humane handling, effective aquaculture practice, and rigorously controlled experimental work. As outlined in this chapter, the successful application of anesthesia in fish depends less on the choice of a single "ideal" agent than on a clear understanding of physiological principles, stress responses, exposure pathways, and procedure-specific requirements. The graded continuum from light sedation to deep anesthesia provides a flexible framework that allows anesthetic depth to be matched precisely to the invasiveness, duration, and welfare implications of the targeted procedure.

Across commonly used immersion, injectable, and physical methods, induction and recovery dynamics are strongly shaped by species-specific traits, environmental conditions, and operational context. Sedative applications play a particularly important role in routine handling and live transport, where partial suppression of activity and metabolism can reduce stress, oxygen demand, and waste production while maintaining basic physiological stability. In contrast, deep anesthesia remains indispensable for invasive or painful procedures, where complete suppression of reflexes and voluntary movements is required to meet ethical and technical standards.

The overview of pharmacological agents highlights that all anesthetics entail trade-offs between efficacy, safety margin, stress modulation, residue considerations, and operator safety. While established compounds such as MS-222, benzocaine, 2-phenoxyethanol, and clove oil remain the mainstays of current practice, emerging plant-derived and experimental agents underscore both the potential for innovation and the necessity for rigorous evaluation. Botanical origin alone does not guarantee safety or superiority, and careful, species-specific validation remains essential.

Overall, effective fish anesthesia is best approached as an integrative process that combines physiological understanding, practical staging, and welfare-oriented decision-making. Continued refinement of protocols, improved standardization, and critical assessment of novel alternatives will remain key to advancing both scientific reliability and animal welfare in aquaculture and aquatic research.

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CHAPTER 11

Alternative Mineral and Vitamin Source in Animal Nutrition: Microalgae

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1. Introduction

In recent years, the exploration of alternative sources for minerals and vitamins in animal nutrition has gained significant interest, particularly with the advent of microalgae as a promising option. These microscopic organisms are recognized for their unique nutritional profiles, which include a rich array of proteins, vitamins, minerals, and essential fatty acids, rendering them highly suitable for fortifying diets across various livestock and poultry (Saadaoui et al., 2021; Costa et al., 2024). The urgency to find sustainable feed alternatives from the growing environmental pressures associated with conventional animal feed production, such as over-reliance on fishmeal and soy, which have raised concerns regarding resource depletion and ecosystem sustainability (Saadaoui et al., 2021; Ma & Hu, 2023). Microalgae offer the dual advantage of being produced on non-arable land and having the ability to utilize carbon dioxide and sunlight for growth, making them an ecologically viable supplement to traditional feed sources (Saadaoui et al., 2021).

Microalgae possess various bioactive compounds that not only enhance nutritional quality but also have promoting effect for animal health. They have been shown to improve growth performance, immune response, and overall vitality in various species, including poultry, ruminants, and aquatic animals (Costa et al., 2024; Ma & Hu, 2023). The presence of specific compounds such as omega-3 fatty acids—particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)—is vital for the metabolic health of animals, highlighting the nutritional potency of these microalgae (Haas et al., 2015; Molino et al., 2018). Furthermore, certain microalgae also contain antioxidants and vitamins, such as those sourced from *Haematococcus pluvialis*, which can serve as natural substitutes for synthetic additives in animal diets, thus aligning with the growing trend toward organic and health-conscious agricultural practices (Mavrommatis et al., 2023; Ma et al., 2020).

The application of microalgae in animal nutrition is not without its challenges. Variability in nutritional composition among different species necessitates careful selection and formulation to optimize their inclusion rates effectively (Zhang et al., 2023). Moreover, ongoing research is paramount to uncover the full potential of microalgae, particularly in terms of their role in enhancing the bioavailability of minerals and vitamins when utilized in livestock and poultry diets (Ribeiro et al., 2020). As research continues to evolve, the integration of microalgae into animal feed represents a transformative shift toward a more sustainable and nutritionally balanced approach to animal husbandry, potentially leading to enhanced productivity and a reduced environmental footprint (Saadaoui et al., 2021; Pătraș et al., 2019).

Microalgae stand out as a viable alternative mineral and vitamin source in animal nutrition, offering a sustainable pathway to enhance the nutrient profiles of animal feeds while supporting health and productivity (Uguz and Sozcu, 2025). The convergence of ecological sustainability and nutritional efficiency positions microalgae as critical players in the future of animal husbandry, paving the way for innovations that meet the demands of both farmers and consumers alike.

2. Description of microalgae

The rise of microalgae technology in animal nutrition is gaining momentum as a sustainable and nutritionally rich alternative for sourcing essential minerals and vitamins. Microalgae, as single-celled photosynthetic organisms, possess remarkable capabilities to convert sunlight, carbon dioxide, and nutrients into biomass that is rich in proteins, vitamins, and minerals, thereby presenting innovative solutions to the challenges faced by traditional animal feed sources (Molino et al., 2018; Souza et al., 2021). In light of increasing global demand for food and the pressing need for sustainable agricultural practices, microalgae can provide a viable pathway by offering high-quality nutritional products without the ecological drawbacks associated with conventional feed sources such as fishmeal and soybean (Mavrommatis et al., 2023; Saadaoui et al., 2021).

Recent research has further highlighted the potential of integrating microalgae cultivation systems into livestock environments, where exhaust air rich in ammonia and carbon dioxide can be utilized for biomass production while simultaneously reducing gaseous emissions (Uguz, 2024). Such approaches demonstrate the dual benefits of nutrient recovery and pollution mitigation, reinforcing the role of microalgae as a circular bioresource in animal production systems. Microalgae have been observed to contain significant levels of key nutrients, including vitamins A, B12, D, and E, alongside essential trace minerals such as iron, zinc, and calcium (Saadaoui et al., 2021; Camacho et al., 2019). The inclusion of microalgae in animal feed not only enhances mineral bioavailability but also contributes to the overall health and productivity of animals. Specifically, certain species such as *Chlorella* and *Spirulina* are noted for their rich content of these essential nutrients, which play critical roles in maintaining animal health, supporting growth, and improving reproductive performance (Chang et al., 2021; Ribeiro et al., 2020). Furthermore, microalgae serve as an effective source of polyunsaturated fatty acids (PUFAs), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are vital for optimal metabolic function and overall health in various livestock and aquaculture species (Gebereyowhans et al., 2023; Ribeiro et al., 2020).

As the quest for sustainable animal nutrition solutions continues, the application of microalgae technology represents an innovative response. By utilizing non-arable land and low freshwater resources, microalgae cultivation can significantly mitigate challenges posed by conventional agriculture, such as land degradation and resource depletion (Mavrommatis et al., 2023; Finamore et al., 2017). Recent studies have demonstrated that the integration of microalgae into animal diets leads to improved weight gain, enhanced immune responses, and better oxidative status among animals, providing both economic and environmental benefits (Nagappan et al., 2021; Altomonte et al., 2018). Despite promising outcomes, larger-scale adoption of microalgae as a feed source hinges on further research to optimize cultivation processes, ensure consistent nutrient profiles, and reduce production costs, thereby making this innovative technology accessible to a broader range of agricultural practices (Zakaria et al., 2020; Liao et al., 2023).

Microalgae seem poised to revolutionize animal nutrition by supplying essential minerals and vitamins through a sustainable, eco-friendly approach. Leveraging their rich nutrient profiles and ability to thrive in diverse conditions, microalgae technology offers a pathway towards improved animal health and productivity while minimizing environmental impacts associated with traditional animal feed sources.

3. Mineral composition of microalgae

Microalgae are gaining recognition as a valuable source of essential minerals in both human and animal nutrition due to their unique biochemical composition and high nutrient density. Characterized by their efficient photosynthetic capabilities, microalgae can yield significant levels of minerals, including calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), and a variety of trace elements such as iron (Fe), zinc (Zn), and copper (Cu) (Sarker et al., 2018; Katiyar et al., 2021). These minerals are crucial for numerous physiological functions in animals, contributing to bone development, metabolic processes, and immune system support (Sarker et al., 2018; Khemiri et al., 2021).

The mineral composition of microalgae varies depending on several factors, including species, growth conditions, and the composition of the cultivation medium. Studies have shown that certain freshwater and marine microalgae species, such as *Chlorella* and *Spirulina*, not only provide an excellent supply of essential minerals but also exhibit higher mineral concentrations than traditional terrestrial feed ingredients (Wild et al., 2018; Tibbetts et al., 2014; Sheikhzadeh et al., 2024). For instance, research indicates that the micronutrient profile in microalgae is substantial and well absorbed by animals, making them an attractive option for enhancing the nutritional quality of animal feeds (Sarker et al., 2018; Sheikhzadeh et al., 2024; Costa et al., 2024).

Moreover, the cultivation conditions, such as nutrient availability and ratios in the culture media, significantly influence the mineral uptake and overall nutritional profile of microalgae (Wild et al., 2018; Sheikhzadeh et al., 2024; Falcão et al., 2023). This variability adds an additional layer of complexity, as optimizing cultivation parameters can lead to significant differences in the mineral content of the harvested biomass. Consequently, proper management of the cultivation environment is paramount for maximizing the nutritional benefits of microalgae (Sheikhzadeh et al., 2024; Abdel-Wareth et al., 2024).

In the context of animal nutrition, the introduction of mineral-rich microalgae into the diet has shown promising results, improving not only the mineral status of livestock but also enhancing product quality, such as meat and milk (Coelho et al., 2020; Costa et al., 2024). The potential of microalgae to serve as a sustainable source of essential minerals offers a compelling strategy to improve animal health and productivity while mitigating the environmental impact associated with conventional feed ingredients (Costa et al., 2024; Khemiri et al., 2021). As research continues to elucidate the benefits and optimize the use of microalgae, their role in animal nutrition may expand, providing a valuable resource for future food production strategies.

The mineral content of different species of microalgae (on dry weight basis) is presented in Table 1, given in previous studies performed by Bertoldi et al. (2008), MišurCoVá et al. (2010), Aouir et al. (2017), Assunção et al. (2017), Shabana et al. (2017), Wild et al. (2018), Altmann et al. (2019), Di Lena et al. (2020), Bélanger et al. (2021), Cerri et al. (2021), Ferreira et al. (2021), Madhubalaji et al. (2021), Martins et al. (2021), Cabrita et al. (2022), Cabrol et al. (2022), Karapanagiotidis et al. (2022), Tibbetts et al. (2023), Neylan et al. (2024), Sathyamoorthy and Rajendran (2022).

Table 1. The profile of macro- and micro minerals in different species of microalgae as animal feed

	Microalgae species				
	Arthrospira sp.	***Chlorella sp.***	***Isochysis sp.***	***Porphyridium sp.***	***Schizochytrium sp.***
Macro minerals (g/kg)					
Calcium (Ca)	0.23-10.3	0.36-53.3	5.83-11.5	6.40-20.7	3.53
Potassium (K)	10.9-29.1	0.01-133	4.10-13.1	6.70-13.5	5.71
Magnesium (Mg)	0.77-4.00	0.41-16.4	3.38-10.0	4.74-13.7	-
Sodium (Na)	4.80-96.2	0.07-16.5	11.1-27.4	8.10-70.7	1.04
Phosphorus (P)	1.50-14.8	5.11-27.1	6.25-27.6	3.17-14.6	4.88
Sulphur (S)	-	0.12	-	6.40-14.8	7.68
Micro minerals (mg/kg)					
Copper (Cu)	0.40-18.7	0-119	6.00-28.0	7.86-45.3	2.08
Iron (Fe)	106-1036	187-5400	15.2-2284	377-11101	13.5
Manganese (Mn)	13.0-550	20.9-1270	36.0-834	22.0-259	-
Zinc (Zn)	0.40-30.1	9.07-530	20.0-940	41.0-392	37.4

4. Vitamin composition of microalgae

Microalgae have emerged as a valuable source of essential vitamins, making them significant players in the fields of nutrition and nutraceuticals. This diverse group of microorganisms, which includes both freshwater and marine species, is noted for its rich composition of various vitamins such as A, B1, B2, B12, C, D, E, and carotenoids like beta-carotene and lutein (Iskusnykh et al., 2022; Mishra et al., 2021; Son & Kwon, 2023). The contribution of microalgae to vitamin intake is particularly relevant, considering the increasing global trend toward plant-based diets, where traditional sources of these nutrients may be limited (Mishra et al., 2021; Son & Kwon, 2023). Utilizing microalgae as a dietary supplement can help bridge nutritional gaps, particularly for vulnerable populations who may lack adequate access to animal products.

Research has established that microalgal biomass contains not only a variety of vitamins but also bioactive compounds that contribute significantly to health benefits. For instance, carotenoids present in microalgae have potent antioxidant properties, which can help mitigate oxidative stress and prevent diseases (Navarro et al., 2016; Mondo et al., 2020). Moreover, some microalgae are recognized for their ability to synthesize vitamin B12, a nutrient crucial for energy metabolism and neurological function, which poses challenges in plant-based diets where such vitamins are generally scarce (Grossman, 2016; Helliwell et al., 2014). The presence of this vitamin highlights the potential role of microalgae as a fortifying agent in food products aimed at enhancing nutritional quality.

Furthermore, the concentration of vitamins in microalgae varies according to species, cultivation conditions, and growth parameters, such as light intensity and nutrient composition of the growth medium (Aguilar-Ruiz et al., 2022; Camacho et al., 2019). This variability presents an opportunity for researchers and food technologists to selectively cultivate microalgae that optimize the yield of specific vitamins, thereby providing tailored nutritional compounds for both human and animal consumption. The use of microalgae not only addresses nutritional deficiencies but also supports the growing demand for sustainable and functional food sources, aligning with global efforts to enhance food security through innovative products (Saadaoui et al., 2021; Hashmi et al., 2023).

The vitamin composition of microalgae manifests their immense potential as functional food ingredients. With their rich nutrient profiles, microalgae can serve not only as dietary supplements but also as high-value components in the development of health-promoting functional foods and animal feed, paving the way for more sustainable and nutritionally complete dietary solutions. According to the previous studies (Borowitzka, 2010; Prieto et al., 2011; Tibbetts et al., 2015; Madeira et al., 2017; Tavernari et al., 2018; Niccolai et al., 2019; Zhu et al., 2020), the vitamin contents of different microalgae species (on basis dry weight) as animal feed is summarized in Table 2.

