Advances in Animal Health, Nutrition, and Sustainability

Immunity, Disease Management, and the Impact of Global Challenges

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ADVANCES IN ANIMAL HEALTH, NUTRITION, AND SUSTAINABILITY: IMMUNITY, DISEASE MANAGEMENT, AND THE IMPACT OF GLOBAL CHALLENGES

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Preface

Dear Readers,

The book in your hands is a comprehensive summary of the rapidly advancing knowledge and practices in animal health, nutrition, and sustainability in recent years. Animal nutrition holds strategic importance in modern livestock management, not only for enhancing production performance but also for strengthening the immune system, preventing diseases, and playing a role in treatment strategies. Additionally, global challenges such as climate change, global warming, and increasing resource pressures profoundly impact the livestock sector, necessitating the development of sustainable solutions.

The primary motivation behind preparing this book was to bring together innovative approaches and findings from researchers across diverse disciplines to create a valuable resource for both the scientific community and practitioners. The articles included in this book provide a wide range of insights, from the role of food in supporting immunity to the effects of global warming on feed supply, from the application of artificial intelligence in livestock farming to the preservation of professions on the brink of extinction.

Key topics covered in this work include:

- Foods and nutrients influencing immunity,
- Clinical applications of serum amyloid A in horses,
- Effects of yeast (Saccharomyces cerevisiae) on the immune system in poultry nutrition,
- The contribution of micronutrients in dairy products to immune health,
- Future applications of artificial intelligence in the livestock sector,
- The role of circular RNA in animal husbandry,
- The impact of climate change on animal nutrition.

We hope this book will serve as a scientific guide for readers, offering new perspectives to address industry challenges and contributing to sustainable

livestock practices. We extend our gratitude to all the authors and researchers who have contributed their inspiring ideas and efforts to this endeavor.

Sincerely,

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CHAPTER 1

FOODS AND NUTRIENTS THAT AFFECT IMMUNITY

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1. INTRODUCTION

Dietary micronutrients and macronutrients are essential for immune cells to maintain their essential functions. Despite compelling evidence that these substances can modulate many immune parameters, nutrient intake and immune status are rarely considered or described in most animal and human immune function studies (Field et al., 2002). This has paved the way for the development of nutritional immunology, a branch of science that emerged from the convergence of the fields of nutrition and immunology, aiming to define the roles of nutrients in immune cell metabolism and function at the molecular, cellular, tissue, and organismal levels (Wu et al., 1999).

The immune system acts as the body's primary defense mechanism by protecting the body from a variety of pathogens. It consists of two main components: the innate (nonspecific) immune system and the adaptive (specific) immune system. The adaptive immune system develops over time and provides a more specific response to infections. The innate immune system acts as the body's first line of defense against many pathogens (Greenberg and Grinstein, 2002).

The immune system is a complex system in the body composed of white blood cells such as monocytes, lymphocytes, neutrophils and macrophages, and specialized immune substances such as antibodies, cytokines and proteins. These components work together to provide resistance and protection against infections and various diseases. The interactions between these various immune cells and mediators are essential for generating effective immune responses (Delves and Roitt, 2000). Adequate and balanced nutritional intake is of great importance for optimal effectiveness of host defense. Studies have identified micronutrients such as amino acids, fatty acids, vitamins and minerals that have an effect on immune responses in animals and humans. Some of these nutrients have been shown to have specific effects on immunity when administered to the body in pharmacological doses (Trichet, 2010).

2. MACRONUTRIENTS AND IMMUNITY

2.1. Proteins

It has long been known that protein deficiency impairs immune function and increases disease susceptibility in animals. Recently, the cellular and molecular mechanisms underlying this effect have become clearer. Dietary

protein deficiency leads to decreased plasma levels of essential amino acids such as arginine, tryptophan, cysteine, and glutamine (Wu et al., 1999). During immune stress, these plasma amino acids are directed not to protein synthesis processes such as growth and lactation, but to tissues involved in inflammation and immune response. For example, they participate in the production of other essential compounds needed for body defense, support the proliferation of immune cells, and contribute to inflammatory protein synthesis (Le Floc'h et al., 2004). Research findings suggest that dietary supplementation with certain amino acids can enhance immune function and reduce morbidity and mortality in animals and humans affected by infectious diseases and malnutrition (Li et al., 2007).

Findings have shown that amino acids play an important role in regulating immune responses in the body and within immune cells (Kaplan and Yildiz, 2012; Li and Wu, 2022). These effects include:

(a) Activation of natural killer cells, B and T lymphocytes and macrophages, (b) Regulation of cellular redox status, gene expression and lymphocyte growth, (c) Production of antibodies, cytokines and other cytotoxic substances by specialized immune cells including B-lymphocytes, CD8+ and CD4+ T-lymphocytes, (d) Ensuring synchronized interaction between the adaptive and innate immune systems.

The innate and adaptive immune systems are regulated by a highly interactive chemical network that includes processes such as antigen presentation, immunoglobulin synthesis, and cytokine production (Calder, 2006). In addition to the production of polypeptides and proteins, these immune systems also depend on the availability of sufficient amino acids for the production of other biologically essential molecules (Kim et al., 2007). Examples of these include important compounds such as superoxide, hydrogen peroxide, nitric oxide, histamine, anthranilic acid, and glutathione. Amino acids play an important role in this process by directly interacting with immune responses or indirectly regulating immune responses through specific metabolites (Li et al., 2007).

2.1.1. Arginine and Citrulline

Arginine can be synthesized from citrulline in almost all cell types. This synthesis process occurs via the citrulline-nitric oxide or arginine-citrulline

cycle (Wu and Morris Jr, 1998). In addition, citrulline can be synthesized in the small intestine of most mammals except cats and ferrets using glutamine, proline, and glutamate (Wu, 1998). The main role of arginine in the immune system is through the catabolism of nitric oxide, which mediates inflammation in macrophages and has a strong cytotoxic function (Fuchs and Bode, 2006). Some research results on arginine are given in Table 1.

Table 1. Some research results on arginine

Medicine	**Effect**	
Mouse	It has been reported that insufficient arginine use in the diet of young rats reduces the amount of plasma arginine and negatively affects nitric oxide synthesis; thus, the immune system is negatively affected.	a
Mouse	It has been reported that 2% arginine supplementation in the diet reduces mortality after microorganism contamination and increases survival rate after infection.	b
Pig	It has been reported that arginine supplementation in the diet increases the immune status and reduces morbidity and mortality in pregnant pigs.	c
Person	Studies show that arginine supplementation may reduce the need for respiratory support and shorten the length of hospital stay in COVID-19 patients.	d

Source; a: (Wu et al., 1999), b: (Adjei et al., 1994), c: (Kim et al., 2007), d: (Fiorentino et al., 2021)

Nitric oxide is a free radical molecule produced in almost all mammalian cells by the enzyme inducible nitric oxide synthase from arginine (Morris Jr, 1999; Bronte and Zanovello, 2005). Expression of iNOS (inducible nitric oxide synthase) in leukocytes is induced in response to stimuli such as IFN-γ and lipopolysaccharide (LPS). It is now well established that nitric oxide plays a critical role in both the innate and adaptive immune systems (Bogdan et al., 2000). The large amounts of nitric oxide synthase synthesized have a cytotoxic effect on viruses and pathogenic microorganisms (Bronte and Zanovello, 2005). Consequently, this free radical serves as a major mediator of the immune response in animals (Kim et al., 2007).

Recent studies have shown that nitric oxide production by inducible nitric oxide synthase (iNOS) in neutrophils and macrophages serves as an

important defense mechanism in lower vertebrates, including mammals, birds, terrestrial animals, invertebrates and fish, protecting them against various threats such as bacteria, viruses, fungi, cancerous cells, intracellular protozoa and parasites (Bronte and Zanovello, 2005).

2.1.2. Glutamine

Glutamine is an amino acid used in large quantities by cells of the immune system. There is ample evidence from in vitro tests that the essential functions of these cells depend on glutamine supply. The increased demand for glutamine, particularly during activation of lymphocytes and macrophages, highlights the essential role of glutamine in the functioning of these cells and in the body's ability to mount a strong immune response (Calder and Yaqoob, 1999).

Glutamine enhances immune system function by increasing the phagocytosis activity of macrophages, stimulating cytokine production by T lymphocytes, and facilitating antibody production by B lymphocytes (Parry-Billings et al., 1990; Field et al., 2002). IL-2 production depends on the glutamine concentration in the medium. IL-2 is crucial for regulating T cell proliferation and is secreted by activated T lymphocytes (Yaqoob and Calder, 1997). In addition, the phagocytosis ability and IL-1 release ability of macrophages also depend on the glutamine concentration in the medium (Calder and Yaqoob, 1999). Some research results on glutamine are given in Table 2.

Glutamine also contributes to the strengthening of the immune system thanks to its effects on improving intestinal health, since glutamine, together with glucose, acts as the main energy substrate for high-turnover cell groups such as enterocytes, fibroblasts and activated immune cells. Therefore, it plays an effective role in maintaining intestinal trophism and reduces the risk of pathogen translocation (Trichet, 2010). In addition, glutamine has the ability to restrict the release of inflammatory cytokines secreted by intestinal cells, such as IL-6 and IL-8 (Coëffier et al., 2001). Thanks to these properties, glutamine is considered an important dietary component in maintaining intestinal integrity (Neu et al., 2002).

Table 2. Some research results on glutamine

Medicine	Effect	
Mouse	It has been reported that dietary supplementation with 4% glutamine reduces mortality after microorganism contamination and increases survival after infection.	a
Chicken	It has been reported that 1% glutamine supplementation to broiler diets causes an increase in the weight of the lymphoid organs thymus and spleen.	b
Chicken	It has been reported that the injection of 40 mg glutamine into eggs of broiler chickens increases the vitality, has an immune system strengthening effect and reduces the mortality rate.	c
Chicken	Addition of 1% L-glutamine to broiler diets has been reported to strengthen immunity and reduce mortality by increasing hormone and cytokine production in response to LPS injection or by reducing oxidative stress.	d
Person	Adding glutamine to the diet in the early stages of COVID-19 infection has been reported to shorten the length of hospital stay and reduce the need for intensive care.	e

Source; a: (Adjei et al., 1994), b: (Bartell and Batal, 2007), c: (Sözcü, 2018), d: (Dilber, 2021), e: (Cengiz et al., 2020)

Glutamine also plays a role in supporting the immune system by protecting against oxidative stress. L-glutamate, one of the products of glutamine metabolism, is very important in this process (Newsholme et al., 2003). Glutamine, an important source of glutamate, plays an effective role in the production of glutathione, a tripeptide necessary to protect cells from oxidative stress (Wu et al., 2004). Glutathione has the ability to combine with various electrophiles and xenobiotics to scavenge free radicals and other reactive oxygen species (e.g., hydroxyl radical, lipid peroxyl radical, peroxynitrite, and H_2O_2), enabling detoxification of these compounds (Fang et al., 2002).

Additionally, evidence suggests that glutamine contributes to the synthesis of arginine, which is needed for nitric oxide production in monocytes and macrophages. Arginine derived from glutamine may play a vital role in maintaining macrophage activity (Murphy and Newsholme, 1998).

2.1.3. Cysteine

In septic patients, the need for sulfur amino acids, especially cysteine, plays an important role. Cysteine is an amino acid whose metabolism during sepsis is well characterized. In addition to its use in acute phase protein synthesis, cysteine's most important effect on the immune system is its role in glutathione synthesis (Grimble and Grimble, 1998).

The metabolism of cysteine, which acts as an important precursor of glutathione in animal cells, shows marked variability in response to infectious conditions (Malmezat et al., 2000).

Glutathione is a vital antioxidant that plays an important role in protecting the cell against oxidative stress, and the presence of cysteine is an important factor that exerts a limiting effect on glutathione synthesis (Wu et al., 2004). Therefore, supplementation of diets with N-acetylcysteine, a stable precursor of cysteine, stands out as an effective strategy to enhance immune functions in the management of various diseases (Grimble, 2001).

2.1.4. Tryptophan

Anthranilic acid, serotonin, melatonin and N-acetylserotonin are among the important end products of tryptophan catabolism (Kim et al., 2007). Tryptophan plays a role in the immune system by mediating these byproducts. Melatonin, serotonin and N-acetylserotonin have the potential to enhance host immunity by reducing the production of superoxides and inflammatory cytokines, neutralizing free radicals and also reducing the production of TNF-α. Anthranilic acid, a byproduct of tryptophan metabolism via indolamine 2,3-dioxygenase, suppresses the production of proinflammatory Th-1 cytokines, thus preventing autoimmune diseases and potentially alleviating neuroinflammation (Perianayagam et al., 2005; Platten et al., 2005; Kaplan and Yıldız, 2012).

2.1.5. Aspartate and Glutamate

Aspartate acts as a precursor in the production of purine and pyrimidine nucleotides. Lymphocytes must proliferate to mount an effective immune response, making aspartate essential for their proliferation (Newsholme and Calder, 1997). In addition, aspartate has an important effect on the immune system by modulating the conversion of citrulline to arginine, which is produced by inducible nitric oxide synthase (iNOS) in activated macrophages

(Wu and Brosnan, 1992). Aspartate thus plays a critical role in maintaining adequate intracellular arginine concentrations to maintain high levels of nitric oxide synthesis required for an effective immune response (Li et al., 2007).

Aspartate and glutamate may affect the immune system by acting as excitatory neurotransmitters on metabotropic and ionotropic receptors in both the central and peripheral nervous systems (Newsholme et al., 2003). Glutamate indirectly regulates immune function by regulating iNOS expression in certain tissues such as the brain (Wu and Meininger, 2002).

The main effect of glutamate on the immune system is due to its leading role in glutathione synthesis. Glutamate plays an important role in the elimination of oxidants by participating in glutathione production and is effective in the regulation of the immune response (Bazer et al., 2004).

2.1.6. Branched Chain Amino Acids

The utilization and transport of branched-chain amino acids (BCAAs) by lymphocytes increases in response to mitogen stimulation. Furthermore, the uptake of these amino acids peaks in the S phase of the cell cycle, i.e., during the DNA synthesis phase (Koch et al., 1990). Since leukocytes cannot synthesize the carbon skeletons of branched-chain amino acids (BCAAs), a deficiency of valine, leucine, or isoleucine in culture medium results in complete cessation of protein synthesis or proliferation in lymphocytes in response to mitogen stimulation (Waithe et al., 1975). This may explain why lymphocyte proliferation is impaired when the extracellular concentration of BCAAs falls below 0.2 mM, a value close to plasma levels; this is often seen in individuals with protein deficiency (Skaper et al., 1976).

Animal studies have shown that inadequate BCAA intake leads to immune system dysfunction (Li et al., 2007). In summary, the demand for BCAAs increases during active proliferation and division of immune cells, and these amino acids play a vital role in immune responses. However, when BCAA concentrations in the medium were adjusted between 0.2 and 1 mM (approximately 1 to 5 times plasma levels), no effect on lymphocyte proliferation was observed (Skaper et al., 1976). A study in broiler chickens showed that feeding the BCAA, valine, at recommended or higher levels did not significantly affect antibody titers to Newcastle virus vaccine (Kaplan and Yildiz, 2017). Based on these findings, it can be concluded that maintaining

normal plasma levels of BCAAs is crucial for immunity, and further research is needed to understand the effects of levels above the normal range on immune function.

