HEALTH AND ENVIRONMENT IN VETERINARY MEDICINE

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PREFACE

Nutrition is a basic need for humans and animals. The prerequisite for producing healthy and highly productive animals is to provide healthy and high quality raw materials. The primary condition for obtaining healthy raw materials is; it is the procurement of animal products from animals raised in healthy conditions in enterprises with modern technology and under the control of veterinarians. Considering that many diseases are transmitted from animals to humans today, veterinary medicine; the importance of human, animal and environmental health is increasing day by day.

In order to feed the ever-increasing world population in a sufficient and balanced way, the need for foods of plant and animal origin is increasing day by day. Arable land in the world; global warming, drought, opening of arable land to settlement etc. because it is decreasing day by day for reasons; in recent years, safflower, flaxseed, lupine etc. have been used as an alternative feed source, especially in poultry farming. The use of feed has increased.

Hunger and diseases caused by inadequate and unbalanced nutrition are one of the most important problems of our day. The cheapest feed sources of livestock enterprises are natural meadows and pastures, and feeds such as plant and industrial product residues. The inadequacy of high quality roughage and concentrate feedstuffs in animal nutrition in our country is the main reason for the low yield in animals.

Conscious feeding is of great importance in raising healthy and disease-resistant animals. In order to get high efficiency from farm animals; animals must be fed rationally and in welfare.

In this book, alternative feed sources in poultry farming; fermentation technology, dietary cation and anion balance and its importance in ruminant feeding probiotics and global warming in ruminants; TMR in dairy cows; in our country, up-to-date information on the existence of meadow-pasture and climate change has been compiled.

We would like to thank our valuable academicians who contributed to the preparation of the book " Health and Environment in Veterinary Medicine ", and we hope that this book, prepared with great devotion, will be useful to interested in the subject researchers, animal breeders, students and Veterinarians.

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CHAPTER 1

FERMENTATION TECHNOLOGY IN ANIMAL NUTRITION

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1. INTRODUCTION

Fermentation is the process in which high-molecular-weight organic substances, primarily carbohydrates, are broken down into smaller molecules by microorganisms. In fermentation technology, the substances targeted for processing or a specific substance undergo a complete transformation from the original material, and there is no resemblance between the main substance in the raw material and the substances resulting from fermentation. Examples of fermentation include wine, beer, spirits, or vinegar. During wine production, the sugar present in grapes and fruits undergoes a complete transformation through fermentation, leading to a significant change in the characteristics and structure of the fruit. A sweet fruit or grape must acquires a completely different taste. The reason for this is the conversion of sugar into alcohol.

In fermentation, not only sugar but also some other components in the must undergo changes. The conversion of starch to ethyl alcohol in starchy raw materials is a more detailed and interesting transformation in beer and spirits technology. The starch in the raw material undergoes fermentation with the action of enzymes, then converts into sugar (maltose), and finally, this sugar is converted into ethyl alcohol by yeast (Arıcı, 2017).

Microorganisms and biological processes play a fundamental role in fermentation technology. Therefore, understanding the microorganisms involved in fermentation technology and the biological processes they cause, and adjusting the processing technique accordingly, are essential aspects of fermentation technology.

2. FERMENTATION TECHNOLOGY

Fermentation technology is used for the production of antibiotics, vitamins, various nucleic acids, organic acids, single-cell protein, various alcohols, ketones, enzyme production, olive processing, yogurt, and alcoholic beverages. The objectives of fermentation technology include:

1. Creating new products through microbiological methods: This includes the production of various alcoholic beverages.
2. Producing various substances by cultivating microorganisms: Using specific environments for yeast production, single-cell protein production, etc.

3. Treating river and industrial wastewater through microbiological methods.
4. Obtaining energy through biological means (Biogas) (Arıcı, 2017).

Carbohydrates are the most important compounds in fermentation technology. The carbohydrates that undergo fermentation are monosaccharides, and starch is broken down into glucose with the help of enzymes. Proteins are important for the nutrition of microorganisms.

3. PEPTONS

Peptone is a compound formed by the breakdown of proteins from proteinaceous material. It is derived from the secondary structure of proteins such as peptides, dipeptides, and amino acids. It serves as an easily accessible nitrogen source. Peptone is water-soluble, does not coagulate when heated, and is particularly suitable for adding to microbiological culture media. It can also be used to some extent as a carbon source in a microbial environment. Peptone is primarily obtained from meat, internal organs, gelatin, milk, plants, and yeast (Bridson, 1970).

Protein enzymes or protein digestive enzymes are generally divided into two groups: one group breaks down proteins into soluble fragments known as peptones, and the other group further breaks down these peptones into smaller fragments known as amino acids (Franek et al., 2014; Heidemann et al., 2000).

Peptone is considered as a different protein derivative that is partially soluble in water and does not coagulate with heat, unlike other protein derivatives, and its solutions do not precipitate when saturated with ammonium sulfate (Poernomo and Buckle, 2002). It is derived from the enzymatic digestion of proteins, such as meat, using chemical fermentation with enzymes like pepsin, trypsin, or hydrochloric acid, resulting in a complex product that is soluble in water and contains peptone and other protein derivatives in the final product, known as a protein hydrolysate. It is primarily used in bacterial nutrition media.

There are three types of pure peptones: amfopeptone, antipeptone, and hemipeptone. The key distinguishing features between peptone and other proteins are that peptone does not precipitate with potassium ferrocyanide and acetic acid, does not coagulate with heat, is easily spread on membranes, and therefore easily absorbed.

3.1. Peptone, Peptide, and Albumin

The primary products of albumin degradation are albumin-like substances that do not coagulate with heat, have greater solubility than albumin, and can be "salted out" with specific salts. Peptone is similar to albumin but less complex than albumin, and its continuous proteolytic analysis of peptone produces peptides.

Peptides are short chains formed by the binding of dipeptides from amino acids. Peptones, on the other hand, are a group of peptides resulting from the hydrolysis of proteins found in animal milk or meat. Peptides are mixtures of dipeptides, tripeptides, tetrapeptides, etc. Additionally, peptides contain fats, salts, minerals, and vitamins. Peptides also function as neuropeptides, antibiotics, and structural molecule hormones. Peptones, on the other hand, serve as a source of nitrogen and carbon. Peptides and peptones are molecules that arise from the hydrolysis of proteins in water. They are composed of amino acids, with carbon, oxygen, hydrogen, and sulfur as their basic elements.

Peptides are classified into five classes: milk peptides, ribosomal peptides, non-pigment peptides, peptides, and peptide fragments. Peptones have three types: amfopeptone, antipeptone, and hemipeptone.

Peptone is a nutrient medium compound used for the growth of bacteria and fungi. The most common type is triptone, which is derived from the digestion of casein and is widely used in molecular biology.

3.2. Peptone Production

Peptone is derived from milk products such as plant protein, casein, whey, and slaughterhouse residues, and is known as the richest result of bacterial growth medium. Peptones can be obtained from secondary meat products such as meat, offal, gelatin, and milk, as well as from fishery industries made with various improvement factors such as time, temperature, pH, and weight/protein weight (E/S ratio) after processing with plants, yeasts, alkalases, and trypsin

Protein is obtained from milk products such as plants, casein, whey, as well as slaughterhouse residues. It is also known as the richest result of bacterial growth medium. Peptones can be derived from secondary meat products such as meat, offal, gelatin, and milk. Additionally, they can be obtained from plants, yeasts, and fishery industries that have been processed with various improvement factors such as time, temperature, pH, and weight/protein weight (E/S ratio) (Dufosse et al., 1997; Green et al., 1977).

Under optimum conditions, the degree of hydrolysis is close to 25% for alkalases and 9% for neutrons. The nitrogen content of peptones prepared under optimal conditions varies between 10% and 12%, similar to commercial peptone. To determine the molecular weight distribution, the obtained peptones are smaller than 15 kilo Daltons (kDa), and this is shown using SDS analysis. In an experiment applied to fish by-products, it was found that the ratio of peptones obtained from protein dissolved using alkalases is higher than peptones obtained from trypsin (Ovissipour et al., 2009).

However, following the emergence of bovine spongiform encephalopathy or mad cow disease, and due to the increasing demand for raw materials, non-meat derived peptones have become more important (Aspmo et al., 2005; Safari et al., 2010).

The increasing demand for bacterial cultures has led to an increased interest in new and cost-effective peptone sources, especially in the biotechnology fermentation industries. Among the materials used in bacterial growth media, nitrogen sources are the most expensive.

Peptone was first proposed for bacterial production in laboratory conditions by Naegel in 1880. Thus, this substance entered the field of bacteriology science as an essential element of culture media with the definition of "meat extract peptone" (Uzeh et al., 2006).

3.3. Peptone Sources

Keratin proteins, which are the main components of hair, feathers, wool, and horns, represent an important raw material source for many applications. Wool contains up to 95% pure keratin. Additionally, keratin protein is rich in sulfur-containing amino acids, particularly cysteine. Keratin protein hydrolysis is usually prepared using acid, alkaline, or enzymatic methods. Some studies have shown that protein hydrolysis from keratinized materials such as horns and feather residues can be used as a peptone source in a microbial environment (Kurbanoglu et al., 2004).

Peptones can be obtained from various animal (fish waste, meat, shrimp heads, casein, carob, milk, etc.) or plant tissues (sunflower, soybeans, etc.) (Parrado et al., 2001; Halkman 2005; Rozman et al., 2005; Uzeh et al., 2006).

Due to the structural properties of fibrous proteins, their degradation is much more challenging compared to other proteins and hydrocarbon-rich materials, so the process steps used during the hydrolysis of these proteins should be performed under more effective conditions. To achieve the desired results during the hydrolysis of fibrous proteins, some of the standard hydrolysis steps need to be modified. These steps include:

- Increasing the concentration of the used acid or base,
- Extending the hydrolysis time,
- Performing hydrolysis processes at high temperatures and pressures.

For example, it has been observed that treating chicken feathers with high-temperature steam hydrolysis results in the conversion of cystine amino acid in its structure to lanthionine, and lysine amino acid to lisinoalanine. Lanthionine and lisinoalanine are used less efficiently by living organisms compared to cystine and lysine. In the hydrolysis of fibrous proteins, enzymes, acids, and bases are also applied together to achieve higher hydrolysis efficiencies (Papadopoulos et al., 1985; Latshaw et al., 1994; Taşkın et al., 2011).

Physical and chemical data alone are not sufficient to evaluate the quality of peptones in the growth medium and to understand their overall effect on microbial growth. Due to the provision of nitrogen and carbon as essential sources in the microbial environment, the effect of peptone can be evaluated through its effects on the growth of microorganisms in the microbial environment (Reissbrodt et al., 1995; Vieira et al., 2005).

Peptones are used as a nitrogen source as well as a carbon source in microbial culture media. Some amino acids and vitamins present in peptones serve as growth factors for certain microorganisms. Additionally, peptones contain a mixture of proteases, peptides, some growth factors, and inorganic salts that provide the essential substances for the development of microorganisms. With all these rich contents and properties, peptones are rare components that can be used as a standalone nutrient in microbiology studies. Many bacterial species can only develop in culture media containing 1% peptone (Bilgehan 2002; Halkman 2005).

In industrial studies aiming to enhance the yields of microbial-derived products, the content of the growth media used by microorganisms has been tested with organic (peptone, malt extract, yeast extract, etc.) and inorganic (urea, ammonium sulfate, sodium nitrate, ammonium nitrate, etc.) nitrogen sources, and ultimately, it has been observed that organic sources provide higher yields (Kunamneni et al., 2005; Cho et al., 2006; Erdal and Taskın 2010).

1. MICROBIAL METABOLISM

Metabolism, which is an integration of numerous enzymatic processes involved in the growth and development of microorganisms, encompasses all the biochemical changes (anabolic, catabolic) occurring in living organisms. Understanding microbial metabolic reactions provides many benefits. Metabolic processes can be approached as two stages of breakdown or digestion processes (Arıcı, 2017).

Extracellular breakdown processes:

The hydrolytic breakdown of large complex molecules such as proteins and starch into sizes that can pass through the microbial cell membrane outside the microorganism's cell is known as extracellular degradation. Complex molecules are too large to enter the cell through the semipermeable microbial cell membrane. These events are catalyzed by extracellular (extracellular) enzymes synthesized within the microorganism cell and secreted into the environment.

Intracellular breakdown processes:

The entry of organically fragmented molecules, which can pass through the cell membrane up to a certain size, into the cell is not a simple diffusion process but occurs through an absorption process facilitated by penetrating enzymes. Energy is required for this process. The further breakdown of large organic molecules occurs within the cell. These processes are carried out by digestive (digestive) hydrolases. The nutrient components formed as a result of the breakdown of intracellular digestive hydrolases are now available for the cell to use. The cell can utilize these nutrients for two vital events that are interrelated. The first event is the synthesis of new cellular components (growth), and the second event is energy-producing reactions (respiration and fermentation) (Arıcı, 2017).

2. ENZYMES

Enzymes are proteinaceous substances produced by living cells that specifically catalyze chemical reactions. Enzymes produced by plant, animal, and microbial cells exhibit activity not only within cells (in vivo) but also outside cells (in vitro). All the breakdown and synthesis reactions in an organism are carried out through the catalytic activities and methods of enzymes. According to this definition, enzymes are essential substances for the formation and continuation of life. The fact that enzymes also exhibit their activities outside of living organisms further increases their importance. Enzymes have become substances that play an important role in our daily lives due to these properties. Nowadays, enzymes are widely used in food, pharmaceutical, and chemical industries, as well as in specialized areas such as leather, dye, and detergent production. They are extensively utilized in the fields of biology, biotechnology, medicine, agriculture, and veterinary medicine (Arıcı, 2017). General characteristics of enzymes:

1. A small amount of enzyme can perform a large amount of work.
2. Each enzyme has a specific substance or group of substances that it affects.
3. Enzyme activity depends on pH, temperature, substrate and enzyme concentration, and the water content in the environment.

3. FERMENTATION MICROORGANISMS

Microorganisms involved in fermentation technology are classified into three groups based on their importance in the industry: yeasts, bacteria, and mold fungi (Arıcı, 2017).

Hygienic compliance and technological efficiency are the characteristics that starter cultures should possess. Hygienic suitability includes the following:

1. The starter organism should not be pathogenic or toxic.
2. The starter culture should be hygienic to prevent contamination and compounds.

Technological effectiveness includes the following:

1. Culture organisms should multiply and regain dominance over spontaneous microorganisms.

2. The culture organism should function effectively in the intended application (such as nitrate reduction, biological acid degradation, etc.).
3. Starter culture preparations should be technologically purified from contamination and compounds.

6.1. Mold fungi

Unlike many yeasts and bacteria, molds grow in complex masses. They exhibit rapid spreading abilities, capable of covering an area of 5 to 10 cm2 in 2-3 days. The resulting mass of filaments is called mycelium, composed of hyphae. In asexual reproduction, spores are formed inside or on top of structures called sporangiophores. Spores formed on the surface are called conidia. The formation of thick walls around any cell of the mycelium results in the structure called chlamydospores (Arıcı, 2017).

6.1.1. Aspergillus

Aspergillus is an important genus of molds. The tips of the vertically rising conidiophores of Aspergillus are spherical or oval in shape. Conidia are single-celled, round, and come in various colors. They form yellow, green, orange, or black colonies on many food surfaces. Their mycelium is fragmented. Some species of this genus produce carcinogenic aflatoxins, while others are used in the industry to produce protease enzymes or citric acid, serving the feed and food industry. They are commonly found on grains and their products, fruits, vegetables, meats, and various other foods. A. flavus and A. parasiticus produce aflatoxins. A. oryzae is used in the production of sake from rice and in alpha-amylase production. It is also utilized as a starter in soy sauce production and koji fermentation (Arıcı, 2017).

Aspergillosis is an infection caused by Aspergillus fungi that can manifest as growth or allergic reactions, playing an important role in ecological systems and human economy. These fungi grow on dead leaves, stored grains, compost piles, or other decaying plants. Aspergillus contains more than 150 species of molds that are widely distributed in indoor and outdoor environments. Scientists studying these molds have reported their usefulness in biotechnology due to their ability to produce numerous cellular enzymes, organic acids, and secondary metabolites (Carroll et al., 1992).

While most species are harmless, certain types of mold, such as Aspergillus fumigatus, Aspergillus terreus, and Aspergillus felis, can cause dangerous diseases, hidden lung diseases, or asthma in humans or immunocompromised pets due to their toxic metabolites.

In classical fungal genetics, an Aspergillus species called Aspergillus (Emericella) nidulans is studied to explain metabolism, regulation, cell cycle, and the metabolism and regulation of filamentous polarity.

4.1.1. Aspergillus spores

Aspergillus spores are found in dust, where they are typically spread through air currents over short and long distances depending on environmental conditions. These spores settle when they come into contact with a solid or liquid surface and can germinate if moisture conditions are suitable. The ability to spread in air currents and grow in any location, i.e., being "ubiquitous," when there is sufficient food and water, is one of the most common characteristics used to describe this mold.

One defining feature of the fungal family is its unique feeding strategy (Samson and Pitt, 1990). These organisms secrete acids and enzymes into the surrounding environment, breaking down polymeric molecules into simpler molecules that can be reabsorbed by the fungal cell. Unlike animals, fungi are not autotrophic. Animals digest their food after consuming it, whereas fungi first digest their food and then consume it. Access to nutrients is supported through mechanical force (Powell et al., 1994).

The decomposition process carried out by Aspergillus is important in directing the natural circulation of elements, particularly in carbon cycling, as it contributes to the replenishment of carbon dioxide and other inorganic compounds. Aspergillus affects the degradation of complex compounds such as starch, cellulose, pectin, and other sugar polymers. Some Aspergillus species can break down more heat-resistant compounds like fats, oils, chitin, and keratin. Maximum decomposition occurs when there are sufficient nitrogen, phosphorus, and other inorganic essential nutrients in the environment. Therefore, decomposition is a means for Aspergillus to acquire nutrients.

4.1.2. Discovery of Aspergillus

Aspergillus, known as a group of fungi, is one of the oldest types of molds. It was first identified by Micheli in 1729 and named Aspergillus because its shape resembled an Aspergillus used for sprinkling holy water in Christian ceremonies (Ainsworth, 1976). Due to its widespread presence in the environment, ease of cultivation in all laboratory settings, and economic importance, Aspergillus has become the most extensively studied mold and the focus of researchers since 1926. Aspergillus rapidly grows in decaying plants, especially rotting straw and compost piles. It also feeds on fertilizers, human tissues, and decomposed skin. Most species have a broad capability to break down complex plant polymers (Polacheck et al., 1989).

The first reported cases of Aspergillus causing serious diseases in animals date back to 1815 by Mayer, and its role in human infections was first reported in 1847 by Sluyter. In the late 1800s, it was discovered that Aspergillus caused asthma in bird keepers and hairdressers. Additionally, it was reported that individuals who were exposed to Aspergillus spores through grain and plant materials developed certain allergic diseases (Rinaldi 1983, Salvaggio 2006).

Although Aspergillus is classified into over 200 species, only around 20 of these species have been reported to cause lung diseases in humans. Other less common species that cause diseases in humans include Glaucus, niger, terreus, nidulans, and Aspergillus niger (Klich, 2006).

The distinguishing feature of Aspergillus is its sporulating structure that resembles Aspergillus. Some cells develop and grow in a shape resembling a foot, forming a cell wall that takes the shape of the letters L or T, and the spores are found at their tips. This is the most important microscopic characteristic that distinguishes Aspergillus. The colors of the spores vary depending on the gender of Aspergillus. For example, the Niger group has black spores, while the Ochraceus group has spores that change color from yellow to brown. Spores of species like fumigatus, nidolanus, and fox are green in color.

Other distinguishing characteristics of Aspergillus species include colony color, growth rate, and heat resistance. Aspergillus species are differentiated from each other by cultivating them in food media under uniform conditions. This is because the growth rate and morphological response of Aspergillus species vary depending on the elements in the environment. They are preferably cultivated in environments containing sucrose as a carbon source and nitrate as a nitrogen source (Powell et al.,.1994).

6.2. Importance of Mold Fungi

Vitamins, antibiotics, various organic acids, and enzymes are obtained from mold fungi. Some mold fungi are used in the ripening of various cheeses. For example, Roquefort and Camembert cheeses are produced using Penicillium roqueforti and Penicillium camemberti, respectively.

Aspergillus niger carries out citric acid and oxalic acid fermentation and is rich in diastase enzyme. It is widely used for industrial purposes. Aspergillus oryzae is used in the production of certain beverages in the Far East. Starch is saccharified with various enzymes and inoculated with this mold (Arıcı, 2017).

6.3. Impact on the Industrial Sector and Biotechnology

Aspergillus strains have significant chemical energy used in production industries such as citric acid production through fumigatus, gluconic, itaconic, and kojic pathways. Citric acid is one of the most commonly used food additives (Currie 1917, Bentley and Bennett 2008). Citric acid, estimated to be produced over 1.6 billion kg annually, is used in the pharmaceutical and cosmetic industries. Aspergillus niger has been used in the metallurgical industry for the production of gluconic acids and in the treatment of calcium and iron deficiencies. Aspergillus terrosus is used in the production of itaconic acid, while kojic acid is produced from fermentation by Aspergillus oryzae (Ruijter 2002, Dodds and Gross 2007).

6.4. Aspergillus Spores and Animal Diseases

Aspergillus spores are ubiquitous and we are frequently exposed to them due to their airborne nature. This exposure is considered normal for humans and generally does not cause any adverse health effects. However, Aspergillus can cause animal diseases through mycotoxin production, allergic reactions, and local or systemic infections. Airborne fungal spores, fungal contamination in food, and direct inhalation or contact with exposed parts of the body, such as through breathing or the eyes, can lead to allergic reactions to Aspergillus.

The respiratory level of spores varies greatly depending on local environmental conditions. Some environments such as compost piles and hangars have high concentrations of spores. Even individuals without allergies or asthma should avoid excessive exposure to spores. This is because repeated contact with high doses of fungal spores can lead to lymphocyte reactions and allergic alveolitis (Deltino et al., 1996; Bush et al., 2006).

6.4.1. Aflatoxin and Aspergillosis

Aspergillus is considered a risk factor in agriculture due to its role in biologically spoiling stored crops, especially as an opportunistic pathogen for field plants under high humidity conditions (Cole and Cox, 1981).

Aspergillosis refers to all diseases caused by Aspergillus, including veterinary and human diseases. Although not a contagious disease, the importance of Aspergillosis is increasing in modern medical care. Aspergillosis has been reported in all domestic animal species and many wild species. Birds are particularly at high risk of this disease. Historically, birds were the first living species known to be affected by Aspergillosis (Ainsworth and Austwick, 1959; Latge, 1999; Latge et al., 2008).

Aflatoxins have been isolated from various sources, including all major cereal crops, peanut butter, corn, cottonseed, millet, peanuts, rice, sorghum, sunflower seeds, tree nuts, and various spices intended for human or animal use. Symptoms of acute poisoning include edema, hemorrhagic liver necrosis, and excessive lethargy.