Table 2. Vitamin profile of different species microalgae as animal feed (on basis of dry weight)

Microalgae species	**Vitamin (mg/kg)**				
	Vitamin B_1	**Vitamin B_6**	**Vitamin B_9**	**Vitamin B_{12}**	**Vitamin E**
Arthrospira platensis	5-50	4-50	0.9	-	50-190
Dunaliella salina	-	-	-	42-49	153.2
Chlorella	15-24	10-17	269	-	200
Nannochloropsis granulata	70	3.6	-	1.7	0.29
Tetraselmis chui	1.09	5.8	-	-	1.57

5. Microalgae usage in animal nutrition

5.1. Poultry nutrition

Microalgae have garnered significant attention as a promising dietary supplement in poultry nutrition due to their high nutritional value and functional properties (Uguz et al., 2025). These microscopic organisms, particularly species such as *Spirulina (Arthrospira platensis*) and *Chlorella*, provide a rich source of proteins, vitamins, minerals, and polyunsaturated fatty acids (PUFAs), particularly omega-3s, which can enhance the overall health and productivity of poultry (Uguz & Sozcu, 2023; Zhang et al., 2025). The integration of microalgae into poultry diets not only helps to meet the growing demand for alternative and sustainable feed ingredients but also addresses concerns of nutrient deficiencies common in conventional feed sources, such as soy and corn (Spínola et al., 2024; Abdel-Wareth et al., 2024).

In poultry nutrition, essential minerals including calcium (Ca), phosphorus (P), magnesium (Mg) which could be identifies as major minerals, and also minor minerals, such as zinc (Zn), iron (Fe), selenium (Se), copper (Cu), are required for vital functions and physiological processes, covering bone development, enzyme functions, immune response and antioxidant defence system, eggshell formation etc. (Smith, 2018; Johnson, 2020; Costa et al., 2024). In commercial nutrition, the minerals are supplemented into diets in organic sources (in chelated forms) and inorganic sources (in form of mineral salts, Brown and Smith, 2019; Liu, 2018). Organic minerals could provide better bioavailability with a higher cost compared to the inorganic forms. Therefore, microalgae have increasingly gained interest as a sustainable and cost-effective alternative instead of organic and inorganic minerals (Costa et al., 2024).

Research indicates that microalgae can significantly improve growth performance, feed conversion ratios, and meat quality in broiler chickens. Studies have shown that the inclusion of Spirulina can lead to improvements in carcass traits, antioxidant status, and overall health, enhancing the nutritional profile of poultry products (Alghamdi et al., 2024; Costa et al., 2024). Furthermore, microalgae contribute positive effects on gut health by acting as prebiotics, potentially improving nutrient digestibility and absorption. This is particularly beneficial in enhancing the resilience of poultry against stressors such as disease and adverse environmental conditions (Mishra et al., 2023).

The ability of microalgae to substitute antibiotics in poultry diets is noteworthy. Investigations reveal that dietary inclusion of species like Spirulina can promote gut health and mitigate pathogenic bacteria without the adverse side effects associated with conventional antibiotic use (Alghamdi et al., 2024). This transition towards antibiotic-free growth promotion aligns with the industry’s shift towards more sustainable practices, responding to consumer demand for natural and healthy poultry products.

Moreover, the cultivation of microalgae is advantageous as it requires fewer resources compared to traditional agriculture; it can be grown in various environments, including brackish or saline water, which aids in conserving freshwater resources (Thapa, 2020). The varied profiles of microalgae, influenced by their growing conditions and species, allow for tailored nutritional formulations that can meet the specific dietary needs of different poultry breeds (Uguz & Sozcu, 2023;2024; Thapa, 2020).

The use of microalgae in poultry nutrition presents a forward-looking solution that combines sustainability with enhanced nutritional benefits. The prospect of integrating these versatile organisms into poultry diets not only aids in improving growth performance and product quality but also supports the broader goals of sustainable agricultural practices.

Performance and product quality

The incorporation of microalgae into animal feed has sparked growing interest among researchers and farmers due to its potential to enhance animal performance and product quality. Microalgae, such as *Chlorella* and *Spirulina*, are recognized for their rich nutritional profiles, containing high levels of essential proteins, vitamins, antioxidants, and beneficial fatty acids, particularly omega-3 and omega-6 (Sikiru et al., 2019; Martins et al., 2021; Saadaoui et al., 2021). Feeding strategies that integrate these organisms aim to optimize growth rates and improve the overall health and product quality of livestock and poultry.

Studies have demonstrated that the dietary inclusion of microalgae can lead to significant improvements in feed efficiency and growth performance in various species, including broilers, rabbits, and pigs. For instance, the supplementation of *Chlorella vulgaris* in rabbit diets has been linked to enhanced growth rates and better oxidative stress status, thereby promoting overall health and productivity (Sikiru et al., 2019; Holman & Malau-Aduli, 2012). Similarly, research indicates that *Spirulina* can elevate the nutritional profiles of meat and eggs, enhancing their market value by improving parameters such as fatty acid composition and antioxidant properties (Bruneel et al., 2013; Martins et al., 2021).

Moreover, the functional properties of microalgae contribute not only to the nutritional enhancement of the feed but also to overall physiological benefits for the animals. Microalgae have been shown to stimulate immune responses, reduce inflammation, and improve gut health, collectively contributing to better performance metrics (Mavrommatis et al., 2023; Holman & Malau-Aduli, 2012). The antioxidants present in microalgae are crucial for mitigating oxidative stress, a condition that can adversely affect growth performance and product quality in livestock. By incorporating naturally derived antioxidants from microalgae, farmers can potentially reduce the need for synthetic additives in animal diets (Fábregas & Herrero, 1986; Mavrommatis et al., 2023).

The use of microalgae in animal feeds promotes enhanced product quality, exemplified by improved meat tenderness, color, and overall palatability. Studies indicate that feeding marine microalgae can enrich the omega-3 fatty acid content of meat products, aligning with consumer preferences for healthier and more nutritious food options (Martins et al., 2021; Bruneel et al., 2013). Furthermore, the sustainability of microalgae production, requiring less land and water than traditional feed sources, underscores its role as a viable alternative in modern animal nutrition practices (Saadaoui et al., 2021).

Microalgae present a multifaceted approach to augmenting the growth performance and health of livestock and improving the quality of animal products, paving the way for more sustainable and effective feeding strategies in the agricultural sector. Continued research into the optimization of microalgal inclusion ratios and types will further enhance their application in animal nutrition, delivering benefits that resonate through food production systems and contribute to overall environmental sustainability.

Health status

Microalgae are nutrient-dense organisms rich in proteins, essential fatty acids, vitamins, and antioxidants, making them an excellent additive that can support and enhance the physiological well-being of animals (Liao et al., 2023; Mavrommatis et al., 2023; Saadaoui et al., 2021). The bioactive components found in microalgae, including their unique polysaccharides and carotenoids, have been linked to antioxidant and anti-inflammatory properties that can bolster immune function and improve overall health in various species, including poultry, ruminants, and aquaculture (Saadaoui et al., 2021; Souza et al., 2019; El-Bahr et al., 2020).

Research has demonstrated that dietary supplementation with microalgae can lead to improved gut health, reduced reliance on antibiotics, and enhanced microbial diversity in the gastrointestinal tract (Liao et al., 2023; Chang et al., 2021). For instance, Mesobiliverdin IXα-enriched microalgae have been shown to promote intestinal health in weaning piglets, demonstrating a potential alternative to traditional antibiotic treatments (Liao et al., 2023). Furthermore, studies indicate that microalgae can significantly enhance the antioxidant status of animals, combat oxidative stress, and support the immune system, which is crucial for maintaining health, especially in high-production settings (Saadaoui et al., 2021; El-Bahr et al., 2020).

One of the critical advantages of incorporating microalgae into animal diets is their ability to improve not only animal health but also the quality of animal products. For example, milk and meat derived from animals fed with microalgae have been shown to have improved nutritional profiles, including higher levels of omega-3 fatty acids and enhanced flavor attributes (Gebereyowhans et al., 2023; Saadaoui et al., 2021). This enhancement in product quality resonates with consumer demand for healthier and nutritionally superior food options.

Moreover, microalgae provide a sustainable feed option that minimizes the environmental footprint associated with conventional feed ingredients. Their cultivation requires fewer natural resources and yields can be obtained without competition for arable land (Mavrommatis et al., 2023; Souza et al., 2021), thus promoting a more sustainable approach to animal production. This characteristic aligns with current trends in animal agriculture, where sustainability and animal welfare are increasingly prioritized concerns.

The inclusion of microalgae in animal feed is a promising strategy not only for improving animal health through enhanced nutritional support and immunity but also for delivering high-quality products to consumers. Continued research and exploration of various microalgal species and their applications will further elucidate their potential benefits and enhance their role in sustainable animal husbandry practices.

5.2. Ruminant nutrition

The utilization of microalgae in ruminant nutrition has gained significant interest in recent years, driven by the growing need for sustainable and nutrient-rich feed alternatives. Microalgae, such as species from the genera *Chlorella, Spirulina*, and *Nannochloropsis*, boast rich compositions, including proteins, essential fatty acids, vitamins, and minerals, which make them highly beneficial for improving ruminant health and productivity (Mota et al., 2024; Amir et al., 2024). The incorporation of microalgae into ruminant diets has been linked to enhanced feed efficiency, increased milk production, and improved meat quality, highlighting their potential as a crucial component of modern livestock feeding strategies (Kalbe et al., 2018; Amir et al., 2024).

One of the primary advantages of incorporating microalgae into ruminant diets is the enhancement of the animal's nutrient profile. Microalgae are particularly rich in long-chain omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have nutritional benefits that can improve the fatty acid composition of both muscle and milk (Kalbe et al., 2018; Amir et al., 2024). Research shows that feeding microalgae can elevate the levels of beneficial fatty acids in meat and milk products, making them more appealing to health-conscious consumers (Amir et al., 2024; Gomaa et al., 2018). Additionally, specific microalgal supplements have been associated with improved gut health by stabilizing the rumen microbial community and enhancing digestion, thus leading to better nutrient absorption and utilization (Wild et al., 2018).

Another notable aspect of microalgae is their ability to reduce methane emissions from ruminants, a significant contributor to greenhouse gas emissions in livestock production. Incorporating microalgal biomass into diets has been shown to mitigate enteric fermentative processes that generate methane, presenting an effective strategy for more sustainable ruminant production systems (Gomaa et al., 2018; Haque, 2018). Given the urgent need to address climate change and its effects, the role of microalgae in reducing methane production is particularly timely and relevant (Haque, 2018).

Despite their potential benefits, the incorporation of microalgae into ruminant diets presents some challenges, including palatability issues that may arise from the specific flavors associated with certain microalgal species (Kholif et al., 2020). Research into the appropriate mixing ratios and feeding strategies is crucial to accommodate these palatability concerns while maximizing the health benefits of microalgae for ruminants (Kholif et al., 2020; Wild et al., 2018). Additionally, the variability in nutrient composition among different microalgae species necessitates careful selection based on the intended dietary formulation and desired health outcomes (Wild et al., 2018).

The usage of microalgae in ruminant nutrition represents a promising avenue for enhancing animal health, improving product quality, and promoting environmental sustainability within the livestock sector. Continued research efforts will help optimize dietary formulations and address challenges related to acceptance and nutrient variability, ultimately paving the way for the wider adoption of microalgae as a staple in ruminant feeding practices.

Performance and product quality

Microalgae, rich in protein, essential fatty acids, vitamins, and antioxidants, provide a sustainable and nutrient-dense alternative to conventional feed ingredients (Mota et al., 2024; Kholif et al., 2020). Their unique biochemical composition supports growth and development in ruminants such as cattle, sheep, and goats, and positively influences the fatty acid profiles and overall quality of meat and milk products (Glover et al., 2012; Kholif et al., 2020).

Research indicates that supplementing ruminant diets with microalgae can significantly improve growth rates and feed efficiency. For instance, studies have shown that adding microalgae, such as *Chlorella vulgaris* and *Nannochloropsis oceanica*, to diets enhances nutrient digestibility and fermentation efficiency in the rumen, leading to improved overall performance in terms of weight gain and milk production (Kholif et al., 2020; Kholif et al., 2020). Additionally, microalgae consumption has been associated with improved health parameters in ruminants, including enhanced immunity and reduced oxidative stress, contributing to lower morbidity rates and improved animal welfare (Kholif et al., 2022; Yusuf et al., 2018).

A crucial aspect of microalgae supplementation is its impact on the fatty acid composition of animal products. Feed formulations that include microalgae are known to enrich milk and meat with beneficial omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Glover et al., 2012; Alves et al., 2018). The increased concentration of these health-promoting fatty acids improves not only the nutritional quality of the products but also aligns with consumer demands for healthier and more sustainable food options (Kholif et al., 2020; Kholif et al., 2016). Furthermore, microalgae have the potential to mitigate methane emissions from ruminants, an important factor in sustainable livestock production and climate change mitigation (Palangı et al., 2022; Fasolo et al., 2020).

However, the successful integration of microalgae into ruminant diets is influenced by several factors, including the type and composition of the microalgae, the proportion used in the diet, and the specific ruminant species being fed (Yusuf et al., 2018; Fasolo et al., 2020). Challenges associated with palatability and digestibility also need to be addressed, as some ruminants may exhibit preferences against certain microalgal feed components (Kholif et al., 2020; Kholif et al., 2022). Thus, ongoing research is critical to optimize the incorporation of microalgae in feed formulations, ensuring that the benefits on performance and product quality are maximized while overcoming potential hurdles (Glover et al., 2012; Fasolo et al., 2020).

The utilization of microalgae in ruminant nutrition presents a compelling opportunity to enhance animal performance and improve the quality of meat and dairy products. By leveraging the rich nutritional profiles of various microalgal species, livestock producers can contribute to the development of more sustainable and healthier food systems while meeting the evolving demands of consumers.

Health

The incorporation of microalgae into ruminant nutrition has emerged as a forward-thinking approach to enhance animal health and productivity in livestock production systems. Microalgae, such as *Spirulina* and *Chlorella*, are rich in essential nutrients, including proteins, vitamins, minerals, and polyunsaturated fatty acids (PUFAs), making them an effective natural supplement to traditional feed sources (Gebereyowhans et al., 2023; Mavrommatis et al., 2023). This nutrient density supports optimal growth and development in ruminants and contributes to better overall health and resilience against various stressors.

Microalgae have significant antioxidant properties, which can help mitigate oxidative stress in ruminants, thus promoting a healthier physiological state. The inclusion of microalgae in the diet has been associated with enhanced immune responses, potentially reducing the incidence of diseases and improving overall herd health (Mavrommatis et al., 2023). Additionally, certain microalgae species can positively modulate the gut microbiota of ruminants, fostering a balanced microbial ecosystem that is essential for efficient digestion and nutrient absorption (Wild et al., 2018). Improved gut health enhances feed efficiency and directly influences animal performance, leading to better weight gain and increased productivity (Wild et al., 2018).

Furthermore, microalgae play a vital role in improving the nutritional quality of dairy and meat products. Research has demonstrated that incorporating microalgae into ruminant diets can enhance the fatty acid profile of milk and meat, particularly by increasing the levels of beneficial n-3 fatty acids, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Gebereyowhans et al., 2023; Liu et al., 2020). This aligns with growing consumer demands for healthier animal products and promotes environmental sustainability by potentially lowering the carbon footprint of livestock production (Gebereyowhans et al., 2023).