2.2. Fats

Fats and fatty acids are essential nutritional components of the diet and, like other nutrients, have important effects on the immune system. Fatty acids, in particular, are vital in regulating immune responses and inflammation due to their anti-inflammatory properties (Fritsche, 2006).

2.2.1. Omega-3 Fatty Acids

The beneficial effects of Omega-3 fatty acids, a type of polyunsaturated fats, on the immune system and inflammation are well known (Barazzoni et al., 2020).

Omega-3 fatty acids positively support immunity by affecting different immune cells. They can affect macrophages in three different ways. The first effect is that omega-3 fatty acids probably affect the membrane structure of macrophages and increase their phagocytic activity. Second, omega-3 fatty acids may be beneficial in reducing brain damage in pathological conditions such as stroke by reducing M1 polarization and increasing M2 polarization in macrophages. The third effect is that these fatty acids can reduce inflammation by reducing the expression and secretion of interleukins, cytokines, and chemokines (Gutiérrez et al., 2019).

Eicosapentaenoic acid (EPA), a type of omega-3 fatty acid, is essential for immune function and has been shown to be effective in managing a number of autoimmune diseases resulting from immune system abnormalities. Additionally, it helps control immunologic disorders and inflammation by inhibiting the production of cytokines and leukotrienes (Narayan et al., 2006).

The effects of docosahexaenoic acid (DHA), a subgroup of omega-3 fatty acids like EPA, on immunity are similar but not identical to EPA. EPA primarily affects cell cycle pathways, while DHA primarily regulates immune response pathways. DHA can also stimulate the production of reactive oxygen species (ROS) by neutrophils (Paschoal et al., 2013). These reactive oxygen species play a critical role in antimicrobial defense and inflammatory processes because they can increase cellular apoptosis and transcriptional signaling (Shaikh and Edidin, 2007).

3. MICRONUTRIENTS AND IMMUNITY

3.1. Vitamins

3.1.1. Vitamin A

A constant supply of vitamin A is needed for the proper functioning of the immune system; therefore, it is considered an essential component of the diet. Vitamin A is found in the diet in different forms such as retinyl-esters, all-transretinol and beta-carotene (Mora et al., 2008).

Of these, retinol is necessary for the regulation of cell-mediated and innate immune functions. In addition, it plays a role in regulating the responses of humoral antibodies produced by the immune system. Retinoic acid, another derivative of vitamin A, has regulatory effects on the innate immune system. Another function of retinoic acid is that it significantly affects the development, differentiation and effective functioning of various immune components (Huang et al., 2018).

Vitamin A deficiency has negative effects on the immune system and cellular functions. It has been observed that phagocytic activity is reduced and B and T cell functions are impaired in organisms with vitamin A deficiency. It has also been reported that vitamin A deficiency reduces interferon production and natural killer cell activity, weakens fixed fat macrophage activity and the response of lymphocytes to mitogen stimulation. In organisms with adequate vitamin A levels, resistance to infections and proper immune function are observed (Meydani et al., 2001).

Carotenoids are pigments found in plants and in colorful fruits and vegetables. Some of these pigments exhibit provitamin A activity and can be converted to vitamin A in the body. Carotenoids act as antioxidants by neutralizing reactive chemical species, including photochemical sensitizers, singlet oxygen, and free radicals (Burton, 1989). Through these effects, carotenoids may directly or indirectly regulate immune function. In vitro and in vivo studies have shown increased immunity in humans and animals receiving carotenoid supplements (Chew, 1993). In addition to protecting immune cells against reactive oxygen species, carotenoids may also regulate immune function by increasing cell-to-cell communication, altering membrane fluidity, and inhibiting arachidonic acid oxidation (Chew, 1996).

3.1.2. Vitamin E

The main effect of vitamin E on the immune system is attributed to its antioxidant properties, especially due to its important role in protecting lipids in immune cells from peroxidation. Furthermore, the concentration of vitamin E in immune cells is directly related to the amount taken in the diet (Trichet, 2010). Some research findings on vitamin E are summarized in Table 3.

Table 3. Some research results regarding Vitamin E.

Medicine	Effect	
Chicken	Dietary supplementation of 150-300 mg vitamin E has been reported to reduce mortality in broiler chicks infected with E. coli.	a
Sheep	It has been reported that dietary vitamin E supplementation in lambs infected with chlamydia does not prevent typical lesions but accelerates healing. In addition, it has been reported that pneumonia is less common in lambs supplemented with vitamin E during autopsy examination.	b
Cattle	It has been reported that vitamin E supplementation increased the immune response in Holstein calves that were given vitamin E from birth to the 24th week.	c
Fish	It has been reported that vitamin E supplementation improved the immune parameters studied in fish whose diets were supplemented with different levels of vitamin E.	d

Source; a: (Heinzerling et al., 1974), b: (Stephens et al., 1979), c: (Reddy et al., 1987), d: (Lin and Shiau, 2005)

Vitamin E interacts with peroxyl radicals produced from polyunsaturated fatty acids in lipoproteins or membrane phospholipids, leading to the formation of stable lipid hydroperoxides. Through this process, vitamin E reduces the harmful effects of lipid free radicals and protects tissues from oxidative damage. This antioxidant effect, which prevents lipid peroxidation, is one of the mechanisms by which vitamin E strengthens immune function (Chew, 1996).

3.1.3. Vitamin C

In addition to acting as a cofactor in various enzymatic processes, vitamin C enhances innate and adaptive immune responses by reducing cellular oxidative stress and strengthens immune function (Carr and Maggini, 2017).

Vitamin C accumulates in leukocytes at levels 50–100 times higher than plasma and is rapidly depleted during infection (Shakoor et al., 2021). Accumulation of vitamin C in neutrophils promotes bacterial cell death by increasing chemotaxis and phagocytosis of neutrophils. It also helps prevent tissue damage by promoting the removal of spent neutrophils from the site of infection (Carr and Maggini, 2017).

It is widely known that vitamin C has protective effects, especially against infectious diseases. Vitamin C supplementation is known to reduce the duration and severity of viral infections by supporting respiratory defense mechanisms. It can even help prevent such infections and has antihistamine properties that relieve flu-like symptoms (Shakoor et al., 2021).

Vitamin C has many beneficial properties on the immune system. These properties can be listed as follows;

Vitamin C is an important antioxidant that protects various biomolecules, including lipids, proteins, and nucleotides, from oxidative damage and dysfunction by inhibiting reactive oxygen species (Shakoor et al., 2021).

It is widely known for its role in stimulating leukocyte activity by promoting neutrophil and leukocyte functions (Alpert, 2017).

Vitamin C exhibits antiviral properties by reducing inflammation, promoting interferon-alpha production, and improving endothelial dysfunction (Carr and Maggini, 2017; Dey and Bishayi, 2018). It has also been shown to reduce proinflammatory cytokine levels while increasing anti-inflammatory cytokine production (Shakoor et al., 2021).

Both animal and human studies indicate that vitamin C is a powerful regulator of the immune system. In animals, vitamin C deficiency has been associated with reduced phagocytosis and antimicrobial activity in macrophages. Conversely, higher vitamin C concentrations have been associated with increased mitogen-stimulated antibody production by peripheral blood lymphocytes and improved neutrophil function (Long and Santos, 1999; Erickson et al., 2000).

Trichlet reported a positive relationship between cellular vitamin C concentration and phagocyte activity in rainbow trout (Trichet, 2010). In another study, Gross and colleagues found that supplementing diets with vitamin C in laying hens increased resistance to E. coli infections (Gross et al., 1988).

3.2. Minerals

Trace elements, which constitute an important category by playing a critical role in various physiological processes, are also critical for the optimum functioning of the immune system (Lukác and Massányi, 2007). Imbalances, deficiencies or excesses in the levels of trace elements can negatively affect the immune system by affecting the activities of immune components. This also affects both cellular and innate immune responses by regulating antibody responses to antigens (Beck, 1999).

3.2.1. Zinc

Zinc, a vital trace element in living organisms, is critical for numerous physiological processes by serving catalytic, structural, and regulatory functions for enzymes, proteins, and transcription factors. Therefore, it plays an important role in both innate and adaptive immune system cells and immune responses. Zinc also helps regulate the production of antibodies and cytokines, as well as the activity of the complement system. Therefore, zinc deficiency can have a significant impact on health by leading to impaired immune function (Chandra, 2004; Weyh et al., 2022).

Zinc, which plays a role in many biological processes in the living body, including the immune system, is of vital importance against viral infections due to its ability to modulate host defense mechanisms, especially during viral diseases (Biaggio et al., 2010; Gammoh and Rink, 2019). Zinc can provide beneficial and therapeutic effects against viral infections by directly affecting viral replication and protein synthesis (Skalny et al., 2020).

Zinc plays a vital role in chemotactic activity and the assembly of neutrophil granulocytes. It is also known to have positive effects on natural killer cells, phagocytosis, CD4+ and CD8+ T cells and oxidative burst processes (Te Velthuis et al., 2010).

In humans and animals, zinc deficiency has a suppressive effect on thymus function, lymphoproliferation, T-lymphocyte development and T cell-mediated B cell functions, impairing lymphocyte number and functions, which negatively affects the immune system (Fraker et al., 1993; Te Velthuis et al., 2010).

3.2.2. Iron

Iron is a trace element recognized for its important role in both host immunity and pathogen proliferation due to its beneficial properties. Since it is an essential component for the survival of living systems, both host cells and invading microbes require sufficient iron to maintain their functions. The immune system has evolved to reduce the amount of iron available to invading pathogens within a few hours of the onset of infection. This process creates a state of hypoferremia in the organism, and iron concentrations in the extracellular fluid and plasma are significantly reduced to limit the pathogenic properties of microorganisms (Ganz, 2009; Weiss and Carver, 2018).

Iron affects the immune system by regulating the proliferation and differentiation of immune cells, including T cell development, and by maintaining the balance between T-helper and T-cytotoxic cells. It also controls immune cell functions and directly interacts with antimicrobial immune defense mechanisms, such as the production of toxic radicals by macrophages (Rosen et al., 1995; Haryanto et al., 2015).

In summary, maintaining adequate iron levels in the living body is important for vital functions such as oxygen transport and energy metabolism, and for a strong immune response against invading pathogens. This plays a critical role in optimal immune system function and proper immune competence (Weinberg, 2009).

3.2.3. Selenium

Selenium, although found in minimal concentrations in the body, plays an important role in the immune system due to its beneficial properties. Selenium, which protects the body against viral infections, exhibits antioxidant properties and protects the cell from oxidative stress as it participates in various enzymatic functions in the catalysis of glutathione peroxidase, deiodinase and thioredoxin reductase enzymes (Razaghi et al., 2021; Sadeghsoltani et al., 2021).

Selenium, known for its anti-inflammatory and antiviral effects, is incorporated into protein structures to form selenoproteins. These selenoproteins strengthen the host defense system by exhibiting antioxidant properties and regulating normal immune function. As a result, selenium increases the activity of natural killer cells and leukocytes in the fight against

infectious pathogens. Selenium deficiency can be a serious risk factor for viral infections (Guillin et al., 2019; Sadeghsoltani et al., 2021).

Studies have shown that selenium activates glutathione peroxidase-1, a cytolytic enzyme responsible for antiviral properties. Studies show that various selenoproteins such as glutathione peroxidase-1 help balance inflammation and oxidative stress levels, especially in the context of SARS-CoV-2 (Seale et al., 2020). In addition to its vital role in immune system regulation, selenium also stimulates the production of interferon-gamma (INF-γ) and T-helper cells, while enhancing immunity by regulating antibody production (Saeed et al., 2016; Moghaddam et al., 2020).

3.2.4. Bor

Boron is an essential trace element that is necessary and vital for the health of plant, animal and human metabolism. Studies on the biochemical mechanisms of action of boron addition to the diet have shown that boron has a positive effect on animal welfare and health. In addition, studies have revealed the role of boron in antioxidant defense mechanisms and improving immunity (Çelik and Çetinkaya, 2019).

It is suggested that the effect of dietary boron on immunity may be due to immunostimulatory effects, such as increasing the activity of natural killer cells in various organisms and positively affecting the proliferation of T cells (Hunt, 2003; Khaliq et al., 2018; Çelik and Çetinkaya, 2019). It is likely that boron exerts these effects through its positive role in immune system organs such as the thymus and spleen (Khaliq et al., 2018).

In summary, the use of boron in the diet is effective in the immune system by increasing cellular immunity, reducing DNA damage and contributing to the preservation of cell membrane stability at high temperatures (Abdelnour et al., 2018). However, an important fact about the use of boron is that it can have immunostimulatory and immunosuppressive effects depending on the dose used. While boron added in appropriate amounts to the diet can have a positive effect on immunity, the use of high doses can lead to toxic effects, increase mortality and negatively affect the development of immune organs (Bulut, 2023).

4. FUNCTIONAL FOODS AND IMMUNITY

4.1. Phytochemicals

The term “phytochemical” is a general term for chemicals or active substances obtained from plants that have unique functions and structures. In addition to the compounds that plants need for growth and reproduction, they also synthesize many phytochemicals that are not essential for their survival. These phytochemicals are synthesized in plants in response to external stimuli such as nutrition, climate change, or infection, and accumulate only in certain parts of the plant (Verpoorte et al., 1999; Tyagi et al., 2010). These phytochemicals produced in plants provide color, aroma, and taste to the plant, as well as a natural defense function. More than 4000 phytochemicals have been discovered to date (Brindha, 2016).

In addition to their protective effects on plants, phytochemicals also have therapeutic potential such as anti-diabetic, antioxidant, cholesterol-lowering, memory-enhancing effects and immunomodulatory activity (Varljen et al., 1989).

4.1.1. Alkaloids

It is known that alkaloids have immune response enhancing effects and therefore may have positive effects on the immune system. While alkaloids such as vinblastine and vincristine stand out with their antitumor effects, it is known that cepharanthine has strong antimicrobial properties and morphine is used in pain management with its analgesic effects. In recent years, research on the immunostimulating effects of alkaloids has increased and the potential roles of these substances in different immunological processes have begun to be examined more closely (Brindha, 2016).

4.1.2. Lectins

Plant lectins obtained from mistletoe have been shown to increase the cytotoxic activity and numbers of natural killer cells and to trigger antitumor activity in animal models. Therefore, it is thought that some plant lectins may have positive effects on the immune system. In addition, lectins can stimulate cytokine gene expression and protein synthesis through sugar-lectin interactions on the surfaces of immunocompetent cells and thus play an immune-enhancing role by affecting immune cells (Hostanska et al., 1996).

4.1.3. Glycosides

Glycosides act on the immune system, particularly by stimulating the central nervous system and cardiovascular system; they also have antimicrobial activity (Brindha, 2016).

4.1.4. Polyphenols

Polyphenols may help reduce inflammation by inhibiting proinflammatory cytokines and may offer immunomodulatory benefits in the management of allergic reactions and autoimmune diseases (Shakoor et al., 2021). Polyphenols, especially compounds such as quercetin, luteolin, and curcumin, have a potentiating effect on the proliferation of T and B cells. These compounds also show significant immunostimulatory effects on macrophages (Gasmi et al., 2022).