6.5. Aspergillus oryzae

Aspergillus oryzae is a fungus used in soy production and does not produce aflatoxins. It is a close relative of Aspergillus flavus and is considered one of the safe species due to its use in fermented foods (Kinghorn and Turner, 1992).

It has been used in Asian countries, especially for making sake, soybean paste, and fermenting soybeans. It has been used for years in the production of fermented foods and beverages, as well as for the production of industrial enzymes for food processing.

The ideal growth temperature for Aspergillus oryzae in a rice-based medium is between 23-36°C, and it cannot grow in an environment exceeding 44°C. The ideal pH range for its growth is 5-6. It can also grow in cornmeal with a moisture content not exceeding 16%. Like most other fungi, it grows as haploid multicellular filaments that give rise to fungal gametes and filaments in culture medium (Klich and Pitt, 1988).

The filaments grow and branch on the surface of the agar, covering it after a few days of incubation. They continue to grow as long as they are not exposed to air and terminate with conidia, which are spore carriers.

The fungal culture initially appears whitish and then turns yellowish-green. Aspergillus oryzae typically becomes olive or brown with age. It appears as a spherical structure with a diameter of 100-200 mm and, unlike other filamentous fungi industrially used, does not exhibit any sexual reproduction during its growth phase. It has eight chromosomes and a genome size of approximately 35 MB.

6.6. The Importance of Aspergillus oryzae in the Food Industry

For over a thousand years, Aspergillus oryzae and Aspergillus sucay have been widely used in Japan and Asian countries in the production of Koji. Koji, a fungal culture, is prepared by sprinkling grains such as wheat, barley, and cooked rice. The growth medium for this mold is usually solid, hence these fungi are called Koji molds. Koji is a complex enzyme preparation process that involves the use of amylolytic enzymes and proteolytic enzymes used in the production of fermented foods and beverages such as sake, shoyu, and miso. It contributes to the color, taste, and aroma, which are important for the overall characteristics and quality determination of fermented products. Aspergillus oryzae is used for the fermentation of grains to produce various grain wines. It is also used in the fermentation of soy to produce soy sauce and miso sauce.

The most important role of oryzae in the food industry is the production of various enzymes that assist in the hydrolysis process of certain raw materials to be fermented (Powell et al., 1994). Among the most important enzymes produced by oryzae spores are amylase and glucoamylase, which play a significant role in the breakdown and solubilization of starch. Alpha-amylase enzyme is a glycoprotein consisting of a single polypeptide chain containing 478 amino acids.

All oryzae strains contain two or three copies of alpha-amylase coding genes, namely amyA, amyB, and amyC (Kitamoto et al., 1994; Machida 2005). They also contain at least two copies of glucoamylase, namely glaA and glaB, which assist in the digestion of raw starch (Hata et al., 1998).

Oryzae is closely related to Aspergillus sugai, slavus, and parasticus. However, genetic analysis of these four strains showed that all oryzae strains contain multiple copies (two or three) of the alpha-amylase gene, while all tested strains of the other three species possess the same gene. Therefore, it can be explained that oryzae has a higher ability to secrete alpha-amylase than the other strains and thus derives the maximum benefit from starch (Klick and Mullaney, 1987).

In addition to producing a large amount of beneficial enzymes, it is crucial that oryzae is safely present in food fermentation. The genes responsible for mycotoxin production are crucial despite not being pathogenic because mycotoxins can be harmful to health and raise concerns about food safety. Furthermore, it has been shown that all investigated species of oryzae so far produce harmless levels of aflatoxin (Klick and Chang et al., 1995; Kusumoto et al., 1998).

7. CONCLUSION AND RECOMMENDATIONS

Clinical research and numerous studies and practical applications in biotechnology have shown that microbial peptone obtained through the fermentation and breakdown of wool proteins, legumes, and plant proteins can be used as a raw material containing nitrogen and carbon. Therefore, it can serve as a good growth medium for microorganisms such as Aspergillus species, including Oryzae and Niger, which are expensive in terms of nitrogen. Peptone can be obtained using this method, which can help save costs associated with microbial growth media, recycle environmental waste, and potentially contribute to some environmental solutions.

Peptone has been derived from various sources such as horn, sheep wool, and soybeans. It has been observed that after the breakdown of wool and horns, which consist of more than 80% keratin proteins, these materials can be used for animal feed and plant fertilizers due to their high protein content, including amino acids, peptides, and peptones.

One of the ways to produce peptone is through the fermentation process performed by microorganisms such as Aspergillus strains. Aspergillus oryzae, a fermented filamentous fungus, is considered a probiotic due to its non-toxin-producing nature (Taşkın and Fırat, 2017). Probiotics can be added to food and health products without the fear of causing harm or disease. The fermentation process produces enzymes that are known to be beneficial for both animals and humans. One of the most important enzymes is amylase, which is recognized as an essential enzyme for the digestive system and gut health.

Enzymes break down food chemically, transforming them into easily absorbable nutrients. This method creates an environment with good digestion, which supports the immune system, intestinal function, and overall digestive health. There are nine different types of enzymes that work directly with probiotics, including amylase, protease, and lipase. Amylase is predominantly found in the pancreas and salivary glands, where it combines with probiotics that can absorb simple sugars secreted by amylase. The function played by amylase is significant because it breaks down sugars into simpler components that bacteria can utilize to convert them into lactic acid, hydrogen peroxide, and other necessary substances for maintaining microbial balance in the digestive system.

The more thoroughly probiotic bacteria break down nutrients from amylase and other enzymes, the faster and more efficiently they work, thus protecting us from diseases.

One of the greatest challenges in cancer treatment is finding an anticancer compound that can kill cancer cells without causing any side effects on healthy cells surrounding the tumor. Therefore, the search for a new anticancer compound from natural sources continues to be a significant scientific effort for many cancer biologists. Among these efforts, there is an enormous field to explore by using the diversity of the 1.5 million fungal species found worldwide, of which only 100,000 have been identified so far, to discover and utilize the best anticancer therapeutic molecules (Hawksworth, 2012; Nadumane et al., 2016).

Fungi in the Aspergillus genus have the ability to produce unique biologically active compounds, as demonstrated by the identification of new anticancer metabolites through the application of standard screening and isolation methods. However, the mechanism by which Aspergillus species combat cancer cells has not yet been thoroughly investigated. Therefore, modern techniques, molecular biology, and bioinformatics can contribute to the identification and resilience of the identified therapeutic molecules. Additionally, focusing on research on Aspergillus species can serve as a resource for future discoveries of anticancer drugs.

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CHAPTER 2

SAFFLOWER SEED *(Carthamus tinctorius)* AS AN ALTERNATIVE FEED SOURCE IN POULTRY BREEDING

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1. INTRODUCTION

For the development of animal husbandry in Turkey, besides the use of high-yielding breeds, it is necessary to feed the animals with rations that will meet their nutrient requirements in an adequate and balanced way. It is an indisputable fact of today that it is impossible to meet the needs of hybrids used in egg, chicken meat and turkey meat production with one or two feeds, it is not enough to meet the nutritional requirements alone in feeding, and the ratios or balances between them must be taken into account. The remarkable increase in poultry production and products is not a coincidence, it is closely related to the developments in nutrition, feed and feed technology as well as genetic progress and is the common output of multidisciplinary studies.

In recent years, in poultry feeding; fibrous feeds, sequential and selective feeding, starch digestibility, small intestinal epithelial tissue barriers, microbiota and probiotic use, use of oat husk and arabinoxylo oligosaccharide in the fight against necrotic enteritis, external parasite control with plant essential oils, nano trace element nutrition, organic in microcapsules in the fight against coccidiosis acid and plant essential oil mixture, enzyme use with cottonseed meal, wheat-based chicken feed, use of carbohydrase-fortified sunflower meal, use of manganese for eggshell strength, polyphenols and synthetic carotenoids to prevent the negative effects of heat stress, isoquinoline alkaloids to combat salmonella, vitamin enriched egg production and sustainable poultry matters are affection come to the fore. It is possible to collect these study subjects, which are carried out in a very wide range, only in a book or book series. Therefore, in this review, the need for our country in the coming years in order to sustain the success achieved in the field of feed, feed technology and nutrition, the importance of which is increasing day by day for poultry (broiler chicken, laying hen, breeding chicken, turkey, goose, duck and quail) production. It has been presented under the following headings in order to examine the research topics and to create suggestions (Kutlu and Şahin, 2017).

The use of agricultural by-products in animal feed is as old a practice as the domestication of animals. The main advantages of using by-products in animal nutrition include reduced dependence of animals on grains consumed by humans and reducing the costs of managing waste products (Keser and Bilal, 2010).

The most commonly used feed materials in poultry feeds are corn and wheat. Corn, which has been largely imported in recent years, is quite expensive. Producers add bread wheat to the rations in order to save on corn. However, these grains are also the main sources of human nutrition. For this reason, the effects of alternative feed plants and feed additives are investigated in order to protect the nutritional resources that have a very important place in human nutrition and to benefit from them in the best way, and to increase profitability by reducing the cost (Çimrin and Tunca, 2012).

2. SAFFLOWER SEED

Safflower (*Carthamus Tinctorius*) is an oilseed plant in the Compositae family, containing 20-40% oil in its seeds (Coşge et al. 2007). It is mainly grown for its oil, and safflower oil is also used in the edible and oil industry. The amount of oil in the seed and the oil content vary according to factors such as variety, climatic conditions, planting time, soil structure. The main factor determining the safflower fatty acid composition is the variety (genotype). Oleic, linoleic, stearic and palmitic fatty acids constitute 96-99% of safflower oil. Oleic and linoleic acid ratios can vary between 10-32% and 58-81%, respectively, according to the varieties (Coşge et al., 2007).

The fatty acid content of safflower oil is given in Table 1, and it has been determined that the oleic and linoleic fatty acid content of the variety varies greatly according to the fatty acid direction in which the variety is bred. For example, the oleic acid content of the domestic safflower variety Remzibey is almost 3 times higher than the local safflower variety Yenice and Dinçer (Coşge et al., 2007; Çamaş et al., 2007).

Safflower has flowers in different colors such as yellow, white, cream, red and orange, which can be 80-100 cm in length, with thorny and thornless forms, the thorny forms contain more oil than the thornless ones. Its seeds are white, brown and in the form of white grains with dark lines on it (in rare cases, black seeds can be found), branching, colorful flowers (petal) can be used in food and fabric dye and have a deep tap. It has 30-50% oil in its seeds, has 2 different types as linoleic (Omega-6) and oleic (Omega-9, olive oil quality), high quality oil as edible, can also be used in the production of biodiesel, whose pulp is considered as animal feed, It is a one-year long-day oil plant that is durable, has a summer character and can grow for an average of 110-140 days (Trakya Agricultural Research Institute, 2023).

2.1. Nutritional Components of Safflower Seed and Safflower Seed Meal

The shell rate of safflower seed is around 40%.(Blair, 2011) it is very difficult to separate the shell from the seed and it is an expensive operation. Seed, variety, soil and climate approximately %, depending on the conditions 15-19 crude protein (CP), 15-20% crude fiber (CS), 30-32% acid detergent fiber (ADF), 40-45% neutral detergent fiber (NDF) (Malakian and Hassanabadi, 2010; İlkdoğan, 2012) and 20-40% ether extract (Coşge et al., 2007) contains. Nutritional effects of newly developed safflower varieties ingredient composition and that of traditional varieties are different from each other. For this reason, especially in recent studies, the nutrient content given for safflower has been compared with the previous years. Among the contents given in the studies carried out, one there is quantity difference.

Safflower meal, from seed oil extraction then the remainder. The quality of its pulp, oil extraction amount of oil remaining after processing and depending on the amount of crust. Safflower oil from seeds by mechanical pressing or solvent obtained by the extraction method.

Solvent extraction method is more effective in oil extraction. Separation of the shells from the seed pressing increases its effectiveness. Main fiber in safflower meal The source is the shell and the CS content in the pulp is depending on the shell content. Crude cellulose its content is obtained from seeds that have been completely peeled. While it decreases up to 2% in the extruded safflower meal, in bagasse from unpeeled seeds It varies between 30-40% (Farran et al., 2010).

3. USE OF SAFFLOWER SEED IN POULTRY FEEDING

It is very important to provide the energy and protein sources needed in the preparation of poultry mixed feeds in a sustainable manner. Our country, which is sufficient in terms of grain sources with high energy content such as corn and wheat, is condemned to import especially in terms of quality protein sources. It is possible to use in poultry mixes that can be produced under the conditions of our country, oilseed meal such as safflower, camelina, guar, fenugreek, lupine, etc. There is a need for research on alternative protein source feeds such as for example; Fenugreek, one of the medicinal and aromatic plants, has the potential to be both a feed raw material and a feed additive due to its high antioxidant capacity, antidiabetic, anticholesteromic, hypoglycemic effects and many other pharmacognosy properties (Olaiya and Soetan, 2014; Singletray, 2017).

The use of these sources in exogenous enzyme supplementation in order to increase the feed value should also be specifically examined. In closing the vegetable protein deficit, the use of oil industry by-products (hazelnut meal, sunflower meal, cottonseed meal, safflower meal, corn gluten meal) suitable for the vegetable production pattern of our country comes to the fore. Feed technologies should be studied to expand the use of alfalfa (alfalfa flour) and dairy industry by-products (whey or powder) as poultry feed (Shariatmadari and Forbes, 2005). In addition, studies showing that even worms are used 10-15 % in poultry feeds (Hwangbo et al., 2009) in countries with protein restrictions appear as an effort to seek alternative sources.

Addition of safflower meal to laying hen rations (Ehsani et al., 2013) and safflower seed (Vashan et al., 2008) can be added at the 10% level however, more than 10% addition it is emphasized that it affects the shell quality negatively (Ehsani et al., 2014). At least 2.5% of the ration addition of safflower seeds, egg unsaturated fatty acids increases the rate (Yakar et al., 2014). Malakian et al., (2011) on broiler rations 20% use of oily safflower seeds reported that it did not adversely affect broiler performance.

Barbour et al. (2016) reported that the addition of 20, 40, 60, 80 and 100% safflower seed meal instead of soybean meal protein to laying hen rations had no effect on egg yolk oil, cholesterol and fatty acid.

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CHAPTER 3

PASTURE AVAILABILITY AND CLIMATE CHANGE

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1. INTRODUCTION

The basic principle in animal nutrition is healthy, continuous, clean and balanced nutrition. In nutrition, the most important source is animal protein. Animal proteins have a very important place in the nutrition of societies. While approximately 33 g of a person's daily protein needs should be met from protein of animal origin, this rate is almost 13-17 grams in our country (Cevheri and Polat, 2009).

The amount and quality efficiency of animal protein in the nutrition of the increasing country population, which is a linear response to the world population, is indisputable; The effect of animal proteins on the growth of a healthy generation is obvious. By increasing the number of animals and the unit amount of animal product per animal in our country, the animal protein requirement of the increasing population can be supplied from within the country. For this purpose, agriculture and livestock policies must be predictive, continuous, need-oriented, accelerating and sustainable. However, these targets for our country have not been realized yet.

2. PASTURE AVAILABILITY

Quality roughage, which is essential for the ration in ruminant feeding, is obtained in two ways. The first is meadow pastures, and the second is forage crops agriculture. Meadow pastures, which form the basis of economic animal husbandry; It provides quality and abundant roughage, has natural plant flora, is a source of genes for biodiversity and endemic species, is a habitat for wildlife, increases land productivity and provides protection. (Açıkgöz, 2001). Animal husbandry in Turkey is largely based on pasture and low-nutrition feeds rich in fillers such as straw. However, the most applicable form of feed supply is to grow forage crops (Kuşvuran et al., 2011).

Access to economic and quality roughage is very important in animal production. In this regard, the most important roughage sources are meadow-pasture and grassland areas in the forest. The existence of these areas in our country is 11.7 million hectares as of 2019 (Ministry of Agriculture and Forestry, 2020). However, they lose their effectiveness due to reasons such as intensive, not paying attention to temporal changes and unconscious grazing, as well as not being able to perform maintenance and drainage operations on time. As a matter of fact, meadow pasture forage plants, which have a high nutritional value and are consumed by ruminants, disappear over time (Budak, 2013).

Meadows and pastures of Turkey have a roughage production potential of 8.7 million tons/year. Because; the ineffectiveness of measures and incentives that increase the efficiency of pasture areas such as preventing excessive and early grazing, alternating grazing, pasture improvement and the use of non-agricultural lands as artificial pasture, or the failure of sustainability policies cause us to benefit from. In addition, the potential for use of plant production residues and by-products, which are released during the production phase of plant products in our country, as an alternative forage source should be professionally examined. Feed sources with high roughage potential should be expanded by using microbial fermentation (such as silage making, fermented feeds), physical and chemical applications, and biotechnological methods (Yaşar and Yıldız, 2022).

It is seen with the roughage imports that a significant part of our meadow-pasture existence has low yield capacity, the forage plant flora is not rich enough, and our country is insufficient to feed the number of animals. It is known that the amount of good quality roughage we produce within the borders of the country cannot meet the nutrient requirement of the number of animals in our country and this deficit has a rate of almost 74% (Okçu, 2020).

3. CLIMATE CHANGE

The rapid rise of atmospheric carbon (CO_2) concentrations throughout the industrial period is due to anthropogenic (human activities). Today, the most important sectors that cause greenhouse gas emissions are energy, transportation, industry and agriculture. Pasture areas, which are vitally important for livestock practices that lead the production of greenhouse gas emissions originating from the agricultural sector, are natural assets and rural-ecological commons that are directly affected by and directly affect climate change. Rangelands are of planetary importance in terms of their biodiversity, high carbon absorption and erosion prevention capacity. In addition, as stated in the Intergovernmental Panels on Climate Change (IPCC), they are important terrestrial carbon sinks, thanks to plant diversity and soil structure. It is known that carbon sinks in the ocean and on land slow the increase of CO_2 in the atmosphere (IPCC, 2019).

In addition to being important terrestrial carbon sinks, pastures are also important for the continuity of rural tradition, food sovereignty and local sustainable development for rural producers who make a living from animal husbandry.

The concept of "food sovereignty", the basic principles of which were determined by the international farmer/peasant movement La Via Campesina, describes the whole of principles and policies that will ensure food security and food safety. The concept, which was put on the agenda of the United Nations and the UN Food and Agriculture Organization (FAO) in 2008, means that peasants, landless farmers, all people who put labor in rural production, and consumers are not subject to markets, and democratically involved in production, consumption and the determination of policies related to them advocates it. Food sovereignty prioritizes "agro ecological production systems" that consider the environment/nature not as a resource, but as a common asset that is open to public access and the right of use is superior to the right of owner ship (Hazar Kalonya et al., 2020). However, today's demand for sustainable, environmentally friendly, agro ecological food production systems by modern consumers seeking both quality and safety contributes to climate change mitigation and protecting biodiversity, while increasing the income of rural producers (TUBITAK, 2021). The diversity of major farming systems associated with semi-natural habitats and rural landscapes is also important for mutually supporting economic sectors such as agriculture, agrotourism and ecotourism (Hazar, 2018).

Due to the effects of climate variability, the increase in meadow and grassland areas due to warming in cold regions will contribute to the development of animal husbandry, and there is high temperature. In other regions, it was stated that the heat stress, which will be shaped as a result of the decrease in forage crops production due to drought and the increase in air temperature, will cause a decrease in feed intake in animals and thus loss of yield (Demir and Cevger, 2007).

Changes in the amount, distribution and intensity of precipitation directly affect the timing and duration of growth of pastures and crops. Occurrence, spread and distribution of diseases in livestock are high influencing the rate of development of pathogens and parasites. Disease vectors are affected by temperatures. These effects cause losses in live weight and milk yield in animals (Malik et al., 2015; Koç et al., 2016).

Animal production has a significant impact on the world's water, soil, and biodiversity resources, and adversely affects climate change in many ways, including land use change (CO_2), enteric fermentation (CH_4), and manure management (N_2O) (Steinfeld et al., 2006). ; Koç et al., 2016).

It is noteworthy that 65% of greenhouse gas emissions come from beef and beef milk. In this context, in order to ensure sustainability in global agriculture and food production and consumption; it is necessary to develop the resources of animal production and increase its environmental performance. In addition, social and economic impacts must be taken into account. It is important to plan the future of the industry in order to reduce its contribution to climate change, to minimize the effects of climate change on livestock productivity and to meet the food needs of the population (Herrero and Thornton, 2013; Koç et al., 2016). In order to minimize all these negative effects, it is necessary to adapt animal production to climate change. To reduce greenhouse gas emissions from agriculture, stopping animal production greenhouse gas growth should be a priority. There are many different ways to do this; some of these are the use of better quality feeds and the improvement of animal nutrition according to changes in temperature, the development of new animal breeds resistant to stress, effective manure transport and stocking management, grazing management and pasture improvement studies. These studies will both eliminate the low yield in animal production due to climate change and reduce the effects of animal production on climate change (Koç et al., 2016).

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CHAPTER 4

DIETIC CATION AND ANION BALANCE AND ITS IMPORTANCE IN RUMINANT FEEDING

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1. INTRODUCTION

Studies have been carried out on the determination of the amount of protein, carbohydrates, fats, vitamins and minerals, which are essential nutrients, necessary for the production and survival of animals. Recently, ruminant breeders have been trying to classify these basic nutrients into more specific subclasses in order to determine the needs of animals more accurately and to prepare a better diet by using chemical analysis of feeds. For example, while proteins are expressed as nitrogen that is degraded and not degraded or non-protein (NPN) in the rumen, carbohydrates can be classified as structural (cell wall material) and non-structural (intracellular material), as well as divided into two parts as those that are degraded and not degraded in the rumen (1).

Essential nutrients are divided into three or four subclasses. This has given researchers the opportunity to examine the dietary supplementation of appropriate levels of essential nutrients and their effects in animals. Due to the small number of subclasses, it was easy to examine the interaction between the feeds. Minerals show a different structure in these main nutrients. Minerals needed by animals are divided into two parts. 1- Micro minerals 2- Macro minerals (2).

This classification is based on the amount of minerals found only in the animal's body and in the diet. This classification is given little consideration in dietary formulations. However, when compared to all other essential nutrients, mineral substances have more biological functions. The main places where minerals take part are acid-base balance, detoxification, osmotic balance, synthesis of genes and enzyme systems that regulate their structural roles (2).

2. THE THOUGHT OF BALANCING ANIONS AND CATIONS IN DIETS.

The idea of balancing anions and cations in diets is not new. This concept was introduced by Mongin (3) in 1981 and was first used in poultry diets. Anion (Cl^-) must be adjusted against cations (Na^+ and K^+) in the diet to optimize the physiological functions of animals. Na^+, K^+ and Cl^- levels of these minerals in the diet should be sufficient due to the continuity of osmotic balance, acid-base balance and acid-base balance in ruminants and their indirect role in the Na+ and K^+ pump in the cell (1).