Despite the promising benefits, the variability in the nutrient composition of different microalgae species, together with potential challenges related to palatability and feed acceptance, underscores the importance of continued research in this area (Wild et al., 2018). Efforts to optimize inclusion rates and improve the overall formulation of ruminant diets incorporating microalgae will be crucial to maximize health outcomes and production efficiency.

Microalgae represent a sustainable and functional feed option that can significantly enhance ruminant health and improve the quality of animal-derived products. Ongoing studies will further clarify their multifaceted benefits and promote broader adoption within the livestock industry, contributing to healthier animals and a more sustainable agricultural system.

6. Conclusions

In conclusion, the application of microalgae as a mineral and vitamin source in animal nutrition presents a compelling case for enhancing the health, productivity, and sustainability of livestock production systems. Microalgae are uniquely positioned to provide essential nutrients that can help bridge gaps in traditional feed sources while promoting optimal animal growth and welfare. As demonstrated in various research studies, the incorporation of microalgae into diets for ruminant species has been associated with improved growth performance, enhanced immune functions, and increased nutritional quality of animal-derived products, such as meat and milk, thereby aligning with consumer demands for healthier food options.

Moreover, the ecological advantages of microalgae cultivation, including their ability to grow in non-arable land and utilize carbon dioxide, further underscore their potential as a sustainable feed ingredient. Ongoing scientific efforts to optimize the use of different microalgal species and address challenges related to palatability and nutrient variability will be crucial for maximizing their benefits in animal nutrition. Ultimately, the integration of microalgae into livestock feeding practices not only supports improved animal health but also contributes positively to broader sustainability goals within the agricultural sector, paving the way for a more resilient and environmentally conscious food production system.

In summary, the exploration and utilization of microalgae in animal nutrition reflect a significant shift toward innovative, sustainable solutions that cater to the health of both animals and ecosystems, representing a vital component of future agricultural practices.

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CHAPTER 12

Direct Effects of *Phloeomyzus passerinii* on Wood Anatomy of Poplar Tree

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1. INTRODUCTION

Forests are among the most important renewable natural resources worldwide and play a vital role in sustaining life in all its forms. The goods obtained from forests affect living organisms throughout their entire life cycles. Forest products, including fuelwood, paper, furniture, cosmetic raw materials, and non-wood forest products, are integral to human life from economic, ecological, and socio-cultural perspectives. In addition, forests offer essential ecosystem services, providing recreational opportunities for people and critical habitats for wildlife and other organisms.

Forest ecosystems are influenced by interconnected ecological, climatic, and biotic factors that interact in a cascading manner (Topaloğlu et al., 2014). Global warming and climate change represent major current and future threats by disrupting temperature and moisture regimes that regulate forest growth and development. Increasing temperature and humidity favor higher insect population densities, and if these trends continue unchecked, insect pests are likely to cause severe damage to forest ecosystems (Jaworski and Hilszczański, 2013). The effect of temperature and humidity changes on insects development their impact on forest ecosystems in the context of expected climate change. Although forests are impacted by global warming, they also play a crucial role in climate regulation through carbon sequestration and other ecosystem functions, thereby influencing global climate dynamics (Flavin, 1990). However, while contributing to climate change mitigation, forests are increasingly subjected to insect damage as an indirect consequence of global warming. Compared with forest fires and other abiotic disturbances, insect infestations represent a more subtle yet potentially more destructive threat to forest ecosystems. The effects of insect damage often remain unnoticed in the early stages and become evident only when populations expand into large-scale outbreaks. Such outbreaks can result in widespread tree mortality, significant degradation of forest stands, and substantial economic costs associated with control and management. Ineffective responses to insect infestations jeopardize forest sustainability and lead to serious ecological and economic losses (Öztürk, 2020).

Poplar species are widely regarded as cornerstone species in plantation forestry due to their exceptionally rapid growth and high productivity. Their short rotation period, typically reaching harvest maturity within 12–15 years, makes poplars particularly suitable for intensively managed afforestation and industrial plantations (Sarıbaş, 1985). Additional advantages include ease of vegetative propagation and hybridization, strong adaptability to a wide range of climatic and soil conditions, and the capacity to produce straight, uniform stems within a relatively brief growth cycle, all of which enhance their economic efficiency in plantation systems (Ünal et al., 2016). Owing to their preference for moist and riparian sites, poplar plantations play a significant role in meeting raw material demands and contributing substantially to the national forestry economy.

Under extreme conditions, aphid infestations lead to a reduction in growth increment and timber yield in some cases, induce alterations in tree architecture (Straw et al., 2000; Kurzfeld-Zexer et al., 2010). Aphid feeding can adversely affect the physiological processes essential for tree growth. Leaves subjected to aphid infestation commonly show a decline in photosynthetic performance due to chlorophyll loss, stomatal impairment, and restricted gas exchange (Zhang et al., 2022; Shen et al., 2023; Poljaković-Pajnik et al., 2024). Furthermore, damage to phloem tissues and the deposition of honeydew on leaf surfaces can modify plant water relations by disrupting transpiration and elevating leaf temperature, thereby intensifying growth limitations. Long-term aphid infestations may also inhibit cambial activity, resulting in decreased cambial cell production and modifications in xylem and phloem development, which are ultimately reflected in reduced annual tree-ring widths (Twayana et al., 2022; Keleş et al., 2024).

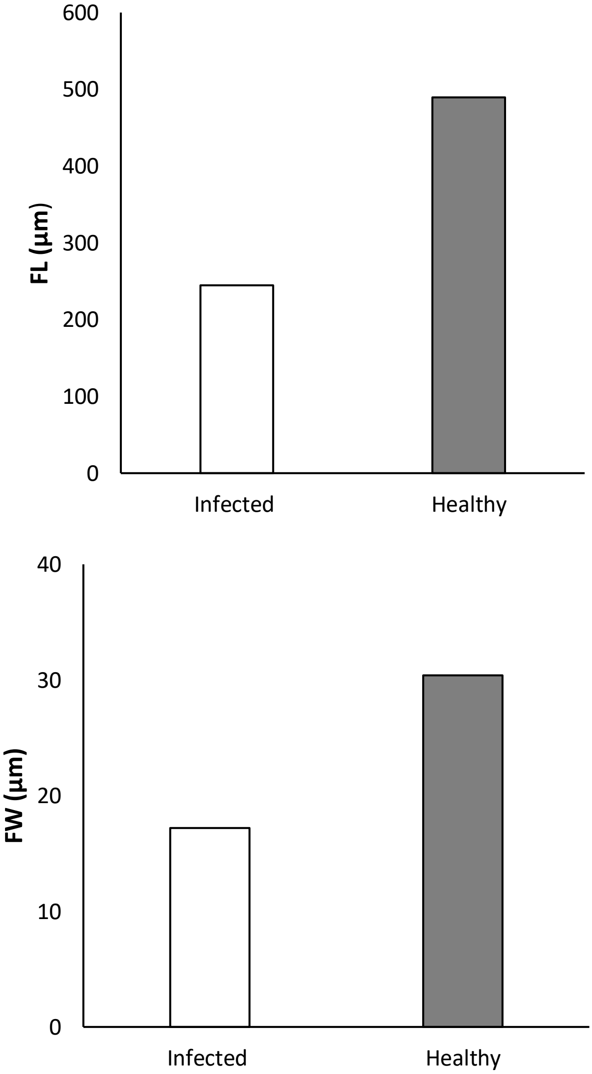
The woolly poplar aphid (*Phloeomyzus passerinii* Signoret) is a highly destructive pest responsible for passerinii disease, which affects the heartwood of poplar trees throughout Europe, Asia, and North America (Allegro & Cagelli, 1996). Despite its broad distribution and the severity of the damage it causes, the effects of passerinii disease on poplar growth and development in Türkiye have not been adequately investigated. Therefore, the primary objective of this study is to assess the impact of P. passerinii infestation on the growth and developmental performance of poplar trees.

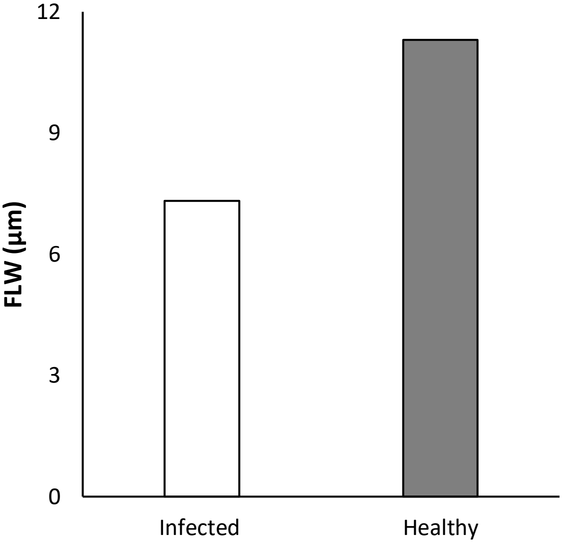
2. MATERIALS AND METHODS

This study was conducted in several different poplar trees in the Daday district of Kastamonu province. Tree age was determined from transverse discs collected at the root collar and breast height (DBH). Sample discs were air-dried, progressively sanded, and visually cross-dated to clearly identify annual growth rings. For anatomical analysis, small wood blocks (approximately 1.5 × 1.5 × 1.5 cm) were excised from the discs. Transverse, radial longitudinal, and tangential longitudinal sections were prepared following standard wood anatomical procedures. Prior to sectioning, samples were boiled in distilled water to ensure complete saturation and removal of entrapped air, then stored in an alcohol–glycerin–distilled water solution (1:1:1, v/v/v) until use (Gerçek, 1997; Merev, 1998). Anatomical measurements were conducted in accordance with IAWA standards and included fibre length, fibre width, fibre lumen width, fibre wall thickness, ray height, and ray width. Measurements were compared between infected and healthy poplar trees.

3.RESULTS

This study evaluated the effects of *Phloeomyzus passerinii* infestation on the wood anatomical characteristics of poplar trees by comparing infected and healthy individuals. The sampled trees had a mean age of approximately 12 years. The anatomical traits of poplar trees varied between infected and healthy trees (Fig. 1).

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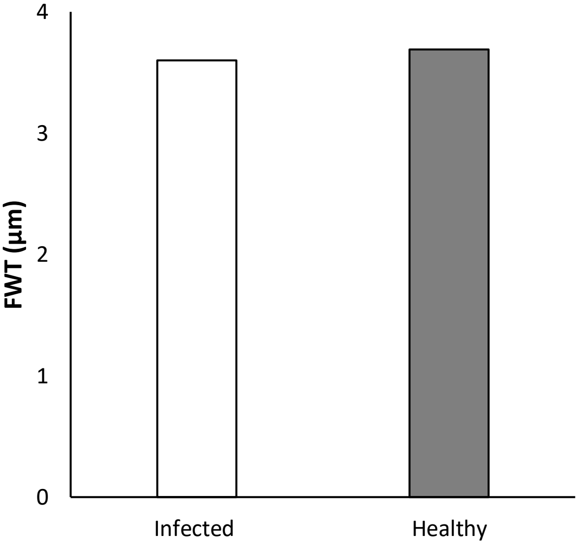
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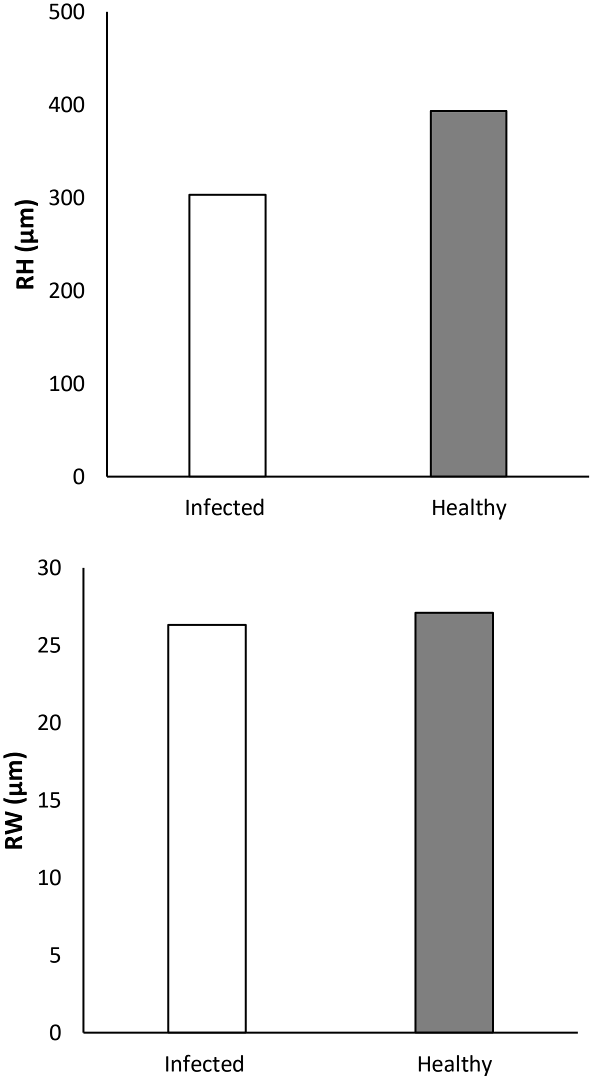

Fig. 1 The anatomical parameters (FL – Fibre Length, FW - Fibre Width, FLW – Fibre Lumen Width, FWT – Fibre Wall Thickness, RH – Ray Height, RW – Ray Width).

Significant differences were observed in mean fibre length, fibre width, and fibre lumen width between infected and healthy poplar trees ($p < 0.05$). Mean fibre length measured 489.5 µm in healthy trees, compared with 244.7 µm in infected trees. Mean fibre width was 30.4 µm in healthy poplars and 17.2 µm in infected individuals. Healthy trees also exhibited a larger mean fibre lumen width (11.3 µm) relative to infected trees (7.32 µm) (Fig. 1). Additionally, mean ray height was higher in healthy trees (393.3 µm) than in infected trees (303.2 µm).

In contrast to the pronounced differences in fibre length, fibre width, fibre lumen width, and ray height, the values for fibre wall thickness and ray width were relatively similar between the two groups (Fig. 1). Mean fibre wall thickness was 3.69 µm in healthy trees and 3.60 µm in infected trees, while mean ray width measured 27.1 µm and 26.3 µm, respectively.