Flavonoids, known as powerful water-soluble super antioxidants, effectively neutralize free radicals and prevent oxidative damage to cells. In addition, they have significant anti-cancer properties that protect against all stages of carcinogenesis. In the body, flavonoids are known to reduce the risk of heart disease (Urquiaga and Leighton, 2000).

On the other hand, tannins are known to have various physiological effects, including host-mediated tumor activity, broad-spectrum anti-infective properties and stimulation of phagocytic cells (Okwu and Okwu, 2004).

4.1.5. Saponins

Due to their ability to promote antibody production and modulate the cellular immune system, the use of saponins as immunological adjuvants is one of their known effects (Sparg et al., 2004; Oleszek and Oleszek, 2020).

Researchers have found that saponins have antitumor effects on cancer cells. Some saponins can inhibit the growth of tumor cells by arresting the cell cycle and apoptosis. Combining saponins with traditional tumor treatment methods may lead to improvements in the success rate of treatment strategies (Sulaiman et al., 2010).

4.1.6. Terponoids

Many terpenoids have been reported to affect immune processes and have anti-inflammatory or anti-arthritic activity. Terpenoids have a twofold

effect on immunity: the first is to suppress the T cell response and the second is to increase antibody production (Brindha, 2016).

4.1.7. Sterols and Sterolins

Studies have shown that sterols and sterolins have immunomodulatory effects in certain types of cancer by increasing natural killer cell activity and affecting T cell proliferation. Additionally, it has been suggested that these compounds may help maintain a balance between Th1 and Th2 cytokines, which may contribute to improved immune responses (Bouic, 2002; Patel, 2008).

4.1.8. Phytobiotics

Phytobiotics, also known as phytogenic feed additives, are plant extracts obtained from spices and herbs. When added to animal feeds, they provide both functional and aromatic properties (Yıldız et al., 2012).

Phytobiotics may include many different bioactive elements such as alkaloids, flavonoids, glycosides, terpenoids, saponins, tannins and polyphenols (Upadhaya and Kim, 2017). The positive effects of phytogenic plants on immunity include antioxidant, antimicrobial and fungicidal properties. These plants have been used to protect foods against spoilage for many years (Yıldız et al., 2012). In addition, it is reported that they can positively affect the stability of feeds and increase storage time with their antioxidant effects (Gheisar and Kim, 2018).

Natural plant extracts (phytobiotics) can be used as an alternative to antibiotics in calf feeds due to their antimicrobial effects in order to prevent pathogenic flora in the intestines of calves. In addition, various phytobiotics have the potential to protect tissues from damage by high reactive oxygen species thanks to their antioxidant effects (Yıldız et al., 2012). Studies conducted with phytobiotics show that they can also contribute to increasing resistance to diseases, preventing diseases and maintaining product quality in poultry (Özbudak, 2019).

4.2. Probiotics

Probiotics are live microorganisms that, when ingested in adequate amounts, confer health benefits on the host (Guarner and Schaafsma, 1998). The most extensively studied and characterized probiotics belong to lactic acid

bacteria, including genera such as Streptococcus, Lactobacillus, Bifidobacterium, Leuconostoc, Lactococcus, and Pediococcus (Batista et al., 2020).

Probiotics maintain the balance of the intestinal microbiota and support the digestive-absorptive activities of nutrients. As beneficial inhabitants of the microbiome, probiotics play a vital role in regulating the immune response by showing beneficial effects in the production of various nutrients, strengthening intestinal immunity, preventing infections, eliminating toxins and destroying microbial pathogens (Corthésy et al., 2007; Duncan and Flint, 2013; Yeşilyurt et al., 2021).

Although the exact mechanisms by which probiotic strains regulate immune responses are not fully understood, their effects on the immune system are listed below:

Probiotics are effective in improving pathogen elimination mechanisms by inhibiting bacterial translocation and adhesion and affect innate immunity. Preventing tissue colonization of pathogens may contribute positively to the treatment of some infectious diseases. For example, Saccharomyces boulardii can significantly reduce some of the adverse effects encountered in eradication therapy of the pathogen by preventing Helicobacter pylori from adhering and colonizing the gastric mucosa (Naghibzadeh et al., 2022).

Probiotics may contribute to the antimicrobial activity of the intestinal microbiota by producing bacteriocins (Kanmani et al., 2013). For example, Lactobacillus species produce bacteriocins that are effective against gram-positive bacteria. Some probiotics have a broader spectrum of activity, acting on both gram-positive and gram-negative bacteria as well as yeasts and molds (Schlee et al., 2007).

Probiotics may modulate immunity by increasing the amount and activity of natural killer cells and enhancing macrophage phagocytosis (Philpott and Ferguson, 2004).

Probiotics can stimulate enterocytes to produce the cytoprotective antimicrobial peptides defensins and mucins, as well as heat shock proteins. Increasing defensin production by probiotics can be considered as an alternative treatment approach to strengthen innate immune defense mechanisms (Schlee et al., 2008).

The effects of probiotics in terms of the acquired immune response include their ability to support IgA production by B cells (Kandasamy et al., 2014).

In summary, probiotics have been reported to have beneficial effects on health in various ways, can alleviate inflammatory bowel diseases, have the ability to regulate allergic reactions, reduce tumor growth in some cancers, and protect the host from bacterial and viral infections (You et al., 2022).

4.3. Prebiotics

Prebiotics are nondigestible food substances that improve host health by selectively promoting the growth or activity of bacterial species in the colon, thus providing host benefits (Schrezenmeir and de Vrese, 2001). These food components are used as growth factors by commensal bacteria, and these bacteria compete with pathogens for the same glycoconjugates found on the surfaces of epithelial cells, preventing pathogens from adhering to and colonizing the colonic epithelium. They also support colonic barrier function, promote the production of short-chain fatty acids, and positively influence cytokine production (Walker, 2000; Korzenik and Podolsky, 2006).

Fermentation of prebiotics by commensal bacterial species results in the release of short-chain fatty acids, which have various effects on immunity. Fermentation of prebiotics in the colonic environment results in acidification of the colonic environment, which is detrimental to some pathogenic bacterial strains. In addition, acidification of the colonic environment is thought to improve mucosal morphology by increasing mucin production, thereby reducing colonization and translocation of pathogenic bacteria (Barcelo et al., 2000; Blaut, 2002; Lomax and Calder, 2008). These short-chain fatty acids support digestive health by nourishing intestinal cells and increasing the length of intestinal villi. In addition to increasing the length of intestinal villi, they also increase the number of epithelial cells in certain villi (De Vries and Stouthamer, 1967). In addition, butyrate, one of these short-chain fatty acids, helps preserve glutamine for use by gastrointestinal immune tissues by reducing the glutamine requirement of epithelial cells (Jenkins et al., 1999).

Prebiotics may also have effects on the immune system independent of the gut microbiota. Prebiotics may modulate immunity by directly affecting Toll-like receptors on gut-associated epithelial and innate immune cells.

Animal studies have shown that prebiotics such as inulin/oligofructose activate immune cells in Peyer's patches, including cytotoxicity of natural killer cells and production of IL-10. Furthermore, the ability of prebiotics to modulate secretory IgA concentrations in the ileum and cecum is an important mechanism of action in regulating immune responses and supporting intestinal health (Watzl et al., 2005).

4.4. Beta-Glucans

Beta-glucans are natural glucose polymer polysaccharides produced by plants such as oats, barley and seaweed. They are important components of the cell walls of plants as well as some bacteria, yeast and fungi (Akramienė et al., 2007). The immune effects of beta-glucans are mediated by various immune cells. Macrophages and dendritic cells are primarily affected by beta-glucans, while other immune cells such as neutrophils, T cells, B cells and natural killer cells are also activated by these compounds (Kerèkgyártó et al., 1996). Some research results on beta-glucan are given in Table 4.

Table 4. Some research results on beta-glucan

Medicine	Effect	
Mouse	Administration of yeast beta-glucans to anthrax-infected mice has been reported to reduce the bacterial load in the lungs and increase the survival rate of infected mice.	a
Chicken	Yeast beta-glucans added to the starter and grower feed of broiler chickens have been reported to improve basic immune responses, such as increasing the phagocytic activities of macrophages, enhancing the lymphoproliferative response, and increasing the size of primary and secondary lymphoid organs.	b
Horse	It has been reported that beta-glucan, applied as an adjunct to treatment in horses with babesiosis, helps blood parameters such as erythrocytes, hemoglobin and hematocrit return to normal in a shorter period of time and is effective in overcoming the disease more quickly.	c
Pig	Dietary supplementation with β-glucans derived from yeast and fungi for 2 weeks after weaning has been reported to reduce susceptibility to enterotoxin-producing E. coli infection (ETEC) and reduce fecal excretion of E. coli.	d

Chicken	Supplementation of low levels of β-glucan (0.014%) to broiler diets has been reported to increase antibody titers after vaccination against Newcastle disease.	e

Source; a: (Kournikakis et al., 2003), b: (Guo et al., 2003), c: (Altıntaş, 2006), d: (Stuyven et al., 2009), e: (Elrayeh and Yildiz, 2012)

Beta-glucans may exert modulatory effects on immunity by activating macrophages, promoting phagocytosis of pathogens, and stimulating the release of pro-inflammatory cytokines (Sato et al., 2006). However, the immunostimulatory effects of beta-glucan depend on the presence of specific receptors on the surface of macrophages and other phagocytic cells. These effects are triggered by the interaction of beta-glucan with selective scavenger receptors that initiate immune responses, such as Dectin-1, complement receptor-3, and lactosylceramide (Akramienė et al., 2007; Trichet, 2010).

In summary, the immunostimulatory and immunostimulating properties of beta-glucan are associated with their ability to activate leukocytes, stimulate phagocytic activity, stimulate the production of reactive oxygen mediators and promote the production of inflammatory mediators, including cytokines such as TNF-α (Akramienė et al., 2007; Keser and Bilal, 2008).

5. CONCLUSION

The immune system is a system that protects the host from various pathogens. The immune system in the body is a system consisting of white blood cells such as macrophages, monocytes, neutrophils, lymphocytes, and antibodies, proteins, and cytokines produced to provide resistance and protection. Adequate, balanced micro and macronutrient intake (vitamins, minerals, fats, proteins, phytochemicals, probiotics and prebiotics, β-glucans in the diet are substances that affect healthy immune response in animals and humans.

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CHAPTER 2

CLINICAL USE OF SERUM AMYLOID A IN HORSES

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1. INTRODUCTION

Controlling the health condition of each animal on the farm is one of the factors in animal production. Early detection of disease is very important for breeders and veterinarians. The acute phase response is a non-specific, systemic physiological reaction to tissue injury, which induces disturbances in homeostatic balance. In this response, acute phase proteins are synthesized. These proteins are recognized as biomarkers of the inflammatory process (Eckersall, 2000; Pollock et al., 2005; Satue et al., 2013; Trela et al., 2022).

The purpose of this systemic response is to supply energy and substrates for combating pathogens, to restrict the availability of critical metabolites to pathogens, and to minimize damage caused by pathogens or facilitate the repair of infected tissues (Petersen et al., 2004; Merhan and Bozukluhan, 2022). During the development of the acute phase response, proinflammatory cytokines acting on different receptors are synthesized by various cells, especially mononuclear cells (Ceron et al., 2005; Crisman et al., 2008; Satue et al., 2013).

Among acute phase proteins, those whose concentrations increase in the blood are classified as positive (Serum amyloid A 'SAA', haptoglobin, ceruloplasmin, fibrinogen) and those whose concentrations decrease are classified as negative acute phase proteins (prealbumin, albumin, transferrin) (Petersen et al., 2004; Merhan and Bozukluhan, 2022). Depending on the severity of the inflammation, acute phase proteins are known as major (10-100 fold increase in the first 48 hours), moderate (2-10 fold increase) or minor (slight increase) (Cray et al., 2009; Trela et al., 2022). In this book chapter, we will describe the structure, function and synthesis of SAA as well as its clinical importance.

2. STRUCTURE, FUNCTION AND SYNTHESIS OF SERUM AMYLOID A

It is an important acute phase protein in many animal species including cattle, sheep, cats and horses (Pollock, 2017). SAA is found undetectable concentration in the serum of healthy horses, but its concentration increases in diseases related to respiratory inflammation, gastrointestinal and musculoskeletal system, thus it becomes an optimal biomarker for assessing

inflammation and evaluating the effectiveness of treatment (Jacobsen and Andersen, 2007; Casella et al., 2012; Satue et al., 2013; Ludwig et al., 2023).

Structure: The molecular weight of SAA is approximately 180 kDa (Petersen et al., 2004) and each of the SAA genes encodes a different protein such as SAA1, SAA2, SAA3 and SAA4. SAA1 and SAA2 proteins are 104 amino acids long (Larson et al., 2003; De Buck et al., 2016). SAA3 is synthesized extrahepatically in mammals except humans (McDonald et al., 2001; De Buck et al., 2016). The SAA4 protein is composed of 112 amino acids and is present in the blood. Unlike SAA1 and SAA2, which are upregulated during inflammation, SAA4 is considered distinct. It is also referred to as 'constitutive SAA' or 'C-SAA', whereas SAA1 and SAA2 are classified as 'acute phase SAA' or 'A-SAA' (Whitehead et al., 1992; De Buck et al., 2016).

SAA combines with HDL, the high-density lipoprotein (HDL_3) fraction, to replace apolipoprotein (Apo) A-I, the major component of HDL in the bloodstream (De Buck et al., 2016). Isolated in 1985 (Husebekk et al., 1986), equine SAA was first measured by Pepys et al. in 1989 (Pollock, 2017). There are 3 isoforms of equine SAA (Hulten et al., 1997).

Function: SAA, whose circulating level increases 1000-fold, inhibits antibody formation by lymphocytes, induces collagenase, is chemotactic for neutrophils and monocytes and increases leukocyte adhesion to endothelial cells. It also has functions such as endotoxin detoxification, endothelial cell and lymphocyte proliferation/inhibition, inhibition of adhesion of T lymphocytes (Petersen et al., 2004; Merhan and Bozukluhan, 2022).

Synthesis: SAA is produced by the liver in response to the SAA-stimulating factor during inflammation, but it is also locally synthesized in the mammary glands, known as 'milk SAA' (MAA) (Petersen et al., 2004; Trela et al., 2022; Bozukluhan and Merhan, 2023). In addition, it is synthesized extrahepatically by adipose tissue, lungs, intestinal epithelial cells, synovial membrane, skeletal muscles, thymus, uterus, and thyroid gland as well as macrophages (Jacobsen et al., 2006; Jacobsen and Andersen, 2007; Trela et al., 2022). The normal plasma concentration of SAA in horses ranges from 0.5-20 mg/L, but in most cases exceeds 7 mg/L. SAA, which reaches concentrations ranging from 100-200 to 1000 mg/L in response to tissue damage (Hulten et al., 1999b; Satue et al., 2013), is synthesized 6-8 hours after the onset of tissue damage, peaks within 36-48 hours, and returns to baseline levels within 1-2

weeks, depending on the healing process. It is degraded in the liver within 30 minutes to 2 hours after synthesis. Therefore, its levels decrease rapidly as the underlying cause of tissue damage is resolved (Satue et al., 2013).