The increasing use of the concept of dietary cation-anion balance (DCAB) in the nutrition of dry dairy cows has raised the question of whether DCAB should be emphasized in the diet formulation of lactating dairy cows and what the optimum DCAB should be in high-yielding dairy cows (4).

Several equations have been used to define DCAB expressed in milliequivalents per kg DM

1.DCAB = (Na + K)/CI
2.DCAB = (Na + K) - (Cl + S)
3.DCAB = (Na + K + 0.15 Ca + 0.15 Mg) - (Cl + 0.6 S + 0.5P)
4.DCAB = (Na + K) - (Cl + 0.6 S) (5)

3. THE ROLE OF DIET CATION-ANION BALANCE IN METABOLIC EVENTS.

Increasing the cation-anion difference in the lactation period increases DM consumption and animal production, and it is seen that the heat stress seen in hot climate conditions is alleviated. Because as a result of production and heat stress, metabolism in the body accelerates and acid formation increases. Increasing the cation-anion difference helps to increase the blood buffering capacity and reduce the increased H^+ ion concentration in the body. The increase in ambient temperature increases the water consumption, there is no relationship between the increase in the cation-anion difference and the water consumption. Metabolic acidosis, laminitis and ketoacidosis seen in lactating animals can be corrected by changes in the diet cation-anion balance and by future studies (2).

The negative effect of high environmental temperature, which has a negative effect on the animal's performance, can be changed by changes in the metabolism of mineral substances in the body (6, 7, 8, 9, 10). Theoretically, with the macro mineral levels in the diet, the performance of dairy cows under heat stress during the lactation period and the balance of the body can be changed. The effects of macrominerals are not completely independent, and their effect on animals under high ambient temperature conditions is different from the effect on animals under normal ambient temperature conditions (11). More work is needed to fully elucidate this situation.

Some authors have suggested that cations in the organism are alkaline and anions are acid-forming (12). This thought is not always true. Because

HPO42- and NH4+ are both proton donors and alkali buffers. Therefore, DCAB mEq = ((Na^+ + K^+) - (Cl^-)) described by the equation does not determine the acidifying or alkalizing properties of the feeds. However, the metabolism and absorption of these ions taken into the body through diets can affect the metabolic process in the animal. The most important role of anions and cations in metabolism is their effects on acid-base balance (2).

Garret (12) and Block (13) take sulfur into account with the equation mEq = ((Na^+ + K^+) - (Cl^- + S)) in calculating the cation-anion balance. Although sulfur is a fixed ion, sulfates acidify biological fluids and, if present in the diet in high quantities, can alter the acid-base balance. Sulfur need not be added to the acid-base equation unless sulfates are added separately to the diet or protein supplements are made.

The Na^+ and K^+ pump mechanism of the cell is a mechanism that requires energy in the form of ATP. The Na^+ and K^+ pump works continuously, independent of other metabolic developments, and takes part in the uptake of glucose into the cell. Considering that glucose is the main source of cellular energy, it is clear that slowing the Na^+ and K^+ pump will not allow the cell to operate at full capacity. This pump system is particularly important in mammary glands where lactose synthesis is high (1).

Urinary excretion of Na^+ and K^+ involves a relationship where Na^+ is retained in the body and K^+ is excreted, depending on the body's need for Na. An excess of one cation against another cation may cause a deficiency. For example, when the amount of K^+ in the diet is higher than Na^+, a Na^+ deficiency may occur due to an excess of kidney K^+, although the dietary Na^+ is in the required amount. In cases where Na^+ is more than Cl^- ion in the intestines, there is a potential exchange of H^+ circulating in the blood with Na^+ ingested, and this event may cause alkalosis. This particular mechanism may explain the increase in the amount of milk corrected for fat in cows supplemented with $NaHCO_3$ or NaCl in their diets. If $NaHCO_3$ is added to the feed, which is practiced in dairy farming today, and if NaCl is not adjusted to limit the amount of Na^+ in the diet, the alkaline property of Na^+ may come to the fore (2).

4. THE RELATIONSHIP OF ANIMAL'S ACID-BASE BALANCE WITH DIET CATION-ANION BALANCE AND ITS EFFECT ON ANIMAL PERFORMANCE

Sanchez et al. (14) in the study in which they kept the diet anion cation balance between +25 and +40 meq in DM by adding mixtures containing $NaHCO_3$, NaCl and KCl in the ratios of 100:0:0, 50:50:0, 33:33:33 to the diet, respectively, in the mid-lactation period of the dairy cows in the summer season. They found that it fell. The fact that the diet cation-anion balance was within narrow limits such as +25 and +40 prevented the occurrence of changes other than parameters such as milk protein and live weight gain in animals. Sanchez et al. (15) compared the studies carried out in different ambient temperatures with the studies in which the environmental temperature factor was removed and found that the buffer substances added to the diet in case of heat stress increased DM consumption and milk yield. In cases where the ambient temperature is high or the heat factor is eliminated, but the DM consumption is limited, there is a decrease in the plasma flow in the portal circulation. The transfer of phosphorus to the organs via portal blood circulation in dairy cows under heat stress in the lactation period was 50% lower than in animals in environmental conditions without heat stress. The increase in respiratory rate in hot environmental conditions causes respiratory alkalosis and this situation is tried to be compensated by the body with metabolic acidosis. Milk production in animals that consume drinking water containing high levels of sulfate and Cl^- is endangered during periods of high ambient temperature.

Erdman et al. (16) found that the acid-base balance in dairy cows shifted to alkaline with the progression of lactation, and blood pH, HCO_3, pCO_2 pressure increased. Erdman (17) evaluated the effect of ambient temperature on blood pH, HCO3 and pCO_2 in animals, but he could not fully elucidate the changes made by diet on these parameters. The investigator suggested that although $NaHCO_3$ tends to increase blood pH, HCO_3 and pCO_2, these values are low when the ambient temperature is high. However, it is controversial whether this effect is shaped by $NaHCO_3$ or just Na. Schneider et al. (10) suggested that the increase in production and feed consumption in dairy cows exposed to heat stress was due to the increase in Na due to $NaHCO_3$ added to the diet, and not related to the increase in HCO_3. Erdman (17) suggested that the response of dairy cows to Na in the form of $NaHCO_3$ is less variable than their response to Na in the form of NaCl, and suggested that HCO_3 in $NaHCO_3$ has a specific role in this.

When the cation-anion balance of the diet is calculated with the formula mEq = $(Na^+ + K^+) - (Cl^-)$, the addition of $NaHCO_3$ to the diet will give a more positive mEq value than the addition of NaCl. Because HCO_3- will not be included in the equation. Therefore, despite the equal addition of Na from two Na sources, $NaHCO_3$ will have a more alkalizing effect than NaCl (1).

In cases of low milk fat from diets with low roughage:concentrated feed ratios, NaHCO3 added to dairy cow diets improves milk fat well. This response is probably due to the reduced saliva and $NaHCO_3$ flow due to the low forage level, filling the feed with $NaHCO_3$ (2).

Kilmer et al. (18) found that dairy cows fed diets depressing milk fat did not affect the level of milk fat in their study when they added $NaHCO_3$ to their diets. When the cation-anion balance in the diets was calculated with the equation mEq = $(Na^+ + K^+) - (Cl^-)$, it was determined that the cation-anion levels were equal since Na from NaCl was replaced by Na from $NaHCO_3$. St Laurent et al. (19), on the other hand, found in a study they conducted that positive responses were obtained from animals when $NaHCO_3$ was added in addition to NaCl, which is the main sodium source, in the control diet. It has been suggested that this result is achieved by increasing the diet cation-anion balance.

Fettmen et al. (20) in their study on the effect of adding Cl- to the diets of dairy cows, they reported that when the amount of Cl in the diet was increased from 1 g/kg to 4.5 g/kg (on a DM basis), an increase was observed in the feed consumption, CAA and milk yield of the animals, but these increases in yield were not at the recommended level. In another study, it was found that dairy cows consuming a cationic diet (20mEq per 100g feed) gave more milk than animals fed diets with a dietary DCAB value of -10, 0, or 10 meq/100 g (19).

Tucker et al. (21) found that animals consuming a diet with a DCAB of 200 mEq gave more milk when they gave diets with a diet cation-anion balance of 200 and –100 mEq/kg in DM to cows in the 3rd and 8th months of lactation, and they found that blood pH and HCO_3 and urine pH increased linearly with increasing DCAB. West et al. (6) They determined that DCAB should be decreased in parallel with these changes, since metabolic activity will decrease with the progression of lactation and decrease in milk yield in dairy cows in the lactation period.

However, the optimal DCAB required to prevent the development of clinical hypocalcemia is vigorously debated. The degree of metabolic acidosis can be easily assessed by measuring urine pH. A urine pH less than 6.0 suggests excessive acidification. At very low blood pH, the cow quickly compensates for this by reducing feed intake, increasing renal proton excretion, increasing reabsorption of potassium and bicarbonate, and respiratory rate, which together reduces variability in urine pH. If the blood pH falls below 7.2, the animal dies (5). Accordingly, a target urine pH between 6.0 and 6.8 was found to be appropriate in the prenatal period for Holstein cows (22). It has been reported that a urine pH below 6.0 (DCAB -240 mEq/kg DM) including grazing flocks (23) improves Ca homeostasis and reduces urinary pH variability within the herd when compared to a positive DCAB diet. These studies did not include the experimental group with a mildly negative DCAB diet (-80 to -120 mEq/kg DM) (24). The urine and serum metabolomics of cows fed antenatal highly negative DCAB diets were reviewed. If a comprehensive analysis of the rational use of anionic salts is made, a hypothesis can be formed for the potential pathophysiological mechanisms of the effects that an overdose of anionic salts may cause in both the cow and the calf (25).

Previous research on metabolic responses to negative dietary DCAB has traditionally focused on usual metabolites (eg, glucose, triglycerides, NEFA=glucose and non-esterified fatty acid). Recently, new information has been identified on blood acid-base parameters and plasma and urine metabolomics in prenatal Holstein cows fed an anionic diet with very low DCAB (26). Cows that consumed enough anionic salts to lower urine pH to 4.96-5.74 had a very low base excess in their blood. This indicated a severe metabolic acidosis. They also had higher blood lactate concentrations than cows consuming a positive DCAB diet. Cows consuming a negative DCAB diet were determined to have urine concentrations of aromatic amino acids, lysine, histidine, and threonine, and low essential amino acids and glucogenic amino acids. However, it was determined that the total circulating non-essential and glucogenic amino acid concentrations were higher. Interestingly, the acids, dietary anionic salts, showed marked effects on a range of plasma glycerophospholipids (25).

It is considered necessary to keep DCAB of lactating animals cationic since these animals have a high metabolic rate and the body is more prone to acidosis. In order to keep the balance positive, Na+ and K+ in the diet should be quite high compared to Cl^-. Therefore, Na^+ and K^+ will alkalize the acidic environment with their alkalizing effects. The ideal DCAB for lactating cows varies with lactation progress and reduction in milk yield. Theoretically, DCAB should be high at the start of lactation and should decrease as lactation progresses. The fact that the addition of $NaHCO_3$ to the diets of dairy cows that consume diets that will not depress milk fat and are in the period after the first 100 days of lactation has little effect indicates that DCAB should be lowered as lactation progresses (2).

As it is fed with more anions, the concentration of H^+ in body fluids, including blood, increases and the pH drops, leading to metabolic acidosis. In response to these changes, the kidneys reduce Na^+ excretion and K^+ and urinary pH as compensatory mechanisms. Therefore, the assessment of urine pH in prepartum cows fed anionic diets is a rapid and low-cost field method to screen for metabolic acidification level (27).

Changes in DCAB will affect urine pH within 48-72 hours (28). Urine pH can be assessed using spot samples without the need for multiple samples over a 24-hour period (29). However, when reporting studies on prenatal urine pH and calcium concentrations at birth, sampling time as days to delivery should be considered as a covariate in models (30).

The optimum degree of metabolic acidosis reflected by urine pH, which can prevent clinical hypocalcemia, is still controversial (31). Strategies using anionic salts focus on achieving specific DCAB and urine pH. However, doses of a particular product are not recommended. Because the dose will depend on the content of cations and anions in the whole diet. Therefore, a dose is not standard. One approach suggests anionic products to achieve a very negative DCAB (<-200 mEq/kg DM) targeting a urine pH below 6.0 (32). This can potentially lead to an uncompensated metabolic acidosis (very low blood pH, very low blood bicarbonate concentration, base excess near 0, and normal $PaCO_2$) (26). The normal urine pH range for cows is between 8 and 8.5 (33). Urine pH < 6.0 suggests that the cow is at the limits of compensatory mechanisms and metabolic hyperacidification (26). It has been shown that cows with a urine pH close to 7.0 have a lower risk of developing clinical hypocalcemia (34). Prepartum, for Holstein dairy cows, urine pH in the range of mild metabolic acidosis (6.0-6.8) will be sufficient to prevent milk fever (22).

Reducing the urine pH by 3 units (from 8.5 to 5.5) means that the kidneys must excrete 1,000 times the extra H+ produced by the body, as the pH scale is logarithmic (5). Charbonneau et al. (34). The lower the DCAB, the lower the urine pH and the lower the DM intake, and the increased risk for uncompensated metabolic acidosis. In fact, cows consuming diets high in anion had an average urinary pH of 5.9, but the same tCa concentration at calving compared with cows with a more moderately low urinary pH of 6.2 (35). Melendez et al. (30) reported that a diet with -100 mEq/kg DM DCAB caused a urine acidity close to 6.5 and blood tCa concentration was close to 8.5 mg/. It is still unclear whether dietary Ca and anionic strategy are more important to meet the calcium needs and maintain the acid-base balance of the cow. In a study using castrated male sheep, the effects of low or high DCAB and dietary Ca levels on various physiological responses were examined (36). Lower DCAB groups were significantly associated with lower urinary pH, higher urinary Ca excretion, and higher serum iCa concentrations. However, blood pH and bone responses did not differ significantly between groups. Based on these results, it was concluded that it is unclear from which compartment the high urinary Ca content in low DCAB groups originates. Low DCAB groups showed a higher abundance of kidney mRNA for parathyroid hormone receptors. They concluded that diets containing low DCAB increased urinary Ca excretion independent of dietary Ca intake (36).

5. RESULT

Further studies are needed to evaluate whether circulatory or urine metabolomic profiles are predictive for cows susceptible to metabolic acidosis and whether these indices return to normal after restoration of normal acid-base status. It can be argued that significantly reducing DCAB is not beneficial. This is because animals will likely excrete excessive amounts of Ca mobilized from the bone and absorbed from the gut.

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CHAPTER 5

DIET CATION-ANION BALANCE IN RUMINANTS AND ITS EFFECT ON ANIMAL HEALTH AND PRODUCTION FEATURES

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1. INTRODUCTION

Hypocalcemia remains a common metabolic disorder in dairy cattle that can lead to reduced milk yield, low fertility and significant economic losses. Although its clinical form has decreased consistently in recent years, it still continues as a dairy business problem. With the available information, producers and nutritionists can protect against milk fever and reduce the adverse effect of subclinical hypocalcemia (SCH) and improve lactation performance of cattle with dietary supplementation of anionic salts. However, such a nutritional intervention must be moderated because of the secondary effects that excess anionic salts can have on physiological adaptations in cows and calves. A moderate metabolic state with a urine pH between 6.0 and 7.0 appears to be sufficient to control and prevent clinical hypocalcemia and reduce the incidence of SCH. A more severe acidotic state has been shown to be more harmful than beneficial to the cow and possibly her offspring.

2. EFFECT OF DIET CATION-ANION BALANCE ON ANIMAL HEALTH AND PRODUCTION CHARACTERISTICS.

Dietary cation-anion balance is directly related to blood acid-base metabolism, and by changing the acid-base balance, biological functions can be changed, and the health and production of the animal can be changed. Small changes in the cation-anion balance in near-birth animals reduce the incidence of hypocalcemia through the hormone that regulates urinary excretion of calcium and the amount of ionized calcium in the blood. These adjustments in the cation-anion balance in dairy cows close to calving reduce the incidence of paralysis caused by calcium deficiency, and although they cause a reduction in the duration of the stroke events, they do not completely eliminate the paralysis. It is associated with changes in the cation-anion balance in edema occurring in the breast region in the prenatal period. However, the effect of acid-base balance on breast edema is not much. Because acid-base balance is mostly formed as a result of biochemical processes (1).

The risk of developing acidosis in these animals is quite high, since it is possible to provide a rapid body weight gain in fattening young ruminants or to meet the energy needs of high milk yielding animals, mostly with the diet consisting of up to 90% concentrated feed. For this reason, various methods have been tried to protect ruminants from acidosis. One of these methods is the addition of buffers to concentrate feeds. The most commonly used substance for buffering is $NaHCO_3$. It has been determined that $NaHCO_3$ added to acidotic feeds prevents the decrease of rumen pH and increases blood pH, pCO_2 and HCO_3- levels. It is reported that $NaHCO_3$ added to acidotic feeds is more effective especially when the rumen pH is at 5.0-6.0 levels, and the rumen pH does not fall below 5.5 thanks to the buffering effect of $NaHCO_3$. It has been suggested that the addition of $NaHCO_3$ to carbohydrate-rich diets provides an increase in animal feed consumption and live weight gain, and decreases in the incidence of rumen hyperkeratosis and rumenitis (2).

Brent (3), in his review of the relationship between acidosis and other nutritional diseases, states that laminitis occurs following lactic acidosis events, and this is due to the increase in histamine in the blood and rumen. The increase in the rapidly fermentable substrate in the rumen is followed by an increase in acidity in the rumen, followed by a decrease in the pH in the rumen, and acidosis occurs. This situation causes rumenitis and liver abscesses as a result of pathogenic microorganisms passing through the rumen wall reaching the liver via portal circulation. Ruminal acidosis disrupts the physiological state of the rumen, and thiamin deficiency causes polyencephalomalacia with the effect of the thiaminase enzyme formed in the rumen.

Ross et al. (4) in their study on 120 young male cattle, they adjusted the diet cation-anion balance to be 0, 15, 30 and 45 mEq in 100 g feed DM using NH_4Cl and $NaHCO_3$. Researchers added $NaHCO_3$ to the diets at the levels of 0.18, 1.21 and 2.49%, respectively, to increase DCAB. As a result of the study, the increase in body weight increased linearly in parallel with the increase in DCAB. They found that daily feed consumption did not change with the increase in DCAB, compared with the acclimatization period, feed consumption increased as DCAB increased in the trial period. While pH and HCO_3 levels of arterial blood increased depending on DCAB ($P<0.05$), ionized Ca, pCO_2 and pO_2 levels in blood did not change. Rumen pH, which increased linearly with DCAB elevation up to day 28, remained unchanged after day 28. Ross et al. (5) in the study where they used NH4Cl and $NaHCO_3$ and DCAB adjusted as 0, 15, 30 and 45 mEq in 100 g feed DM for animals in the last period of feeding, daily DM consumption increased due to arterial blood pH and HCO_3 DCAB elevation, and ionized Ca, pCO_2 and pO_2 levels were not affected.

The addition of $NaHCO_3$ to the diet leads to a significant reduction in hepatic abscesses, resulting in a reduction in economic damage from digestive and hepatic diseases, which adversely affect the health of animals and often cause insufficient production. In calves, $NaHCO_3$ is used in the weaning phase. This substance is recommended to reduce major economic damage from birth deaths and postpartum diseases. It is known that a good nutrition and a normal development in the new birth stage are a guarantee in terms of future productivity and health of the animal (6).

In recent years, especially in ruminant fattening, it has been seen that animals are fed with diets consisting of concentrated feeds under the name of intensive fattening in order to reach the desired slaughter weight in a shorter time. However, it has been reported that pathological lesions such as acidosis, rumenitis, abnormal rumen papillae, hyperkeratosis, abomasum displacement, liver abscess and dystrophic and necrotic findings in the heart, kidney, liver and fore-stomach are observed in these animals fed with highly concentrated diets, and milk fat decreases in dairy cows (7).

Administration of $NaHCO_3$ to animals is particularly effective in stimulating the appetite of female cattle raised in warm environments. Because during the hot summer months, there is an alarming decrease in animal productivity and feed consumption. Likewise, the use of $NaHCO_3$ will be positive, since the composition of the feed needs to be changed in line with the new needs of the animal after birth, which is a very sensitive period. The addition of $NaHCO_3$ to the feed of ruminants seems to increase feed consumption levels, especially during the most sensitive times of the rearing period, and increase the digestibility of feed in diets with high energy concentration (6).

2.1. EFFECT OF VERY LOW DIET CATION-ANION DIFFERENCE ON COW PERFORMANCE AND HEALTH

A second-order polynomial relationship has been reported between prenatal urine pH and the risk of hypocalcemia in dairy cattle (8). Cows with a urine pH between 5.5 and 6.5 show a lower risk of hypocalcemia. Targeting the more moderate pH below 6.0 will not be helpful. Santos et al. (8) reported that the risk of abomasum displacement decreased from 12% in cows consuming a diet containing DCAB - 200 mEq/kg DM (corresponding to urine pH 5.7) to

6% in cows consuming a diet containing +200 DCAB (mEq/kg DM corresponds to urine pH 8.0). Another study reported a relationship between displaced abomasum formation and blood and urine pH and serum tCa concentration. Dairy cows with left displacement of the abomasum had lower prenatal urine pH (6.11 ± 0.2 - 6.65 ± 0.1) and blood pH (7.27 ± 0.01-7.32 ± 0.01) compared to cows without digestive problems (9).

Vieira-Neto et al. (10) Prepartum cows consuming DCAB +110 mEq/kg DM diet, cows consuming DCAB -70 mEq/kg diet and another group consuming DCAB -180 mEq/kg DM diet were compared. It was determined that DCAB -180 mEq/kg DM intake was 1.1 kg/day lower than -70 mEq/kg DM and DCAB -70 mEq/kg DM intake was 0.8 kg/day lower than the control diet.

Urine pH was approximately 6.5 and 5.5 in cows fed DCAB -70 and -180 mEq/kg DM, respectively. In addition, blood pH decreased linearly when DCAB fell from -70 and -180 mEq/kg DM. Also, cows fed -180 mEq/kg DM gave less milk at the first milking than cows fed -70 mEq/kg DM. Cows fed a more acidogenic diet had increased levels of hormone-sensitive lipase, which tended to have less insulin release after a glucose load and increased release of fatty acids after an insulin load. Since the expression of genes related to gluconeogenesis in adipose and liver tissues was not altered by the DCAB level, these changes could not be explained at the mRNA level. Vieira-Neto et al. (10) concluded that diets inducing a more aggressive acidosis (DCAB -180) can alter insulin secretion and tissue response, and consequently alter the protein profile in adipose tissue to favor lipolysis over lipogenesis. This higher lipolysis may be a result of lower DM intake of cows consuming a diet with more negative DCAB.