4. CONCLUSION

Forest ecosystems are directly influenced by ecological, climatic, and biotic factors, all of which are closely interconnected and function in a domino-like manner. One of the most critical challenges of the present and future is global warming and climate change, which threaten the balance of temperature and moisture key climatic factors governing forest growth and development. Increases in temperature and humidity contribute to a rise in insect populations per unit area. If such increases cannot be controlled in the future, insect pests are expected to cause severe damage to forest ecosystems. The poplar cottony scale, *Phloeomyzus passerinii* (Signoret) (Hemiptera: Aphididae), is a major monophagous insect pest in poplar plantations. Originating from Eurasia, this species causes substantial damage to poplar stands, particularly in Central Anatolia, Türkiye. In Kastamonu, this aphid species also commonly impaired the growth and developmental performance of poplar trees. The present study investigated the effects of *Phloeomyzus passerinii* on the wood anatomical properties of poplar trees. The results showed different patterns between healthy and infected poplar trees. From an ecological wood anatomy perspective, healthy poplar trees exhibited greater fibre length, fibre width, and fibre lumen width compared with aphid-infected individuals. In contrast, fibre wall thickness and ray width remained largely unaffected by infestation. These patterns suggest that *Phloeomyzus passerinii* primarily constrains cambial activity and cell expansion rather than secondary wall deposition, resulting in an overall reduction in wood anatomical dimensions in infected poplar trees.

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CHAPTER 13

Gut Microbiata and Welfare in Farm Animals

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Introduction

The gastrointestinal tracts of animals comprise a complex and different microbial community that is essential for immune system development, pathogen removal, meat quality, and nutrient digestion and absorption (Chen et al. 2022). Bacteria, fungi, viruses, archaea, and protozoa appear in the intestines with a rich population. The intestinal micro-ecosystem is made up of a variety of microorganisms, the most of which are bacteria, making up approximately 70% of the microbial population (Sekirov et al. 2010; Sender et al. 2016). Bacteria, protozoa, fungus, viruses, and archaea create the microbiome, an ecosystem that is ten times larger than the host's cell count. The microbiome functions both to decompose or transform nutrients, and to provide a protective effect, as it competes with pathogenic microorganisms for nutrients (Khafipour et al. 2016). The number and diversity of population in the intestine show a rapid increment and then gradually become stabilize while growing of animals. The intestine acts as a stable ecological niche for these resident bacteria's, depending on the host's physiological processes for essential biological functions such as nutrition and reproduction (Fetissov 2017). Interactions between host-microbiota-environment and adaptation between these interactions are important for the health and productivity of animals (Wang et al. 2024).

Different species have different dominant gut microbial group compositions and relative proportions (Richards et al. 2005). In ruminants, the rumen has a function for being an anaerobic fermentation chamber, 95% of which consists of bacteria. Both of the number of microorganisms and diversity are important factors for ruminants' digestive system (Li et al. 2015; Young 2016). The rumen microbial ecosystem consists of different kinds of bacteria, protozoa, fungi, archaea and bacteriophages. Bacteria constitute approximately 40–60% of the rumen microbial mass. The rumen environment harbors a wide variety of bacteria capable of digesting cellulose, hemicellulose, lignin, starch, protein, and lingo-cellulosic feed components (Alataş and Umucalılar 2011). On the other hand, the gastrointestinal microbiota of poultry is a complex ecosystem comprising mainly bacteria, then virus, protozoa, fungi and archaea (Smulikowska, 2006; Wei et al., 2013). The gut microbiome includes different bacterial populations, mainly phyla *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria* in poultry (Naeem and Bourassa, 2025).

The gut microbiota forms a secretory-immunomodulatory network with the host animal, which is intimately related to cognitive functions, responses against stress, and social behavioral patterns. Furthermore, the microbiota has significant roles and also mediates information exchange across the gut-brain axis through multiple pathways, comprising the enteric nervous system, immune system, gut endocrine signals, and microbial metabolites (Song et al. 2021). In this process, the intestinal microbiota significantly influences the host brain and behavior by transmitting information to the central nervous system via different pathways, comprising the vagus nerve, peripheral circulatory system, and immune system, through the signaling molecules they produce (Yu et al. 2024). The structure and function of the intestinal microbiota can be altered by various complex factors such as disease, stress, environment, diet, and drugs (Sender et al. 2016; Lopez-Garcia et al. 2021; Chen et al. 2022; Li and Guan 2017; Difford et al. 2018). Therefore, dysbiosis of the rumen and intestinal microbiota directly affects methane release, performance, feed efficiency, health status, and welfare status of animals (Richards et al. 2005; Xu et al. 2013; Zhang et al. 2021; van Kuijk et al. 2021).

In this chapter, the composition of the gut microbiota in farm animals, its functions, host-microbiota interactions, its relationship with welfare, the effects of management factors on the microbiota and microbiota management are discussed.

Development and Importance of Gut Microbiota in Ruminants

The ruminant gastrointestinal microbiome starts to develop during postnatal period, when it is colonized by the maternal microbiome and the surrounding (Jami et al. 2013). Early colonizers (Streptococcus and Enterococcus) utilize the available oxygen in the gut, creating the anaerobic environment necessary for anaerobic gut inhabitants such as *Bifidobacterium* and *Bacteroides*. These two bacteria are key intestinal bacteria that have beneficial effects on the mucosal immune system. Their presence plays a vital role in the development of immunological tolerance to commensal microbiota (Malmuthuge et al. 2015). Therefore, neonatal gut colonization is a critical period for the developing gut and immune system, and has long-term health implications as it impacts multiple systems such as metabolism and neurodevelopment (Hansen et al. 2012).

Because ruminants' immune systems are not fully developed at birth, colostrum intake plays a vital role in passive immunity. Colostrum is rich in immunoglobulins (especially IgG), growth factors, vitamins, and antimicrobial compounds. In ruminants, immune transfer occurs via colostrum, and its beneficial microbial content, particularly *Lactobacillus* and *Bifidobacterium*, supports the development of the gut microbiota (Işık Soytürk et al. 2025). These bacteria create lactic acid and short-chain fatty acids, which have nutritional and regulatory effects on intestine cells called colonocytes. Colostrum also includes complex carbohydrates and a variety of proteins that control intestine growth and early immunological responses (Rostoll-Cangiano et al. 2025). These encourage the digestive tract's rapid cell division, development, and maturation (Blum and Baumrucker 2002). The luminal environment, which is rich in bacteria, and the intestinal immune system are physically separated by the intestinal barrier. These growth factors accelerate the proliferation of epithelial cells, increase mucus production, and stimulate the secretion of antimicrobial peptides, all of which help to produce a protective layer that restricts direct contact between bacteria and epithelial cells. This distinction is crucial for minimizing potential harmful effects by avoiding needless immune reactions to commensal microbes (Chase and Kaushik 2019). Therefore, inadequate colostrum intake in newborn ruminants could disrupt intestinal development, inhibiting the proliferation and adherence of beneficial bacteria, and potentially leading to persistent negative effects on the gut microbiota (Song et al. 2021). Microorganisms from the meal eventually make their way into the rumen when the animal grows and starts eating. A portion of the bacteria that had previously colonized the digestive system start to work, starting fermentation and greatly expanding the microbiome. For ruminant growth and performance, microbial fermentation in the rumen is essential (Jami et al. 2013).

Bacteria found in the digestive tracts of all ruminants are primarily *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Fibrobacteres*. The most prevalent bacteria in the ruminant digestive tract are *Actinobacteria*, *Proteobacteria*, *Spirochaetes*, and *Tenericutes*, which break down cellulose, hemicellulose, fatty acids, polysaccharides, and aromatic compounds after *Bacteroidetes* and *Firmicutes* (Khafipour et al. 2016; Forcina et al. 2022). The rumen microbial community of 14-day-old calves includes all main rumen bacterial groups, including cellulolytic and proteolytic species (Li et al. 2012). According to Jami et al. (2013), certain rumen bacteria that are essential for rumen function could be found as early as one day after birth. According to Guzman et al. (2015), Dill-McFarland et al. (2017), and Yeoman et al. (2018), bacteria colonize the rumen on the first day following birth, however pre-weaned calves' rumens are underdeveloped, and the microbial makeup of the gut gradually changes as cows get older. In young animals, the gut microbiota develops quickly, and as animals age, it stabilizes. Thus, another element influencing the rumen microbiome is host age. For instance, newborn, two-month-old, six-month-old, and two-year-old cows have different dominating rumen bacteria (Jami et al. 2013). Guo et al. (2020) reported that bacterial composition is influenced by the intestinal segment in which the animal is located, as well as its age. The distribution of diverse microbiota is influenced by the distinct biological and chemical conditions found in each segment of the intestine. The jejunum has a mixture of *Butyrivibrio* and *Firmicutes* bacteria. Populations of *Anaeroplasma*, *Butyrivibrio*, *Faecalibacterium*, *Paludibacter*, *Prevotella*, *Pseudobutyrivibrio*, and *Succinivibrio* are found in the rumen (Thacharodi et al. 2024). The rectum had the fastest microbiota growth rate, indicating that the bacterial community there matures early in life, followed by rumen and abomasum bacteria. The duodenum, which matures later, had the slowest microbiota growth rate. Young animals' microbiota is more comparable to adjacent intestinal segments than that of older animals, indicating that early intervention may be an effective means of modifying an animal's gut microbiota and enhancing intestinal health and function (Guo et al. 2020).

Bacteriophages, abundant in the gastrointestinal tract, help maintain gut microbial balance by influencing bacterial diversity, antimicrobial resistance, and metabolic functions (Lin et al. 2021). Fungi contribute to fiber digestion in ruminants, forming a small but functionally important component of the gut microbiota. Members of the phylum *Neocallimastigomycota*, which includes fungi like *Piromyces* and *Neocallimastix*, produce strong hydrolytic enzymes that break down plant matter. These enzymes work in concert with fibrolytic bacteria and methanogenic archaea to improve the rumen's ability to break down lignocellulose (Krol et al. 2023; Jyothi et al. 2024). Archaea are a significant part of the gut microbiome, especially in the rumen, where they contribute to methanogenesis (Danielsson et al. 2017). The large intestine has the greatest amount of microorganisms in young ruminants before the rumen develops. (von Engelhartd 2011; Rey et al. 2014; De Nardi et al. 2016). *Lactobacillus* (*L. acidophilus*, *L. plantarum*, *L. fermentum*, *L. salivarus L. casei*, *L. helveticus and L. brevis*) is prevalent in the rumen of young ruminants, particularly during weaning, and in older animals during concentrate feeding (Stewart et al. 1997). *Butyrivibrio fibrisolvens* and *Prevotella ruminicola* are the most important proteolytic bacteria in the rumen (Sales et al. 2000). Approximately 1500 species of archaea are present in the digestive tract of ruminants, 90% of which are methanogenic, constituting 3–4% of the rumen ecosystem. *Methanobrevibacter ruminantium*, Methanosarcina barkeri, and *Methanobrevibacter* species are the most important methanogens in the rumen microbial ecosystem of sheep and cattle (Efenberger et al. 2014). Acetogenic bacteria such as *Acetytomaculum ruminis* and *Eubacterium limosum* reduce methane excretion by oxidizing oxygen to hydrogen to acetic acid and increase the energy efficiency of ruminants (Alataş and Umucalılar 2011). Methanogenic archaea are found in the immature rumen of lambs long before solid substrate enters the rumen (2-4 days), and they reach concentrations comparable to those in adult animals about 10-14 days after birth (Morvan et al. 1994). Goats consume a wider variety of feedstuffs than cattle and sheep, which is reflected in their gut microbiota composition, even though they share a core microbiome with these animals (Cremonesi et al. 2018). The predominant bacterial species in the goat microbiota, Ruminococcaceae and Prevotellaceae, provide effective fiber breakdown and metabolic flexibility (Wang et al. 2022). Zou et al. (2020) investigated the colonization process of microbiota adhering to rumen and intestinal tissue in goats from birth to adulthood and reported that the formation and dynamic fluctuations of the gut microbiome undergo three distinct microbiome progression stages throughout life (in the fetus, in late pregnancy, from newborn to weaning, and from weaning to adulthood). However, ruminants have a strong host-microbiota specificity (Kittelmann et al. 2014), which means that the microbial community composition and fermentation profile will revert to their pre-treatment composition following any modification (such as rumen digestion, exogenous bacterial administration, or antibiotic treatment) (Weimer et al. 2010). Thus, a potentially unique opportunity to manipulate the microbial community through dietary adjustments is presented by the developing rumen in the infant.

Development and Importance of Intestinal Microbiota in Poultry

The intestinal microbiota has vital roles for maintaining homeostasis and providing a protection against pathogens (Diaz Carrasco et al., 2019). In birds, the gut microbiota interacts with immune system by maturating the immune cells in order to ensure that commensal microorganisms are tolerated, identify and control pathogens, such as Clostridium perfringens and Escherichia coli, and keep the pathogens under the immunity control (Adil and Magray, 2012; Klindworth et al., 2013). Additionally, it increases the epithelium absorptive surface and produces metabolites such as short chain fatty acids, indoles, vitamins, and antimicrobial compounds, which aid in the formation of the lamina propria, mucosal layer, and intestinal epithelium (Dibner and Richards, 2005; Shakouri et al., 2009; Oakley et al., 2014).

The colonization of bacteria's in the gut of chicks begins immediately hatching process with contacting to environmental materials (hatching baskets, transportation boxes etc.), management issues by people or other materials in growing houses (feed, water, bedding material etc.; Kizerwetter-Swida and Binek, 2008; Stanley et al., 2014). As the chicks grow, the microbiota become more complex and comprises a range diverse of microorganisms (Wei et al., 2013).