3. CLINICAL SIGNIFICANCE OF SERUM AMYLOID A

Inflammation is a complex protective response of body tissues involving defensive cells and inflammatory mediators to pathogens and cell damage. Early detection of inflammation is important for effective treatment. Therefore, systemic inflammation can have serious and fatal consequences (Borges et al., 2007; McGovern, 2018). Inflammation in horses can be caused by bacterial (Cohen et al., 2005), viral (Hulten et al., 1999b) as well as operation (Pollock et al., 2005) and colic (Vandenplas et al., 2005; Westerman et al., 2016).

Various studies have investigated the impact of age and sex on SAA levels in horses and reported different results. In newborns, there is an increase 72 hours after birth and the reason for this increase is related to tissue damage during birth and the release of cytokines in maternal blood (Nunokawa et al., 1993; Satue et al., 2013). In one study, SAA levels were reported as 21.23 μg/mL in foals younger than 12 months and 14.93 μg/mL in foals older than 18 months (Satoh et al., 1995). In another study, SAA levels were reported as 19.37 μg/mL in foals younger than 12 months and 21.53 μg/mL in foals older than 18 months (Nunokawa et al., 1993). Nunokawa et al. (1993) and Satoh et al. (1995) reported that SAA levels remained within the physiologic reference range ranging from 16.6-23.6 mg/L in the last 4 months of pregnancy. It is difficult to diagnose the disease in newborn foals because the clinical signs of the disease are not obvious. Therefore, SAA measurement in foals can help the diagnosis and high SAA concentrations in foals are indicative of infection. Neonatal sepsis is a critical condition, and delaying treatment can be life-threatening. As a result, measuring SAA can aid in diagnosis and help initiate the most suitable treatment more quickly (Stoneham et al., 2001; Hulten and Demmers, 2002; Satue et al., 2013).

The SAA level in operated horses is a useful parameter in monitoring patient prognosis and is a sensitive indicator of the severity of surgical trauma. The extent of the inflammatory response can be affected by factors such as the length of the surgical incision, the degree of tissue disruption, and the type of tissue involved (Satue et al., 2013). The operation alters the level of SAA in the

blood by stimulating an acute phase response (Jacobsen et al., 2005; Jacobsen, 2022). A sustained increase in SAA concentration postoperatively is an indication of postoperative infection. SAA measurement can also play a role in treating acute abdominal pain, as its levels are linked to the severity of the condition and generally normalize within 11-22 days in uncomplicated cases (Hulten et al., 1999b; Witkowska Pilaszewicz et al., 2019). In pony mares experimentally infected with equine influenza virus, SAA concentration was reported to increase by 100 mg/L 48 hours after infection, while it decreased to its normal concentration approximately 10 days after infection (Witkowska Pilaszewicz et al., 2019). In a study conducted in foals infected with *Rhodococcus equi*, it was reported that SAA levels were high but SAA levels decreased with treatment and clinical improvement (Giguere et al., 2003). In addition, acute phase protein levels can also help determine when a horse with respiratory infection can return to training after recovery (Hulten et al., 1999b; Jacobsen and Andersen, 2007, Jacobsen, 2022).

SAA in serum has been isolated from the synovial fluid of horses with arthritis (Jacobsen et al., 2006; Pollock, 2017) as well as from the peritoneal fluid of horses with gastrointestinal diseases (Pihl et al., 2015; Pollock, 2017). In a study, it was reported that the serum and peritoneal fluid levels of horses with colic increased compared to healthy horses, but the concentration of SAA in peritoneal fluid was low (Copas et al., 2013; Pihl et al., 2013; Ludwig et al., 2023). Different intestinal diseases in horses are significantly associated with the concentration of acute phase proteins in plasma and peritoneal fluid, depending on the duration of the disease and the degree of obstruction (Pihl et al., 2015; Ludwig et al., 2023). Serum SAA levels were significantly elevated in horses with acute colitis and Clostridium difficile-induced enterocolitis, compared to both healthy horses and those with obstructive intestinal lesions (Pihl et al., 2016; El-Deeb et al., 2020; Minamijima et al., 2022; Ludwig et al., 2023).

The serum level of SAA has been reported to be above 2000 mg/L in adult horses with bacterial infections, while in viral infections a lower increase is observed. Therefore, the extent of the response could assist in distinguishing the species of microorganism involved (Jacobsen and Andersen, 2007; Satue et al., 2013). SAA concentrations reaching 1000 mg/L have been reported in horses with Streptococcus zooepidemicus infection (Hobo et al., 2007;

Witkowska Pilaszewicz et al., 2019). In another study, it was reported that SAA, which is not a specific indicator of viral infection in horses, is an important diagnostic tool for the control of viral infections and monitoring of treatment (Hulten et al., 1999a). Used as an auxiliary parameter for early diagnosis of respiratory infections, SAA is a useful parameter for monitoring response to treatment. Bacterial infections or severe influenza cases lead to sustained increases in SAA levels (Hulten et al., 1999a; Petersen et al., 2004; Cohen et al., 2005; Satue et al., 2013).

4. CONCLUSION

SAA, an important acute phase protein in horses, increases in plasma concentrations shortly after tissue damage during the acute phase response. Its concentration decreases shortly after, in parallel with healing. As an objective marker of clinical, subclinical inflammation, and tissue damage, the use of SAA in equine medicine is significant, though more comprehensive studies are needed.

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CHAPTER 3

EFFECTS OF GLOBAL WARMING ON FEED SUPPLY

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1. INTRODUCTION

One of the most striking results of climate change in a global sense is the concern that access to clean, continuous, sufficient and balanced food sources will be. The main reason for this situation is that a clean, continuous, sufficient and balanced feeding model for animals in the livestock sector is becoming more and more difficult every day and access to the right feed at the right time is decreasing day by day.

One of the biggest challenges of the 21st century is to develop sustainable practices in the face of increasing population and decreasing natural resources. Since the world population is expected to reach 9.7 billion by 2050 (United Nations 2019), increasing agricultural and animal production is becoming a necessity. The primary factor that will be affected by global warming is the decrease in the level of usable water, which is our source of life. Plant production is negatively affected by climate change, which makes it difficult to supply the feed needs of animals (Aydar, 2021). A 60-70% increase in animal product consumption is expected by 2050. This increase in consumption will demand various resources, and feed will be the most difficult resource to find due to the limited natural resources, ongoing climate change and food-feed-fuel competition (Makkar et al, 2014).

*TOB, 2023

The release of greenhouse gases such as CO2, CH4, N2O, CFC, and aerosols into the atmosphere has caused an unnatural increase in ecological temperature, and this phenomenon has been called "global warming" (Aydar, 2021).

Global warming is a problem that is increasing day by day with the developments and importance of agricultural and animal husbandry activities. Plant production is negatively affected by climate change, which strengthens the supply of animal feed needs (Mirón et al, 2023). Climatological features such as temperature and precipitation have important features in the use of pastures and other nutritional care (Koyuncu and Nageye, 2020). The effects of greenhouse gases on global warming and their emission sources are given in Table 1.

Table 1. Effects of greenhouse gases on global warming and emission sources

Greenhouse Gases	Contribution rate	Emission sources
CO_2	50	Fossil fuels Destruction of forests
CFC	22	Sprays Air conditioning and cooling systems Materials used in the electronics industry
CH_4	14	Rice fields Animal digestive systems Biomass burning Landfills Mines Natural gas pipeline leaks
O_3	7	Traffic Burning incidents in thermal power plants Destruction of tropical forests
N_2O	4	Artificial fertilizers Fossil fuels Nylon production
Water steam	3	Oceans, seas, rivers, lakes Thermal sources Respiration and sweating of plants and animals

*Edemen et al., 2023; CFC: Chlorofluorocarbons

The increase in greenhouse gases causes the permeability of the atmosphere to decrease, causing the earth to warm more than normal. The

primary factor that will be affected by global warming is the decrease in the usable water level, which is our source of life (Aydar, 2021).

According to the RCP4.5 model, in which the General Directorate of Meteorology examined the impact of climate change on Turkey between 2016 and 2099, a very low drought risk is expected in the provinces of Kars, Ardahan and Iğdır, a very high drought risk is expected in the provinces of Şanlıurfa, Mardin and Şırnak in the future, while a moderate drought risk is expected for the entire Central Anatolia Region (Dellal et al, 2024).

2. POSSIBLE EFFECTS OF GLOBAL WARMING ON FEED SUPPLY

The nature of the effects of climate variables on the livestock food supply chain, the scope and level of impact are indicated according to some factors listed below (Godde et al., 2021).

a. **Productivity:** It is expected that water-stressed areas will be most adversely affected by climate change. In some high latitude areas, yields will increase due to longer growing seasons due to reduced cold stress. Soil salinity in coastal areas may increase due to rising sea levels and increased frequency and intensity of storm surges. Irregular rainfall, especially in arid regions, increases soil salinity. Rapid weather events and rising temperatures may increase stress on important pollinator species because they cause changes in disease distribution. Post-harvest losses are likely to increase on farms with poor storage conditions under hotter and wetter conditions.
b. **Variability in feed availability:** Global climate variability is generally thought to have an increasing negative impact on annual forage production values. Changes in seasonal climate patterns have context-specific effects that can be positive or negative. Extreme and sudden weather events can limit livestock access to pasture and cause greater disruptions in forage production.
c. **Water resources:** Unusually hot weather and low rainfall due to climate change could increase the water needs of plants and animals, and is expected to increase the pressure on water resources, especially in regions that are otherwise water scarce. In addition, these increases in air temperature will cause more glacier melt, disrupting historical

surface water flows. Extreme and sudden weather events such as floods and droughts resulting from higher temperatures will increase the concentration of pathogens, sediment, salt, nutrients or pollutants in the water, resulting in water quality that is unfit for consumption by animals.

d. **Animal health and animal production:** Animal welfare and well-being are adversely affected by reduced access to feed and reduced feed quality, heat stress, diseases (epidemics and weakened animal immune systems) and mortality from extreme climate events such as storms, floods, heat and cold waves. Globally, in some regions where cold winters are experienced, higher temperatures are likely to reduce animal cold stress and maintenance energy requirements, as well as the cost of heating shelters.
e. **Processing, storage, transport and retail:** Higher temperatures, increased humidity and rising sea levels are options in addition to capital (machinery, transportation, power grids, telecommunications, etc.). Rising air temperatures can also increase the risk of heat stress in animals during transport. Food and feed storage and supply can also deteriorate, leading to reduced standards, durability and shelf life. Increased variability in production and extreme climate conditions can make refrigeration less regular and complex logistics systems.
f. **Animal product consumption:** Climate change is reducing the availability, quality and safety of animal products through contamination with pathogens or pesticides and reduced nutritional quality and sensory appeal. Prices may increase and are likely to become more volatile. Changing social norms are expected to affect diets, particularly in high-income countries.
g. **Labor:** Labor availability and productivity are likely to be adversely affected by climate change due to heat stress, increased risk of new disease outbreaks and extreme events such as heat waves, floods and severe storms. Labor will also be adversely affected by exposure to declining air quality associated with rising temperatures and nutritional deficiencies resulting from changes in food supply.

h. **Prices:** Costs, commodity prices and price volatility along the supply chain are likely to increase under climate change. The impacts of climate change on animal product prices can be observed primarily through changes in feed costs and feed availability.

Various studies have been conducted in our country to reveal the economic effects of climate change.

It has been reported that climate change will cause a 10-50% cost increase in dairy cattle farms by 2044. It has been determined that 48-71% of this increase in production costs is due to heat stress and 24-52% is due to increases in feed prices (Koç and Uzmay, 2019).

Increased temperature can increase lignin and cell wall components in plants (SanzSaez et al, 2012; Polley et al, 2013), which leads to a decrease in digestibility and nutrient content (Polley et al, 2013). Heat stresses can cause a decrease in the amount of harvested product, change nutritional value and allow deterioration in the composition of the species (Koyuncu and Nageye, 2020).

It is also known that the characteristics of meadows and pastures have started to change due to ecological change with global warming, and the dominance of shrubby plants in nature has increased, and it is reported that shrubby plants, tree branches and leaves and cactus trunks, which have a strong chance of survival in this environment, can be evaluated as feed. It has been determined that cacti can be used in ruminant feeding without any problems, and that a significant portion of the roughage requirement of animals can be met from cactus branches and trunks by supplementing with urea (Gebremariam et al, 2006; Tegegne et al, 2006). In a study conducted to evaluate the trunks of cactus, which is the plant best adapted to arid climates, as feed, it was determined that the use of cactus as a roughage source during the finishing period of lambs fed positively affected fattening performance and reduced feeding costs (Osorio et al, 2006).

3. CONCLUSION AND RECOMMENDATIONS

It is thought that the methods to be developed can reduce the effects of climate change (Fitzgerald et al, 2009; Gauly and Amber, 2020). Studies to prevent negative situations that will occur due to heat stress of feed additives should be increased.

In order for animal husbandry activities to continue uninterruptedly, it is necessary to take precautions and develop new strategies regarding global warming and the problems it will bring.

Innovative steps should be taken to meet the needs of our age in terms of feed supply against global warming and drought and the problems it brings, which are experienced today and will increase in the future.

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CHAPTER 4

THE EFFECTS OF YEAST (*Saccharomyces cerevisiae*) ON THE IMMUNE SYSTEM IN POULTRY NUTRITION: CELLULAR STRUCTURE, MECHANISMS OF ACTION, AND PHYSIOLOGICAL FUNCTIONS

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Introduction

Energy and protein losses are among the most significant challenges observed in animal nutrition. Growth promoters, ionophores, and antibiotics had proven effective in reducing these losses prior to their ban by the European Union due to the potential for residues in meat or dairy products. This situation has driven researchers to explore feed additives that could serve as alternatives (Elghandour et al., 2017). For this reason, the use of natural additives in animal nutrition has increased. In this context, yeast and yeast derivatives, with their biologically active components, have the potential to improve both animal health and performance. Yeast is particularly noteworthy for its positive effects on the immune system, its support for growth performance, its enhancement of resistance against pathogens, and its contributions to overall health improvement (Peng et al., 2020). Yeasts and yeast derivatives, consisting of 60 genera and 500 different species—most notably Saccharomyces cerevisiae (SC)—play a significant role in animal nutrition, although only a few are commercially utilized (Mahasneh et al., 2023). Yeast species like SC, in particular, positively impact overall health and performance by supporting the immune system. Yeast products are commonly used as feed additives in various forms, including live yeast (LY), yeast cell wall (YCW), hydrolyzed yeast (HY), and autolyzed yeast (AY) (Bortoluzzi et al., 2018a; Pascual et al., 2020; He et al., 2021; Yang et al., 2023). Today, the use of yeast and its derivatives is becoming increasingly important in enhancing sustainability in livestock production and reducing the use of antibiotics. This chapter will explore the effects of yeast and its derivatives, their outcomes across different animal species, and future research perspectives. The discussion will focus on their mechanisms of action on the immune system, organ-level impacts, and effects on physiological parameters.