The adverse effects of extremely negative DCAB were also demonstrated in a study in which a diet with a DCAB of −154 mEq/kg DM had a urine pH <6.0 from 20 days before expected delivery until delivery (11). Compared to a group of cows fed antenatal diet with moderately positive DCAB (+89 mEq/kg), it produced similar serum tCa concentration at calving, higher urinary Ca excretion, higher prenatal serum NEFA (450 µEq/L) and lower percentage of milk fat in DM.

3. CATION-ANION BALANCE IN PREVENTION OF MILK FEVER

In the past, SCH predominantly occurred in the first 2 days after birth (12). Recently, postpartum 7-8 days in dairy cattle. SCH cases were reported 2 days after birth, with a prevalence of close to 19% (13) on days (14). Strategies to prevent hypocalcemia include the use of anionic compounds (Chlorides and Sulfates) that cause a mild metabolic acidosis to alter the dietary cation-anion difference (DCAB) (15).

There is no known mechanism to increase the intestinal absorption of Ca by decreasing DCAB. Lomba et al. (16) determined that the absorption of Ca from the intestines increased due to the (-) acid-forming nature of the negative ions in the intestine. However, Léclerc and Block (17) did not detect an increase in the absorption of Ca added to the diet. If dietary calcium levels are low compared to the animal's needs, Ca absorption in the intestines is by active transport, which is regulated by 1.25 $(OH)_2D_3$ produced in the kidneys (18).

In dairy cows near calving, Ca^+ from the blood begins to be drawn into the udders for colostrum synthesis, in this case the animals respond to the increased production of 1.25 $(OH)_2D_3$ in the kidneys by increasing the active absorption of Ca from the intestines. If it is true that the excess of anions relative to cations forms an acidic environment in the intestines, the active absorption of Ca from the intestines will decrease during the birth period, which is the period when the need for Ca is greatest (19).

1.25 $(OH)_2D_3$ and PTH (Para Thyroid Hormone) levels are not insufficient in the blood of cows close to birth. However, milk fever can be prevented when vitamin D3 and its metabolites are given to cows near calving in pharmacological doses. When near-birth dairy cows are fed diets high in anions versus cations, there is a possibility that the animals will not respond to Ca resorption regulated by hormones, due to excessive influx of HCO^{3-} or H^+ into bone cells. Excess K^+ ions in the plasma can be replaced by intracellular H^+, and the deficiency of Cl- can prevent the entry of HCO_3 into the plasma. An excess of HCO_3 or H^+ ion flux may inhibit osteoclasts in resorbed bones, since the resorption process is clearly a result of increased acidity (1).

It seems unlikely that Ca absorption will increase as a result of decreased DCAB. However, in cases where anions (-) are high, both active and passive absorption of Ca from the intestines may actually decrease. Therefore, even in cases where dietary Ca is high, the animal may perceive the dietary calcium level to be low due to low Ca absorption. In this case, since there is not enough information about the 1.25 $(OH)_2D_3$ and PTH levels in the kidneys and the absorption of Ca, it is difficult to say that this is the case (20).

Léclerc and Block (17) and Block (19) showed that when DCAB is reduced, there is an increase in bone mobilization that starts about 3-4 days before birth. This roughly coincides with the theoretical increase of 1.25 $(OH)_2D_3$ and PTH in the blood in preparation for lactation.

Milk fever was found in 47% of newly born dairy cows consuming diets with a DCAB value of 330.5 mEq / kg DM calculated with the formula mEq=(Na^+ + K^+) - (Cl^- + SO4), and it was observed that milk fever completely disappeared when these diets were adjusted as -128.5 mEq/kg DM (19).

In their study, Lérlerc and Block (17) fed dairy cows close to calving 4 diets containing +400, +200, +100 and +50 mEq DCAB coefficient in kg DM calculated with the formula mEq= ((Na^+ + K^+) - (Cl^- + SO_4). and there was no change in the digestibility of calcium, which is in contrast to the findings of Lomba et al. (16).

Gaynor et al. (21) mEq= ((Na^+ + K^+) - (Cl^-) calculated with the formula and high concentration Ca-containing diets (more than 10 g/kg) 4 different DCAB (in 100 g DM) 22.0 (anionic), 59.9 (moderate) and 125.8 (cationic) diets were reported to be fed to Jersey cows in the prenatal period with the lowest amount of Ca and urinary excretion. Researchers found that animals that ate the anionic diet for three days before birth had high levels of 1.25 $(OH)_2D_3$ in their blood. Gaynor et al. showed a study that showed that tissues did not respond to the hormone PTH in the case of metabolic alkalosis. In the study, it was determined that there was a decrease in the production of 1.25 $(OH)_2D_3$ in case of alkalosis.

Fredeen et al. (22) showed in their study that milk fever can be prevented with anionic DCAB. In their study, in which the researchers compared diets with DCAB of 40-50 mEq and greater than 85 mEq in 100g DM, they found that when DCAB is increased, urinary Ca excretion decreases and Ca, P absorption and turnover in bones decrease. In addition, it was determined that metabolic acidosis occurred in animals consuming diets containing less than 40 mEq DCAB, and metabolic alkalosis occurred in animals consuming diets at the level of 50µmEq in DCAB 100g DM.

3.1. ANIONIC SALTS, DIET CATION-ANION DIFFERENCE, URINARY PH AND HYPOCALCEMIA

In normal blood, the pH is slightly alkaline, as cations exceed anions. Herbivores who generally consume feeds high in K^+ and Ca^{2+} and relatively low in anions such as Cl^-, SO_4^{2-} and PO_4^{3-} have a slightly higher blood pH than non-herbivores. This puts ruminants in a compensable state of metabolic alkalosis with no adverse health effects. In this case, the kidneys excrete excess K+ in the urine, preventing alkalosis from becoming life-threatening. The high cation content of urine results in the formation of typical alkaline urine in cattle consuming K-rich feeds (15). This metabolic alkalosis is exacerbated when dairy cows are fed diets high in cations. This metabolic alkalosis state reduces the cow's ability to maintain calcium homeostasis during calving (23).

Therefore, to correct the cation-anion imbalance, dietary anion supplementation in the prenatal period has been used as an effective way to restore calcium homeostasis (23). This mild metabolic acidosis is associated with increased sensitivity of parathyroid hormone receptors in the bone and kidney (15). As a result, mobilization of calcium reserves from bone and reabsorption of calcium from the kidney and absorption from the gut result in increased circulating calcium concentrations. Therefore, the use of anionic compounds in prepartum diets has become a common preventive strategy to correct hypocalcemia in dairy cows.

7. RESULT

It is difficult to talk about an ideal DCAB value for dairy cows in the early lactation period. The DCAB value of most grain feeds is considered to be around 100 mEq/kg per DM. It has been shown that milk fever can be prevented in naïve dairy cows by feeding diets containing low calcium and phosphorus levels (17, 19, 21, 24). Therefore, DCAB should be 350-450 mEq/kg DM when the diet contains 50% grain. For low-yielding cows in the middle of lactation, a diet containing more meadow grass and less legumes and a DCAB of 250-300 mEq/kg DM is not a problem. Considering that cows consuming alfalfa-corn mixture that does not depress the fat ratio in milk, respond to NaHCO3 in the first period of lactation and not in the last period of lactation, it can be said that DCAB should be less than 350 at low production levels and higher than

350 mEq in cases of high milk yield. In the dry period, DCAB should be less than 200 mEq / kg (1).

Almost all of the roughage sources are cationic. Among them, alfalfa is the most cationic (25). Therefore, prenatal use of alfalfa predisposes animals to milk fever. During this period, meadow grass is a better option for these animals.

The diets of newly born dairy cows are generally in the direction of high cation balance, regardless of their roughage source. Although reducing calcium and phosphorus levels in the diet during the prenatal period reduces the incidence of milk fever, it does not completely eliminate the presence of this disease in the herd. In this case, the cation-anion balance of the diet is an additional factor to consider in eliminating the disease. In other words, in calcium-rich diets, cation-anion balance should be adjusted in the first degree to control the disease, while cation-anion balance should be considered as a secondary factor in dairy cows fed with diets with low calcium and phosphorus levels. Therefore, dietary calcium level is not as important a factor as the metabolic status of dairy cows at birth in the development of milk fever (20). We can say that lowering DCAB above -100 mEq/kg DM and lowering urine pH below 6.0 does not improve the performance of cows in terms of lactation, on the contrary, it may negatively affect health parameters such as feed intake and insulin sensitivity.

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CHAPTER 6

TMR AND PARTICLE SIZE FOR HEIFERS AND COWS

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What do TMR mean?

Total Mixed Ration (TMR): It is called TMR to mix roughage and concentrate feeds that all additives are such as minerals, vitamins and salts together and keep them in front of the cows for 20-24 hours a day as a single feed. “complete ration” or TMR was originally described in the Journal Dairy Science in 1917, meaning it has a history of more than 100 years (1). *Free or Ad Libitum Feeding*: In Latin, "ad" means “according to”, and "libitum" means “unlimited”. It is used more in the field of music in English; It means optional, plentiful and spontaneous. Abbreviation for “ad lib.” is in the form. To mean “ad libitum” in the veterinary field; means that animals can freely consume the feed at all times, eat as much as they want whenever they want, and always have feed in front of them (2,3).

Preparation of TMR for cows

When preparing TMR, cows should be evaluated according to milk yield, condition score, dry period, lactation period (Dry Matter Intake (DMI) during the first month, 2.2% of Live Weight (LW) at calving, 2.8% on day 14 and 3.3% on day 30). They are grouped separately according to the yield periods such as (4). Average feed intake is determined for each group, and 5-10% more TMR feed is left with feed mixer and feeder machines once a day or once during milking, then 2 times in order not to spoil the silage, especially in summer heat. In some cases (weekends, holidays, etc.) feeds can be left once every 2 days. Since the keratin plaque formation on the teats closes within the first 1-2 hours after milking, each new feed should be placed in the manger during milking in order to keep the cows standing (3). Cows can splash the feed 0.75-1.5 m away while receiving feed, it is necessary to push the TMR towards the feeder 6 times a day or more. For this purpose, pushing can be done manually or with a "automatic feed pushing machine or robotic feed pusher". Cows eat the closest feed first. If the TMR is not regularly within reach of the cows, the cows will become more aggressive and their feed intake may not reach the desired level. There should be a feeder length of at least 50 cm per cow (4,5).

If cows moved to a high yielding group do not reach the average milk production of the group in about 5 weeks or before 60 days, it is necessary to move them to a lower group. There should not be very large differences in the nutrient content of TMR between groups. TMR feeds should be given at the same time, and the cows should be brought to milking at the same time in order to prevent the fights of the animals especially during feed intake. In the USA, this feeding system has been applied in 70% of dairy cow farms in closed barns since the 1950s (1,4). In recent years, unmanned TMR robots that taking the feed, weigh, mix, grind and distribute feed materials have also been used in the world (Photo 1).

Photograph 1. Robotic TMR Machines (6)

Nutrient amounts in TMR for heifers and dairy cows

If silage is used in TMR, the total dry matter of the mixture should not fall below 50%, ideally it should be 55-60% Dry Matter (DM) content should not exceed 60% DM or moisture analysis for TMR can be determined within minutes with devices such as weekly simple probe NIRS (Near Infrared Spectroscopy). In particular, it should be noted that the DM percentage of corn silage may vary within a farm or between farms and may decrease each month depending on maturation (4,5).

Table 1. The ratio of daily DMI to live weight in dairy cows according to milk yield, (4)

Milk Yield, kg/day (4% Fat)	Daily DMI of Large Dairy Breeds such as Holstein, % / kg LW	Daily DMI of Small Dairy Breeds such as Jersey, % / kg LW
45	4.30	5.80
41	4.10	5.40
36	3.80	5.00

34	3.65	4.80
32	3.50	4.50
30	3.40	4.25
27	3.25	4.00
25	3.13	3.85
23	3.00	3.70
20	2.85	3.50
18	2.70	3.30
16	2.60	3.10
14	2.50	2.90
9	2.10	2.50

The length of 50-65% of the roughage particles in the TMR should be between 8 and 19 mm. Cows consuming the finer particles of the ration actually reduce their neutral detergent fiber (NDF) intake as well (7). DMI of cows should be within ±5% of expected daily dry matter intake. If DMI is significantly higher or lower than anticipated, the nutrient supply to the animal may be excessive or insufficient and potentially adversely affect animal health or performance. In general, TMR feed rejection should not exceed 3-4%. If the amount of feed remaining for the next day is higher than normal, it means that the dry matter of the feeds has increased, and if there is no feed for the next day, it can be said that the dry matter content of the feeds decreases or the moisture content increases. TMR residues rejected by cows can be fed to aged heifers, calves or beef cattle, respectively. These feeds should not be given to heifers and cows in the transition period (last 3 weeks of pregnancy and first 3-4 weeks of lactation) (5,7). Cows do not eat the same amount of feed every day. Air and environmental temperatures greatly affect daily feed intake. If the daily feed intake differs by 5% (approximately 2 kilograms) in a TMR, the TMR ration should be reformulated using the new feed intake value (4).

Table 2. Nutrient amounts in TMR for calves, female calves and heifers, (4)

	1-6 months	7-11 months	12-24 months
Concentrate feed rate in TMR, %	60 – 65	20 – 25	10 – 15
Daily DMI, % / kg LW	2.6	2.2	1.7
Crude protein (CP), % DM	16-21	13-14	12 –below
Soluble protein, % Crude protein (CP)	25 – 30	30 – 35	30 – 38
Degradable protein, % CP	45 – 55	33 – 37	25 – 30
Undegradable protein, % CP	45 – 55	63 – 67	66 – 72
NEM, Mcal/kg DM	1.69	1.58	1.43
NEM, Mcal/kg DM	1.07	0.96	0.85
Total NDF, % DM	25-33	25-33	25-33
Calcium, % DM	0.59-0.78	0.44-0.58	0.37-.039
Phosphorus, % DM	0.32-0.45	0.21-0.26	0.18-0.19
Magnesium, % DM	0.22	0.22	0.22
Potassium, % DM	0.8	0.8	0.8
Sulfur, % DM	0.2	0.2	0.2
Salt (NaCl), % DM	0.25 - 0.30	0.25 - 0.30	0.25 - 0.30
Sodium, % DM	0.10 - 0.12	0.10 - 0.12	0.10 - 0.12
Chlorine, % DM	0.20 - 0.24	0.20 - 0.24	0.20 - 0.24
Manganese, ppm	50	40-44	38-43
Copper, ppm	15 – 25	15 – 25	15 – 25
Zinc, ppm	70 – 80	70 – 80	70 – 80
Iron, ppm	100	100	100
Added Selenium, ppm	0.3	0.3	0.3
Added Cobalt, ppm	0.2	0.2	0.2
Added Iodine, ppm	0.5	0.5	0.5
Vitamin A, µ/kg DM	3392-5506	3392-5506	3392-5506
Added Vitamin D, µ/kg DM	925-1519	1046-1167	1277-1453
Total Vitamin E, µ/kg DM	18-85	55-61	68-77

Table 3. Amount of nutrients in TMR for lactating dairy cows, (4)

	Early Lactation (first 4 months)	Mid Lactation	Late Lactation
Concentrate feed rate in TMR, %	50 – 60	40 – 50	35 – 45
Daily DMI, % / kg LW	4.0+	3.5+	3.0+
Crude protein (CP), % DM	17 - 17.5	16 – 17	15 – 16
Soluble protein, % Crude protein (CP)	30 – 34	32 – 36	32 – 38
Degradable protein, % CP	62 – 66	62 – 66	62 – 66
Undegradable protein, % CP	34 – 38	34 – 38	34 – 38
NEL, Mcal/kg DM	1.58 - 1.81	1.72 – 1.81	1.58 – 1.72
NDF from roughage, % DM	19 – 25	25 – 26	27 – 28
Total NDF, % DM	25 – 33	25 – 35	35 – 38
Oil, maximum, % DM	5 – 7	4 – 6	4 – 5
Calcium, % DM	0.81 - 0.91	0.77 - 0.87	0.70 - 0.80
Phosphorus, % DM	0.35 - 0.39	0.35 - 0.37	0.35 - 0.40
Magnesium, % DM	0.28 - 0.37	0.25 - 0.34	0.22 - 0.28
Potassium, % DM	1.00 - 1.50	1.00 - 1.50	1.00 - 1.50
Sulfur, % DM	0.23 - 0.24	0.21 - 0.23	0.20 - 0.21
Salt (NaCl), % DM	0.45 - 0.50	0.45 - 0.50	0.45 - 0.50
Sodium, % DM	0.20 - 0.25	0.20 - 0.25	0.20 - 0.25
Chlorine, % DM	0.25 - 0.30	0.25 - 0.30	0.25 - 0.30
Manganese, ppm	44	44	44
Copper, ppm	11 – 25	11 – 25	11 – 25
Zinc, ppm	70 – 80	70 – 80	70 – 80
Iron, ppm	100	100	100
Added Selenium, ppm	0.3	0.3	0.3
Added Cobalt, ppm	0.2	0.2	0.2
Added Iodine, ppm	0.5	0.5	0.5
Vitamin A, IU/kg DM	9911	9911	9911
Added Vitamin D, IU/kg DM	1651-2422	1651-2422	1651-2422
Total Vitamin E, IU/kg DM	66	66	66

Table 4. Amount of nutrients in TMR for dairy cows in the last 42-60 days of pregnancy or in the dry period, (4)

	Far off (First 21 or 39 days)	Close up (late 21 days)	Close up (late 21 days), anionic
Concentrate feed rate in TMR, %	12 – 15	22 – 25	22 – 25
Daily DMI, % / kg LW	2	1.8	1.8
Crude protein (CP), % DM	12 – 13	13.5 - 14.5	13.5 - 14.5
Soluble protein, % CP	30 – 38	30 – 38	30 – 38
NEL, Mcal/kg DM	1.27 - 1.40	1.36 – 1.49	1.36 – 1.49
NDF from roughage, % DM	19-25, minimum	19-25, minimum	19-25, minimum
Total NDF, % DM	25-33, minimum	25-33, minimum	25-33, minimum
Calcium, % DM	0.45 - 0.55	0.45 - 0.55	1.40 - 1.60
Phosphorus, % DM	0.30 - 0.32	0.30 - 0.32	0.32 - 0.35
Magnesium, % DM	0.24 - 0.28	0.28 - 0.32	0.28 - 0.32
Potassium, % DM	0.80 - 1.00	0.80 - 1.00	0.80 - 1.10
Sulfur, % DM	0.2	0.2	0.35 - 0.40
Salt (NaCl), % DM	0.25 - 0.30	0.25 - 0.30	0.25 - 0.30
Sodium, % DM	0.10 - 0.12	0.10 - 0.12	0.10 - 0.12
Chlorine, % DM	0.20 - 0.24	0.20 - 0.24	0.70 - 0.80
Manganese, ppm	38-43	38-43	38-43
Copper, ppm	18 – 25	18 – 25	18 – 25
Zinc, ppm	70 – 80	70 – 80	70 – 80
Iron, ppm	100	100	100
Added Selenium, ppm	0.3	0.3	0.3
Added Cobalt, ppm	0.2	0.2	0.2
Added Iodine, ppm	0.5	0.5	0.5
Vitamin A, IU/kg DM	7709	7709	7709
Added Vitamin D, IU/kg DM	1650-2422	1817-2422	1817-2422
Total Vitamin E, IU/kg DM	86	180	180

Advantages of TMR

- This system is very effective in the first 1 month early period of lactation. Because the uterus of a cow that has given birth should be able to recover, there should be no fat accumulation in the liver, more milk production and an open appetite. In this period, NEB (Negative Energy Balance) occurs when the blood circulation is excessive with the effect of NEFA (non-esterified fatty acids) and BHB (β-hydroxy butyric acid). The excess of NEB causes LH (Luteinizing Hormone) deficiency, reduction of the diameter of the first dominant oocyte follicle in the ovary, delays in its discharge into the oviduct, and ultimately an increase in the calving interval (8).
- If roughage and grain-based concentrates are given separately without mixing (traditional feeding) twice a day, rumen pH decreases after feeding. In TMR, on the other hand, pH changes of the rumen decrease, rumen ammonia level decreases, rumen movements are regular, microbial protein synthesis increases, the amount of saliva increases, feed consumption increases, the risk of acidosis and the occurrence of other metabolic diseases are reduced by 5%. Feed Conversion Rate (FCR) decreases by 4% in TMR. Depending on these, with the increase in microbial protein synthesis, 5-8% or 1-2.5 kg more milk per day is obtained (4,8).
- Milk obtained from cows with TMR; Fat, protein, casein, lactose, dry matter, acidity (pH value), temperature value, shortening of clotting time with rennet, α-lactalbumin, β-lactoglobulin, albumin, lactoferrin, lysozyme and fatty acid profiles may increase (7).Cow owners can use a wide variety of by-product feeds with TMR and thus save on ration cost. Because feeds such as urea, limestone, straws, bran, husks, husks, cobs, oils and some bypass protein sources are not palatable, they can be added to the TMR in moderate amounts by blending with little or no reduction in feed intake. Cereal straws such as wheat straw, barley straw, oat straw can be added to the TMR wagon daily up to 0.25% of the LW (1,4).
- Prior to TMR, concentrated feed was given to cows during milking in milking parlors, this was no longer necessary. Since the TMR is given ad libitum, the cow can move around after taking the feed and can

come back and continue the feed intake. Daily DMI does not decrease (1.5).

- Since forages and concentrates are prepared at the same time, labor and time loss can be minimized. In TMR, feed wastage is less because the roughage remains and sticks to the roughage, especially silages (1,8).
- A TMR, if properly managed, provides greater accuracy in formulation and feeding. In TMR, it is ensured that the amount of each ingredient added with feed scales is strictly controlled. Total daily feed intake can also be easily determined (5).
- When a TMR is properly mixed, the cow cannot consume more or less roughage or concentrate than planned (1).

Disadvantages of TMR

- It causes additional costs as equipment such as mixing and chopping wagons and weighing scales are needed (1).
- In some cases, existing buildings, feeders, and mangers can make the use of a TMR system impossible or add additional costs (1,3).
- For those with small herds (20 heads and down cows) and those who are pasture-based for a long time, it may not be economical to install and daily implement a TMR system (4).
- It must be chopped before being put into the TMR wagon because straws and silages are baled or stored in long form. TMR blades that shred long fodder can reduce the particle size of most tall grasses and straws, while also reducing the size of other feedstuffs and silage. This leads to metabolic problems such as subacute ruminal acidosis (SARA). As a solution, long-particle hay and straw should be shortened to the desired length before being placed in the chopping and mixing wagon (1,7).
- Working times (usually 3-5 minutes) of chopping and mixing wagons should be done according to the manufacturer's recommendation. While excessive mixing can cause serious problems due to grinding and powdering of feed, insufficient mixing can cause less feed consumption by cows and increase selectivity (1,7,8).