The major microbiota of major organs in the gastrointestinal tract could be categorized as given here (Bindari and Gerber, 2022):

Crop	*Firmicutes*: *Lactobacillus* (dominant), *Enterococcus*, *Streptococci* *Actinobacteria*: *Bifidobacterium* *Proteobacteria*: *Enterobacter*
Gizzard	*Firmicutes*: *Lactobacillus* (dominant), *Enterococcus*, *Streptococci*, *Clostridium*
Small intestine	*Firmicutes*: *Lactobacillus* (dominant), *Clostridium*, *Ruminococcus*, *Streptecossus*, *Candidatus arthromitus* *Proteobacteria*: *Escherichia*, *Enterococcus* *Bacteroidetes: Bacteroides*
Ceca	*Firmicutes: Ruminococcus, Clostridium, Faecalibacterium, Pseudobutyrivibrio, Maegamonas, Lactobacillus, Subdoligranulum, Sporobacter, Acetanaerobacterium, Peptococcus, Eubacterium* *Bacteroidetes: Bacteroides* *Proteobacteria*: *Escherichia*, *Bilophila*

Cloaca	*Archaea: Methanobacterium, Methanobrevibacter, Methanothermobacter, Mathanosphaera, Methanopyrus, Methanothermus, Methanococcus* *Firmicutes: Ruminococcus, Clostridium, Faecalibacterium, Ruminococcus, Bacillus, Eubacterium, Turicibacter* *Fusobacteria:Fusobacterium*

The Relationship between Microbiota and Animal Welfare

Because of its extensive microbial community, the rumen in ruminants is frequently compared to a "black box." The ruminal microbiota is thought of as a separate organ made up of many bacteria with a gene pool far greater than the host's cells. Through certain metabolic pathways, this microbiota affects the host's health and nutritional absorption. As a result, the host's digestive and metabolic processes depend heavily on the ruminal microbiota (Tardiolo et al. 2025). The intricate web of endocrinological, immunological, and neurological mediators that connect the gut and the brain is known as the gut-brain axis. The behavior of farm animals is greatly influenced by this network (Grenham et al. 2011). For example, metabolites produced by the gut microbiota, particularly short-chain fatty acids, bile acids, and amino acid derivatives, play an important role in appetite regulation in ruminants. Intestinal microorganisms contribute to appetite regulation by regulating food perception, nerve signaling, and hormone secretion within the digestive tract (Yu et al. 2024). Furthermore, the gut microbiota is considered a crucial element in regulating fat accumulation. Excessive fat accumulation is closely linked to quality and is considered a serious health and economic problem in animal production (Zhao et al. 2022), as an imbalance in the gut microbiota contributes to lipid accumulation (Zhao 2013). Portune et al. (2017) reported that obesity is closely associated with the gut microbial profile. In livestock, the microbiota in the gastrointestinal tract digests and ferments the feed consumed by animals, converting it into nutrients used by the host for milk and meat production (Liu et al. 2021c). The rumen is a specialized compartment containing diverse populations of microorganisms, including bacteria, protozoa, anaerobic fungi (and methanogels) (Matthews et al. 2019). Rumen fermentation is crucial for the digestion and utilization of fibrous feed in ruminants. The digestibility of lignocellulosic feed can be improved by increasing the number or activity of rumen lignocellulosic organisms, which positively influences rumen fermentation (Muck et al. 2018). In ruminants, the rumen microbiota ferments feed and produces volatile fatty acids, which are the primary energy source for the host (Miura et al. 2021). Short-chain fatty acids, such as acetate, propionate, and butyrate, are produced when commensal gut bacteria in the colon ferment food fiber. These acids are quickly absorbed by colon cells through mono-carboxylate transporters, passive diffusion, or bicarbonate exchange (Liu et al. 2021d). In addition to boosting insulin secretion, preserving energy homeostasis, and enhancing the function of the intestine, liver, skeletal muscle, and adipose tissue, colon-derived short-chain fatty acids that reach systemic circulation have anti-inflammatory and immunomodulatory effects (Liu et al., 2021a). Short-chain fatty acids, in particular, are among the metabolites generated by the beneficial gut microbiota and are crucial for both illness prevention and recovery (Sanderson 2004). Short-chain fatty acids have also been reported to play a crucial role in maintaining the morphology of the small intestinal wall and providing energy to host cells and intestinal microflora (Mannelli et al. 2019; Jiao et al. 2021).

Nutrient utilization, immunological response, and host health are all impacted by the interaction between the gut microbiota and intestinal epithelial cell metabolism (Liu et al. 2021). Disturbances in the homogeneity of the gut microflora have serious effects on the digestive system and various other organs. This disharmony in commensal relationships also leads to various metabolic disorders. The dominance of methanogens leads to bloating, while high-starch feeds cause rumen acidosis. Disrupted microflora also leads to diseases such as reticuloperitonitis, ulcers, diarrhea, and so on (Xu et al. 2021). As a result, the gut bacteria and microbial functions of an unwell host are different from those of a healthy person (Clavel et al. 2017). Similar to an immune-active organ, the rumen microbiome is continuously stimulated by both internal and external factors, such as conditionally dangerous microbes like *Salmonella* and *E. coli* that are constantly present in the gastrointestinal tract and microorganisms in feed. The appropriate composition and abundance of the microbiome influence the proper development of intestinal capillaries, which in turn facilitates nutrient absorption. Reductions in the abundance or changes in the composition of the microbiome can reduce host immunity and increase the risk of metabolic diseases (Cholewińska et al. 2020). Similarly, Spiljar et al. (2017) demonstrated that the activation or suppression of the Toll-like receptor produced by microbial signals may impact the modulation of immune responses. According to Liu et al. (2021), the rumen microbiota is therefore crucial for the growth and control of the newborn's immune system as well as for sustaining long-term health and high production. According to Malmuthuge et al. (2013), the expression of host genes encoding mucosal immune responses and epithelial barriers depends on the gut microbiota and mucosal immune functions during the weaning and newborn periods.

On the other hand, the rumen microbiota is also associated with both rumen and intestinal fermentation processes that lead to methane emission in ruminants. The final result, which is regarded as a byproduct of fermentation, is methane. By converting fiber to me-thane, methanogens in the rumen use methanogenesis pathways to aid in the digestion of fiber. Nevertheless, the host loses a substantial amount of dietary energy as a result of this process (2–12%) (Moss et al. 2000). Methylotrophs like *Methanosphaera*, Methanosarcinales, and *Methanomassiliicoccacea*, which use acetate, methylamines, and methanol to create methane gas, are found in adult ruminants. Methanogenesis is further aided by other methanogenic archaea in the rumen, including Methanomicrococcus blattioca, *Methanobacterium ruminantium*, and *Methanosarcina barkeri* (Mizrahi et al. 2021). Additionally, methanogenic microorganisms react with hydrogen-producing rumen organisms, such as protozoa, bacteria, and fungi. Dietary modification can improve ruminant performance by controlling methanogen levels (Malmuthuge 2017). Thus, manipulating the digestive microbiome also improves feed absorption and reduces methane emissions, a greenhouse gas (Khafipour et al. 2016; Malmuthuge 2017).

Recent studies have focused on the relationship between gut microbiota and welfare in poultry production (Kers et al., 2018; Iannetti et al., 2020; Di Marcantonio et al., 2022). Especially when an imbalance occur in gut microbiota, the population of beneficial bacteria shows decline and the harmful bacteria population (such as *E. coli, Salmonella, Campylobacter*) increases, that could be accepted as and stressors and increase the fear level of birds (Kogut, 2019; Lyte et al., 2025). Gao et al. (2025) indicated that birds having longer tonic immobility duration had a poor microbial diversity with a lower population of beneficial bacteria in their gut. In a field study, broiler farms with lower cumulative welfare score based on health status, feeding, housing conditions, and behavioral patterns) had poorer microbiota diversity, consisting with a higher percentage of pathogenic bacteria (Di Marcantonio et al., 2022).

Effects of Management and Environmental Factors on Microbiota

Generally, the physical environment—such as access to indoor and outdoor spaces, the number of animals per stocking density, etc.—is a crucial factor that influences phenotypic development and, ultimately, animal welfare (Zeineldin et al. 2019). Raising systems and management practices in ruminants change the gut microbiota's makeup, which impacts the animals' immune systems and meat quality (Thoene-Reineke et al. 2014; Chen et al. 2019; Wiersema et al. 2021; Yan et al. 2021). Microbial diversity has shown inter-individual heterogeneity in response to feeding regimes and grazing patterns. Compared to sheep maintained in grassland settings, where nutritional diversity promotes a richer microbial ecosystem, sheep raised in intensive systems typically have decreased microbial diversity (Zhou et al. 2017). According to Chen et al. (2022), pasture grazing also improves livestock meat quality and immune system activity by changing the gut microbiota's composition and increasing the number of beneficial bacterial species like *Lactobacillus*, *Actinobacteria*, *Bacteroidetes*, *Clostridium spp*., and *Proteobacteria*. Similarly, Zhang et al. (2021) found that the intestinal microbiota composition of Angus cattle fed in feedlots and those grazed in pastures differed significantly, which had an impact on the quality of the meat. According to Kim and Wells (2016) and Mwangi et al. (2019), grazing has an impact on the metabolic characteristics of the gut microbiota (*Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*) in dairy and beef cattle, which in turn influences the characteristics of skeletal muscle and meat components. The amylolytic bacterium *Streptococcus bovis* is usually found in low numbers in cows fed high-grain diets or in cows that have long adapted to grain diets, and in high numbers in cows fed high-grain diets that have not yet adapted, according to Fernando et al. (2010), who also reported that a high-grain diet increases rumen starch. According to Khafipour et al. (2009), increasing the amount of concentrate feed poses a threat to rumen metabolic processes, especially in high-yielding ruminants, because it hinders the microbiome's ability to digest cellulose, which causes rapid starch degradation and a drop in rumen pH to 5.5. This is because the altered microbiome (reduced numbers of *Firmicutes*, *Streptococcus* and *Bacillu*s species) leads to a slower synthesis process, a weakened digestion process, an accelerated flow of digesta from the rumen to the abomasum, and a decrease in milk fat (Jami et al. 2014; McCann et al. 2014). A meal high in roughage promotes the growth of *Bacteroides*, *Fibrobacter*, and *Ruminococcus* in the rumen as well as their cellulolytic activity as compared to concentrate diets. On the other hand, ruminants moving from rich grazing to concentrated diets show a decrease in bacterial diversity, but a movement in the opposite direction shows an increase in the diversity and concentration of bacteria involved in lactate generation, amylolysis, cellulose, and proteolysis (Belanche et al. 2021). Hungate (1966) reported that ruminant bacterial composition is predominantly Gram-negative when fed diets high in roughage, while animals fed a high-grain diet contain more Gram-positive bacteria such as *Lactobacillus*, and that rumen pH levels decrease after consumption of readily fermentable carbohydrates. Furthermore, the ruminal microbiome-host interaction stimulates ruminal epithelial development. Acetate and butyrate production driven by the rumen microbiome in lambs and kids after starter feeding suggests that growth-related signaling pathways in the rumen epithelium are mediated by growth-related genes. Papilla morphology and rumen epithelial growth are just two of the physiological processes in the epithelium that are regulated by this symbiotic interaction between the host and the ruminal microbiome (Lin et al. 2019). Conversely, limited exposure of lambs to their mothers or other animals delayed the establishment of cellulolytic bacteria compared to lambs raised in close contact with their mothers during the first few weeks of life. This observation suggests that early environmental exposure also plays an important role in the establishment of a host-specific microbiota (Fonty et al. 1989). Additionally, castration increases the amount of subcutaneous and intramuscular fat in ruminants via altering the microbiota in the small intestine (Whon et al. 2021). Similarly, Zheng et al. (2022) reported that the gut microbiota affects meat quality by increasing intermuscular and intramuscular fat status. Fat accumulated in muscles affects meat properties such as tenderness, juiciness, and flavor and is of economic importance in animal husbandry (Fang et al. 2017). Animal meat quality is impacted by alterations in the gut microbiota, according to other research (Lahiri et al. 2019; Qi et al. 2019; Wen et al. 2019; Chen et al. 2021; Liu et al. 2021a).

In ruminants, rapid dietary changes (especially when switching to high-protein diets) and the short time required for microbiome adaptation lead to disruption of microbiota composition. Inadequacies in the feeding of animals related with un-adapted feeding system and inappropriate feed quality lead to metabolic disorders such as acidosis in ruminants (McCann et al. 2014; Khafipour et al. 2016). Acidosis occurs when ruminants are fed high-sugar feeds (i.e., feeds containing starch) or silage with low-quality. Excessive lactic acid accumulation in the rumen leads to a decrease in rumen pH from 6.2–7.2 to 5 or even 4. Protozoa are absent from the rumen's contents as a result of the pH drop, and Gram-negative bacteria belonging to the *Bacteroidetes* species proliferate (Khafipour et al. 2009). Along with acidosis, mastitis and even lameness occur, impairing animal welfare (Khafipour et al. 2016). Ketosis is another metabolic disorder resulting from a poorly adjusted diet and, consequently, a disrupted microbiome composition. The transition from dry to lactation, typically in ruminants, makes it particularly difficult for the gastrointestinal microbiome to adapt to dietary changes. During this time, bacterial composition also changes, with increased levels of bacteria such as *Anaerovibrio lipolytica*, *Prevotella bryantii*, and *Megasphaera elsdenii* and decreased numbers of fibrolytic bacteria such as *F. succinogenes* and *Butyrivibrio proteoclasticus* (Brown 2018).

Seasonal changes significantly affect microbiota composition (Liu et al. 2022). Ribeiro et al. (2017) reported that the microbial composition in the digestive tract changes when host habitats change. Jiang et al. (2020) reported that the core gut microbiota in goats consists of *Bacteroides*, *Oscillospira*, *Alistipes*, *Ruminococcus*, *Clostridium*, and *Oscillibacter* species, and that gut microbial species are similar across goat breeds under the same rearing environment and nutritional conditions. Cui et al. (2023) reported that microbial diversity in Tibetan sheep was higher in the pasture-grazing group. The relative abundance of microorganisms changed significantly when sheep were switched from pasture to enclosure (using a diet of natural pasture grasses or oat straw). The prevalence of pathogenic bacteria, which was higher during grazing, decreased in enclosures, benefiting sheep health. They also demonstrated that feeding strategies have strong effects on the rumen microbiota of sheep. Cholewinska et al. (2021) reported that a sudden environmental change caused by transport also altered the gut microbial composition of young ruminants. Six hours after transport, a decrease in the populations of *P. britain*, *Anaerovibrio lipolyitica*, *P. ruminicola* and *Succinivibrio dextrinosolvens* was noted, along with an increase in acid production and a decrease in pH, characterized by the proliferation of *Ruminococcus flavefaciens, R. amylophylus, Prevotella albensis* and *Fibrobacter succinogenes*.

There are many factors affecting the microbiota in the gastrointestinal tract, mainly bird genotype, age, sex, diet, following liter quality and management, housing condition and hygiene, medication treatments during production period (Kers et al., 2018). Previous studies highlighted a huge difference in the cecal microbial composition of different breeds of fast growing broilers (Pandit et al., 2018; Ji et al., 2020; Tumova et al., 2021), and between fast and slow-growing broiler genotypes (Richards et al., 2019). On the other hand, it was reported that the dominant bacteria could change with increasing of age. Kers et al. (2020) reported that the phylum of *Clostridiaceae* was predominantly found in the cecum of broilers at 0 days of growing period, whereas Lu et al. (2003) indicated that *Lactobacillus* comprised the 25% of the cecum bacteria at 3 days of age. Additionally, sex-linked variations in glycan and lipid metabolism by the cecal microbiota may be responsible for the sex-related microbiota difference (Lumpkins et al., 2008; Lee et al., 2017; Cui et al., 2021). In a previous study, Cui et al. (2021) found a higher population of *Bacteroides*, *Megasphaera*, *Phascolarctobacterium Megamonas* in male broilers, whereas the phylum of *Akkermansian* was higher in cecum of females at 35 days of age.

Nutrition, in the manner of both diet composition and form (mash, crumble and pellet) has a crucial impact on gut microbiota as changing microbial population and the phylum content (Apajalahti et al., 2004). Compared to mash form, the wheat-based feed in pellet form increased the number of *Coliforms* and *Enterococci* in the ileum and *Clostridium perfringens* in the broiler cecum (Engberg et al., 2002). Besides, some different feed additives (such as probiotics, prebiotics, and enzymes) are largely used in commercial poultry feeds. These additives are efficient in modulation of gut microbiota and stimulation of immunity (Yadav and Jha, 2019, Bindari and Gerber, 2022).