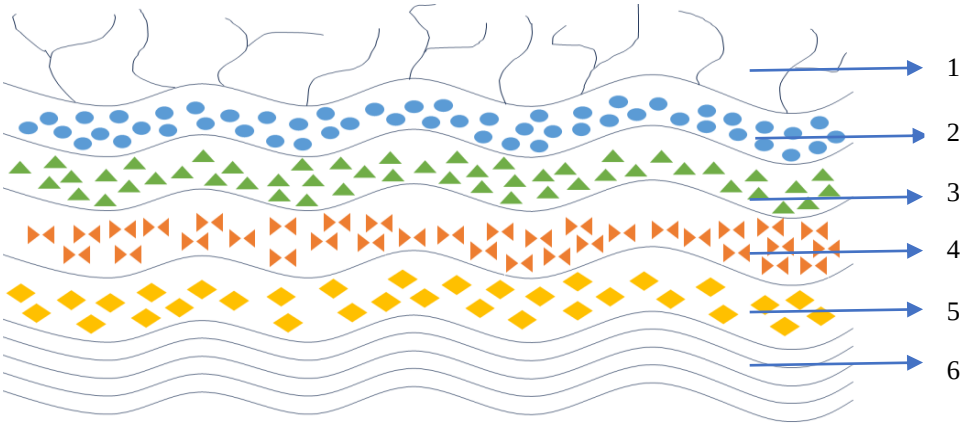
• Structure and Function of *Saccharomyces cerevisiae*

YCW is primarily composed of polysaccharides and proteins (Kogan and Kocher, 2007). Polysaccharides account for approximately 75–85% of the cell wall, while proteins make up the remaining 15–25%. Among the long-chain polysaccharides in the cell wall are water-soluble mannans, water-soluble and insoluble glucans, and chitin (Nguyen et al., 1997). α-D-mannans and β-D-glucans represent the two main types of polysaccharides (Perricone et al.,

2022). Cell wall polysaccharides are present in varying proportions depending on the yeast species; however, in SC, the amounts of mannans and glucans are nearly equal (Amin et al., 2021). Proteins, on the other hand, covalently bind to mannans, forming mannoproteins. Mannan is a general term for the polysaccharide component of glycoproteins and is typically represented as a linear polymer of linked mannose residues. Yeast mannan has a structure consisting of an α(1–6)-linked backbone with α(1–2)-linked and α(1–3)-linked branches (Perricone et al., 2022). α-D-mannans possess antioxidant properties and have the ability to inhibit the harmful effects of mycotoxins by interacting with their dangerous radical metabolites. This feature suggests that α-D-mannans not only support the immune system but also help prevent oxidative damage caused by mycotoxins (Križková et al., 2001). This mechanism can be a particularly important strategy for reducing the effects of mycotoxins in animal nutrition. Many intestinal bacterial pathogens are known to possess mannose-specific lectins that allow them to adhere to mannose-rich epithelial surfaces in the gut and stomach, leading to colonization and subsequent infection (Bäumler et al., 1997). These lectins facilitate the adhesion and proliferation of pathogens in the intestine, thereby contributing to the development of various intestinal diseases (Perricone et al., 2022). Therefore, mannans and mannose-containing compounds are thought to play a significant role in maintaining gut health and have the potential to limit the harmful effects of pathogens. Glucans, as D-glucopyranosyl-based polysaccharides, are formed by the covalent bonding of (1→3)-β-D-glucan and (1→6)-β-D-glucan (Kapteyn et al., 1995). These structures determine the biological activities of glucans and their effects on nutritional value. Specifically, the bonds between (1→3)-β-D-glucan and (1→6)-β-D-glucan support various biological functions of these compounds, such as modulating the immune system, exhibiting antioxidant properties, and interacting with mycotoxins (Perricone et al., 2022). Chitin is a linear polysaccharide made up of N-acetylglucosamine (N-acetyl D-glucosamine) monomers, and it is found in much lower concentrations in the yeast cell's external skeleton compared to mannans and glucans (Hofmann et al., 1994). Chitin is a rigid compound typically found in the exoskeletons of insects, fungi, and certain microorganisms. In yeast, this component helps maintain the structural integrity of the cell wall, providing protection against external factors. Mannan oligosaccharides (MOS), which are classified as

prebiotics, serve as nutrient sources for specific microorganisms in the gastrointestinal system. These compounds can stimulate the growth of beneficial bacteria in the intestine. MOS binds to pathogens in the gastrointestinal system, limiting their colonization, strengthens intestinal mucosal integrity, affects immune system activity, and plays a role in antioxidant and antimutagenic defenses (Perricone et al., 2022). The structure of cell wall of SC is indicated in Figure 1.

Figure 1. Structure of Yeast Cell Wall



1: Fiber layer, 2: Mannoprotein, 3: ß-glucan, 4: ß-glucan+Chitin, 5: Mannoprotein, 6: Plasma membrane

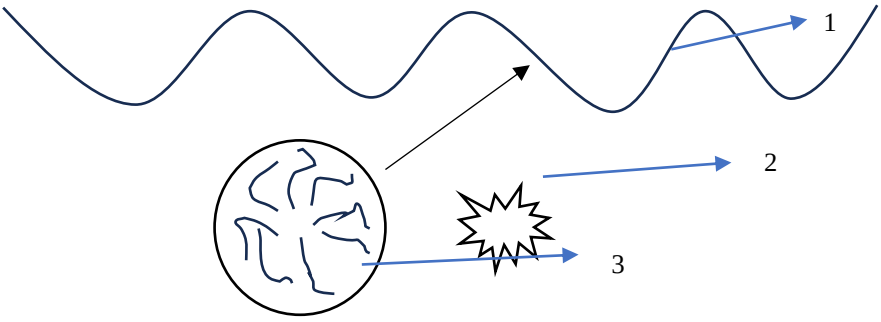
- **Mechanism of Action of *Saccharomyces cerevisiae***

Yeast can exhibit beneficial effects on living animals through multiple mechanisms. Its bioactive components, such as Mannan Oligosaccharides (MOS) and β-glucans, support the immune system. β-glucans activate the immune response through immune cells, such as macrophages (Petravić-Tominac et al., 2010). MOS regulate immune function by acting like a non-pathogenic antigen with adjuvant-like activity (Świątkiewicz et al., 2014). The most well-known of these mechanisms is its competitive ability with pathogens. The literature also highlights other effects such as improved gut health, induction of immune system function, enhanced nutrient digestion, increased metabolite production, toxin reduction, improved intestinal morphology, and stress-reducing effects (Sampath et al., 2023).

I. Mechanism of Action of *Saccharomyces cerevisiae* Against Pathogenic Agents

SC has the ability to compete with pathogenic agents for binding sites on the intestinal epithelium. By attaching to the epithelium, SC prevents harmful pathogens from adhering to the intestinal wall (Figure 2), thereby minimizing the damage these pathogens can cause to live cells (Bilal et al., 2023).

Figure 2. Competitive Feature of *Saccharomyces cerevisiae*

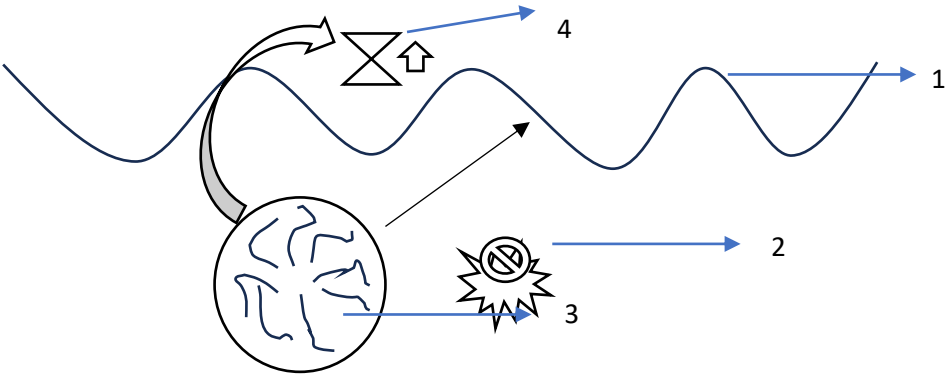


1: Intestinal epithelium, 2: Pathogenic agent, 3: *Saccharomyces cerevisiae*

II. Mechanism of Action of *Saccharomyces cerevisiae* on Gut Microflora

When stimulated by probiotics and prebiotics, the gut microflora can stabilize gastrointestinal (GIT) barrier function, enhance enzymatic activity, and promote microbial growth. Similarly, the inductive effect of SC can increase the proliferation of beneficial bacteria in the GIT microflora (Figure 3), while also reducing the number of harmful pathogens (Ezema et al., 2015). The balancing of gut microflora in this way contributes to overall gut health and function.

Figure 3. *Saccharomyces cerevisiae*'nin bağırsak mikroflorasını iyileştirici etkisi

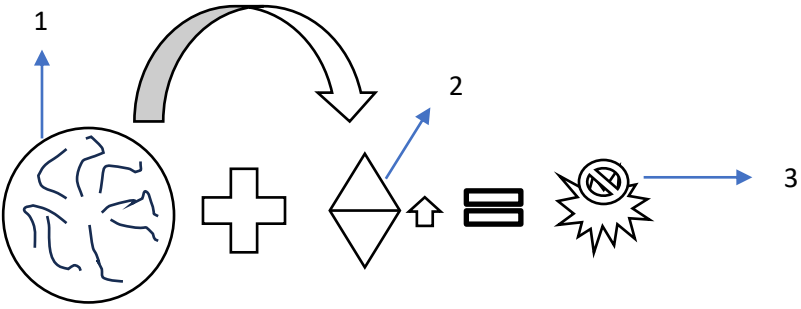


1: Intestinal epithelium, 2: Pathogenic agent, 3: *Saccharomyces cerevisiae*, 4: Intestinal microflora

III. Mechanism of Action of *Saccharomyces cerevisiae* on the Immune System

SC contains cell wall components (Figure 1) that stimulate the immune system. These components can trigger various immune responses, including the production of immune system cells that help recognize and combat potential pathogens (Figure 4) (Stier et al., 2014).

Figure 4. Effect of *Saccharomyces cerevisiae* on the Immun System



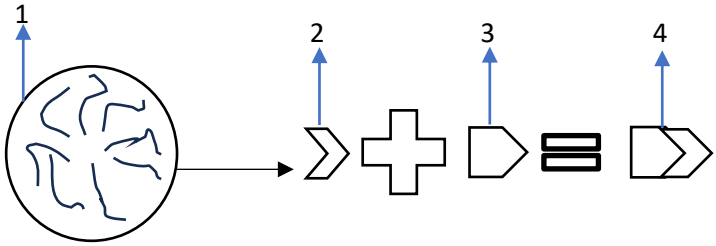
1: *Saccharomyces cerevisiae*, 2: Immune cell, 3: Pathogenic agent

IV. Mechanism of Action of *Saccharomyces cerevisiae* on Nutrient Digestion

SC and its by-products contain enzymes that aid in the digestion of nutrients bound in the form of phytate, particularly the phytase enzyme, which is often deficient in broilers. In this context, SC can help improve the

digestibility and absorption (Figure 5) of essential nutrients, such as minerals and amino acids, for live animals (Sampath et al., 2023).

Figure 5. Effect of *Saccharomyces cerevisiae* on Nutritional Digestion

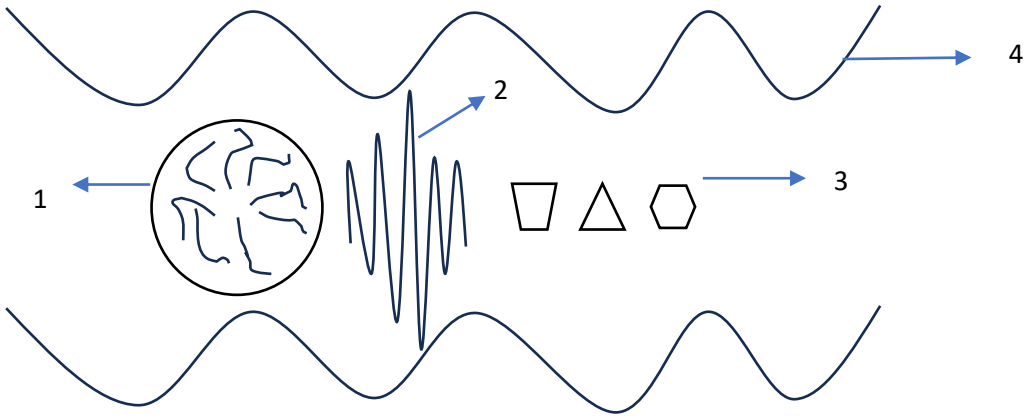


1: *Saccharomyces cerevisiae*, 2: Enzyme, 3: Nutrient, 4: Digested Nutrient

V. Mechanism of Action of *Saccharomyces cerevisiae* on Metabolite Production

As a result of digestion in live animals, significant metabolites are produced in the gut microbiota. These metabolites provide important energy sources for the animals. During fermentation, SC and its by-products can promote the production of metabolites, such as short-chain fatty acids (Figure 6), which contribute to the development of a suitable intestinal environment and assist in energy metabolism (Pang et al., 2022).

Figure 6. Effect of *Saccharomyces cerevisiae* on metabolite production

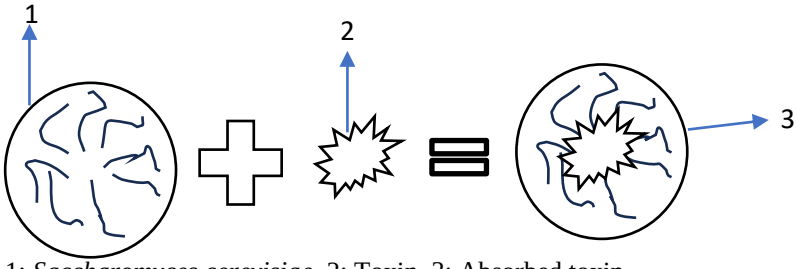


1: *Saccharomyces cerevisiae*, 2: Fermentation process, 3: Metabolite production, 4: Intestinal epithelium

VI. Saccharomyces cerevisiae'nin Toksin Absorbsiyonu Üzerine Etki Mekanizması

Consumption of toxin-contaminated feed by animals can reduce growth performance in poultry (Xu et al., 2023). SC can bind to toxins and mycotoxins present in external factors (such as feed), preventing their absorption in the intestines and thereby reducing their negative effects on animal health (Bruinenberg et al., 2021).