Feeds and ratios to be used in TMRs of heifers and dairy cows

Cows are good at using their noses to separate grain from feed in TMR, eating grain first and leaving long feeds for later. They disrupt the balance of TMR and cause acidosis. To avoid this problem, it may be necessary to chop long roughages finer or replace them with feeds that are more difficult to sort out. Multiple high protein supplements can be used to meet rumen undegradable protein (UDP) needs for dairy cows. Methionine and lysine are the most limiting essential amino acids. In rations of ruminants are mostly contained corn-based silage, grains or corn by-products. Animal protein sources such as meat meal, meat bone meal with high UDP content, fish meal, soybean meal or cottonseed meal should be used. If rations with a high grain corn content are used, the use of brewer's yeast, distillate products such as dried distillers grain solubles (DDGS), corn gluten meal and feather meal may be limited. If the milk yield is more than 30 kg/day, rumen-inert or by-pass fats up to 5% of the TMR, DM can be used. Minerals, especially calcium sources, can be increased if oil is used in TMR. In hot summer months and when the humidity in the air is more than 70%, the amount of potassium in the TMR may be slightly higher. In general, if there is low (10-12 ppm) copper in the blood serum, the amount of copper in TMR can be increased. Copper deficiency; may result from excessive intake of iron, manganese, molybdenum and sulfur (9,10).

Table 5. Ratios of feed in TMR to be prepared for dairy cows, % (5)

Feeds	Normal Feed Ratios, %
Roughages	50-70
Energy-containing feeds (cereals, oils, etc.)	3-30
Protein sources	5-20
Grain by-products (brans, etc.)	0-30
Urea	0-1
Molasses	5-10
Mineral resources	1-2
Vitamin premixes	10 g/100 kg

Table 6. Feed amounts for 100 kg TMR prepared daily for dairy cows and heifers according to their yield, kg, (5)

Feeds	Dairy cows with milk yield of 20 kg/day or more	Dairy cows with milk yield below 20 kg/day and heifers with good growth	Cows in dry period	Heifers with Average Daily Gain (ADG) of 700 g/day
Corn, barley, sorghum	10	4	6	5
Silages, grass, pulpes, straws	30	40	60	50
Soy bean meal	15	15	6	10
Cotton seed meal, Sunflower meal	10	5	5	4
Brans such as wheat, rice	22	23	10	18
Molasses	10	10	10	10
Mineral mixture	2	2	2	2
Salt	1	1	1	1

Table 7. Example 1: TMR content for early lactation dairy cows with a milk yield of 25 kg/day (11)

	Feeds	DM basis, %
Milk yield: 25 kg/day,	Corn silage	36.44
Live weight: 680 kg	Corn flake	18.29
Milk fat: 3.5%,	Soybean meal, expeller	7.65
DMI: 13.5 kg/day	Soybean meal, solvent 48%	2.53
ADG loss: -0.9 kg/day	Legume dried herb	20.17
NEL, kcal/day: 27900	Lintered whole cottonseed	8.41
NEL in kcal/kg DM: 2060	Calcium fatty acid	0.65
RDP, g/day: 1421	Blood meal	1.02
RUP, g/day: 947	Limestone	0.56
CP, g/day: 2368	Monophosphate	0.40
CP % DM: 17.4	Salt	0.70
	Mineral-vitamin	3.18

DMI: Dry matter intake, ADG: Average daily gain, NEL: Net energy lactation, RDP: Rumen degraded protein, RUP: Rumen undegraded protein, CP: Crude protein

Table 8. Example 2: TMR content for early lactation dairy cows with a milk yield of 35 kg/day, (12)

	Feeds	DM basis, %
Milk yield: 35 kg/day,	Corn silage	26.9
DM,%: 52.5%	Alfalfa silage	13.0
NDF, %/ DM: 43.0	Whole cottonseed	18.0
ADF, %/ DM: 21.5	Soy husk	9.10
CP, %/ DM: 15.8	Corn gluten meal	8.90
NDF, %/ DM: 43.0	By-pass product (blood meal, fish meal, etc.)	4.40
NEL, Mcal/kg DM: 1.6	Corn meal	15.9
RUP, %/ DM: 6.9	Soybean meal,48%	1.20
	Limestone	0.55
	Sodium bicarbonate	0.75
	Salt	0.43
	Bentonite	0.65
	Mineral-Vitamin	0.12
	Potassium carbonate	0.02

DMI: Dry matter intake, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, NEL: Net energy lactation, RUP: Rumen undegraded protein, CP: Crude protein

Particle lengths of forages to be used in TMR

In rations with TMR to be prepared for dairy cows, the particle lengths of roughages such as hays, silages, straws should comply with the 4-boxes Penn State Particulate Separator (PSPS) criteria. Four sieves or screen (19 mm-8 mm-4 mm-bottom box) are placed on top of each other. Approximately 500 g of TMR sample is taken and placed on the top 19 mm sieve. And all the sieves are shaken together for about 30 seconds. The sieves are separated and the remaining samples are weighed one by one. The evaluation is made as follows:

The amount of TMR remaining on the top sieve with a diameter of 19 mm should be 2-8% in sample feed. If this ratio is higher, it means that the long grass is weeded and consequently grain consumption increases. In our example, we took a sample of 500 g of feed, if 30 g of feed remained on a 19 mm sieve, our rate would be 6%. And this value is in the normal range. The amount of TMR remaining on the 8 mm diameter sieve should be 30-50% in sample feed. In our example, we took 500 g of feed sample, 150-250 g of feed particles should remain on the 8 mm sieve. The amount of TMR remaining on the 4 mm diameter sieve should be 10-20% in sample feed. In our example, we have took a sample of 500 g of feed, 50-100 g of feed particles should remain on a 4 mm sieve. The final amount of TMR poured into the bottom collection container should be 30-40% in sample feed. In our example, we have took 500 g of feed sample, 150-200 g of feed particles should be poured at the bottom (13).

Table 9. Optimal ratios of forages in TMR above 4 boxes of PSPS (13,14)

Pore diameter in the box	Particle length of roughage	Corn Silage, %	Grass Hay, %	Wheat Straw, %	Alfalfa Hay,%	TMR,%
19 mm	greater than 19 mm	3-8	27	6	38	2-8
8 mm	8 mm-19 mm	45-65	36	51	20	30-50
4 mm	4 mm-8 mm	20-30	27	35	27	10-20
Free pore bottom of sieve	smaller than 4 mm	<10	10	8	15	30-40

Photograph 2. Using the Penn State Particulate Separator (15)

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CHAPTER 7

THE EFFECT OF RUMINANT ANIMALS ON GLOBAL WARMING

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Global Warming

Some major climate changes have occurred in the past in the world, and these major changes have led to the extinction of some living species. There is a prevailing view that global warming, which is the subject of recent times, changes in climates and that if necessary precautions are not taken, the life of living things will be endangered. Fundamental studies to investigate the causes of global warming and to determine solutions could only start at the end of the 1970s at the international level (Kadıoğlu, 2001). Recently, methane (CH_4), carbon dioxide (CO_2), nitrous monoxide (N_2O) and chlorofluorocarbon (CFC) gases have increased in the atmosphere due to reasons such as the use of fossil fuels, the increase in industrialization, the rapid increase in population, deforestation and the increase in consumption trends in societies. As a result of the strengthening of the natural greenhouse effect due to the increase of these gases, the temperature rise that occurs in the world and in the lower troposphere is called "Global warming". In other words, global warming is the process of systematically increasing the temperature all over the earth. Global climate change, on the other hand, changes in weather movements, humidity, precipitation, drought, etc. It is expressed as changes in climate elements due to global warming (Akın, 2006). The rays from the sun pass through the atmosphere and warm our world. Earth's atmosphere consists of various gases. Methane (CH_4), carbon dioxide (CO_2), water vapor (H_2 O), nitrous monoxide (N_2 O), ozone (O_3), chlorofluorocarbon (CFC) etc. in the atmosphere. gases trap a part of the heat coming to our world from the sun and keep the world at a certain temperature. Thanks to the ability of the atmosphere to retain heat, the temperature of the waters on earth (rivers, streams, seas, oceans, etc.) stays in balance and prevents freezing. This heat retention and warming feature of the atmosphere is called the “greenhouse effect” (Kadıoğlu, 2001; Türkeş et al., 1999; Sandal, 2007; Türkeş et al., 2000).

Causes of Global Warming

Global warming, population growth and industrialization created by human beings at the level of 90% in the last 50 years, and the continuous increase in greenhouse gases in the atmosphere cause the natural balance to deteriorate gradually and become unsustainable. save (IPCC, 2007; Çavdar, 2007). Among the main causes of global warming, fossil fuels, burning of agricultural wastes, areas opened for agricultural activities, decrease in forest areas and energy use and industry are among the main reasons for the increase in ruminant animals in parallel with human needs (Oruç, 2016).

Gases Causing Global Warming

With the rapid rise of greenhouse gases, especially since the second half of the 20th century, natural disasters that are caused by global warming and that seriously threaten life, especially human and plant and animal species, have begun to emerge (Öztürk, 2002; Bozoğlu, 2003).

Carbon dioxide Gas (CO_2)

The greenhouse gas that exists in the atmosphere at the highest levels and should be handled with care is carbon dioxide (Steinfeld, 2006; Sirohi and Michaelowa 2007). Carbon dioxide is an odorless and colorless gas formed by the reaction of organic substances with oxygen, and has an acidic taste due to carbonic acid formed when dissolved in water It occurs as a result of the combustion of any material containing carbon (wood, coal, oil, etc.) and as a result of aerobic respiration in living things. Today, on the one hand, the rapid increase in the use of fossil fuels (petroleum, coal, etc.), on the other hand, the destruction of vegetation and forests causes the rate of carbon dioxide in the atmosphere to increase gradually (Clarke, 2001; Çepel, 2010). It is stated that one of the important sources of carbon dioxide gas occurring today is caused by animal production and is equivalent to approximately 9% of all emissions (Clarke, 2001). Fossil fuels are used to obtain artificial fertilizers used to produce animal feed, and approximately 41 million tons of CO_2 is produced annually (Steinfeld, 2006). The CO_2 amount, which was 600 billion tons in total before the industrialization revolution, increased to 750 billion tons with the industrial reform (Kadıoğlu 2001).

Methane Gas (CH_4)

Another gas that creates a greenhouse effect in the atmosphere is methane. Methane gas is a gas that occurs as a result of the decomposition of organic wastes in an anaerobic environment (Steinfeld, 2006). Methane creates a primary greenhouse gas effect, while the CO_2 effect is secondary. The heat capture capacity of methane in the atmosphere is 21 times higher than that of CO_2, and its lifespan is shorter than other gases. While natural resources account for 30% of the total methane emissions, human activities account for 70%. Methane is included in the atmosphere during the production and transportation of coal, natural gas and oil. Methane gas is produced as a result of the storage of manure and related activities, especially in ruminant animals. It is stated that there is a small amount of methane release in the fertilizers stored in the pasture (Sherlock et al., 2002). It is stated that its concentration in the atmosphere has increased approximately twice since the industrial revolution (Atalık, 2005). Therefore, high cost technologies are needed to control anthropogenic methane emissions. Methane occurs as a by-product of digestive activities in some animals, especially in ruminants, and it also occurs in waste places (garbage, animal manure, etc.) (Bauher, 1994; Atalık, 2005). Ruminants are defined by their structures with a special digestive system. In this way, ruminants are an important methane producer with the greenhouse gases they produce as a result of easily digesting low-quality cellulose-rich materials. Bolle et al. (1986) reported that the effect of ruminants on methane gas formation was 18%. In fact, animals individually produce very little methane. For example; a cow produces about 80-110 kg of methane per year. However, at this point, the main problem for ruminants to be held responsible is not the amount of gas they produce, but the digital presence they reach around the world.

Nitrogen Dioxide Gas (N_2O)

Nitrogen Dioxide gas is mainly produced as a result of the burning of fossil fuels and the processing of agricultural lands. Nitrogen dioxide gas is an important emission in the atmosphere that creates a strong greenhouse effect. The main nitrogen oxides originating from animal shelters are nitrogen dioxide (NO_2), nitrogen monoxide (NO) and nitrous oxide (N_2O) (Anonymous, 2001). The emission of nitrous oxide has 300 times more heat retention than the same amount of carbon dioxide. About 80% of the corn produced in the world is used in animal feed, that is, in animal production. Urine and fertilizers accumulated as a result of grazing in pastures or fertilizers applied to pastures are important sources of N_2O (Janzen et al., 1998). Steinfield et al. (2006) stated that today the intensity of nitrogen fertilizer use is approximately 16% higher than in the 18th century. To reduce nitrogen monoxide (N_2O) gas; They make recommendations to increase the productivity of animals and reduce the number of animals, to reduce the amount of nitrogen removed by manure, to reduce the amount of nitrogen given to the fields for crop production, to reduce the amount of nitrogen consumed in animal feeding rations (Clarke, 2008; Gworgwor et al., 2006).

Ozone (O_3) Gas

Ozone gas, which consists of three oxygen molecules, is a colorless, poisonous gas and is found in the upper layers of the atmosphere. This gas is what gives the sky its blue color. It plays a very important role in making the planet a livable world by absorbing the ultraviolet rays coming from the sun to the earth by forming the ozone layer of the atmosphere. At the same time, since it is a gas with a greenhouse effect, it contributes to the creation of a suitable environment for life on earth by keeping the world temperature at certain intervals. The deterioration of the integrity of the ozone layer causes more ultraviolet rays to reach the earth. Intense ultraviolet rays can cause skin rashes, burns, skin cancer, and eye diseases. It can also cause weakening of the immune system in humans (Oruç, 2016).

Water Vapor (H_2O)

The greenhouse effect of water vapor in the atmosphere is around 3%. As a result of climate changes, an increase in water vapor occurs and causes global warming. However, people cannot be directly involved in the increase in the effective water vapor, and climate change causes it (Oruç, 2016).

Relationship Between Livestock Production and Ecosystem

While the increase in the world population has increased the demands for animal products such as meat, milk and eggs, the globalization in the market has accelerated the international food entry and exit, which has led to the rapid growth of the industry based on animal production. At this point, the livestock sector is going through a complex process of technical and geographical change (Anonymous 2015). On the other hand, the shift of cities towards rural areas has led to a decrease in the production areas of pasture and fodder crops where animal husbandry is maintained. In particular, growth in pig and poultry production (usually industrial) has resulted in a pause in cattle, sheep and goat production potential in some regions. It is stated that

80% of the estimated growth in livestock today is based on industrial livestock enterprises. At this point, these changes have put the livestock sector in direct competition with the decreasing soil, water and other natural resources. Every year, approximately 56 billion land animals are slaughtered for human consumption (Oruç, 2016). When the greenhouse gas emission originating from agriculture is considered on the basis of industrial livestock, it has been determined that it is 2 times higher than traditional mixed production systems and 6 times more than pasture-based production systems (Verge et al., 2007). 70% of the cultivated agricultural land and 30% of the world's land area are directly related to animal production (FAO, 2006). While forage crops production requires approximately one-third of all arable land, grazing covers 26% of the world's terrestrial area. It is stated that approximately 70% of grazing lands in arid regions are eroded, usually as a result of deterioration due to overgrazing. FAO (2009) states in a report that animal production is one of the important causes of the world's most important environmental problems such as global warming, land degradation, air and water pollution and loss of biological diversity. It is estimated that by 2050, livestock production will grow twice as fast in the developing world compared to other sub-sectors of agriculture. It is stated that industrialized animal production contributes to environmental problems such as increased greenhouse gas emissions, infertility in soils, and water pollution. It is stated that these problems will continue to increase unless the enterprises can transform from intensive enterprises with more animals to traditional and extensive enterprises. Intensive enterprises; They stand out with their high animal density, economic criteria, mechanization and biotechnology applications. While intensive animal production has existed in Europe and North America for a long time, it has also started to develop in Asia and Latin America. Although intensive farming practices are observed in some parts of Africa and Asia, traditional farming is still maintained (Anonymous, 2010).

Effects of Global Warming on Animals

Possible effects of global warming on animals prevail as chemical environment, biological environment, physical environment or direct effects of climate. Maintenance and feeding conditions manifest themselves with possible effects that may occur in physical environmental conditions. Due to extreme climatic conditions, the cost of housing increases and yields such as meat, milk and reproduction may decrease. It has been demonstrated with structured periods that extremely hot conditions cause shortening of the lactation period in lactating animals and have an effect on milk quality and quantity (Chase and Sniffen 1988; Bucklin et al., 1991; Alnaimy et al., 1992). In addition to the level of feed efficiency, there are reports about the animal that causes a decrease in the fattening period and feed consumption (Silanikove , 2000; Davis et al., 2001; Göncü and Özkütük 2003). Spending water life, drought, flooding planted enclosures close to home, etc. Since it will reduce the plant production areas due to its cultivation, it will be aimed to raise primarily those for human nutrition in the existing addable points, and there will be reductions in pasture or forage crops for animals.

The Impact of Ruminant Animals on Global Warming

Recently, with the intensification of poultry, dairy, beef cattle and dairy industry, there has been a serious increase in environmental pollution caused by farms (Sirohi and Michaelowa 2007). The most produced greenhouse gases originating from agriculture and livestock in Turkey are CH_4 and N_2O, and the global warming potentials of these gases are 21 times and 310 times higher than carbon dioxide (CO_2), respectively (Forster et al., 2007). Ruminant is the name given to the group of animals that have a stomach structure with four compartments and ruminate. Ruminants are all herbivores (feeding on plant sources), and there are many types of animals in this group. Ruminants play a very important role in the food chain because they evaluate cellulose and NPN (Non-Protein Nitrogen-Protein-free nitrogen) compounds that animals such as cattle, sheep, goats, buffaloes, humans and other farm animals can digest very little or not at all. (Ozturk, 2007). It is reported that 80% of methane emissions from farm animals originate from large ruminants. In researches, it has been seen that methane gas from ruminant animals causes greenhouse effect and ammonia causes acid rains and causes worldwide problems (Bauher, 1994). It is stated that 16.4% of the annual methane gas consists of ruminant and animal manure, and this 16.4% rate constitutes approximately 2.9% of all greenhouse gases that cause global warming (Johnson et al., 1992). For example, an adult cattle produces approximately 80-110 kg of methane gas per year (O'Mara, 2004). However, considering that the number of ruminant animals is around 1 billion worldwide, it contributes significantly to the greenhouse effect (FAO, 2008).

Formation of Methane Gas in the Rumen

Methane gas is produced as a result of the fermentation activities of microorganisms in the rumen and intestines of ruminants. However, 90% of the total methane gas produced is produced in the rumen (Kamra, 2005). Many researchers have reported that carbohydrates used as diets are fermented by bacteria and protozoa in the rumen, and essential fatty acids, CO_2 and CH_4 gas are formed as a result of this fermentation (Öğün, 1995).

As a result of fermentation of carbohydrates by bacteria and protozoa present in the rumen, H_2 and CO_2, the main components of methane gas, are formed together with essential fatty acids. In the rumen of ruminant livestock (sheep, goats, cattle), methane is produced by microorganisms through methanogenesis (methane formation) during the anaerobic fermentation of roughage-based rations containing soluble and structural carbohydrates (Kurihara et al., 1999). This causes energy loss in ruminants. In other words, it is known that there is a negative relationship between energy use and methane production in ruminants. The production of methane gas in ruminants fed with poor quality roughage can reach approximately 15-18% of the digestible energy taken. The representation of methane gas production in the rumen is shown in figure 1.

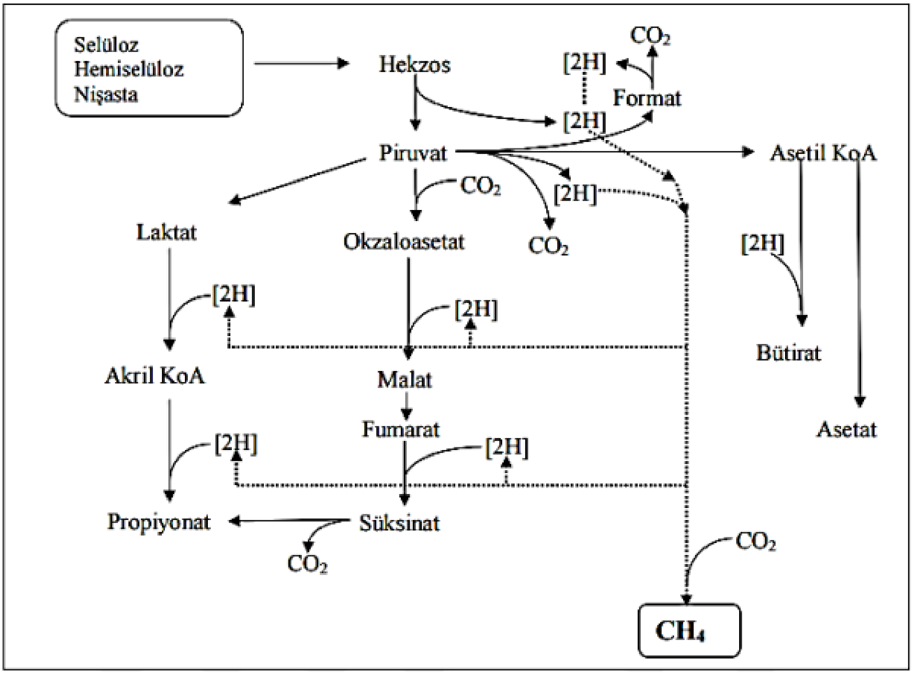

Figure 1. Demonstration of Methane Gas Production in Rumen (Mitsumori et al., 2008).

Ration-Based Feeding Strategies for Reducing Methane Emission in Rumen

- ✓ Methane emission decreases in animals fed with a rich source of carbohydrates and concentrated feeds in the ration (Johnson, 1995). The roughage source in the ration also has an effect on methane emissions. In ruminants fed with cereal roughage, methane gas production is higher than in ruminants fed with legume roughage. Because there is less structural carbohydrate (cellulose) content in legume roughage compared to legume roughage, and more propionate production occurs due to its low residence time in the rumen (Johnson, 1995).
- ✓ Increasing the amount of dry matter consumed increases the rate of passage in the rumen and decreases the residence time of the feedstuffs in the rumen. Studies have shown that the rate of passage of liquid and solid rumen content through the rumen increases from

54% to 68%, resulting in a 30% decrease in methane formation (BIaxter, 1967).

- ✓ Adding oil to ruminant rations reduces the formation of methane gas (Dohme and Machmüller 2000).

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CHAPTER 8

ALTERNATIVE FEED ADDITIVES USED IN POULTRY NUTRITION AND THEIR IMPORTANCE

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Introduction

Antibiotics are widely used in the poultry industry. Although formerly used therapeutically to improve animal health and welfare, it has been introduced into diets at sub-therapeutic levels for prophylactic purposes or to promote growth (Huyghebaert et al., 2011). The use of antibiotics as growth stimulators in livestock for many years has led to the contamination of animal products and the environment with antibiotic residues and the spread of antimicrobial resistance in zoonotic bacterial pathogens (Gong et al., 2014; Yang et al., 2015). The international spread of resistant pathogens through food from one country to another, especially with increasing global trade in food products of animal origin, has raised serious concerns (Brenes and Roura, 2010; WHO, 2012). The emergence of microorganism strains with high antibiotic resistance in animals and people consuming animal products due to the continuous use of antibiotics in animal feeds for the purpose of protection from diseases and increasing yield has led to the prohibition of the inclusion of antibiotics in feed as a growth accelerator in many countries (Bach Knudsen and Jørgensen, 2001). In animal nutrition, intensive researches are carried out on "Alternative Feed Additives", which are not needed under normal growing conditions, but which allow the nutrients in the feed to reach the animals without spoiling when added to the feed, to be digested more easily by the animal, and to be absorbed from the intestines and transported to the body cells, they reach the animals without spoiling, increase the amount of product, improve feed efficiency, change the appearance of the product, affect its quality, and increase its quality (Kutlu and Özen, 2009). Probiotics, prebiotics, organic acids, probiotics, and essential oils are used as alternative growth stimulators in poultry nutrition (Öztürk, 2009).