Microbiota Management

Animal gut microbiota composition depends on nutrients (Lanng et al. 2021). A high rice diet improved the meat quality of goats while altering the gut microbiota (increasing Oscillibacter) (Wang K. et al. 2021). According to Miura et al. (2021), one of the most crucial elements in the production of high-quality meat is the rise of *Prevotella spp.* and *Bacteroidales* species during the fattening phase in cattle, which provides a consistent rumen fermentation. By controlling the ruminal and intestinal microbiota of lambs, dietary L-carnitine supplementation enhanced ruminal fermentation and raised short-chain fatty acid contents (Martin et al. 2022). According to Jiao et al. (2021), intra-ileal administration of short-chain fatty acids enhanced the quality of meat by preventing the production of fatty acids and the expression of Acetyl-CoA carboxylase mRNA in *Longissimus dorsi*. Chen et al. (2022) reported that antioxidants such as magnolol, butyrate, essential oil, etc. supplemented with diet will reduce oxidative stress by changing the intestinal microbiota composition and improve the meat quality of farm animals.

However, since antibiotics are prohibited, feed additives known as direct-fed microorganisms (DFMs) have been identified as one of the antimicrobial substitutes that include naturally occurring living microbes and improve animal health and productivity (Krol et al. 2023). Microorganisms utilized as DBMs for ruminants often include fungus like *Saccharomyces* and *Aspergillus*, bacteria like lactic acid-producing and lactic acid-utilizing bacteria, and other bacterial groupings. DFMs are largely based on their effects on rumen pH stabilization, rumen fermentation, and feed digestion, which improve rumen function, thereby improving feed efficiency and ruminant productivity (Ban and Guan 2021).

To lessen acidosis, antimicrobials such monensin are used to stop the pH decline brought on by abrupt dietary changes (Clemmons et al. 2019). When choosing microbial strains for use as DFM in ruminants, factors like enhancing host health, bolstering intestinal barrier function, boosting host immune responses, lowering stress and enhancing animal welfare by controlling gut-brain communication, competing with pathogens for nutrients in the gut and promoting desired microbial fermentation, generating antimicrobial compounds like organic acids, bacteriocins, and hydrogen peroxide, and promoting enzyme secretion (Yoon and Stern 1995; Seo et al. 2010; FAO 2016). The main lactic acid-producing bacteria (LAB) in the rumen include *S. bovis*, *Lactobacillus* species, and *S. ruminantiu*m, which can proliferate quickly in low pH environments. Acidosis is brought on by *S. bovis* overgrowth. As a result, LAB like *Lactobacillus* and *Enterococcus* can be employed as DFMs due to their capacity to boost lactic acid-utilizing bacteria and enhance ruminal microorganisms that are acclimated to the presence of lactic acid in the rumen (Hernandez et al. 2008). Because it may use lactate to create propionate, the primary precursor of gluconeogenesis in the ruminant liver, *Propionibacterium* is another possible lactic acid-using DFM. P*ropionibacterium* supplementation has been shown to boost energy efficiency, provide additional substrate for lactose synthesis, and possibly lessen ketosis in early-lactation dairy cows (Weiss et al. 2008). Furthermore, anaerobic rumen fungi, found among the rumen microbiota, possess the most potent fibrolytic activity when added to the diet, aiding the utilization of roughage by breaking lignocellulosic bonds. Forage is the most important feed source for ruminant diets, but its high fiber content is a limiting factor. Therefore, anaerobic rumen fungi, acting as fiber degraders, physically penetrate plant cell walls and enzymatically attack them, contributing to the breakdown of rumen fibers to convert cellulose to glucose (Agustina et al. 2020).

The DFMs, like yeast, have been reported to have the potential to support the maturation of rumen microbial flora and gut microbial balance in young ruminants (Chaucheyras-Durand et al. 2019). According to Kraimi et al. (2019), by changing the gut microbial composition and the release of microbial metabolites (such as short-chain fatty acids, neurotransmitters, and catecholamines), DFM given to farm animals may have an impact on anxiety-like behaviors, memory capacity, social behavior, and feeding behavior. DeVries and Chevaux (2014) reported shorter feeding intervals and longer rumination duration in ruminants fed diets supplemented with *Saccharomyces cerevisiae* yeast. Broadway et al. (2015) reported that yeast supplementation increased rumen pH and volatile fatty acid content, reduced methane formation, and increased total microbiota and cellulolytic bacterial counts. As a natural feed additive, yeast has also been reported to promote the growth of lactic acid-utilizing microorganisms and minimize rumen lactic acid accumulation (Marden et al. 2008).

Manipulating the rumen microbiome early in life can lead to lower methane emissions (Abecia et al. 2018). This intervention, without targeting the removal of protozoan populations colonized by methanogens, has reduced methanogenesis by 13–31% (Hegarty 1999; Guyader et al. 2014). During the development of the rumen microbiome, a shift in feeding practices (from a high-starch to a fiber-rich diet) resulted in a rise in *Methanobacteriaceae* and a fall in the hydrogen-utilizing family *Succinivibrionaceae*. Therefore, manipulating the feeding regimen early in life may be a potential way to achieve lower methane emissions during animal development (Furman et al. 2020). Because ruminants in the developing stage are carriers of fully developed rumen function, they can receive microbial transmission from adult rumen microorganisms. Transplanting rumen microorganisms may accelerate colonization processes compared to some microbial preparations, and the host's immune system may also be impacted (Li et al. 2023). Palma-Hidalgo et al. (2021) demonstrated that inoculating young ruminants with fresh rumen fluid from adult animals early in life resulted in a more complex and diverse bacterial community during the pre-weaning period and accelerated the formation of methanogenic, protozoal, and fungal communities. This microbial complexity facilitated the host ruminant's adaptation to nutritional challenges, facilitating the transition from milk to solids during weaning. Furthermore, kids showed a higher daily live weight gain at 8 weeks (immediately after weaning). This suggests that kids experience less growth retardation and weaning shock, thus manipulating the rumen microbiota with fresh rumen fluid leads to positive health and welfare outcomes.

In poultry nutrition, gut microbiota could be modulated by nutritional strategies as administration of feed additives (Jha and Berrocoso, 2015). Probiotics could be described as microbial substances containing single or multi cultures of living beneficial microorganism (especially lactic acid bacteria - *L. bulgaricus, L. acidophilus, L. lactis, L. casei, L. plantarum*, *L. salivarius*, *Enterococcus faecium, E. faecalis, Streptococcus thermophilus, Bifidobacterium sp.* (Huang et al., 2004), fungi and yeast. This additive takes part in multiple mechanisms, primarily by promoting gut integrity, supporting the microbiota, boosting immunity, and then improving growth performance in birds (Singh et al., 2015; Wang et al., 2017).

Prebiotics is defined as non-digestible source and could potentially regulate the composition of gut microbiota selectively beneficial phylum of bacteria, and improve growth performance and health status of birds (Yadav and Jha, 2019). Commercially most used prebiotics are oligo-saccharides, fructo-oligosaccharides, mannan-oligosaccharides, galacto-oligosaccharides, soya-oligxo-saccharides, xylo-oligo-saccharides, pyro-dextrins, and lactulose The prebiotic supplementation could increase the bifidobacteria population affecting the host's health in a positive manner (Huyghebaert et al., 2011), also lactic acid bacteria suppressing pathogens (Alloui et al., 2013; Ganguly, 2013) .

Other feed additive is organic acids including lactic acid, propionic acid, butyric acid, acetic acid, fumaric acid etc. (Ricke, 2003), provide a stimulating effect for gut health and also performance of birds (Saki et al., 2012). Organic acids provide a suppressing effect growth of pathogenic bacteria such as *Enterobacteriaceae* and *Salmonella*, therefore prevent intestinal diseases, improve immunity, nutrient digestibility and productivity (Smulikowska et al., 2010).

In poultry nutrition, many exogenous enzymes (phytase, β-glucanase, amylase, protease, lipase etc.) is largely used to counter the anti-nutritional factors in feed ingredients (Costa et al., 2009; Jha et al., 2015), minimize the environmental pollution originating excreta, support the growth of beneficial bacteria in the gut, optimize the fiber utilization, providing a better growth performance (Singh et al., 2017). Also, the enzyme supplementation is effective to control the horizontal contamination of Salmonella (Yang et al., 2008; Adeola and Cowieson, 2011).

Conclusion

It is challenging to properly determine the regulatory targets and mechanisms of the gut microbiota and its metabolites in farm animals due to their richness and complexity. Furthermore, because the makeup of the gut microbiota varies among individuals and is influenced by a variety of environmental and managerial factors, it is very difficult to define. These microbes have the capacity to form what is known as a healthy microbiota as long as they are advantageous to the host. Thus, it is essential to comprehend how variations and changes in the gut microbiota affect host health. Maintaining this research will support the sustainable growth of animal production in addition to enhancing animal welfare and productivity.

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CHAPTER 14

The Effects of Nanotechnology-Based Essential Oil Microencapsulations on Aphids and Whiteflies

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1. INTRODUCTION

Aphids (Aphididae) and whiteflies (the *Bemisia tabaci* complex) cause severe economic losses worldwide in vegetable, field, and ornamental crops due to both direct feeding damage and their ability to transmit plant viruses. The intensive and prolonged use of chemical insecticides has accelerated the development of resistance in these pests, leading to additional problems such as pesticide residues, environmental toxicity, and the disruption of beneficial entomofauna. Consequently, research efforts have increasingly focused on the development of safer, target-specific, environmentally friendly, and long-lasting plant-derived bioinsecticides.

In this context, essential oils have long been investigated as promising bioinsecticidal agents. However, their practical application under field conditions is limited by several intrinsic drawbacks, including high volatility, photodegradation, short persistence, and poor adhesion to plant surfaces (Akhtar & Isman, 2018; Pavela, 2015). Many of these limitations have begun to be overcome through the rapid advancement of nanotechnology-based formulation techniques in agriculture (Benelli, 2018; Rai et al., 2021). Nanotechnology enhances the bioavailability of essential oils by reducing droplet size to the nanometer scale and improves photostability (Silva et al., 2020; Sharifi-Rad et al., 2020).

Moreover, nanoemulsion systems formed with surfactants facilitate penetration through the insect cuticle, while nanoencapsulation using polymeric matrices or solid lipid nanoparticles protects essential oils against environmental degradation and prolongs their biological activity (Campos et al., 2019; López et al., 2021; Gupta et al., 2024). This review evaluates the insecticidal, repellent, and physiological effects of essential oil nanoformulations on aphids and whiteflies, and critically analyzes the advantages and limitations of nanoemulsion and nanoencapsulation techniques based on more than forty recent academic studies.

2. CONTRIBUTIONS OF NANOTECHNOLOGY TO ESSENTIAL OIL FORMULATIONS

2.1 Fundamentals of Nanoemulsions

Nanoemulsions are typically oil–water dispersions with droplet sizes ranging from 20 to 200 nm. Surfactants play a crucial role in stabilizing these droplets, thereby reducing oxidation and degradation of essential oils under ultraviolet (UV) radiation (Lutfi et al., 2024; Teixeira et al., 2021). The reduction in droplet size significantly enhances cuticular penetration, which is a key factor in the efficacy of contact insecticides (Sharma et al., 2022).

Pascual-Villalobos et al. (2017) demonstrated that essential oil nanoemulsions caused 30–60% higher mortality in *Aphis gossypii* compared to conventional emulsions. Similarly, Ghosh et al. (2019) reported a three- to five-fold increase in the storage stability of nanoemulsified essential oils.

2.2 Nanoencapsulation Techniques

Nanoencapsulation involves entrapping essential oils within polymeric, lipid-based, or biopolymeric shells to achieve:

- controlled release,
- protection against photodegradation, and
- prolonged persistence on plant surfaces (Mendes et al., 2020; Sánchez-Ortega, 2021).

Christofoli et al. (2015) showed that polymer-encapsulated *Zanthoxylum* oil formulations exhibited prolonged bioactivity and enhanced resistance to UV degradation. Likewise, Salama et al. (2025) reported that nanoencapsulated geranium oil displayed significantly higher toxicity against sap-sucking insect pests compared to its conventional form.

In addition, nanoencapsulation has been shown to improve the stability of terpenoid compounds responsible for repellent activity, which is particularly important for monoterpenes with short half-lives (Sharifi-Rad et al., 2022).

2.3 Modes of Action

The effects of essential oil nanoformulations on insect pests are mediated through multiple biochemical and physiological mechanisms, including:

- inhibition of acetylcholinesterase (AChE) activity (Huang et al., 2023),
- disruption of cuticular integrity (Sharma et al., 2022),
- impairment of the respiratory system,
- induction of antifeedant behavior (Isman, 2020), and
- behavioral repellence mediated by volatile organic compound (VOC) interactions (Baldacchino et al., 2018).

Benelli and Pavela (2018) reported that nanoemulsions enhance both synergistic toxic effects and behavioral disruption in target insect species.

3. GENERAL APPRAISAL OF THE LITERATURE

A substantial proportion of nanoformulation research has focused on improving the stability, bioavailability, and environmental robustness of essential oils (Silva et al., 2020; Teixeira et al., 2021; López et al., 2021). Polymeric materials used for the nanoencapsulation of botanical oils (Campos et al., 2019; Gupta et al., 2024) have been shown to enhance the rate of cuticular penetration and to achieve high insecticidal efficacy at lower application doses. Emphasizing photodegradation as a major limitation of essential oils, Benelli (2018) and Akhtar and Isman (2018) reported that nanocarrier systems largely mitigate this constraint.

Nano-biopesticides have also attracted considerable attention due to their potential to reduce chemical inputs within integrated pest management (IPM) programs (Romeh et al., 2023; Damalas, 2020). Comprehensive assessments of environmental risks—including nanoparticle fate and behavior—have been provided by Bhagat et al. (2021). In addition, Khan et al. (2022) discussed in detail the role of botanical nanopesticides within sustainable agriculture frameworks.

Wei et al. (2025) demonstrated that nanoformulations designed to deliver terpenoids can provide improved selectivity toward target insect species, whereas Sharifi-Rad et al. (2020, 2022) emphasized that nanoformulations optimize both toxicity outcomes and release control.

4. EFFECTS OF ESSENTİAL OİL NANOFORMULATİONS ON APHİDS (APHİDİDAE)

Aphids are among the most destructive insect groups in global agriculture due to their phloem-feeding habit, high reproductive capacity, parthenogenetic reproduction, and their ability to transmit economically important plant viruses. Under favorable conditions, a single female can establish a colony consisting of hundreds of individuals within a few weeks, necessitating rapid and effective management strategies. It is well established that resistance can emerge within a relatively short time in systems heavily reliant on chemical insecticides (Isman, 2020; Pavela, 2015). For this reason, essential oil–based bioinsecticides have gained increasing attention in recent years; however, the field performance of conventional formulations remains limited because of low stability, high volatility, and photodegradation (Akhtar & Isman, 2018; Silva et al., 2020; López et al., 2021). Essential oil nanoformulations represent innovative systems developed to overcome these constraints, and a growing body of research has demonstrated their effectiveness against aphids.