Figure 7. Effect of *Saccharomyces cerevisiae* on toxin absorption

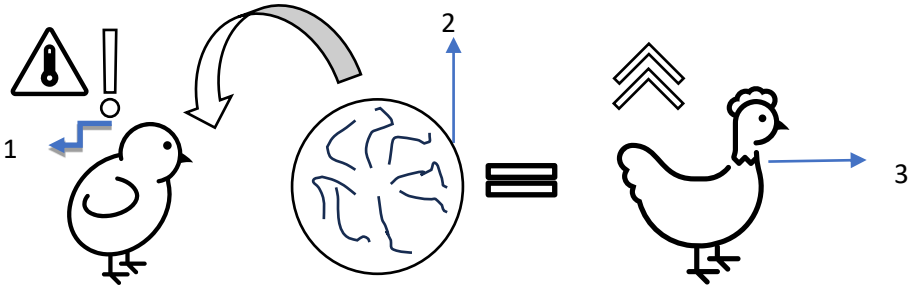


1: *Saccharomyces cerevisiae*, 2: Toxin, 3: Absorbed toxin

VII.Mechanism of Action of *Saccharomyces cerevisiae* on Stress Factors

Yeasts contain cell wall components that can enhance appetite and improve feed intake (Figure 1). By supporting gut barrier function and the immune system, SC contributes to the ability of poultry to cope with various stress factors (Figure 8) (Haldar et al., 2011).

Figure 8. Effect of *Saccharomyces cerevisiae* on reducing stress factors

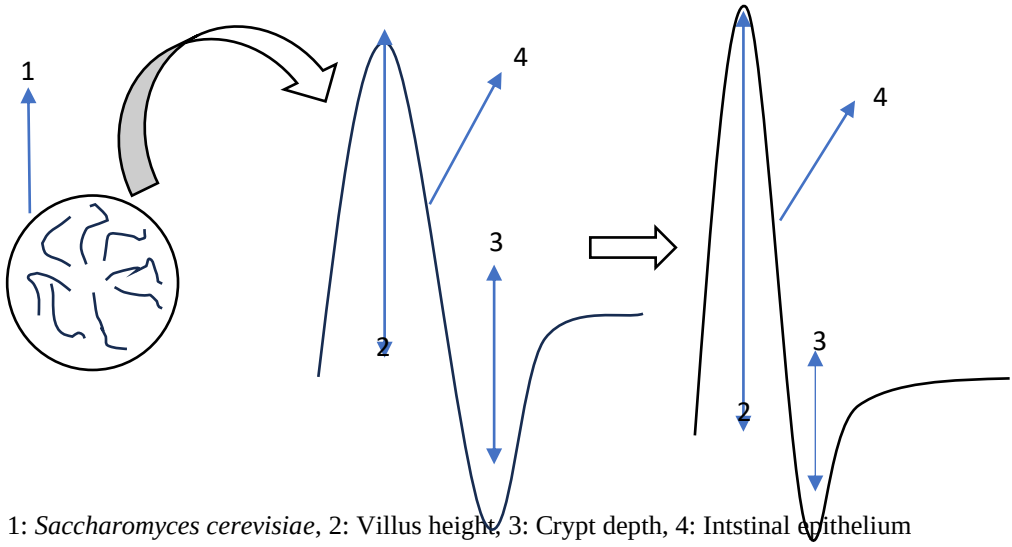


1: Chick exposed to heat stress, 2: *Saccharomyces cerevisiae* supplement, 3: Healthy growing chicken

VIII. Effect of *Saccharomyces cerevisiae* on Intestinal Morphology

SC can improve intestinal structure, including a reduction in villus height and crypt depth in the intestinal epithelial tissue (Figure 9). These changes help enhance nutrient absorption and contribute to the maintenance of intestinal integrity (Sampath et al.,2023).

Figure9. Effect of *Saccharomyces cerevisiae* on intestinal morphology



1: *Saccharomyces cerevisiae*, 2: Villus height, 3: Crypt depth, 4: Intstinal epithelium

- **Nutrient Content of *Saccharomyces cerevisiae***

In terms of nutrient content, SC contains high levels of protein and low levels of crude fat. The crude protein content of SC ranges from 39.3% to 52.04%. When examining the crude ash content, it includes important minerals such as calcium, phosphorus, and sodium, with values ranging from 4.87% to 9.3%. SC can also be beneficial as a feed additive in poultry rations, particularly when plant-based sources are deficient in essential amino acids. As previously mentioned, the glucan and mannan content follows a similar pattern (Table 1).

Table 1. Nutrient Content of *Saccharomyces cerevisiae*

Contents, %	Minimum	Maximum
Dry Matter	89.1	96.9
Crude Protein	39.3	56.8
Crude Fiber	0.1	4.4
Nişasta	0	17.5
Starch	0	20.7
ADF	0	5.7
Ether Extract	0.35	8.2
Crude Ash	4.87	9.3
Calcium	0.1	0.54
Phosphorus	0.96	2
Sodium	0.04	0.37
Lysine÷Protein	4.6	7.6
Methionine÷Protein	1.3	2.2
Cysteine÷Protein	0.33	1.9
Threonine÷Protein	3.7	5.6
Triptofan÷Protein	1	1.4
Glucan÷Glucose	6.46	14.34
Mannan÷Mannose	0.55	14.8

(Feedipedia.com, 2024; Patterson et al., 2023)

• Effects of *Saccharomyces cerevisiae* on the Immune System in Poultry

Poultry products are among the most consumed animal products globally, providing humans with rich nutrition, including protein and other essential nutrients. With the growing human population, the demand for these products has naturally increased. In this context, while various genetic selections have been made over the years to meet the increasing demand, maintaining animal health is a critical factor in maximizing the genetic potential of poultry (Perricone et al., 2022).

In poultry, SC plays a role in regulating immune responses, both through cell-mediated and humoral mechanisms (Yalçın et al., 2014). As previously mentioned, various yeast derivatives have been used in the past years. Yeast and its products balance immune and stress responses, while also regulating

blood antioxidant parameters (Perricone et al., 2022). General information about the**se products is shown in Table 2.**

Table 2. Characteristics of yeast used in poultry

	Hydrolyzed Yeast	**Live Yeast**	**Yeast Cell Wall**	**Autolyzed Yeast**
Activeness	Inactive	Live	Inactive	Inactive
Contents	Free amino acids MOS	Active yeast cells	ß-glukan MOS	Peptides Free amino acids ß-glukans, MOS
Production Process	Controlled chemical disintegration process	Live yeast production	Extraction of yeast cell contents	Breaking down yeast cells by their own enzymes through autolysis
Function	Immune support Gut health	Fermentation Immune support	Gut health Toxin binder	Digestive support Immune support

(Bortoluzzi et al., 2018a; Pascual et al.; 2020; He et al., 2021; Yang et al., 2023)

I. Effect of *Saccharomyces cerevisiae* on Blood Serum and Antioxidant Levels

SC has garnered attention for its positive effects on antioxidant capacity and blood parameters in poultry nutrition. Studies have shown that yeasts can improve biochemical parameters in blood serum and support antioxidant levels by reducing free radical production (Wang et al., 2021). Moreover, immunoglobulins, which are one of the key components of the immune system, belong to the antibody class and are produced by B lymphocytes (LeBien et al., 2008). HY, by increasing the antibody levels in the blood, can create a defense mechanism against foreign pathogens in both broilers and laying hens (Yalçın et al., 2010; Yalçın et al., 2013). Yeasts play a critical role in combating disease agents. Against pathogens such as Newcastle disease (ND), Avian influenza (AI), Eimeria tenella, C. perfringens, and Salmonella spp., AY, LY, HY, and YCW can enhance immune responses by increasing specific antibody titers in the blood serum (Gao et al., 2009; Attia et al., 2017; Roto et al., 2017; Ahiwe et al., 2019; Gingerich et al., 2021). The addition of HY to broiler diets can increase the number of white blood cells, lymphocytes, and heterophils (Attia et al., 2017). An increase in serum levels of immunoglobulin A (IgA) and immunoglobulin M (IgM) generally indicates enhanced immune function and

better protection against infections. IgA is primarily found in mucosal areas and prevents pathogenic bacteria from entering subepithelial regions (Bortoluzzi et al., 2018b). IgM is the first antibody produced in response to infection and plays a crucial role, especially in the early stages of immune response (Liu et al., 2019). Studies have proven that SC can support animals by increasing the levels of IgA and IgM in the blood serum against pathogens such as Newcastle disease (ND), Eimeria tenella, and Salmonella spp. (Gao et al., 2008; Gao et al., 2009; Ahiwe et al., 2019; Pahlavanzadeh et al., 2021).

Malondialdehyde (MDA) serves as a biomarker for understanding oxidative stress and lipid peroxidation levels (Aluwong et al., 2013). It has been proven that the addition of LY to broiler (Attia et al., 2022) and laying hen (Qıı et al., 2024) diets decreased the blood MDA levels. Additionally, the inclusion of LY in laying hen diets can also enhance the total antioxidant capacity (Liu et al., 2021). Toxic agents can contaminate animal products, leading to diseases in humans. By delaying the proliferation of these toxic agents as much as possible by increasing antioxidant levels in blood parameters, it can indirectly extend the shelf life of poultry products, such as white meat and eggs, in the poultry industry (Shaltout et al., 2016).

II. The Effect of *Saccharomyces cerevisiae* on Intestinal Morphology

Yeasts can support intestinal barrier functions by reducing inflammation and helping to maintain intestinal homeostasis (Sızmaz et al., 2023). Changes in the depth of intestinal crypts serve as a compensatory mechanism against apoptosis during a pathogenic attack, indicating that enterocytes are attempting to regenerate by progressing toward the tip of the villus (Kudupoje et al., 2022). The ratio of intestinal villus height (VH) ÷ crypt depth (CD) is an important measure used to evaluate intestinal structure and can provide insight into the regeneration process. When compared to CD, a slower growth rate of VH indicates an abnormal structure, which can lead to impaired nutrient absorption and weakened intestinal barrier functions (Turner, 2006). HY and YCW have been shown to protect intestinal morphology against ND by increasing the villus height to crypt depth (VH ÷ CD) ratio (Muthusamy et al., 2011). Reactive oxygen species (ROS) can increase under high environmental temperatures, leading to enhanced intestinal permeability. This can cause damage to cell

membranes and impair intestinal barrier functions (Perricone et al., 2022). Under heat stress, LY can protect intestinal morphology by increasing villus height (VH) (Haldar et al., 2011). Even in the absence of any stress factors, both HY and LY can enhance the villus height to crypt depth (VH ÷ CD) ratio, strengthening the impact of the intestinal wall structure on the digestive system (Chuang et al., 2019; Sampath et al., 2021).

III. Effect of *Saccharomyces cerevisiae* on Immune Organs

Changes in the weight of immune organs can provide significant insights into the status of the body's immune response and overall health. These alterations are often observed in relation to the activity of the immune system, the severity of existing inflammatory responses in the body, and the proliferation of immune cells (Song et al., 2021). An increase in the weight of immune organs is generally associated with a stronger immune response and increased proliferative activity, whereas a decrease in organ weight indicates immune system weakness and increased cellular death processes within the organs (Sikandar et al., 2017). A review of the literature reveals that, particularly against ND and AI pathogens, HY has been shown to increase the weight of the thymus and bursa of fabricius in broilers (Attia et al., 2017). The stability of organ weight in response to disease agents may indicate proper functioning of the body's immune system. AY, by maintaining the weight of the spleen and bursa of fabricius against Salmonella spp. pathogens, can help prevent the proliferation of cell deaths (Ahiwe et al., 2019). In an environment free of environmental pathogenic agents, high organ weight is one of the indicators of immune adequacy. The addition of LY to broiler diets can increase the weight of the spleen, thymus, and bursa of fabricius, even in pathogen-free conditions.

IV. Effects of *Saccharomyces cerevisiae* on Intestinal Microflora

The stability of the intestinal microflora is related to age (Oakley et al., 2014). As microflora gains diversity and stability in the early stages of life in poultry, its protective mechanisms against pathogens strengthen, and nutrient absorption and metabolic processes are optimized (Rinttilä et al., 2013). SC has been proven to be effective in improving intestinal microflora and promoting the growth of beneficial bacteria through various studies (Sun et al., 2020; Liu

et al., 2021). In the poultry intestinal microflora, there are two dominant bacterial phyla. The Bacteroides phylum is known for its role in the digestion of polysaccharides and oligosaccharides and is primarily composed of gram-negative bacteria (Stanley et al., 2014). The Firmicutes phylum, on the other hand, predominantly contains Lactobacilli species and is particularly known for its role in the production of short-chain fatty acids (SCFAs) (Sun et al., 2020). SC, by aiding the fermentation of polysaccharides and oligosaccharides, plays a crucial role in the production of beneficial metabolites such as SCFAs, thus providing energy to the host (Park et al., 2017) (Figure 6). The addition of LY to layer hen diets and HY to broiler diets can increase Bacteroides and Firmicutes bacteria in the ileum, reducing the presence of Escherichia coli in the feces (Park et al., 2020; Sampath et al., 2021). Similarly, the inclusion of HY and YCW in broiler diets increases Bacteroides in the feces and ileum, while the addition of LY to layer hen diets boosts Firmicutes in the feces and ileum, helping to reduce Escherichia coli levels (Muthusamy et al., 2011; Liu et al., 2021). Yalçın et al. (2013) demonstrated that the addition of AY to broiler diets reduces Escherichia coli levels in the feces. SC achieves these benefits by enhancing the energy absorption rate in the intestines through the stimulation of Bacteroides and Firmicutes bacteria, and by supporting the production of SCFAs (Sun et al., 2020).

V. Effects of *Saccharomyces cerevisiae* on Redox Biology

The immunological effects of SC can support the immune system through various mechanisms. Redox biology is a field that studies oxidation and reduction reactions in cellular processes, regulating the immune system through various signaling pathways (Surai et al., 2021). The NF-κB signaling pathway (Nuclear Factor Kappa B) plays a role in regulating both innate and adaptive immunity. NF-κB activity is essential for lymphocyte survival and activation, as well as for the initiation of normal immune responses. NF-κB can be rapidly activated in response to pathogenic stimuli, pro-inflammatory cytokines like interleukin-1 (IL-1), and stress factors (Li and Verma, 2002). Many pathogens are recognized by "pattern-recognition receptors (PRRs)" that have evolved to detect pathogen-associated molecular patterns. The most well-known PRRs are the "toll-like receptors (TLRs)," a group of transmembrane proteins that govern the activation of cellular signaling pathways after directly

or indirectly recognizing pathogens (Wang et al., 2018). Among these, TLR-2 plays a crucial role in recognizing different bacterial cell wall components (Imler and Hoffmann, 2000). T lymphocyte cell responses are divided into two types which are Th-1 responses (associated with cellular immunity) and Th-2 responses (associated with humoral immunity). TLR-2 plays an immunologically inductive role, while IFN-γ (Interferon Gamma) enhances immune responses by promoting the maturation of Th-1 cells (Song et al., 2024). This process strengthens the immune reaction. NF-κB is involved in the production of IFN-γ, which is essential for the function of Th-1 cells (Aune et al., 1999). In summary, activation of TLR-2 triggers the NF-κB signaling pathway, leading to the production of IFN-γ. At the same time, NF-κB is one of the key regulators of pro-inflammatory gene expression and is involved in initiating the transcription of genes such as inducible nitric oxide synthase (iNOS) (Tak and Firestein, 2001).