Feed Additives Used in Poultry

Probiotics

Probiotics are live microbial supplements that positively affect host health by regulating the microbial balance of the digestive system. Lactic acid bacteria constitute the most important group of probiotic microorganisms (Billoo et al., 2006; Kim et al., 2006; Lee and Salminen, 1995; Salminen et al., 1998). Probiotics reach the villi of the intestinal wall much earlier than pathogenic bacteria and prevent the pathogens from sheltering in the digestive tract (Bahadıroğlu, 1997).

Probiotic bacteria prevent the increase of these toxic substances by preventing the proliferation of toxic amine and other pathogenic bacteria that produce ammonia (Alp et al., 1993; Jones and Thomas, 1987; Lyons, 1987; Nemeskery, 1983). The proliferation of probiotics in intestinal epithelial cells suppresses their growth by reducing the reduction-oxidation potential and preventing aerobic pathogenic microorganisms from making use of oxygen (Yalçın et al., 1996). Probiotics also play an important role in the prevention of intestinal inflammation and in the treatment of cancer (Shahani,1980; Kim,1988; Fuller,1989). The mode of action of probiotics varies according to the type of probiotic microorganism used, its strain, the type of animal, whether there is a stressful situation in the animal, and the amount given to the animal (Fuller, 1989; Lyons, 1987; Wu, 1987). Commercial probiotic preparations contain live cultures of bacteria, fungi, yeast, and yeast cultures, and various enzymes. These preparations not only consist of one microorganism strain but also contain up to 8 different microorganism strains (Fuller,1989; Yalçın ve ark.1996). Microorganisms commonly used in probiotic production are Lactobacillus and Streptococcus (Wu,1987; Fuller,1989; Yalçın ve ark.1996).

Considerations when using probiotics

Since probiotic microorganisms are sensitive to environmental conditions, attention should be paid to storage conditions, feed processing techniques, interaction with feed additives added to mixed feed, the characteristics of the carrier used, and the pH of the environment. Commercial probiotic preparations are prepared in different forms such as powder, granule, pellet, liquid suspension, and capsule. When the microorganisms produced are dried in accordance with the freezing technique, they can maintain their vitality for a long time. Probiotic preparations should be stored at 22-250^{o}C and in a dry place. Bacteria lose their viability when the storage temperature rises above 300^{o}C (Jones and Thomas, 1987). Although the number of probiotics decreases over time in mixed feeds with low moisture content, since they remain viable for a longer period of time in this type of mixed feed, feeds with probiotic additions should be stored appropriately in a dry and cool place (Yalçın et al. 1996).

Prebiotics

Prebiotics encourage the development of beneficial microflora in the intestine, have a positive effect on regular and healthy digestion, increase mineral absorption, strengthen the immune system, and thus contribute to overall health. Prebiotics are naturally found in vegetables, fruits, and grains such as asparagus, bananas, chicory, garlic, onions, wheat, and tomatoes (Zhang, 2005). Prebiotics, unlike probiotics, are non-living food additives and must be taken in minimal doses to be effective. However, it is not possible to reach this level by consuming these foodstuffs naturally. For this reason, prebiotics is specially added to biscuits, confectionery, cereals, dairy products, beverages, baby foods, and children's foods (Nakakuki, 2003).

Organic acids

Microorganisms that make up the natural microflora of the digestive system produce organic acids such as propionic acid, acetic acid, and lactic acid. As a result of using them as feed additives, the balance of microflora in the digestive tract is turned in favor of beneficial microorganisms, thus preventing the growth of pathogenic microorganisms. Organic acids such as lactic acid, acetic acid, propionic acid, fumaric acid, citric acid, and formic acid are used as feed additives in animal nutrition (Alp et al., 1999; Çakmakçı and Karahan, 1999; Karademir and Karademir, 2003). Organic acids are mainly used to prevent mycotoxin growth in feed and feed raw materials and to protect animals against mycotoxication in this way, to extend the storage period of feed and feed raw materials, to prevent aerobic deterioration in silages and to increase the aerobic stability of silages (Karademir and Karademir, 2003; Canibe et al., 2001). Since organic acids have strong bacteriostatic effects, they are used as Salmonella control agents in feed and water sources of poultry (Tüzün and Çiftçi, 2010). The addition of organic acids to the feed also causes the pH of the crop to decrease. The addition of a 1% mixture of formic and propionic acid leads to a decrease in pH in the cecum. The decrease in pH in the cecum makes colonization of Salmonella difficult (Waldroup et al., 1995; Nir and Şenköylü, 2000).

Phytobiotics

Phytogenics, also called phytobiotics, are natural bioactive compounds of botanical origin used as an alternative to antibiotics in animal nutrition and added to feeds to improve performance in animals (Windisch et al., 2008; Kiczorowska et al., 2017). Although phytogenics contain a wide variety of substances in terms of biological origin, formulation, chemical description, and purity, they can be classified into four groups (Windisch and Kroismayr, 2006):

1) Herbs (products from flowering, woody and non-permanent plants);
2) botanicals (whole or processed parts of a plant, such as roots, leaves, bark, spices, etc.);
3) Essential oils (hydrodistilled extracts of essential plant compounds);
4) Oleoresins (anhydrous solvent-based extracts).

Many studies have shown that phytogenic feed additives have antimicrobial, antiviral, antioxidative, anthelmintic, anti-inflammation, antistress, and nutrigenomic effects and various functions, including improvement in feed flavor and intestinal development or health (Hashemi and Davoodi, 2010; Yang et al., 2015). In addition to these properties, it is reported to stimulate growth, immune modulator, feed intake, and endogenous enzyme secretion, and increase production (Upadhaya and Kim, 2017).

Essential oils

Essential oils, also called essential oils, can be synthesized by all plant organs such as flowers, buds, seeds, leaves, branches, bark, herbs, fruits and roots. They are also secondary plant metabolites stored in secretory cells, cavities, ducts, epidermic cells or glandular trichomes (Bakkali et al., 2008). Essential oils are also mixtures of many secondary metabolites such as phenols (anethol, timol, öjenol, karvakrol, chavicol, etc.), terpenes (monoterpenes and sesquiterpenes etc.), alcohols (borneol, isopulegol, lavanduol, α-terpineol, nerolidol, santalol, α-santalol, etc.), aldehydes (citral, myrtenal, cumin aldehyde, citronellal, cinnamaldehyde, benzaldehyde, etc.), ketones (carvone, menthone, pulegone, fenchone, camphor, thujone, verbenone, etc.), esters (bomyl acetate, linalyl acetate, citronellyl acetate, geranyl acetate, etc.), oxides (1,8-cineol, bisabolone oxide, linalool oxide, sclareol oxide, etc.), lectins, polypeptides or polyacetylenes (Bakkali et al., 2008; Thacker, 2013; Adaszyńska-Skwirzyńska and Szczerbińska). The antimicrobial properties of essential oils have been widely reported in the literature (Dorman and Deans, 2000; Cristani et al., 2007; Yitbarek, 2015). The mechanism of antibacterial action of the majority of them is in the form of denaturing and coagulating proteins by acting in the bacterial cell wall structure. In addition, essential oils have properties such as feed consumption activation, secretion of digestive secretions, immune stimulation, antioxidant, coccidiostatic, anthelmintic, antiviral, anti-inflammatory activity, morphological and histological modifications of the gastrointestinal tract (Kumar et al., 2014).

Enzymes

Enzymes are biocatalysts produced by living cells and serve in specific biochemical reactions. Adding enzymes to the feeds, it is aimed to make better use of the indigestible structural carbohydrate elements and other organic and inorganic elements in the feed and to neutralize some undesirable substances by providing the enzymes that animals cannot secrete sufficiently or at all. The use of enzymes as feed additives is more effective than other species because the rate of passage of feed through the digestive tract is high in poultry and they do not have a developed microbial digestion as in ruminants. Thus, fragmentation of indigestible polysaccharides in the cell wall of grain feeds, lowering of intestinal viscosity caused by non-digestible polysaccharides, and increasing the use of phosphorus are provided and the usefulness and ME values of feeds are increased (Baliga et al., 2011; Özden, 2008).

Current Studies on Alternative Feed Additives in Poultry

Inci (2019) used 150 Japanese quails (*Coturnix coturnix Japonica*) at 56 days of age in his experiment to determine the effect of probiotics added to mixed feeds at different rates (0%, 0.5%, 1%) on some blood parameters of Japanese quails. In the study, blood parameters such as total protein, glucose, cholesterol (LDL), LDL-C, chlorine, sodium, malondialdehyde (MDA), alanine transaminase (ALT), lactate dehydrogenase (LDH), and Magnesium (Mg) values were examined. According to the trial result; It was determined that the use of probiotics added to the diets of Japanese quails exposed to heat stress significantly affected the chlorine, total protein, and MDA values. On the other hand, it was determined that the probiotic supplement used at different rates on glucose, LDL, LDL-C, sodium, ALT, LDH, and Mg values did not have any effect and there was no statistical difference.

Erdoğmuş et al. (2019), in their study, gave a probiotic containing *Pediococcus acidilactici* and *Bacillus subtilis* to broiler chicks at a dose of $1x10^7$ cfu/mL with drinking water continuously from the 14th day to the 35th day. Feed consumption, body weights, and feed conversion rates were followed on a weekly basis. Villus height and crypt depth in the cecum and ileum and antibody titer analysis were performed in 28-day-old chicks. They found the positive effect of probiotics on villus height and crypt depth in the cecum and ileum to be superior to salinomycin. They found that the body weight gain was higher in the probiotic and probiotic+salinomycin groups than in the control and salinomycin groups. They determined that the feed consumption and feed efficiency values were superior in the probiotic and probiotic + salinomycin groups than in the control and salinomycin groups. No difference was observed between the groups in terms of salinoantibody titers.

Aydın and Yıldız (2020), in their study, investigated the effect of adding probiotics to the drinking water of breeding quails on some short-chain fatty acids. In the study, 160 breeder quails (6 females and 6 males in each subgroup in the control group, 5 females and 5 males in each subgroup in the experimental groups) at the age of 8 weeks were randomly divided into 3 groups, and each group into 5 subgroups. They kept 60 quails in the control group and 50 quails in each of the other two groups. They continued the experiment for 56 days and during the experiment the animals were fed with a ration based on corn and soybean meal. In the study; The K group did not add to the drinking water fed with the basic ration, the P1 group added 0.5 g / 10 lt of probiotics to the drinking water in addition to the basic ration, and the P2 group added 1 g / 10 lt of probiotics to the drinking water in addition to the basic ration. At the end of the study, acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, caproic acid, total SCFA (Short Chain Fatty Acids) and BCFA (Branched Chain Fatty Acids) values of the cecum content were not statistically significant. As a result, they concluded that the addition of probiotics in breeding quails has a positive effect on intestinal health.

Daş et al (2020), in their study, investigated the effects of peppermint oil addition to quail diets on growth performance, meat quality, color, and blood oxidative stress characteristics. For this purpose, they used 40 Japanese quails at the age of 10 days and continued the experiment for 35 days. The research groups were divided into 4 groups, each of which had 10 quails individually. In the rations prepared for quails, no feed additives were used in the control group, while 0.1%, 0.2%, and 0.3% Peppermint Oil (NY) were added to the feeds of the other groups. There was no difference between the groups in terms of body weight (BW), daily live weight gain (DLWG), daily feed consumption (DFC), feed conversion ratio (FCR), carcass and slaughter characteristics, color, and pH throughout the experiment. However, they found that it significantly decreased the total oxidative status (TOS) and increased the total antioxidant status (TAS) in the blood. As a result, they reported that the addition of peppermint oil to quail rations did not change the fattening performance and carcass characteristics, but it would be beneficial to add 0.1% peppermint oil to the ration as a feed additive since it increased TAS values and decreased TOS values.

Şahin et al. (2020) determined the effect of a mixture of thyme oil and carob powder in broiler-mixed feeds on fattening performance, carcass parameters, and some visceral weights. In the study, 192 mixed-sex chicks of daily age were used. In the experiment, they formed a total of 4 groups, 1 control group, and 3 experimental groups, each consisting of 48 chicks. Each group was divided into 4 subgroups of 12 and they continued the study for a total of 42 days. While the basal ration was given to the control group, rations containing a mixture of thyme oil and carob powder were given to the other three experimental groups, respectively (0.5, 1, and 2 g/kg). At the end of the experiment, there was no statistical difference in terms of body weight (BW), live weight gain (LWG), feed consumption (FC), feed conversion ratio (FCR), carcass and visceral weights in the experimental groups compared to the control group.

In his study, İpçak (2020) aimed to reveal the effectiveness of the addition of encapsulated fennel seed essential oil (FSEO) to compound feeds on the growth performance, small intestine microflora, and morphology of broiler chickens, and to examine the genetic and molecular mechanisms in these biological processes through small intestine transcriptomic profiling using nutrigenomic technologies. In the study, a total of 400 1-day-old Ross-308 genotype male chicks were randomly distributed into 5 groups with 16 replications in each group. The experiment consisted of a control group fed with standard feed without any feed additives and broiler groups fed with the addition of 50 mg (FSEO50), 100 mg (FSEO100), 200 mg (FSEO200), or 400 mg (FSEO400) encapsulated FSEO per 1 kg of standard feed. When the performance parameters obtained were evaluated, it was determined that the live weights (CA) and European production efficiency factor (EPEF) values tended to increase in the FSEO50 group but significantly increased in the FSEO100, FSEO200, and FSEO400 groups compared to the control group. It was determined that the body weight gain (BWG) increased in parallel with the increase in FSEO level and was highest in the FSEO400 group. It was determined that feed consumption (FC) was not affected by the increase in FSEO level, and the feed conversion ratio (FCR) improved in all FSEO levels compared to the control group, but there was no difference between the FSEO -supplemented groups. It was found that FSEO improved the morphology of the duodenum, jejunum, and ileum, and increased the thickness of the mucosal layer in the duodenum and jejunum, and the muscular layer thickness in the jejunum and ileum. In addition, by suppressing pathogenic microorganisms in the jejunum and ileum, *Lactobacillus spp*. contributed to the increase in the population. In the transcriptome profile obtained as a result of microarray analysis of samples taken from small intestine tissues, it was determined that the mRNA expression levels of 261 genes in the FSEO 50 group, 302 genes in the FSEO 100 group, 292 genes in the FSEO 200 group and 348 genes in the FSEO 400 group changed compared to the control group.

In their study, Bingöl (2020) investigated the effects of adding different levels of peas and peas to the laying hen's rations instead of soybean meal on the performance and egg quality criteria. The experiment was carried out with laying hens at the age of 30 weeks. The trial consisted of 7 groups, one control, and 6 treatment groups, each group from 7 subgroups and each subgroup from 7 laying hens. A total of 343 laying hens were used. At the end of the study, it was observed that the egg weight was significantly lower in the 10% pea (D1) group compared to the other groups ($P<0.05$). In terms of egg production, there was no difference between the groups except for the 1st and 4th weeks, including the last week. Although there was a difference in feed consumption between the groups in the 5th week, no difference was found in the other weeks in general. The differences between the groups were found to be insignificant in the rate of feed conversion, except for the 1st week. The differences between the groups in terms of diameter and shape index, which are egg quality criteria, were found to be insignificant. Differences between groups in terms of white index, Haugh unit, color scale, and shell thickness were found to be statistically significant ($P<0.05$). As a result, it has been reported that meeting 30% of the protein of soybean meal, which is used as a protein source in laying hen rations, with or without the addition of enzymes from peas, does not have any negative effect on performance. It has been reported that the addition of enzymes to diets containing 20% and 30% pea protein has a significant positive effect on egg internal quality criteria such as white index, haugh unit, and egg yolk.

Kırar et al. (2020), in their study, aimed to determine the effects of sumac added to the feed of Japanese quails at different levels on body weight gain (BWG), feed consumption (FC), feed conversion ratio (FCR), oxidative stress parameters and meat quality. In the study, 120 mixed-sex Japanese quails at the age of 10 days were randomly divided into 4 groups, one of which was the control group. The animals in each group were divided into 15 repetitions in pairs. 0%, 1%, 2%, and 3% sumac powder were added to quail feeds, respectively, and the rations were prepared as isocaloric and isonitrogenous. In the study, they determined that the addition of sumac had no effect on BWG, FC, and FCR.

Yıldız et al. (2021), in their study, investigated the effect of adding a probiotic-enzyme mixture to rations containing different levels of metabolic energy on performance, egg quality, and serum parameters in laying quails. They were created in a 3 x 2 randomized plot design with 6 treatment groups with 4 replications, each containing 5 female quails, formed by the addition of 3 metabolic energy levels (2900 (control), 2775, and 2650 kcal/kg), and two probiotic-enzyme mixtures (0, and 1 g/kg). They fed 120 female quails with trial rations for 10 weeks. They reported that reducing the ration metabolic energy level to 2650 kcal/kg as the main factor affects the feed conversion ratio and shell thickness negatively, but positively affects the Haugh unit and increases the serum cholesterol concentration. They observed that reducing the metabolic energy level of the diet by 2775 kcal/kg increased the specific gravity and serum calcium concentration. It was observed that the addition of a probiotic-enzyme mixture to the ration positively affected egg production, feed efficiency, shell thickness, serum calcium and phosphorus concentrations, and decreased serum glucose concentration. As a result, they reported that laying quails could be fed with rations containing 2775 kcal/kg metabolic energy without affecting performance, and the addition of probiotic-enzyme mixture to the ration affected the performance, shell quality, and serum parameters positively, but lowering the ration energy level by 2650 kcal/kg negatively affected the investigated parameters.

Olgun et al. (2021), in their study, determined the effects of adding cardamom powder at different levels (0, 1, 2, 3, and 4 g/kg) to layer quail rations on performance, egg quality, and serum parameters. In the experiment, a total of 120 laying quails (4 in each) at the age of 20 weeks were randomly allocated to 5 treatment groups with 6 replications. The quails were fed with treatment rations for 10 weeks. In the experiment, the feed consumption of quails increased significantly with the addition of 3 g/kg cardamom powder to the ration. Eggshell thickness increased significantly compared to other groups with the addition of cardamom powder at the level of 1 g/kg. It was reported that the egg yolk L* value increased, while the egg yolk a* value decreased with the addition of 4 g/kg cardamom powder to the ration. At the end of the experiment, they reported that the addition of cardamom powder at the level of 3 g/kg to the ration increased the feed consumption of quails, while the addition of 1 g/kg improved the eggshell quality and serum cholesterol level.

Şen et al. (2021), in their study, investigated the effects of adding grape pomace, which is a by-product of the wine industry and rich in structural carbohydrates, to broiler rations with enzymes on the performance values of live weight, live weight gain, feed consumption, and feed conversion ratio. For this purpose, 120 0-day-old Ross 308 broilers were used. They were distributed as Control, Grape, and Enzyme groups with 40 chicks in each group and 10 chicks in each subgroup. The control group consumed the basic ration, the grape group consumed the ration with 5% grape pulp added to the basic ration, and the enzyme group consumed the ration with 0.1% enzyme and 5% grape pulp added to the basic ration. At the end of the study, they reported that the addition of enzymes had a positive effect on live weight and live weight gain, feed consumption increased in grape and enzyme groups, and feed utilization was similar in all groups.

Yıldırım et al. (2022), in their study, aimed to eliminate the morphological effect of heat stress on the ovaries and oviducts, which are the organs where egg production and structuring take place, and to eliminate this effect with probiotics. For this purpose, 40 female laying Japanese quail

(*Coturnix coturnix Japonica*) were randomly divided into four groups. In the first group, the ambient temperature was kept at 14-25°C all day for eight weeks. During the period when the quails in the second group were kept at 14-25°C, they added 0.5 cc/L of fermented natural LAB (Lactic Acid Bacteria) liquid to their drinking water. In the third group, heat stress was created in such a way that the hen environment was kept at 30-34°C for 8-12 hours a day. In the fourth group, they added 0.5 cc/L fermented natural LAB liquid to the drinking water of quails for eight weeks when they were exposed to heat stress. They measured that the number of yellowish follicles and the mean diameter of yellow follicles decreased in quails under heat stress.

Aydın (2022), in his study, examined the effect of enzyme addition to corn-weighted broiler rations on performance. 162 broiler chicks were used in the study. In the trial, which lasted for 6 weeks, the animals were fed 3 different rations with 0, 0.5, and 1 kg/tonne enzyme (Xylanase: 12.000 µg/g, b-glucanase: 5.000 µg/g and Pectinase: 7 µg/g) added to corn-weighted rations. While the body weight gains were found to be insignificant in all weeks, a relative increase was achieved in the last week as the enzyme addition increased. The differences between the groups in terms of feed consumption and feed efficiency were found to be insignificant. In terms of carcass characteristics, except for the wing weight, they reported that the weights increased significantly with the addition of 1 kg/tonne enzyme to the rations. They reported that the application of probiotic-effective fermented lactic acid bacteria as feed additives in quails exposed to heat stress reduced the number of yellow follicles and prevented live weight loss.

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CHAPTER 9

THE EFFECT OF PROBIOTICS ON METHANE GAS RELEASE IN RUMINANTS

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Introduction

The rapidly increasing population in the world leads us to face many environmental problems such as food shortages, climate change and global warming. Global warming due to greenhouse gases remains a global problem that is recognized as a major threat to the world ecology and can affect almost all sectoral activities. Although the share of the livestock sector in total human-induced greenhouse gases released into the atmosphere is estimated to be 11%, the contribution of meat and dairy animals to greenhouse gas formation is 41%, respectively. The digestive events occurring in the rumen are carried out by microorganism activities and the digestion of nutrients results in animal productivity. However, as a consequence of these activities, some undesirable metabolites are formed and these cause nutrient loss and environmental problems. Ruminal methane release results in a loss of 2-12% of the energy obtained from feed. Since methane is a gas with a greenhouse effect, it also poses an ecological problem by contributing to global warming. In the rumen of a ruminant, 3.5 L of methane is released from 100 g of digestible cellulose and 650 L of methane is released from a dairy cow per day. A cow capable of producing 9000 kg of milk per year releases 120-130 kg of CH_4 per year (Powers et al., 2014). The share of methane gas in global warming is 23 times higher than that of CO_2, with an annual production of 80-115 million tons by ruminant animals in the world (IPCC, 2007). The role of CH_4, which is among the greenhouse gases, in global warming is undeniable due to its greater heat retention capacity compared to carbon dioxide. Since methane emission, which is a greenhouse gas, cannot be prevented in ruminant animals, it is aimed to reduce undesirable end products such as carbon dioxide, methane and hydrogen sulfide formed in the rumen in order to increase the productivity of animals in recent years. Therefore, strategies and studies to reduce methane emission are taking priority.