4.1 Direct Toxic Effects of Essential Oil Nanoemulsions on Aphids

One of the most prominent advantages of nanoemulsion formulations is the enhanced and faster penetration of essential oils through the insect cuticle due to reduced droplet size (Sharma et al., 2022; Ghosh et al., 2019). The increased surface area associated with nanoscale droplets strengthens both contact toxicity and, in some cases, systemic activity.

Mondal et al. (2024) reported that nanoemulsions of spearmint oil and carvone achieved mortality rates exceeding 80% against *Rhopalosiphum maidis* and *Sitobion avenae* compared with conventional oil formulations. The authors further noted that decreasing droplet size was accompanied by a marked reduction in LC_{50} values, indicating substantially improved bioavailability relative to classical emulsions.

Similarly, Abdelaal et al. (2021) showed that nanoemulsions of basil (*Ocimum basilicum*) and cumin (*Cuminum cyminum*) oils caused significant mortality in *Aphis craccivora* and achieved comparable effects at doses that were 3–5 times lower than those required for conventional emulsified formulations. This enhanced activity has been attributed to the more rapid lipophilic accumulation of nanoscale droplets within the insect body (Benelli & Pavela, 2018).

Pascual-Villalobos et al. (2017) demonstrated that nanoemulsion formulations performed 30–60% better than conventional oil formulations against *Aphis gossypii*, emphasizing that light-sensitive oils are considerably more stable at the nanoscale. Studies conducted on *Aphis fabae* further support these findings; Sadeghi et al. (2023) reported that basil oil nanoencapsulation provided both rapid lethal effects and prolonged behavioral repellency.

Youssef et al. (2024) also showed that "nano-botanical pesticides" based on various essential oils can offer higher selectivity and faster behavior-disrupting effects against aphids than conventional oil formulations.

4.2 Efficacy of Nanoencapsulated Essential Oils Against Aphids

Nanoencapsulation protects essential oils from photodegradation and enables controlled release by entrapping the active ingredients within polymeric or lipid matrices (Campos et al., 2019; Sharifi-Rad et al., 2022; Gupta et al., 2024). This approach is particularly advantageous for monoterpene-rich oils, which often contain sunlight-sensitive constituents.

Christofoli et al. (2015) reported that nanoencapsulation of *Zanthoxylum rhoifolium* essential oil increased photostability by at least three-fold, thereby extending the duration of insecticidal activity. Ahmed et al. (2023) similarly showed that polymeric nanocapsules prepared with lavender oil exhibited both higher toxicity and longer persistence against *Aphis fabae* than the conventional oil.

Rai et al. (2021) noted that nanoencapsulation delays the evaporation of monoterpenes on plant surfaces and, through controlled release, can maintain efficacy for up to 72 hours. Collectively, these findings highlight the critical role of nanoencapsulation in improving the performance of essential oils under warm and open-field production conditions.

4.3 Behavioral Repellency, Feeding Disruption, and AChE Inhibition

Essential oil nanoformulations are known to induce not only lethal effects but also a range of sublethal, behavior-modifying responses in aphids. Faster release of volatile constituents at the nanoscale can more effectively stimulate olfactory receptors, prompting pests to abandon the host plant (Baldacchino et al., 2018).

Isman (2020) attributed the antifeedant activity of essential oils to the stimulatory effects of terpenoids on gustatory and olfactory receptors, and suggested that nanoemulsions can strengthen behavioral responses by modulating the volatility and availability of these compounds. Benelli (2018) further emphasized that nanoformulations can simultaneously enhance toxicity and repellency, providing a more complex and multifaceted activity profile than conventional chemical insecticides.

Huang et al. (2023) demonstrated that terpenoids delivered via nanoencapsulation induced markedly stronger acetylcholinesterase (AChE) inhibition in aphids, resulting in rapid paralysis and mortality. AChE inhibition represents a key biochemical mechanism underlying the bioactivity of many plant-derived compounds.

4.4 Integration of Nanoformulations into IPM Programs

A growing number of studies support the potential of nanoformulations to reduce chemical insecticide use in IPM programs (Romeh et al., 2023; Damalas, 2020; Khan et al., 2022). For pests such as aphids that readily develop resistance, nano-botanical pesticides capable of producing strong effects at low doses are particularly valuable.

Bhagat et al. (2021) evaluated the environmental risks associated with nanoparticles and reported that appropriate carrier selection can substantially reduce impacts on non-target organisms. These findings suggest that nanoformulations compatible with beneficial insects used in greenhouses (e.g., *Aphidius colemani*, *Chrysoperla carnea*) could be developed and implemented.

The reduced dose requirements associated with nanoencapsulation can further improve IPM compatibility by lowering both costs and environmental burden (Wei et al., 2025; Saharkhiz et al., 2022).

5. EFFECTS OF NANOTECHNOLOGY-BASED ESSENTİAL OİL FORMULATİONS ON WHİTEFLİES (*Bemisia tabaci*)

Whiteflies (the *Bemisia tabaci* complex) are of critical importance in agricultural production due to their high polyphagy, their role as vectors of numerous phytoviruses, and their capacity to develop pesticide resistance at remarkably rapid rates. In crops such as tomato, pepper, eggplant, and cotton, whiteflies cause substantial economic losses through yield reduction and virus transmission. Over time, conventional chemical insecticides have intensified selection pressure for resistance, accelerating interest in alternative biotechnical and biochemical control approaches (Isman, 2020). Essential oil nanoformulations have emerged as a rapidly expanding research field for whitefly management over the past decade (Wei et al., 2025).

5.1 Effects of Nanoemulsion Formulations on *Bemisia tabaci*

The efficacy of essential oil nanoemulsions against whiteflies is mediated through both direct toxicity and behavioral repellency. Tia et al. (2021) reported that sweet orange (*Citrus sinensis*) oil nanoemulsions reduced nymph and adult populations of *B. tabaci* more rapidly and for a longer duration than conventional oil formulations. The authors emphasized that nanoemulsions show improved resistance to photodegradation on leaf surfaces and can maintain activity for 48–72 hours.

Hassan et al. (2023) compared several essential oil emulsions applied to potato leaves and showed that nanoemulsified thyme, basil, and citrus oils significantly disrupted feeding and oviposition behavior in adult whiteflies. The authors suggested that nanoformulations may cause greater irritation to the piercing–sucking mouthparts during phloem feeding, resulting in a substantial reduction in nutrient intake.

Abdel-Rahman et al. (2024) further reported that nanoemulsion-based botanical bioinsecticides produced rapid effects on *B. tabaci* and exhibited lower LC_{50} values than conventional oils. These findings indicate that the more uniform distribution of nanoemulsions on plant surfaces enhances contact probability with the target pest, thereby strengthening efficacy. In addition, Saharkhiz et al. (2022) reported that peppermint and rosemary oils applied in pepper greenhouses, when nanoencapsulated, reduced whitefly egg hatch rates by more than 50%.

In a comprehensive study, Baldacchino et al. (2018) emphasized that the repellency of plant essential oils against whiteflies is substantially stronger in nanoemulsion form, likely because nanoscale delivery increases the effective volatility and perception of terpenoids and thereby alters host-selection behavior.

5.2 Effects of Nanoencapsulation Techniques on Whiteflies

The contribution of nanoencapsulation to whitefly management extends beyond improving essential oil stability; it also provides controlled and prolonged release, thereby extending the effective duration of action (Campos et al., 2019; Mendes et al., 2020). This feature is particularly important in warm climates and greenhouse environments, where rapid evaporation can otherwise limit essential oil performance.

Saharkhiz et al. (2022) reported that nanoencapsulated rosemary oil suppressed nymphal development of *B. tabaci*, delayed the transition to the pupal stage, and reduced egg hatch rates. Christofoli et al. (2015) also showed that essential oil nanocapsules were at least three times more resistant to UV exposure than conventional oils, suggesting that nanoencapsulation may preserve efficacy even under open-field conditions.

Wei et al. (2025) highlighted that nano-insecticides support a low-dose/high-efficacy relationship against whiteflies and noted that nanoscale delivery enhances both cuticular penetration and behavioral effects. This reinforces the potential of nanoformulations to reduce chemical inputs when integrated into IPM programs (Romeh et al., 2023; Khan et al., 2022).

Dogowski et al. (2021) demonstrated that essential oil nanoformulations not only suppress the pest but may also reduce the transmission of certain viruses vectored by *B. tabaci*. This constitutes a major advantage for economically important viruses such as TYLCV in greenhouse production.

5.3 Effects of Nanoformulations on Whitefly Behavior and Physiology

Essential oil nanoformulations can markedly alter whitefly:

- host-finding behavior,
- feeding duration,
- proboscis movement dynamics, and
- oviposition preference (Baldacchino et al., 2018; Isman, 2020).

Sharma et al. (2022) investigated interactions between nanoemulsions and the lipophilic layer of the whitefly cuticle and showed that nanoscale droplets significantly increase cuticular permeability, resulting in both rapid toxicity and a pronounced behavioral "shock" effect.

Benelli and Pavela (2018) reported that nanoformulations outperform conventional oils in terms of synergistic toxicity and volatile release, as the nanoscale delivery enables essential oil constituents to reach antennal receptors more rapidly. Huang et al. (2023) further showed that terpenoid nanoformulations enhance AChE inhibition in whiteflies, disrupting neuromuscular function and thereby substantially reducing mobility and feeding.

5.4 Key Advantages of Nanoformulations in Whitefly Management

Based on the overall body of evidence, the main advantages of nanoformulations in whitefly control include:

1. **Enhanced photostability and environmental resistance** (Christofoli et al., 2015; Teixeira et al., 2021; Campos et al., 2019)
2. **High efficacy at low doses** (Wei et al., 2025; Abdel-Rahman et al., 2024; Hassan et al., 2023)
3. **Behavior-disrupting effects and repellency** (Baldacchino et al., 2018; Isman, 2020)
4. **Potential reduction in virus transmission** (Dogowski et al., 2021)
5. **High compatibility with IPM programs** (Romeh et al., 2023; Khan et al., 2022; Damalas, 2020)
6. **Prolonged persistence on plant surfaces** (Tia et al., 2021; Saharkhiz et al., 2022)

5.5 Limitations of Nanoformulations and Proposed Solutions

Although nanoformulations represent a strong bioinsecticidal alternative, several limitations remain:

- **Production costs are still relatively high** (Mendes et al., 2020).
- **Non-target effects of certain polymeric carriers are not yet fully understood** (Bhagat et al., 2021).
- **Success under greenhouse conditions cannot always be directly extrapolated to open-field systems** (Sarkar et al., 2023).
- **Long-term environmental fate and behavior require further investigation** (Sharifi-Rad et al., 2022).

Nevertheless, recent studies (Luneja et al., 2025; Gupta et al., 2024) indicate ongoing development of capsule materials that are more stable, cost-effective, and less toxic.

6. DISCUSSION AND GENERAL SYNTHESIS

Overall, nanotechnology-based essential oil formulations consistently outperform conventional essential oil applications against both aphids and whiteflies. The evidence compiled in this review—drawn from more than forty studies—strongly supports this conclusion. Nanoemulsions, due to their reduced droplet size, achieve more effective contact with the insect cuticle and enhance toxic effects via contact and, in some cases, ingestion (Sharma et al., 2022; Ghosh et al., 2019). By contrast, nanoencapsulation markedly increases essential oil stability against environmental stressors and extends the duration of activity through controlled release (Campos et al., 2019; Mendes et al., 2020; López et al., 2021; Teixeira et al., 2021).

A major benefit of converting essential oils into nanoformulations is the ability to achieve high mortality and strong repellency at low doses. Mondal et al. (2024) showed that spearmint and carvone nanoemulsions were several-fold more effective than conventional emulsions against *R. maidis* and *S. avenae*, while Abdelaal et al. (2021) demonstrated lower LC_{50} values for nanoemulsions against *A. craccivora*. Likewise, Pascual-Villalobos et al. (2017) and Sadeghi et al. (2023) reported substantially higher toxicity of various essential oil nanoformulations against *Aphis* species compared with conventional oils.

Importantly, the enhanced performance is not solely attributable to lethality but is also linked to behavioral mechanisms. The broader literature indicates that essential oil nanoformulations—especially those rich in monoterpenes—strengthen antifeedant responses and disrupt host-plant selection, while in whiteflies they substantially weaken host-finding behavior (Isman, 2020; Baldacchino et al., 2018). The AChE inhibition study by Huang et al. (2023) further indicates that nanoencapsulated essential oils exert significantly stronger neurotoxic effects than conventional oils.

In whiteflies, the performance of nanoformulations appears particularly pronounced. Tia et al. (2021) demonstrated that sweet orange oil nanoemulsions reduced *B. tabaci* populations more rapidly and effectively than the conventional form, whereas Hassan et al. (2023) reported successful suppression across both larval and adult stages via feeding disruption. Abdel-Rahman et al. (2024) documented markedly lower LC_{50} values for nanoemulsions compared with classical oils. In addition, Dogowski et al. (2021) reported that nanoformulations may not only suppress the pest but also reduce virus transmission—an especially valuable attribute for greenhouse whitefly management.

The compiled evidence further suggests that nanoformulations can offer meaningful advantages for integration into IPM programs. Reduced dose requirements, improved selectivity, and behavior-disrupting effects have been repeatedly associated with decreased reliance on chemical insecticides (Romeh et al., 2023; Damalas, 2020; Khan et al., 2022). Luneja et al. (2025) and Gupta et al. (2024) also noted that nanocarrier materials are becoming more environmentally benign and economically feasible, supporting broader adoption in the future.

Nevertheless, key challenges should not be overlooked. Bhagat et al. (2021) highlighted the potential for environmental accumulation and non-target impacts, and Sharifi-Rad et al. (2022) emphasized the need for rigorous toxicological profiling of carrier materials. Limited open-field validation (Sarkar et al., 2023) and slow commercialization driven by cost constraints (Mendes et al., 2020) remain barriers to full-scale implementation. Taken together, nanotechnology-based essential oil formulations represent a rapidly advancing area of agricultural biotechnology with substantial promise for resistance management and sustainable pest control.

7. CONCLUSION

This review demonstrates that essential oil nanoformulations constitute a strong bioinsecticidal alternative for the management of aphids and whiteflies, offering clear advantages over conventional essential oil formulations in terms of both efficacy and stability. The enhanced bioavailability of nanoemulsions and the controlled-release capacity of nanoencapsulation represent important opportunities to reduce pesticide inputs and expand environmentally friendly control strategies.