Researches has indicated that SC plays a beneficial role in redox biology. Chou et al. (2017) demonstrated that the addition of LY to broiler diets stimulates PRRs in the spleen, leading to an increase in TLR-2 receptors, which in turn results in an increase in IFN-γ production. Similarly, the addition of LY to diets has triggered an increase in IL-1 levels, leading to the activation of the NF-κB signaling pathway. As a result, an increase in the production of iNOS and IFN-γ was observed (Chuang et al., 2021). Nitric oxide (NO), derived from iNOS, is a compound that contributes to inflammation. Excessive NO production, resulting from iNOS activation, can cause damage while breaking down pathogenic agents (Han et al., 2020). While NO plays an important role in immune response at normal levels, excessive production of iNOS should also be carefully monitored (Shah et al., 2020). Finally, Bortoluzzi et al. (2018b) demonstrated that the addition of OY to broiler diets can assist the immune system by modulating TLR-2 receptors and increasing IL-1 levels.

- **Conclusion**

SC stands out as a valuable additive in poultry nutrition due to its positive effects on improving biochemical parameters in blood serum and enhancing antioxidant capacity by reducing free radicals. By preserving intestinal barrier functions and optimizing the VH/CD ratio, it enhances the health of the digestive system and improves nutrient absorption efficiency. It strengthens

immune responses by increasing the weight of immune organs and helps animals develop resistance to pathogens. While promoting the proliferation of beneficial bacteria in the gut microbiota, it also aids in suppressing harmful pathogens. Through its regulatory role in redox biology, it battles oxidative stress while supporting the immune system and maintaining cellular health by regulating the expression of proinflammatory genes.

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CHAPTER 6

CORNEA ULCER IN HORSES AND TREATMENT OPTIONS

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1. INTRODUCTION

Corneal ulcer, also known as ulcerative keratitis, is one of the most common serious eye problems in horses (Brooks and Matthews, 1999; Maile, 2012; Hellander-Edman et al., 2013; Hartley, 2014; Bazay, 2024). Corneal ulcers occur due to erosion of the corneal epithelium, the outermost layer of the cornea, due to various etiologies (Hellander-Edman et al., 2013; Hartley, 2014). The severity of corneal ulcers in horses can vary widely, from mild superficial corneal scratches or damage to full-thickness corneal perforations that can cause prolapse of the iris (Brooks and Matthews, 1999). The cornea contains many nerve endings. Therefore, ulceration or keratitis formed in the cornea is very painful. Additional signs of keratitis include redness, excessive tearing, eye discharge, and a cloudy appearance to the eye (Williams and Pinard, 2013). If corneal lesions are not treated promptly and appropriately, they can lead to bacterial or fungal infection and potential vision loss (Hartley, 2014, Çatalkaya et al., 2023). Other complications include rupture of the bulbus oculi (Brooks and Matthews, 1999; Bazay, 2024). Prompt veterinary intervention is critical to maintaining visual function. With appropriate treatment, most horses can recover fully, maintaining both their health and quality of life.

The large and lateral aspect of horse eyes makes them prone to injury. The cornea is most often affected by this condition. Corneal ulcers or keratitis occur as a result of damage to the corneal epithelium, the outermost layer of the eye, and the accompanying corneal inflammation. Corneal lesions in horses are usually caused by trauma, foreign bodies or infections (Brooks and Matthews, 1999; Brooks, 2010; Hellander-Edman et al., 2013; Hartley, 2014). This injury irritates or damages the corneal epithelium, leaving the cornea and underlying structures unprotected (Brooks and Matthews, 1999). This condition is quite painful and can lead to a deeper infection (Brooks, 2010).

2. HEALING AND STRUCTURE OF THE CORNEA

To better understand the different types of corneal ulcers, it is important to know the structure of the cornea. The cornea is the clear and transparent front part of the horse's eye. It covers the iris, pupil, and anterior chamber. It is a transparent and transparent dome-shaped structure on the front surface of the eye that is 0.8 to 1 mm (0.03 to 0.04 inches) thick. The cornea protects the eye, allows light to pass through, and helps focus light on the retina at the back of

the eye (Williams and Pinard, 2013; Gelatt, 2019). It is made up of three main layers (Williams and Pinard, 2013; Brooks and Plummer, 2022):

Epithelium: The epithelium is the outermost layer that repels tears and prevents microorganisms from entering the deeper layers of the cornea.

Stroma Layer: The stroma layer is located beneath the epithelium and makes up about 90% of the thickness of the cornea. The stroma layer is primarily composed of water.

Deep Endothelium: Deep endothelium is located under the stroma layer. Descemet's membrane is the basement membrane of the deep endothelium.

Corneal epithelial healing occurs in three phases: a lag phase, a migration phase, and a proliferation phase. During the lag phase, the attachments of neighboring cells of the wound to nearby cells and the underlying basement membrane change. The epithelium surrounding the lesion thins and an epithelial layer begins to migrate toward the center of the wound. This migration is accompanied by the formation of new temporary attachments and the disassembly of attachment complexes. Once the epithelial layer covers the lesioned area, epithelial cells proliferate to restore the thickness and differentiation of the anterior epithelium. Stromal defects stimulate keratocytes to undergo apoptosis. Loss of the epithelial basement membrane is considered a critical factor in determining the fibrotic response of keratocytes and subsequent scarring. Endothelial cells do not actively divide in horses. Therefore, cell losses due to corneal perforation or surgical trauma are compensated for by thinning and spreading of existing cells. Corneal decompensation occurs with cell loss, producing diffuse corneal edema (Hartley, 2015).

3. TYPES OF CORNEA ULCERS

Three types of corneal ulcers are observed in horses: simple, recurrent and complex.

3.1. Simple Corneal Ulcers

Simple corneal ulcers are superficial inflammations involving the outer layer of the cornea and up to one-third of the stroma.

Simple corneal ulcers may not be noticeable to the naked eye. However, they can be easily identified with fluorescein stain during ophthalmic evaluation (Michau et al., 2003; Bazay, 2024).

Simple ulcers do not have deep infection, but the eye can still be infected with bacteria or fungi (Michau et al., 2003; Williams and Pinard, 2013). Simple corneal ulcers usually heal rapidly without complications (Peyrecave-Capo et al., 2022).

3.2. Recurrent or Refractory Corneal Ulcers

Recurrent ulcers are also known as indolent ulcers. This type of ulcer occurs because the corneal epithelium fails to adhere to the underlying stroma, preventing it from healing properly (Michau et al., 2003; Gilger, 2012).

Recurrent ulcers can be detected with fluorescein staining. They differ from simple ulcers because staining is also seen below the periphery of the corneal epithelium. This indicates that the cornea has separated from the stroma (Michau et al., 2003; Gilger, 2012; Williams and Pinard, 2013, Bazay, 2024).

3.3. Complex Ulcers

This category of corneal ulcers includes deep ulcerations that reach the stromal layers of the cornea. In these cases, bacterial or fungal agents are usually present in the damaged corneal tissue and cause moderate to severe inflammation of the cornea (Williams and Pinard, 2013). Complex ulcers are known for their long healing times (Peyrecave-Capo et al., 2022).

4. ETIOLOGY

Corneal ulcers in horses usually begin with lesions such as thin (small) scratches or abrasions on the eye. It is usually caused by small foreign objects entering the eye. Horses that are exposed to environments with a lot of branches, dust or sand are predisposed.

In corneal lesions, the healing process is negatively affected when bacterial or fungal organisms settle in the tear film (Maile, 2012; Williams and Pinard, 2013).

The healthy corneal epithelium and tear film layer act as a strong barrier against pathogens. However, the corneal tissue is very sensitive to physical injuries, which makes the entire eye more susceptible to infections.

While there are non-pathogenic bacteria in the eye flora and these do not pose a threat to the eye, pathogenic bacteria such as *Staphylococcus* spp., *Listeria* spp. and *Streptococcus* spp. can multiply when the cornea is injured and cause infectious keratitis. Such infections are particularly common in

young horses, whose immune systems may be weaker (Maile, 2012; Brooks and Plummer, 2022; Çatalkaya and Karacan Sever, 2023).

Fungi such as *Aspergillus* spp. and *Fusarium* spp. also commonly cause corneal ulcers (Çatalkaya and Karacan Sver, 2023; Bazay, 2024). These fungi settle in damaged corneal tissue and cause the severity of ulceration to increase further.

5. SYMPTOMS

Symptoms may vary depending on the severity of the lesion in the cornea. Common corneal ulcer symptoms include pain, squinting, eye swelling, excessive blinking, increased tear volume, tear discharge, sensitivity to light, pupillary constriction, cloudy eyes, eye redness or swelling, and a bluish tint in the cornea (Williams and Pinard, 2013; Wickens, 2023). Corneal ulcers may also be accompanied by uveitis. As the infection progresses, microorganisms progress to the stromal layers and give the eye a molten or gelatinous appearance (Maile, 2012; Williams and Pinard, 2013). If a corneal ulcer cannot be treated with aggressive medical treatment or surgical intervention, the cornea can be significantly damaged and perforated (Maile, 2012; Williams and Pinard, 2013; Brooks and Plummer, 2022). In some cases, this can lead to the formation of a descemetocele, which resembles a crater-like defect in the cornea, absorbing fluorescein dye at the edges but remaining clear in the center. The presence of a descemetocele is a critical indicator that the integrity of the bulbus oculi has been severely compromised and usually requires surgical repair to prevent rupture (Brooks and Matthews, 1999).

Sudden changes in vision are considered a medical emergency. Intervention is critical to preserve a horse's vision and prevent further complications.

6. DIAGNOSIS

It is important to perform a complete ophthalmic examination to diagnose corneal ulcers. A good ophthalmic examination should include sedation and nerve block and should ideally be performed in a dark room. Sometimes the horse may need to be referred to a specialist (Maile, 2012; Conrado et al., 2023).

In addition to physical examination of the eye for lesions and problems, the Schirmer tear test can be performed to detect and eradicate

keratoconjunctivitis sicca, which occurs due to inadequate tear film (Hartley, 2015).

Fluorescein staining is used to determine the exact location of the corneal ulcer. Corneal cytology may be required for the diagnosis of chronic and complex corneal ulcers (Maile, 2012; Hartley, 2015; Bazay, 2024).

These diagnostic tests help determine the etiology of the corneal ulcer and the severity of the corneal ulcer, allowing the physician to determine appropriate and effective treatment (Hartley, 2015, Çatalkaya et al., 2023). Rapid diagnosis of corneal ulcers is important for rapid application of effective treatment and minimizing tissue loss (Williams and Pinard, 2013; Conrado et al., 2023).

7. TREATMENT

The aim of treating corneal ulcers in horses (Ollivier, 2005; Brooks and Plummer, 2022):

- Determine the etiology of the ulcer
- Treat existing infections and prevent new ones
- Slow down the breakdown of corneal collagen
- Prevent possible secondary uveitis
- Provide structural support to the weakened cornea if necessary
- Provide analgesia

Aggressive treatment is applied to reduce the likelihood of infection and reduce inflammation in the eye in the treatment of corneal ulcers (Brooks and Plummer, 2022). Treatment usually lasts several weeks. The type of medication and frequency of dosage vary depending on the case. In severe cases, surgery may be recommended (Ollivier, 2005; Hellander-Edman et al., 2013).

It is also important to physically protect the affected eye with a fly mask or cup mask during treatment.

Treatment of Simple Ulcers

The aim of treatment in simple ulcers is to reduce contamination and prevent bacterial colonization in the stroma. Treatment involves the use of topical antibiotic therapy until the corneal ulcer is determined to have healed by fluorescein staining (Williams and Pinard, 2013).

Treatment of Recurrent Ulcers

In the treatment of recurrent ulcers, surgical correction of eyelid and/or conjunctival (third eyelid) anomalies is usually recommended. In addition, it may be necessary to remove dead or damaged tissue with a dry cotton swab before the procedure in the damaged cornea (Bazay, 2024).

The appropriate topical antibiotic is preferred according to the cytology and culture results to be performed.

Treatment of Complex Ulcers

Other surgical interventions such as corneal grafts may be required for complete healing of complex ulcers. Corneal grafts include conjunctival flap, corneal transplantation or horse amnion (Williams and Pinard, 2013).

7.1. Medical Treatment

Ophthalmic problems are difficult to treat in many horses because of the difficulty of topical application of eye medications. There are multiple options for treating corneal ulcers in horses. The veterinarian may use either a combination of treatment options or individually, depending on the individual condition of the horse.

7.1.1. Drug administration

Although the cornea may seem simple to treat, it is often difficult due to the extreme sensitivity of the area and the presence of tissue barriers that can prevent the penetration of the medication (Bazay, 2024).

Medications are most often applied to the horse's eye by the following methods. Each of these methods has its own advantages and disadvantages (Brooks and Plummer, 2022):

- Topical ocular application
- Ocular injection or implant
- Systemic application

With topical application, the ability of medications to penetrate the cornea and deeper structures of the eye may be limited. They may also be diluted and drained from the eye due to tears (Brooks and Plummer, 2022).

Topically applied medications usually have a short contact time with the treatment area. Therefore, they must be applied frequently to the eye to be effective. The horse keeper is usually responsible for applying these medications, and failure to do so will result in unsuccessful treatment.

7.1.2. Subpalpebral lavage system

The subpalpebral lavage system consists of an ophthalmic catheter that allows topical liquid medication to be applied to the surface of the cornea.

The subpalpebral lavage system consists of a silicone tube that passes through the upper or lower eyelid and is fixed to the horse's face. The tube is passed through the horse's mane and advanced along the neck and ends in a closed port. The medication is then injected through this catheter (Dwyer, 2013; Bazay, 2024). This system is generally preferred in horses that are difficult to treat or require frequent treatment. This application method provides convenience in long-term treatments that require frequent application. Because the application of medications is easier and safer (Brooks and Matthews, 1999; Williams and Pinard, 2013).

The subpalpebral lavage system in horses is critical in the treatment of infected and deep ulcers and serious eye conditions (Dwyer, 2013; Bazay, 2024). This system can be used both in the hospital environment and at home. It can be left in place for 10-12 weeks if necessary. Many horses tolerate the subpalpebral lavage system well. The most commonly reported complications include catheter leaks or breakage. Using a fly mask or hood can help prevent damage to the subpalpebral lavage system (Maile, 2012; Dwyer, 2013).

7.1.3. Control of infection

All types of corneal ulcers may be accompanied by uveitis. Treatment with topical atropine sulphate and systemic NSAIDs (non-steroidal anti-inflammatory drugs) is usually required to control inflammation. Oral or intravenous NSAID therapy with drugs such as phenylbutazone and flunixin meglumine may help with analgesia (Williams and Pinard, 2013). Other drugs that can be used to reduce inflammation and prevent stromal lysis include acetylcysteine and sodium ethylenediamine tetra-acetic acid (EDTA). The veterinarian may prescribe additional treatments according to the specific needs of the horse (Brooks and Matthews, 1999). Some studies have suggested that the use of corticosteroids and topical NSAIDs should be avoided because they may delay epithelial healing (Brooks and Matthews, 1999; Bazay, 2024).

7.1.4. Clearing Infections

In order to treat corneal ulcers completely, bacterial and fungal growth infecting the cornea must be effectively controlled and treated. Broad-spectrum topical antibiotics are generally preferred according to culture results (Bazay, 2024).

In bacterial infections, topical antibiotics such as Chloramphenicol, Gramicidin-neomycin-polymyxin B, Gentamicin, Ciprofloxacin, Tobramycin are usually prescribed (Brooks and Matthews, 1999).