Animal feeding practices to reduce ruminal methane release are based on two main principles. These are; 1. Reducing the number or activity of methanogenic microorganisms in the rumen, 2. Directing the hydrogen formed as a result of rumen fermentation to the synthesis of other compounds containing hydrogen without participating in methane formation. In animal feeding strategies to reduce ruminal methane release, alternatives such as the inclusion of fat in the ration, increasing the ratio of rough concentrate feed in favor of concentrate feed, increasing feed intake and feeding frequency, probiotics, ionophore antibiotics, organic acids, various medicinal and aromatic plants themselves or extracts or essential oils obtained from them, methane inhibitors and biotechnological methods are emphasized. Although positive results are obtained from many of these implementations, the fact that the effect obtained is not long-lasting and that the microorganisms in the rumen gain resistance to the practice over time, that some implementations have a negative effect on digestion or are toxic to the organism makes it difficult to carry these implementations into practice. These strategies are a subject that urgently requires attention in terms of improving animal performance in the short term, preventing energy loss, and environmental importance in the long term (Anonymus, 2010).

Rumen Microorganisms and Methane Producing Bacteria in Rumen

The microbial ecosystem of the rumen consists of bacteria (10^{10}-10^{11} cells/ml), archaea (10^{7}-10^{9} cells/ml), protozoa (104-106 cells/ml), fungi (103-105 zoospores/ml) and bacteriophages (10^{8}-10^{9} ml) (Kamra, 2005; Klieve et al., 2005). Approximately more than 200 bacterial species have been isolated in the rumen and at least 20 species were identified to be present in the rumen at the level of 10^{7}-10^{10} cells/ml. In addition, bacteria present in the rumen at more than 10^{7} cells/ml are considered as dominant bacteria. (Martin, 1994).

Methanogenic bacteria convert H_2 and CO_2 to methane (Wolin et al., 1997). Methanogens constitute 0.5-3% of the total bacterial population of ruminants and are often present in high quantities (10^{6}-10^{8} per g of wet feces) (Joblin et al., 1990). Methanogens live in strongly anaerobic conditions and provide all their metabolic energy by reducing hydrogen and carbon dioxide to produce methane. There is a symbiotic relationship between methanogenic bacteria and ciliated protozoa (Ohene-Adjei et al., 2007). It is recognized that there is an inverse relationship between the number of bacteria responsible for most of the fermentative activities in the rumen and the number of protozoa (Ohene-Adjei et al., 2007). Methanogens are classified under archaea and are divided into 5 major classes: *Methanobacteriales*, *Methanosarcinales*, *Methanococcales*, *Methanomicrobiales* and *Methanopyrales*. *Methanobacteriales* are dominant in the rumen. *Methanobrevibacter* *ruminantium*, *Methanomicrobium mobile* and *Methanosarcina* are the most important methanogens in the rumen microbial ecosystem isolated from sheep and cattle rumen (Yanagita et al., 2000). Among the 28 genera and 113 species of methanogens known to exist in nature, only seven are widely cultivated from the rumen. These are *Methanobacterium formicicum*, *Methanobacterium bryantii*, *Methanobrevibacter ruminantium*, *Methanobrevibacter millerae*, *Methanobrevibacter olleyae*, *Methanomicrobium mobile* and *Methanoculleus olentangyi* (Janssen and Kirs, 2008).

Methane Gas Formation in Rumen and Methane Reduction Method

Anaerobic fermentation of feeds in the rumen is achieved by microorganisms that settle there and have a symbiotic relationship with the ruminant. The volatile fatty acids (VFA) formed as a result of this digestion are rapidly absorbed from the rumen epithelium and meet approximately 50-75% of the animal's energy requirement (Faverdin, 1999). There are many bacterial, protozoan and fungal species in the rumen that are involved in the fermentation of feed to provide their own energy requirements. As a result of their collective activities, carbohydrate sources taken with feed are fermented in the rumen and converted into volatile fatty acids (VFA), H_2 and CO_2. The main VFAs formed as a result of fermentation are acetic, propionic and butyric acid, which are used to meet the energy needs of animals. Hydrogen is the most important energy source for methanogens (CH_4 producing microorganisms). The H_2 and CO_2 released as a result of rumen fermentation are subjected to the reduction reaction $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ by methanogenic microorganisms (bacteria, archaea, protozoa) and cause methane (CH_4) gas formation (methanogenesis) (Hegarty and Klieve, 1999; Moss et al., 2000; Görgülü et al., 2009). Generally, methane (CH_4) formation is produced by two types of methanogens; slow growing (about 130 hours) methanogens that produce CH_4 from acetate and fast growing (about 4-12 hours) methanogens that reduce CO_2 with H_2. In the rumen, CH_4 is mostly made by fast reproducing species (Weimer, 1998). It is also reported that 9-25% of methanogenesis in the rumen is associated with protozoa (Newbold., et al 1995).

The utilization of hydrogen ions by methanogenic (methane producing) bacteria is very essential for the sustainability of microbial digestion in the rumen. Due to high hydrogen ion concentrations in the rumen, it is not possible to oxidize NADH (nicotinamide adenine dinucleotide), which is reduced during the glycolysis reaction, and in such a case, metabolism shifts towards ethanol and lactic acid production by entering an undesirable metabolic pathway that is harmful for the ruminant (Breves and Leonhard-Marek, 2000). This may cause subacute rumen acidosis.

Methane formation in the rumen is affected by rumen conditions (physiological and chemical), ration, animal species, passage rate of rumen contents, rumen volatile fatty acid profile and pH (Utpala et al., 2006). CH_4 formation (methanogenesis) in the rumen is critical in preventing hydrogen accumulation that suppresses dehydrogenase enzyme activity and in ensuring that the rumen operates at optimum performance. Therefore, the objectives for determining strategies to reduce CH_4 formation can be summarized as: a) Reducing hydrogen production without impairing feed digestion, b) Stimulating hydrogen utilization to release alternative end products beneficial to the animal, c) Suppressing the number and/or activity of *archeae* methanogenics in a way that does not lead to hydrogen accumulation in the rumen or in a way that accompanies pathways to stimulate hydrogen consumption (Martin et al., 2010).

Probiotics and Probiotics Mode of Action

Probiotics can be defined as "biological products containing a group of living bacteria, fungi and yeasts or their cultures, which are mostly Gr (+) and facultative anaerobes, which grow implanted in the intestines of animals, are not absorbed from the digestive tract, show antagonistic effects against pathogenic microorganisms, increase feed utilization in animals" (Antunovic ve ark., 2005). Microorganisms generally used as probiotics are Lactobacillus, Bacteriodes, Enterococcus, Streptococcus, Pediococcus, Bacillus and Bifidobacterium spp bacteria, Aspergillus spp fungi and yeast cultures (Saccharomyces cerevisiae) (Öztürk, 2008b).

Live bacteria, fungi and yeasts used as probiotics must maintain their viability in storage, administration and intestinal environment in order to be effective (Wu, 1987). Probiotic preparations consisting of *Lactobacillus*, *Bifidobacterium* and *Streptococcus spp* should be stored at 22-25°C and in a dry environment. They lose their viability when the storage temperature rises above 30 °C. While *Saccharomyces cerevisiae* yeast and *Bacillus spp* bacteria used as probiotics are resistant to pelleting temperature, *Lactobacillus*, *Bifidobacterium* and *Streptococcus spp* bacteria exhibit significant losses due to pelleting temperature. (Aldous and Alexander, 2001; Kim, 1988; Thunes, 2017)

Probiotics used in ruminants are present in many forms such as powders, pastes, gels, liquid suspensions, pills or capsules and can be administered to animals by dissolving in drinking water, milk, milk substitute feed or by mixing well with feed or by sprinkling on feed, directly into the rumen or by direct drinking (Anonymous, 2016; Lettat et al., 2012; Astuti et al., 2022). Probiotics given with feed or water in ruminant animals first proliferate in the rumen, affecting and changing the microbial ecosystem and fermentation properties of the rumen. The effects of probiotics may vary according to the strain of bacteria, the dose given, the time of use and the conditions of use. Probiotic mixtures containing more than one bacterial strain are effective in more animal species. It is also reported that probiotics will be more effective if given continuously. (Sarıca, 1999).

Among the microorganisms that make up probiotics, the use of lactic acid-producing bacteria i) creates continuous and constant lactic acid production in the rumen (*Enterococus faecum* and *Lactobacillus spp*), ii) microorganisms in the rumen adapt to the presence of lactic acid, iii) stimulate the growth of lactic acid utilizing bacteria (*Megashaera elsdenii*, *Selenomonas ruminantium, Propionibacterium spp*) in the rumen environment, iv) have a positive effect through stabilization of ruminal pH (Beauchemin et al., 2003; Ghorbani et al. , 2002; Raeth-Knight et al., 2007). It has also been reported that bacteria that utilize lactic acid in the rumen i) increase propionic acid production (*Streptococcus bovis, Fibrobacter succinogenes*, *Propionibacterium spp*.) rather than lactic acid production by i) converting lactic acid to volatile fatty acids (*Megasphaera elsdenii*) ii) increasing the propionate level in the rumen with their conversion of lactic acid to propionic acid will reduce CH_4 production (Grainger and Beauchemin, 2011).

Propionic acid forming bacteria (*Streptococcus bovis, Fibrobacter succinogenes*), nitrate-nitrite reducing bacteria (*Wolinella succinogenes*, *Selenomonas ruminantium, Propionibacterium spp*.), sulfate reducing bacteria (*Desulfovibrio spp, Desulfotomaculum spp., Fusobacterium spp*.), homoacetogens (*Peptosterptococcus productus*), methylotroph bacteria (*Nitrosomonas spp., Methanomicrococcus spp, Methanosarcino spp*.), capnophilic bacteria (*Actinobacillus succinogenes, Manheimia succiniproducens, Succinivibrio dextrinosolvens*) are microoganisms used in in vitro and in vivo studies on CH_4 inhibition of *Fibrobacter succinogenes* in the cellulolytic bacterial population responsible for cellulose digestion in rumen by producing succinate and producing low hydrogen leading to propionate formation (Chaucheyras-Durand et al., 2010; Elanthamil and Bandeswaran, 2017).

Another group of probiotics used to suppress methane production are reducing acetogenesis bacteria. These bacteria produce acetic acid by reducing the hydrogen and carbon dioxide that accumulate there, especially in the intestines. Thus, the substrate required for methanogens can be reduced by the presence of another organism that uses the H ions required for methane production. However, these bacteria are not abundant enough in the rumen to compete with methanogens and positive and consistent results have not been obtained from the studies conducted so far (Boadi ve ark., 2004). Nevertheless, increasing the amount of acetogens in the rumen or supplementing the ration externally is considered as a strategy that may be effective in reducing the effect of methanogens (Valdez ve ark., 1996).

Another type of bacteria, grouped under the name of methane oxidizers, also restricts ruminal methane production *in vitro*. "Methane release" can be briefly defined as the difference between methane produced and methane expended in metabolism. Methane oxidizing bacteria utilize methane and prevent its release from metabolism, thus limiting the energy excreted by methane. There is a limited number of methane oxidizing bacteria in the rumen (Moss et al., 2000). Valdez et al. (1996) reported that methane production was suppressed when they inoculated a bacterial strain obtained from pig intestine in *in vitro* rumen environment. However, the *in vivo* effects of methane oxidizers have not been investigated yet (Moss et al., 2000).

The reducing effect of yeast cultures on CH_4 production is explained in three ways: 1) By reducing the number of protozoa in the rumen, *Aspergillus oryzae* caused a direct reduction of 45% in the protozoa population, resulting in a reduction of up to 50% in CH_4 production (Frumholtz et al., 1989), 2) By increasing butyrate and propiate production, and 3) By increasing the growth and number of acetogenic bacteria that utilize metabolic hydrogen (Elanthamil and Bandeswaran, 2017). It is reported that the use of yeast as a probiotic increased the population of lactic acid-utilizing bacteria in the rumen by 75%, but the number of lactic acid-producing bacteria decreased significantly, while the number of cellulotic bacteria increased by 50%. As a result, the production of propionic acid from lactic acid improves and thus more ration energy can be utilized. According to the results of the studies, yeasts (*Saccharomyces cerevisae* and *Aspergillus oryzae*) suppress methane production (Boadi et al., 2004). *In vivo* (Grainger and Beauchemin, 2011) and *in vitro* (Mutsvangwa et al., 1992) studies have reported that yeasts suppress methane production. Although the mechanism of action of yeasts in suppressing methane production has not been fully elucidated, it is thought to affect the types of bacteria in the rumen environment (McGinn et al., 2004). Carro et al. (1992) reported that methane production increased with the addition of yeast to rumen contents *in vitro* and this effect may be due to the decrease in rumen protozoa and acetate:propionate ratio. In the experiment in which *Saccharomyces cerevisiae*, *Trichosporon sericeum*, *Candida norvegensis* yeast cultures, which are the most studied yeast cultures, were evaluated, a decrease in CH_4 production was observed. In addition to these studies, some other *in vitro* studies have shown that yeast cultures can give ineffective or negative results regarding CH_4 formation (Wang et al., 2016; Oeztuerk et al., 2016). It is believed that these different results regarding CH_4 production are probably due to strain differences in yeast cultures, changes in ration type, dose used, physiological conditions of animals and species differences (Newbold and Rode, 2006; Patra, 2012). *Saccharomyces cerevisiae* and *Aspergillus oryzae* decreased the rumen lactic acid level (Erasmus et al., 1992), (Alexander et al., 1997; Amarasinghe et al., 2018; Kim, 1988) and this decrease in rumen lactate level was observed in *Selenomonas ruminantium*, It has been reported that the increase in the activity of rumen lactic acid-utilizing bacteria such as *Selenomonas lactilytica* and *Megasphaera elsdenii* (Erasmus et al., 1992) (Aldous and Alexander, 2001; Kennedy, 2001; Kim, 1988) leads to a 4-31% reduction in methane production by improving feed utilization (Sarıpınar Aksu and Sulu, 2005).

Bacteriocins are peptide or protein compounds produced by bacteria (Meral and Biricik, 2013) and their effects on reducing methane release have been studied. Nicin is one of the most widely studied bacteriocins and is produced by the bacterium *Lactococcus lactis*. In two different studies conducted under *in vitro* conditions, it was determined that the addition of nicin, a natural and safe bacteriocin, reduced methane release by 36% (Hegarty and Klieve, 1999; Callaway et al., 1997; Lee et al. 2002). In an *in vitro* study conducted by Lee et al. (2002) with HC5 bovisin, a bacteriocin produced from *Streptecoccus bovis*, it was observed that methane production decreased by 50%.

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CHAPTER 10

EPIDURAL ANESTHESIA AND ANALGESIA IN HORSES

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INTRODUCTION

Epidural anesthesia is a form of regional anesthesia (Muir and Hubbell, 2009; Michielsen and Schauvliege, 2019). In regional anesthesia, a part of the body or parts of the body where insensitivity is desired are desensitized (Tranquilli and Grimm, 2015). Epidural injections are preferred for surgical procedures in the pelvis, hindlimb, tail, perineum and abdominal folds. For this purpose, analgesia and anesthesia are provided by injecting drugs into the extradural space, under the ligamentum flavum (Natalini and Driessen, 2007; Borer Weir, 2014; Campoy et al., 2015; Michielsen and Schauvliege, 2019). Epidural anesthesia can be performed repeatedly/continuously with a single injection or using an epidural catheter (Natalini, 2010). Intrathecal (spinal, subdural or subarachnoid) analgesia drugs are injected into the subarachnoid space containing cerebrospinal fluid (CSF). CSF helps the diffusion of drugs (Borer-Weir, 2014).

1. EPIDURAL ANESTHESIA AND ANALGESIA IN HORSES

Epidural injections can be performed in horses cranially (lumbosacral space) or caudal (sacro-coccygeal or 1st and 2nd intercoccygeal space) (Robinson and Natalini, 2002; Natalini and Driessen, 2007; Natalini, 2010; Love, 2012).

1.1. Anatomy of the epidural space

The spinal cord is located within the vertebral canal and extends from the brain to the caudal lumbar region (Otero and Campoy, 2013). The spinal canal, spinal cord and brain are protected by the meninges and CSF (Otero and Campoy, 2013; Borer-Weir, 2014). It forms the meninges around the spinal cord, namely three layers: the pia mater, the arachnoid membrane and the dura mater (Natalini and Driessen, 2007; Otero and Campoy, 2013). The pia mater is the innermost membrane and is attached to the spinal cord. The arachnoid is the middle layer. Its outer surface is attached to the dura mater (Otero and Campoy, 2013; Borer-Weir, 2014). The subarachnoid space is located between the pia mater and the arachnoid membrane and contains CSF (Otero and Campoy, 2013). The epidural space is the space between the dura mater and the vertebral canal wall (Otero and Campoy, 2013; Borer-Weir, 2014). The epidural space contains blood vessels, nerves, fat, and lymphatics.

Each of the associated dorsal and ventral nerve roots is initially covered by an extension of the dura mater and arachnoid membrane (Natalini and Driessen, 2007; Otero and Campoy, 2013). Spinal nerves exit the spinal canal through the intervertebral foramen. Diffusion of drugs into the neural tissue is required to obtain good epidural anesthesia after epidural injection (Borer-Weir, 2014; Michielsen and Schauvliege, 2019).

1.2. Indications for Epidural Anesthesia and/or Analgesia

The aim of epidural anesthesia is to desensitize the caudal and last sacral nerves and to cause sensory loss in the innervation areas (Skarda et al., 2009). Caudal epidural injection provides anesthesia in outpatient surgeries of the rectum, anus, perineum, tail, urethra, bladder, vulva, or vagina of sedated horses (Doherty and Valverde, 2006; Skarda et al., 2009; Vigani and Garcia-Pereira, 2014; Carpenter and Bryon, 2015). In this anesthesia, correction of rectal prolapse, recto-vaginal fistula or uterine torsion, laparoscopic cryptorchidectomy and fetotomy can be performed (Robinson and Natalini, 2002; Natalini and Driessen, 2007). Epidural anesthesia can be used for the same anatomical regions under general anesthesia (Doherty and Valverde 2006), as part of a multimodal analgesic plan (Borer-Weir, 2014), or for postoperative analgesia (Doherty and Valverde, 2006; Carpenter and Bryon, 2015).

1.3. Contraindications of Epidural Anesthesia and/or Analgesia

Conditions such as hypovolemia, septicemia, bacteremia, skin trauma/infection or neoplasia are contraindications for epidural anesthesia (Doherty and Valverde, 2006; Dugdale, 2010; Love, 2012; Campoy et al, 2015, Steagall et al., 2017). Alternative anesthesia techniques should be considered to provide analgesia in these patients (Dugdale, 2010; Love, 2012; Campoy et al., 2015, Steagall et al., 2017). In addition, in traumatized patients with pelvic or lumbar vertebral injuries, it may be difficult to perform the procedure due to the deterioration of anatomy (Dugdale, 2010; Steagall et al., 2017). In patients with increased intracranial pressure, brain herniation may occur due to accidental intrathecal injection. Therefore, it is not preferred with such patients (Michielsen and Schauvliege, 2019).

1.4. Complications of Epidural Anesthesia and/or Analgesia

Various complications related to epidural injection have been reported. Failure to obtain good anesthesia and analgesia is mostly associated with incorrect technique, anatomical anomalies, and fibrous scar tissue formation in this region due to previous epidural injections (Doherty and Valverde, 2006; Skarda et al., 2009; Carpenter and Bryon, 2015). Severe ataxia or lying down may occur in case of overdose (Doherty and Valverde, 2006; Skarda et al, 2009; Natalini, 2010; Carpenter and Bryon, 2015). High doses and/or rapid injection may also cause ataxia or general discomfort in the horse. In the case of lying down and motor blockage, the horse may need to be sedated until the motor blockage in the hind legs is over (Doherty and Valverde, 2006; Skarda et al., 2009; Natalini, 2010). The risk of breaking the needles is very low. However, it can be broken. Therefore, the horse must be properly restrained and sedated prior to injection. The use of styled flexible needles can further reduce this risk (Skarda et al., 2009). Systemic use of drugs, especially with the use of an alpha2-agonist, may cause sedation and cardiovascular depression (Doherty and Valverde, 2006; Skarda et al., 2009). If the procedure is performed without following the rules of asepsis, infection may occur at the injection site (Otero and Campoy, 2013). Systemic administration of opioids or accidental injection into the subarachnoid space may cause central stimulation (Natalini, 2010). Epidural administration of opioids may cause systemic pruritus (Doherty and Valverde, 2006; Carpenter and Bryon, 2015). Local sweating or perineal edema may occur in epidural anesthesia with lidocaine or xylazine (Michielsen and Schauvliege, 2019).

1.5. Cranial epidural injection

Cranial epidural anesthesia performed by administering anesthetic to the lumbosacral space in horses is not a preferred technique. Caudal epidural injection is very difficult (Skarda et al., 2009; Love, 2012). In horses, the spinal cord terminates at the level of the caudal half of the second sacral vertebra (Carpenter and Bryon, 2015). This means that injection into the lumbosacral space carries the risk of dura mater puncture and accidental injection into the subarachnoid space, as well as an increased risk of motor blockade and ataxia (Doherty and Valverde, 2006; Carpenter and Bryon, 2015). By applying finger pressure to the lumbosacral intervertebral space, a depression can be palpated between the dorsal spinous process of L6 and S1. Rectal palpation may be helpful in locating the L6-S1 intervertebral space (Skarda et al., 2009).

1.6. Caudal epidural injection

Caudal epidural injection is made into the first coccygeal space (Co1-Co2). This technique is simple, inexpensive and the most commonly used technique (Skarda et al., 2009; Carpenter and Bryon, 2015). There is no risk of spinal injection in this region (Natalini, 2010; Carpenter and Bryon, 2015). In addition, it is less likely to cause motor blockage in the hind legs (Love, 2012).

The Co1-Co2 range can be determined by raising and lowering the tail. In addition, this space can be palpated caudal to the sacrum as the first movable joint (Doherty and Valverde, 2006; Skarda et a., 2009; Carpenter and Bryon, 2015)

1.7. Technique and equipment

During epidural anesthesia, good handling of the horse and preparation of the area are mandatory (Doherty and Valverde, 2006; Skarda et al., 2009; Love, 2012). If possible, the horse should put equal weight on both hind legs (Love, 2012). Regardless of whether a single injection is made or an epidural catheter is placed, strictly aseptic work is required (Doherty and Valverde, 2006). Hypodermic needles or spinal needles can be used for a single injection. Local anesthetic injection around the injection site may be helpful to minimize the pain of the procedure (Skarda et al., 2009).