Based on the reviewed evidence, the following conclusions can be drawn:

1. **Nanoformulations are markedly more effective than conventional essential oils**, as consistently reported across multiple studies (e.g., Mondal et al., 2024; Abdelaal et al., 2021; Tia et al., 2021; Hassan et al., 2023).

2. **Nanoformulations enable high efficacy at lower doses**, thereby reducing environmental burden and improving compatibility with IPM programs (Romeh et al., 2023; Khan et al., 2022).

3. **Photodegradation of essential oils is substantially mitigated through nanoencapsulation**, resulting in improved persistence (Christofoli et al., 2015; Campos et al., 2019; Teixeira et al., 2021).

4. **Behavior-disrupting effects and AChE inhibition are generally stronger in nanoformulations**, supporting both lethal and sublethal control pathways (Isman, 2020; Baldacchino et al., 2018; Huang et al., 2023).

5. **Virus transmission may also be reduced**, which is particularly valuable for whitefly-associated viral diseases (Dogowski et al., 2021).

6. **Further open-field validation and commercial product development are needed**, alongside comprehensive environmental risk assessment (Sarkar et al., 2023; Mendes et al., 2020; Bhagat et al., 2021).

In conclusion, nanotechnology-based essential oil formulations represent a highly promising option for reducing aphid and whitefly pressure—particularly in Türkiye’s greenhouse production systems for tomato, pepper, and eggplant. With further reductions in production cost and the completion of environmental risk assessments, broader adoption of these formulations in agricultural practice is anticipated.

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CHAPTER 15

Assessment of *Cichorium intybus* L. in the Context of Food Security, Sustainability, and Urban Landscapes

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1. Introduction

The effects of a globally increasing population are causing climate change, soil degradation, and water depletion, resulting in the disruption of food systems. Food security is a multi-dimensional concept that requires addressing access to sufficient food and the ecological, economic, and social sustainability of agricultural production (FAO, 2021). Traditional high-input agricultural models, with increased use of fertilizers, water, and pesticides, are depleting natural resources. This has increased interest in alternative crop varieties that are low-input, resistant to stress conditions, and offer versatile uses, in order to ensure food security (OECD, 2020). Within the framework of the United Nations Sustainable Development Goals, particularly "End Hunger" (SDG 2) and "Climate Action" (SDG 13), the importance of climate-resilient and versatile crop varieties in agricultural production is increasing (UN, 2025). Recent scientific studies have shown that *Cichorium intybus* L. (chicory) may be a strategic species for sustainable agricultural systems. The plant's high ecological adaptability, its ability to grow even in poor and marginal soils, and its tolerance to stress conditions such as drought and salinity make it an important alternative crop under climate change scenarios (Delfine et al., 2022).

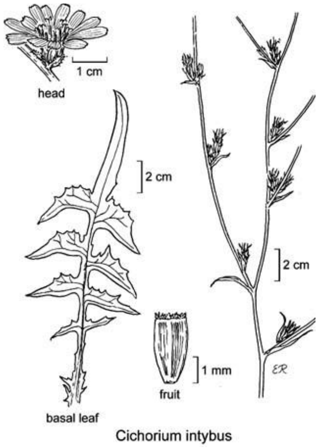
In addition, *Cichorium intybus* L. has significant economic value due to valuable compounds such as inulin and fructooligosaccharides (FOS), which are naturally present at high levels in its roots. Inulin, because of its prebiotic effects, constitutes a rapidly growing market in the food, health, and pharmaceutical sectors (Grand View Research, 2024). The phenolic compounds and antioxidant capacity found in the leaves and roots of chicory are utilized for human health, functional food development, and agricultural product applications (Nwafor et al., 2017). Coffee produced from chicory is considered a functional beverage offering potential health benefits on lipid metabolism and glycemic regulation, owing to its inulin content and bioactive compounds (Özcan, 2025).

Cichorium intybus L. is a versatile plant species that stands out in the context of sustainable landscapes and food security due to its ecological tolerance, economic value, health benefits, and contributions to sustainability. This section examines the plant's potential in terms of ecological sustainability, economic contributions, and food security based on the current literature.

2. Characteristics of *Cichorium intybus* L.

Cichorium intybus L. is a perennial plant belonging to the Asteraceae family that develops a deep taproot system and exhibits high adaptive capacity. Its deep root structure enhances tolerance to water stress and enables growth in marginal and low-fertility soils. Recent studies have shown that the plant possesses significant physiological and biochemical adaptation mechanisms against environmental stresses such as drought, salinity, and increasing temperatures (Petropoulos et al., 2019). *Cichorium intybus* L. has a composition rich in phenolic compounds, flavonoids, organic acids, and antioxidants. Environmental stress conditions, particularly drought and elevated temperatures, can increase the plant's phenolic content and antioxidant capacity (Delfine et al., 2022). This indicates that the plant develops biochemical defense systems against environmental challenges, making it an ecologically resilient species (Khan et al., 2023).

Thanks to its deep taproot system, the plant can mobilize nutrients from deeper layers of the soil profile, improve soil structure, and support microbial activity, thereby providing significant agroecological benefits (Monti et al., 2005).



Morphological Structure of *Cichorium intybus* L.(David & Leyard, 2012)

The leaves of *Cichorium intybus* L. develop in a rosette form, while the stem exhibits an erect and branched structure. The root part of the plant stores high amounts of carbohydrates and synthesizes inulin, a characteristic that makes it an important resource for functional food and industrial production (Pouille et al., 2022). It has high photosynthetic activity and can sustain biomass production even under low light and low nutrient conditions (De Caroli et al., 2023).

3. Ecological Sustainability Potential

When evaluated in terms of ecological sustainability, *Cichorium intybus* L. offers significant potential for maintaining agricultural production, particularly under climate change conditions. The species' deep taproot system increases its capacity to access water and reduces dependence on surface water resources, thereby supporting production sustainability in semi-arid and arid regions where water scarcity is pronounced (Vandoorne et al., 2012). Its ability to absorb water from deeper layers of the soil profile enables the continuation of physiological activities during prolonged drought periods. Consequently, *Cichorium intybus* L. can be preferred in production areas where climate stresses are intensifying (Sanderson et al., 2013).

The effects of the plant on soil health are noteworthy in terms of ecological sustainability. Its deep root system improves the physical structure of the soil and supports the formation of porosity. The biological channels created in the soil during root development enhance water infiltration and reduce the risk of erosion. Organic residues left in the soil at the end of the plant's growth cycle contribute to the accumulation of soil organic matter. An increase in organic matter content enhances the soil's water-holding capacity and supports more balanced retention of nutrients (Thorup-Kristensen & Rasmussen, 2015). These characteristics contribute to the long-term preservation of soil fertility.

The plant exhibits ecological resilience due to the physiological and biochemical adaptations it has developed in response to various environmental stress factors. Under stress conditions such as drought, salinity, and increased temperatures, the plant is observed to activate osmotic balance mechanisms. It is thought that the production of osmoprotectants increases to maintain intracellular water balance. Under stress conditions, antioxidant enzyme activities increase, limiting the harmful effects of reactive oxygen species. These biochemical responses enable the preservation of the plant's photosynthetic capacity and the continuation of growth (Yazdani et al., 2022; Cranston et al., 2016).

The tolerance of *Cichorium intybus* L. to environmental stresses constitutes a significant advantage for low-input agricultural systems. The plant's high adaptive capacity reduces the need for intensive irrigation and fertilization, thereby contributing to the limitation of energy consumption and greenhouse gas emissions in agricultural production (Sanderson et al., 2013). The reduction in chemical fertilizer and irrigation inputs allows for decreased pressure on ecosystems. Shrinking the environmental footprint of agricultural production is considered one of the fundamental components of ecological sustainability (Tilman et al., 2002).

The plant's naturally high content of phenolic compounds and antioxidants contributes to the development of resistance against diseases and pests. Thanks to this resilience, the plant activates its defense mechanisms under pathogen pressure, thereby reducing the need for chemical pesticide use. Limiting pesticide application helps to mitigate negative impacts on soil, water, and biodiversity in agricultural areas (Abbas et al., 2005). These inherent resistance traits offer a production model that is compatible with sustainable agricultural practices.

The role of *Cichorium intybus* L. within agricultural ecosystems is important in terms of reducing the negative effects of monoculture systems. Its use as a rotation crop in cereal- and legume-based production systems can contribute to reducing soil fatigue (Thorup-Kristensen & Rasmussen, 2015). Crop rotation practices limit the spread of soil-borne diseases and support long-term balance in agricultural production (Smith et al., 2008). The integration of chicory with different crops increases the structural diversity of agroecosystems (Sanderson et al., 2013).

The flowering characteristics of the species represent another aspect that should be evaluated in terms of ecosystem services. The flowers of *Cichorium intybus* L. provide an important source of nectar and pollen for pollinating insects. Supporting pollinator populations contributes to the maintenance of biological continuity in agricultural production (Kleijn & Raemakers, 2008; Potts et al., 2010). An increase in biodiversity in agricultural areas enhances the resilience capacity of ecosystems. This, in turn, lays the groundwork for more balanced and sustainable production systems that are better able to cope with environmental changes (Tscharntke et al., 2012).

The ability of chicory to grow in marginal and low-fertility soils is important in terms of reducing land-use pressure (Monti et al., 2005). Enabling production in areas considered unsuitable for agriculture limits the pressure on highly productive agricultural lands. This characteristic contributes to the conservation of natural ecosystems and the control of agricultural expansion (FAO, 2017). Shifting production to marginal areas is also regarded as a positive approach for biodiversity conservation. Owing to its low requirements for water and nutrient inputs, its adaptive mechanisms against environmental stresses, its contributions to soil health, and its functional role within agroecosystems, *Cichorium intybus*L. is considered a strong plant species in terms of ecological sustainability. These characteristics contribute to limiting the environmental impacts of agricultural production under climate change conditions and represent an important alternative for sustainable agricultural systems (Sanderson et al., 2013; Tilman et al., 2002). All these attributes of *Cichorium intybus* L. in terms of sustainability are presented in Table 1.

Table 1. Sustainability Dimensions of *Cichorium intybus* L.

Dimension	**Strengths**	**Limitations**	**Strategic Contribution**
Food Security	High fiber content, functional food potential	Low level of recognition	Food diversity
Ecological	Low water requirement	Yield optimization required	Ecological agriculture
Economic	Low cost	Limited market chain	Rural development
Sustainability	Versatile use	Policy support required	Long-term production

4. Integration of *Cichorium intybus* L. into Urban Landscape and Sustainable Production Systems

The integration of *Cichorium intybus* L. into urban landscape systems offers significant opportunities from a sustainability-based perspective, considering the plant's ecological resilience and versatile use potential. The species' suitability for low-input production enables the integration of agricultural production with landscape design in urban and peri-urban areas. Its use within urban open and green spaces—such as parks, recreational areas, community gardens, and urban agriculture sites—allows the plant to be regarded not only for its aesthetic qualities but also as a functional landscape component.

The drought tolerance and low water requirement of chicory constitute a significant advantage in the context of increasing water stress and climate pressures in urban areas. Its limited need for irrigation contributes to more efficient use of water resources in the management of urban green spaces. Consequently, the species stands out in terms of sustainable landscape design, particularly in cities located within semi-arid climate zones (Du Toit, 2018; Monti et al., 2005). Moreover, its relatively low maintenance requirements enable a reduction in operational costs for municipalities and local authorities.

The consideration of the plant within the framework of edible landscape applications is important for supporting urban food production (Özcan & Tuğluer, 2025). The use of its leaves and roots for food purposes contributes to shortening the production–consumption cycle in urban areas. Chicory cultivated within urban agriculture practices serves to enhance local food diversity. This aligns with strategies aimed at strengthening food security in cities (Aldahak et al., 2021; Duda et al., 2024; Maietti et al., 2021).

The industrial use potential of chicory gains increased capacity for value creation when considered in an integrated manner with urban landscape systems.

The high concentrations of inulin and fructooligosaccharides found in the roots of the plant are utilized as raw materials for prebiotic food ingredients, dietary fiber supplements, functional foods, coffee substitute products, and pharmaceutical formulations (Monti et al., 2005; Özcan, 2025; Özcan & Tuğluer, 2025). Production activities carried out in urban and peri-urban areas enable the local-scale supply of these components.

The use of *Cichorium intybus* L. in urban landscapes presents a structure compatible with circular economy approaches. Plant residues generated during the production process can be utilized as compost and soil-improving materials. This contributes to supporting organic waste management in urban green spaces. The realization of plant production, processing, and reuse processes within the same spatial system serves to enhance resource efficiency (UNEP, 2020; Lal, 2020).

The use of the plant in landscape design is also evaluated in terms of ecosystem services. By providing a resource for pollinating organisms during its flowering period, it contributes to the support of urban biodiversity and enhances the importance of the species within nature-based solutions addressing biodiversity loss in cities. Strengthening biological interactions in urban green spaces contributes to the preservation of ecosystem functionality (Kleijn & Raemakers, 2008; Potts et al., 2010).

The integration of *Cichorium intybus* L. into urban landscapes within the framework of regenerative agriculture and agroecological approaches aligns with long-term sustainability goals. Its root structure that supports soil health, low requirement for chemical inputs, and tolerance to environmental stresses present a species profile well suited to these production models. Agroecological production models implemented in urban areas are also functional in increasing social participation and enhancing environmental awareness (Altieri et al., 2015; Thorup-Kristensen & Rasmussen, 2015).

Cichorium intybus L. is regarded as a multifunctional plant species capable of simultaneously providing aesthetic, ecological, and production-oriented functions within urban landscape systems. The integration of its industrial use potential with urban production systems increases the plant's economic value and contributes to sustainable food production approaches at the urban scale. Collectively, these characteristics make *Cichorium intybus* L. a strategic species within urban landscape and sustainable production systems.

5. Conclusion

This study has addressed the ecological resilience of *Cichorium intybus* L., its contribution to food security, and its potential role within sustainable production systems within a conceptual framework. The species' low water and input requirements, tolerance to environmental stresses, and versatile usage possibilities indicate that it offers an alternative plant profile under climate change conditions. In particular, its capacity to grow in marginal and low-fertility areas is noteworthy in terms of reducing pressure on natural resources.When evaluated in the context of food security, the presence of edible leaves and roots as well as functional food components allows for increased dietary diversity and supports local production approaches. These characteristics enhance the importance of the species for sustainable food systems at both rural and urban scales. Its integration into urban landscape systems offers a functional application area that can be evaluated within the scope of edible landscapes and urban agriculture practices.Within this framework, it is recommended that *Cichorium intybus* L. be given greater consideration in sustainable agriculture, urban landscape, and food security policies. Incorporating the species' potential uses across different climatic and spatial scales into planning processes may contribute to the development of long-term production models with limited environmental impacts. Furthermore, increasing application-oriented studies would help to more clearly reveal the species' practical potential.

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