After the site is prepared and the injection site is determined, the needle is placed perpendicular to the skin in the center of the palpable cavity (Natalini, 2010; Carpenter and Bryon, 2015). A popping sensation can be detected as the needle passes through the Ligamentum flavum (Natalini, 2010; Love, 2012; Carpenter and Bryon, 2015). The needle is slightly withdrawn 5 mm to prevent injection into the intervertebral disc (Natalini, 2010; Love, 2012). An upright approach can be used for cranial and caudal epidural injections (Natalini, 2010; Carpenter and Bryon, 2015). Another technique for caudal epidural anesthesia is insertion of the needle at an angle of 30° with the horizontal plane (Skarda et al., 2009; Natalini 2010; Carpenter and Bryon 2015). In adult horses, the needle can be advanced approximately 3.5 to 8 cm to reach the intervertebral space (Carpenter and Bryon, 2015). Several methods can confirm whether the needle is correctly placed in the area. First, a drop of liquid is aspirated from the hub of the needle just after the ligamentum flavum penetration with the hanging drop technique. This aspiration occurs due to negative pressure in the epidural space (Doherty and Valverde 2006; Love 2012; Otero and Campoy 2013; Carpenter and Bryon 2015). A second method is to lose resistance when injecting air or liquid (Doherty and Valverde, 2006; Otero and Campoy, 2013; Carpenter and Bryon, 2015). After a successful epidural injection, clinical effects such as loss of tail or anus tone can be observed (Doherty and Valverde, 2006). Aspiration should be performed before slow injection to ensure there is no intravascular injection (Natalini, 2010; Michielsen and Schauvliege, 2019).

1.8. Continuous epidural analgesia

In some cases, repeated epidural drug administration may be required to provide prolonged analgesia. Continuous epidural anesthesia may be indicated in patients with a variety of clinical symptoms, such as painful conditions (fractures, wounds, or lacerations) in the hind limbs or, as a result, persistent tenesmus with rectum or uterine prolapse (Carpenter and Bryon, 2015). Epidural catheters can remain in place for up to twenty days (Martin et al., 2003).

An aseptic technique is required for every injection through an epidural catheter (Doherty and Valverde, 2006; Love, 2012). The catheter should preferably be sterile flushed with saline after each drug administration (Natalini, 2010).

1.9. Drugs Used in Epidural Anesthesia and Analgesia

Drugs used in epidural analgesia should provide analgesia or anesthesia with minimal systemic effect and minimal motor blockade (Valverde, 2008). Drugs or drug combinations are selected depending on the desired effect and duration of action. Drugs that can be administered epidurally are alpha2-agonist, local anesthetics, opioids, ketamine, tramadol or tiletamine-zolazepam (Doherty and Valverde, 2006; Skarda et al., 2009; Carpenter and Bryon, 2015) (Table 1). In high-dose epidural injections, drugs may spread cranially and a more cranial epidural blockade may form (Doherty and

Valverde, 2006). The choice of drug depends on the indication for which epidural anesthesia or analgesia is used (Michielsen and Schauvliege, 2019).

Local anesthetics prevent depolarization of nerves. Thus, it prevents the transmission of any sensory input, including the sense of pain (Carpenter and Bryon, 2015). In addition to sensory blockade, motor blockade may also occur with epidural administration of local anesthetics (Robinson and Natalini, 2002). Sensory blockade of the anus, perinea, rectum, vagina, and vulva can be useful in surgery on the vagina or vulva, such as correcting a recto-vaginal fistula. Fetotomy or reduction of rectum, vagina or bladder prolapse to avoid ongoing tenesmus are other indications for the use of local anesthetics in epidural injections. Adding epinephrine to local anesthetic solutions can provide a faster onset of action and a longer duration of action (Carpenter and Bryon, 2015).

Most epidural medications provide analgesia. However, it does not provide complete anesthesia. This includes alpha-2 agonists that bind to their receptors in the spinal cord after epidural injection, thereby providing analgesia (Robinson and Natalini, 2002; Carpenter and Bryon, 2015). With these drugs, motor blockade may not occur and ataxia and/or throwing may occur. Opioids are potent analgesics (Robinson and Natalini, 2002). They provide longer duration of analgesia and can be used alone or in combination with local anesthetics and/or alpha-2 agonists for acute or chronic pain (Carpenter and Bryon, 2015). Produces good epidural analgesia by administration of an opioid alone. However, good anesthesia cannot be provided. Tramadol, ketamine, and tiletamine-zolazepam can also be injected into the epidural space, but more studies are needed to recommend these combinations for epidural use in horses (Carpenter and Bryon, 2015).

Drug combinations can prolong the duration of analgesia. However, careful dosage is required to avoid adverse effects of drugs (Doherty and Valverde, 2006; Carpenter and Bryon, 2015). Opioids combined with alpha-2 agonists may be useful for long-term pain management in cases of hindlimb pathology with extreme lameness. Combination of alpha-2 agonists with local anesthetics may provide a longer lasting effect than local anesthetic alone (Doherty and Valverde, 2006; Michielsen and Schauvliege, 2019).

Table 1. Drugs used in epidural injection (Michielsen and Schauvliege, 2019)

Group	Drug	Dosage (mg/kg)	Onset (minutes)	Duration (minutes)	Remarks
Local anesthetics	Lidocaine	0.2-0.25	5-20	60-120	Careful with re-dosing since overdose can cause ataxia, recumbency and hypotension.
	Ropivacaine	0.02-0.1	10	180	Minimal ataxia and cardiorespiratory effects.
	Mepivacaine	0.2-0.25	20	80	
	Bupivacaine	0.06	< 6	>300	Rapid onset and long duration.
Opoids	Morphine	0.1	30-80	>300	Mild systemic effects.
	Methadone	0.1	15	300	Rapid onset but intermediate time of action. Can be diluted in 10 ml 0.9% sterile saline for a horse of 500 kg.
	Tramadol	1	< 30	240-300	Can be diluted in 10-20 ml 0.9% sterile saline for a horse of 500 kg.
Alpha-2 agonist	Xylazine	0.17-0.22	15-30	150-210	Minimal sedative effects. Can be diluted in 10ml 0.9% sterile saline for a horse of 500 kg for perineal analgesic/anesthetic effects or in 20-30 ml for a more rostral spread and analgesic effect. Preferred alpha-2 agonist for epidural use since a more potent antinociceptive effect is observed with minimal sedative and cardiovascular side effects.
	Detomidine	0.03-0.06	10-15	120-160	Minimal to excessive sedative effects. Can be diluted in maximal 10 ml 0.9% sterile saline for a

					horse of 500 kg to limit rostral spread and its side effects.
Other	Ketamine	0.5-2	5-10	30-80	Systemic effects can occur with higher dosages. Dilution of ketamine in 10 to 30 ml 0.9% sterile saline for a horse of 500 kg.
	Tiletamine-zolazepam	1	< 30	240-300	Can be diluted in 10-20 ml 0.9% sterile saline for a horse of 500 kg. Increase in noxious pressure stimulus by epidural administration but further studies should be conducted.

2. CONCLUSION

Epidural anesthesia and analgesia are one of the effective techniques in horses as part of balanced anesthesia and for postoperative pain management. Caudal epidural anesthesia is a simple, inexpensive and effective method in equine medicine. It can be performed together with sedation when general anesthesia is not desired in cases such as surgical procedures in the horse's hind extremities, perineal and sacral regions. Providing longer duration of analgesia in pain management is possible due to the availability of epidural catheters. Complications may occur, but the benefits are greater.

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CHAPTER 11

WITH THE HANDWRITING OF DOĞAN ÖZDEMİR, FARRIER ABDULLAH ÖZDEMİR AND HIS GENERATION IN AFYONKARAHİSAR

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Introduction

Healthy hoof is essential for the horse welfare and use of the horse for different purposes. It is therefore declared that equine performance is directly influenced by hoof health. When viewed from this point, the importance of hoof management in horses should be understood detailed. The statement; "No Foot, No Horse" is the most explanatory word in this subject (Kaplan, 2020; Yücel, 2020; Thirkell and Hyland, 2017).

Traditional farriery has almost disappeared with the advent of automobiles and tractors. There are an estimated 116 million equines (donkeys, horses and mules) globally, with 36 million in the 38 lowest-income countries according to a report informed by the Food and Agriculture Organization of the United Nations. Major declines in working equines populations have been reported in different parts of the world. But accurate population data is lacking for many countries (Anonim).

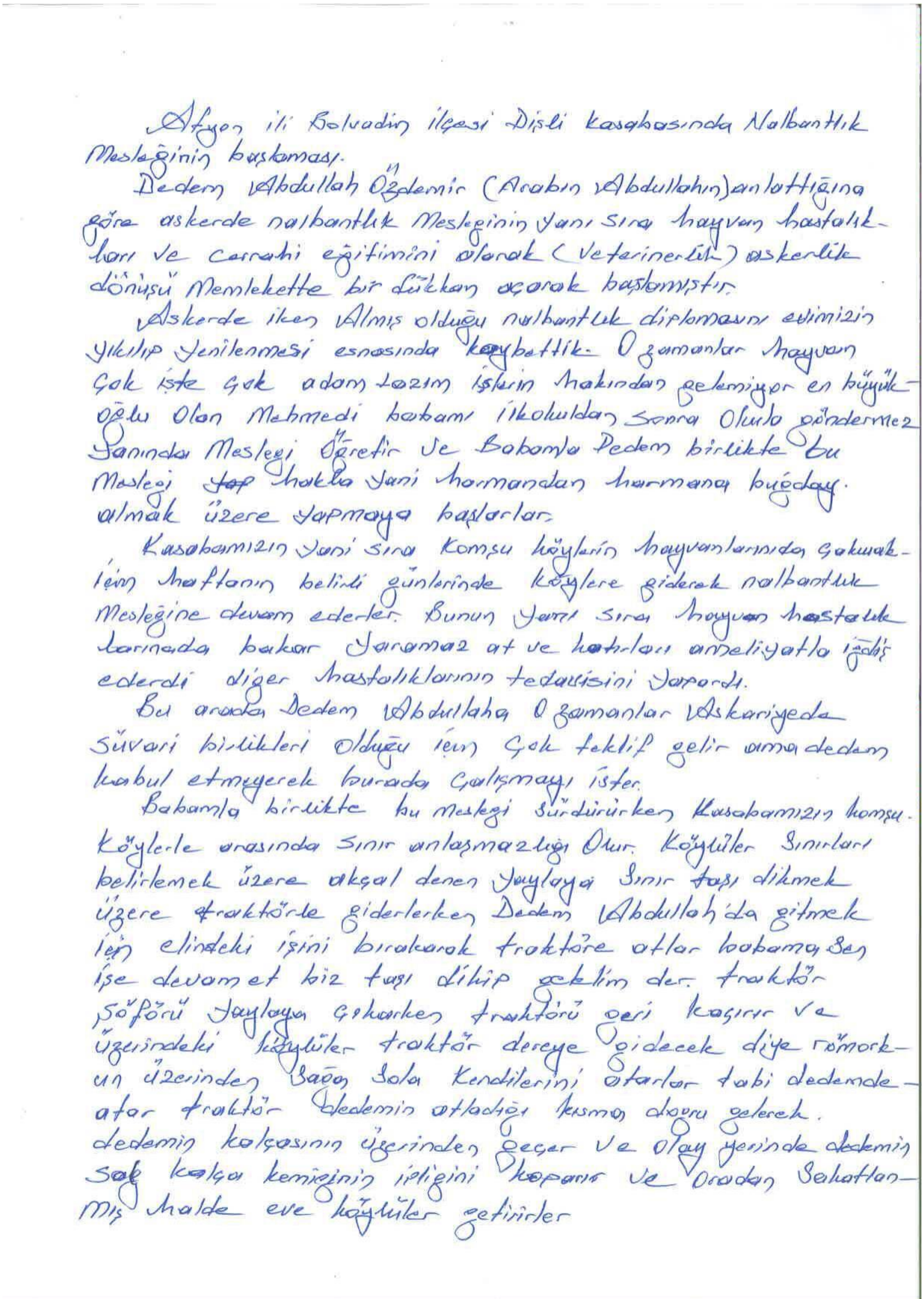
Afyonkarahisar is among of Turkey's important provinces with both of geographical position and present livestock potential (Cicek, 2005). The buffalo breeding also survives as an economic source and buffalo farming is common business in Afyonkarahisar (Çiçek and Tandoğan, 2010). There was also great number of working equines in and around Afyonkarahisar. Formerly, farriery was the most respected occupation in Afyonkarahisar. when working equines, oxes and buffalo were being used for transportation and agricultural activities.

In this chapter, Doğan Özdemir, from Dişli, a town and municipality in the Bolvadin District, Afyonkarahisar Province, wrote the history of farriery in his own town with his handwriting (Fig. ..), Mr. Doğan himself, his father (Mehmet Özdemir) and grandfather (Abdullah Özdemir, Arabın Abdullah) was famous and respectable farriers in the region. The text is concerned about their activities and exemplary life journeys. Many thanks to Doğan Özdemir for this valuable handwriting.

We also want to mention about Afyonkarahisar Agriculture, Livestock and Farriery Museum. This museum has been established with collaboration of Afyonkarahisar Governorship, municipality and Afyon Kocatepe University in 2021 (Biricik et al., 2020). Doğan Özdemir donated his family's older farrier equipments to this museum.

Below, Doğan Özdemir's handwritten text and the museum pictures are presented.

A- Doğan Özdemir's Handwritten text about his family



Afyon ili Bolvadin ilçesi Dişli kasabasında Nalbantlık Mesleğinin başlaması.

Dedem Abdullah Özdemir (Arabın Abdullahın) anlattığına göre askerde nalbantlık Mesleğinin yanı sıra hayvan hastalıkları ve cerrahi eğitimini alarak (Veterinerlik) askerlik dönüşü Memlekette bir dükkan açarak başlamıştır.

Askerde iken Almış olduğu nalbantlık diplomasını evimizin yıkılıp yenilenmesi esnasında kaybettik. O zamanlar hayvan çok işte çok adam lazım işlerin hakından gelemiyor en büyük oğlu olan Mehmedi babamı ilkokuldan sonra okula göndermez yanında Mesleği öğretir ve Babamla Dedem birlikte bu Mesleği ~~top~~ hakla yani harmandan harmana buğday almak üzere yapmaya başlarlar.

Kasabamızın yanı sıra komşu köylerin hayvanlarınıda çokluğu için haftanın belirli günlerinde köylere giderek nalbantlık Mesleğine devam ederler. Bunun yanı sıra hayvan hastalıklarınada bakar yaramaz at ve katırları ameliyatla iğdiş ederdi diğer hastalıklarının tedavisini yapardı.

Bu arada Dedem Abdullaha O zamanlar Askeriyede süvari birlikleri olduğu için çok teklif gelir ama dedem kabul etmeyerek burada çalışmayı ister.

Babamla birlikte bu mesleği sürdürürken Kasabamızın komşu köylerle arasında sınır anlaşmazlığı olur. Köylüler sınırları belirlemek üzere akçal denen yaylaya sınır taşı dikmek üzere traktörle giderlerken Dedem Abdullah'da gitmek için elindeki işini bırakarak traktöre atlar babama sen işe devam et biz taşı dikip gelelim der. traktör şöförü yaylaya çıkarken traktörü geri kaçırır ve üzerindeki köylüler traktör dereye gidecek diye römorkun üzerinden sağa sola kendilerini atarlar tabi dedemde atar traktör dedemin atladığı kısma doğru gelerek dedemin kalçasının üzerinden geçer ve olay yerinde dedemin sağ kalça kemiğinin iliğini koparır ve oradan sakatlanmış halde eve köylüler getirirler

Dedem Abdullah günlerce süren tedavinin fayda etmediğini
görünce sonunda Babam Eğridirde bulunan kemik hastanesine-
götür Orada ameliyatla Sağ kalça Kemiğini Sabit bükülmeye-
cek şekilde tedavi ederler ve dedem Ondan Sonra nalbant-
lık Mesleğini Sürdüremez. Ancak Sandalyede dükkanın Önünde
Oturur babamı kontrol ederdi.

Artık nalbantlık Mesleğini babam devam ettiriyor babamın
büyük oğlu ben olduğum için ilkokul dörtde iken babam.
nal kesiyor bende ona gücüm yetmedi için iki elimle bir-
likte çekiç vuruyorum. Babam bu mesleğin Sonu yok Okula.
gidip okumamızı boş zamanlarda Ona yardım yapmamızı ve
bu mesleğide devam ettirmemi istiyordu.

Dedem dükkanda Oturuyor babamın hayvanı yatırmasına.
ayaklarını bağlamasına tırnak tutma, nal verme, çivi verme
gibi işleride ben yapıyordum boş zamanlarda dedemin en -
büyük erkek torunu olduğum için beni yanına çağırıyor
hayvan hastalıklarını anlatıyor ne yapmam gerektiğini
anlatırdı. Harman zamanı geldiğinde babam dükkanda
hayvan nallıyor bende eşekle harman yerlerini dolanıp alacak
olduğumuz buğdayı topluyordum.

Günler haftaları, haftalar ayları aylarda yılları
Kovaladı ben Lise çağlarına geldiğimde Mesleği Öğrenmiştim
Dedem 1986. Yılında Vefat etdi artık babamda bana
ne yapılması gerektiğini Öğretti ama işler giderek azalıyordu-
Zaman . zaman Motorlu taşıtlar Çıkınca Kasabada hayvan
azaldı bende Meslek Lisesini bitirdim Babam bana.
oğlum ikimizin dükkanda durmasına gerek yok geleni ben-
iyi kötü nallarım Sen kendine bir iş bul diyerekten beni
iş aramam için gönderdi. Bende 1987. Yılında Orman işletme
Müdürlüğünde işe başladım_ Ondan Sonrada işler durma
noktasına geldi artık Malzeme Parasını kazanamaz hale
geldik ve ve ~~di~~ babamda kalp rahatsızlığı nedeniyle.
1996 yılında tamamen işi bıraktık 2001. Yılındada babam
hakkın rahmetine kavuştu.

Fig. 1: Abdullah Özdemir

Fig. 2: Mehmet Özdemir

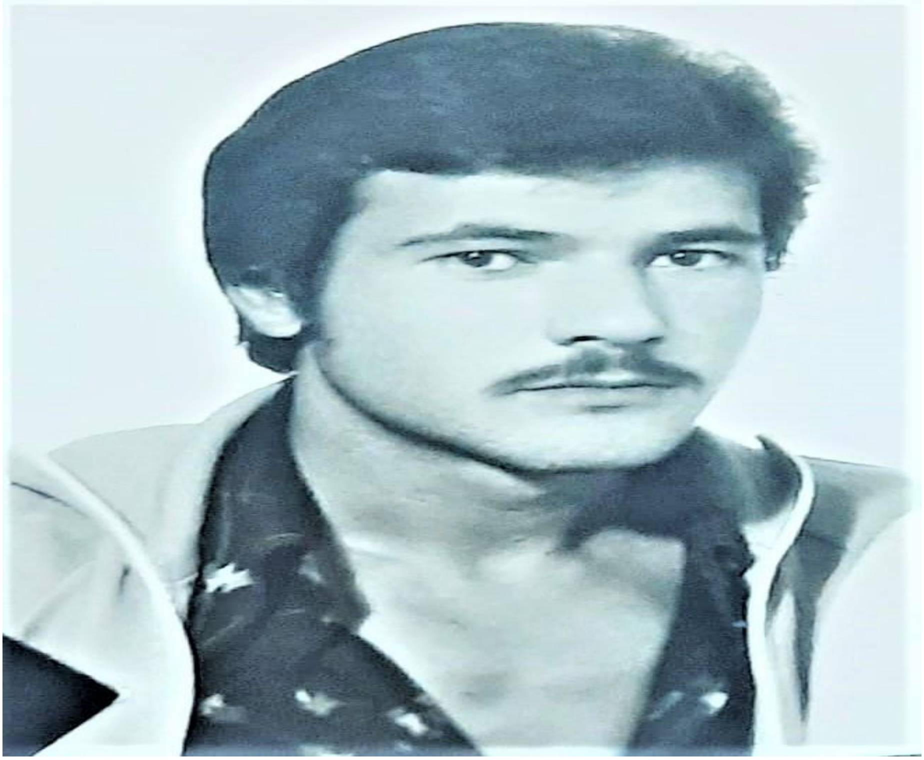


Fig. 3: Doğan Özdemir

B: Afyonkarahisar Agriculture, Livestock and Farriery Museum Equipments

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CHAPTER 12

AFYONKARAHİSAR AGRICULTURE, LIVESTOCK AND FARRIERY MUSEUM

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Introduction

The agricultural activities in Turks are seen in all the periods in which we can follow the history. Agricultural activities have been practiced in Anatolia since the early periods of history. The main source of livelihood of the people was agriculture and livestock just like today (Atmaca, 2018; Bülbül, 2017)..

Farriery or blacksmithing is the art of hoofing, which is used to protect the soles of the hooves of pack and riding animals such as horses, donkeys, mules, and oxen. A person who nails the hooves of ungulates is called a farrier. Farriers and farriery practices play a crucial role in equine husbandry (Khan et al, 2022). There are an estimated 116 million equines (donkeys, horses and mules) in the world according to a report informed by the Food and Agriculture Organization of the United Nations (Anonim).

The words "If a nail falls, the shoe falls, if the shoe falls, the horse falls, if the horse falls, the cavalry falls, if the cavalry falls, the army falls and the war is lost" attributed to Genghis Khan are still spoken today. From this perspective, farriery was also a valid profession in the military field in ancient times. Horseshoes served the same function then as automobile tires do today. In 1883, a "Horseshoe Factory" was established in the Nuruosmaniye district of Istanbul to supply the horseshoes needed by the animals in the regiments and battalions of the army. Modern farrier courses were given in the Military Veteran's School in 1888 to train farriers for the Ottoman army, and a school that trained farriers was opened in Konya during the War of Independence.

Since the 1990s, with the proliferation of motor vehicles in parallel with the development of technology, interest in this profession has decreased and blacksmithing has come to an end (Balcı, 2020).

Afyonkarahisar is among of Turkey’s important provinces with both of geographical position and present livestock potential (Cicek, 2005). Afyonkarahisar Agriculture, Livestock and Farriery Museum has been established with collaboration of Afyonkarahisar Governorship, municipality and Afyon Kocatepe University in 2020 (Biricik et al., 2020), under scope of Erasmus+ Vocational Education project titled as “***Reviving of Farriery; Disappearing Profession***”. The museum has impressive historical collections and dedicated preservation of cultural heritage, to trace the history of agriculture, livestock and farriery. This museum has been mentioned in the local press (https://twitter.com/nalbantprojesi/status/1462110694950719488).

Below, Afyonkarahisar Agriculture, Livestock and Farriery Museum and equipment pictures are presented.

A: Afyonkarahisar Agriculture, Livestock and Farriery Museum

B: Afyonkarahisar Agriculture, Livestock and Farriery Museum Equipments

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