In fungal infections, an antifungal preparation must be prescribed. If the treated corneal ulcer does not respond to antibiotic treatment, antifungal treatment should also be added to the prescription.

In horses, 5% natamycin, 1% miconazole, 1% itraconazole, 30% dimethyl sulfoxide, and 1% voriconazole are commonly used topical antifungal drugs. Anterior uveitis may initially worsen after starting antifungal treatment. However, these medications should be used 4-6 times a day for at least 2-4 weeks to eliminate the fungal infection. For deep infections, this period may extend up to 6-8 weeks (Williams and Pinard, 2013).

7.2. Surgical Treatments

Depending on the type and severity of the corneal ulcer, surgical treatments may be required in addition to drug therapy. Surgical intervention is preferred, especially if the corneal ulcer affects deeper parts than one-third of the thickness of the corneal stroma or does not respond to medical treatment (Ollivier, 2005).

7.2.1. Conjunctival Flap

Conjunctival flaps are a common surgical procedure used to treat corneal ulcers. This procedure involves suturing healthy conjunctival tissue over the damaged corneal area to promote healing and provide structural support (Bazay, 2024).

7.2.2. Cornea Transplant (Keratoplasty)

Keratoplasty may be required to treat deep or perforated corneal ulcers, complex and/or deep stromal abscesses. This procedure involves removing the damaged tissue in a way that favors the eye (Maile, 2012; Bazay, 2024). During surgery, the damaged portion of the cornea should be removed without touching

Descemet's membrane, and the donor cornea should be sutured over the defect with a one-millimeter overlap at the edges. In cases of erosive or severely infected ulcers, a conjunctival pedicle or amniotic membrane graft can be sutured to the transplanted corneal area to minimize necrosis of the graft. Enucleation is the best option in cases of perforated ulcers that do not respond to treatment (Maile, 2012).

7.2.3. Enucleation

If surgery is necessary to treat a corneal ulcer, but surgery is not possible due to financial or other reasons (temperament of the horse, lack of time on the part of the caregiver, etc.), enucleation of the eye may be recommended. This procedure can be performed under either general anesthesia or sedation and nerve block (Williams and Pinard, 2013).

Many horses adapt well to having only one eye. Enucleation of the eye is also a way to stop the severe pain caused by corneal ulcers.

7.3.Additional Current Therapies

Given the frequency of eye injuries in horses, there is interest in developing new medical technologies to simplify management of the lesion for both horses and caregivers. These therapies can complement and/or replace existing medications, depending on the individual needs of the horse (Bazay, 2024).

7.3.1. Autologous Serum

Another treatment for corneal ulcers is the application of serum obtained from the horse's own blood to the eye. Studies have shown that this procedure supports and accelerates corneal healing (Wickens, 2023, Bazay, 2024). In addition, autologous serum application can prevent or prevent stromal melting (Brooks and Matthews, 1999).

7.3.2. Corneal Collagen Cross-Linking

Corneal collagen cross-linking is a treatment that involves applying eye drops containing vitamin B2 to the cornea and then exposing it to ultraviolet light. This process stabilizes the cornea by creating "cross-links" in the corneal stroma and prevents further melting.

Corneal collagen cross-linking can be an alternative to antibiotics. This helps avoid the risk of developing antibiotic resistance. However, this method carries the risk of direct damage to corneal cells and intraocular structures due to UV radiation (Hellander-Edman et al., 2013). To reduce these risks, corneal collagen crosslinking protocols use vitamin B2 drops to block and limit the transmission of UV light to deeper eye structures (Bazay, 2024).

7.3.3. Corneal Debridement

A procedure that involves superficially removing dead and damaged corneal tissue using dry, sterile cotton swabs can treat recurrent corneal ulcers in horses. Sedation, frontal nerve blockade, and topical anesthesia should be administered for this procedure. After debridement, a keratotomy is performed to expose the healthy corneal stroma and promote healing. However, deep or infected ulcers should not be treated in this manner. To accelerate corneal healing, biological "bandages" made from horse amnion or porcine small intestinal submucosa can be applied to the debrided area. These bandages provide both corneal healing and additional structural support (Williams and Pinard, 2013).

8. PROGNOSIS

Corneal ulcers are a common condition in horses and usually present as superficial corneal ulcers. These ulcers usually heal within 3-7 days without complications. However, treatment may be continued for 1-2 weeks (Wickens, 2023). Severe corneal ulcers may take weeks or even months to heal completely. Antibiotic resistance, delays and difficulties in eye treatment can often lead to slow healing and/or failure to heal (Williams and Pinard, 2013; Prucha et al., 2020). Repeated fluorescein staining during treatment may help monitor healing (Brooks and Matthews, 1999; Bazay, 2024). For the treatment and management of corneal ulcers, simple and recurrent ulcers should be re-evaluated 5-7 days after starting treatment. Complex ulcers require follow-up within 24-48 hours. In addition, worsening symptoms may require re-evaluation of all ulcer types.

9. PROTECTIVE MEASURES

Although corneal ulcers are common in horses, horse owners can take important steps to minimise the risk. It is important to keep stables and paddocks free of hooks or sharp points. Keeping fences in good condition and clearing overhanging tree branches from pastures can also help prevent scratches and abrasions to the eyes. Using fly masks on horses is another way to protect the horse's eyes from insects, dirt and seed heads. In addition, regular eye checks can help to prevent corneal lesions from developing and worsening, allowing for early detection and treatment of potential problems (Wickens, 2023).

10. CONCLUSION

Corneal ulcers are among the most common eye lesions in horses. If not treated early and appropriately, they can lead to serious infection, severe pain, and vision loss.

- Sudden changes in vision in horses are a medical emergency. Prompt evaluation is important to preserve vision.
- Suspected corneal ulcers require a comprehensive eye examination, including cytology, bacterial and fungal cultures, and sensitivity testing.
- The eye should be monitored continuously during treatment to determine the effectiveness of treatment.
- Treatment of corneal ulcers should be aggressive. If the ulcer does not heal, worsens, is at high risk for perforation, or has perforation, surgery should be considered.
- If medical or surgical treatments (conjunctival flap, keratoplasty, keratectomy, etc.) fail or surgical treatment (conjunctival flap, keratoplasty, keratectomy, etc.) is not possible, enucleation should be offered to stop the infection and relieve pain.

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CHAPTER 7

MICRONUTRIENTS IN IMMUNE HEALTH OF DAIRY COWS

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Introduction

In cattle, immunoglobulins (IgG) are not transferred to the fetus via the placenta during pregnancy. As a result, calves are born immunologically naive and must begin developing their immune systems through the passive transfer of IgG provided in colostrum. During the first 24 hours of life, the calf's intestines are considered "open," allowing enterocytes in the small intestine to absorb molecules directly into the bloodstream (Brady & Eckelkamp, 2024).

Role of Colostrum in Calf Development and Immunity

Newborn calves are particularly susceptible to environmental pathogens during this period, as reflected in their high rates of neonatal mortality and morbidity. Effective colostrum management is critical for ensuring calf survival and has significant long-term health and productivity implications. While antibody-mediated protection, particularly through IgG, remains the primary focus, colostrum also contains growth factors, immune cells, and bioactive molecules that contribute to pathogen defense and regulate the development of the calf's immune system (Cid de la Paz & Rostoll-Cangiano, 2024).

Colostrum quality is traditionally defined by its IgG concentration, but researchers are now exploring the relative importance of other macronutrients and bioactive components. Early consumption of high-quality colostrum not only supports the calf's high metabolic demands but also promotes the ontogenetic development of its gastrointestinal system. This broader understanding of colostrum quality aims to improve calf development by modulating colostrum composition through tailored nutritional strategies during the prepartum period. Studies suggest that both nutrient restriction and overfeeding during late gestation can reduce colostrum volume and IgG yield, emphasizing the role of prepartum feed management in colostrogenesis (Hare et al., 2024).

Moreover, heat treatment of colostrum has been shown to preserve IgG effectively. However, it adversely affects other important colostral components, such as IgA, insulin, insulin-like growth factors (IGF), complement proteins, enzymes, and live cells, thereby reducing their concentration or viability. On the other hand, freezing preserves the natural antimicrobial properties of bovine colostrum, which are significantly diminished by heat treatment (Mann, 2024).

Gut Microbial Development and Immune Function

Holstein calves are especially vulnerable to gastrointestinal disorders in their first week of life. This highlights the importance of understanding gut bacterial colonization and immune function changes in newborn calves. A study investigating mucosa-associated bacteria and host immune function in the colon collected tissue biopsies from calves within two hours of their first colostrum feeding (D0) and again at five days of age (D5).

Metagenomic sequencing revealed that diarrhea-associated bacteria were more abundant in the colon mucosa at D0 compared to D5, while beneficial bacteria such as *Bifidobacterium*, *Lactobacillus*, and *Faecalibacterium prausnitzii* increased by D5. Functional gene analysis indicated that pathways related to viral infection, such as interferon signaling, were activated in the colon mucosa at D5. These findings suggest that environmental changes in the gut during early life reduce opportunistic pathogens and promote the growth of beneficial bacteria, alongside the activation of immune-related pathways (Nishihara et al., 2024).

IMMUNE HEALTH OF DAIRY COWS

Dairy cows are highly vulnerable to metabolic and infectious diseases during the pre-partum and postpartum periods. Because changes occur in the immune system mechanisms during these periods. During these periods, the incidence of many economically important infectious and metabolic diseases such as uncontrolled inflammation, mastitis, metritis, displacement of the abomasum and ketosis increases. These situations endanger both the health of the cows and the continuity of the enterprises due to losses in milk production. Therefore, early intervention and effective management strategies should be activated at this point.

Metabolic and infectious diseases in early lactating cows often interact with each other. For example, a relationship is known between retained placenta and the development of mastitis. The incidence of mastitis in cows in ketosis is twice as high as in healthy cows . The most important diseases that occur during this transition period are immune disorders. Special rations should be created to meet the needs for energy and nutrients in the pre- and post-partum periods. Otherwise, the metabolism and immune responses of cows may be affected (Sordillo, 2016).

Studies have observed that during the transition period, there are many critical changes in both innate and adaptive immunity that affect susceptibility to new diseases (Mallard et al., 1998; Aitken et al., 2011; Ingvartsen and Moyes, 2013). Cows during this period are susceptible to infection and an increase in the severity of diseases is observed. Both systemic and local infection directly affect the development of diseases in cows during the transition period. Delayed inflammation may lead to a hyporeactive state characterized by delays in the migration of adequately functioning neutrophils and other innate immune elements. Some studies have shown a significant decrease in neutrophil functions during the transition period. This decrease includes important functions such as reactive oxygen (ROS) production, myeloperoxidase activity, chemotaxis and phagocytosis (Cai et al., 1994; Kimura et al., 1999; Mehrzad et al., 2001). The decrease in chemotaxis and ROS production of neutrophils from cows with mastitis and metritis in early lactation is more pronounced compared to healthy cows (Cai et al., 1994). Some studies have also shown that the ability of early lactation udders to respond to E. coli endotoxin is reduced compared to mid-lactation cows (Grommers et al., 1989). Delayed migration of neutrophils and decreased antimicrobial activity are said to be among the reasons for the more severe course of coliform mastitis in the transition period (Hill, 1981; Shuster et al., 1996).

The causes of dysfunctional inflammation during the transition period have been a subject of curiosity for researchers. The role of the interaction between the endocrine and immune systems is not ignored in this process. Both in vitro and in vivo studies have shown that immune cells can produce not only hormone receptors but also various neuroendocrine factors (Lamote et al., 2006a; Kelley et al., 2007; Weigent, 2013). For example, it has been stated that the increase in steroid hormones at the time of birth may be responsible for some of the changes in the functions of bovine neutrophils (Burton et al., 2005).

Glucocorticoid receptors are found on a variety of immune cell types, and increased plasma glucocorticoid levels at the time of birth exert a potent immunosuppressive effect (Anderson et al., 1999; Lippolis et al., 2006a; Kelley et al., 2007). The mechanism by which glucocorticoids impair bovine neutrophil function involves downregulation of L-selectin and CD18 adhesion molecules, which are required for efficient activation and migration at tissue injury sites (Burton et al., 1995). In addition, changes in estradiol and

progesterone levels in the period immediately before birth have been reported to have direct and indirect effects on both lymphocyte and neutrophil functions (Roth et al., 1982; Lamote et al., 2006b).

plasma steroid hormone concentrations may not cover the entire transition period. This suggests that other hormones specific to the transition period may also contribute to immune and inflammatory disorders. For example, several homothetic hormones such as prolactin, growth hormone, IGF and insulin are said to cause significant fluctuations in metabolism and dry matter intake (DM) during the transition period (Ingvartsen and Andersen, 2000). In summary, changes in homothetic hormones at parturition and early lactation affect critical immunity of cows in the transition period.

Physiological and metabolic changes at parturition and the onset of lactation are thought to play a role in the disruption of appropriate immunity and inflammation. For example, one study investigated the effects of mastectomy on various immune parameters of milk production in pregnant cows. This study aimed to examine the effects of endocrine changes in late pregnancy and parturition on milk production (Kimura et al., 1999, 2002b; Nonnecke et al., 2003). During the pre- and postpartum periods, the increase in free fatty acids (NEFA) in mastectomized cows was moderate compared to non-mastectomized cows. It was observed that the immunity of mastectomized cows was impaired for a short time at parturition, but lymphocyte and neutrophil functions were reduced for a longer period in non-mastectomized cows (Kimura et al., 1999; Nonnecke et al., 2003). In this study, it was also observed that lactation has a negative effect on the composition of peripheral leukocyte populations (Kimura et al., 2002b). The main conclusion of these studies is that the main immunosuppressive factor in periparturient cows is not the changes in parturition and steroid hormone profiles but the increased metabolic demands of early lactation. Changes in nutritional intake and availability of certain nutrients may constitute a common link between decreased immunity and increased incidence of both metabolic and infectious diseases (Sordillo, 2016).

Nutritional status of dairy cows is also associated with optimal immunity and general health status. Nutritional needs of dairy cows change throughout the lactation cycle and unbalanced diets cause functional disorders and health problems. For example, some studies have observed that both overfed and

underfed dairy cows have a higher incidence of disease at the beginning of lactation compared to normal cows (Heuer et al., 1999; Roche et al., 2009). BMT and energy balance during the dry period may lead to abnormally high or low BCS during the prepartum period. Underfed cows (birth BCS < 3 on a 5-point scale) lack the energy and protein reserves necessary for optimal milk yield, milk fat and disease resistance (Hoedemaker et al., 2009; Roche et al., 2009). Sudden decrease in BMT in high BCS cows results in increased plasma NEFA (free fatty acid) concentrations due to severe negative energy balance (NEB) and fat mobilization . Continuous increases in plasma NEFA cannot be tolerated and various health problems occur in early lactation (Heuer et al., 1999; Hoedemaker et al., 2009). Therefore, controlling the pre- and postpartum nutrition of cows is extremely important for lactation performance and immunity.

During parturition, decreased BMT and increased NED decrease immunity. In some studies, it has been observed that decreased BMT and increased plasma NEFA concentrations are associated with the deterioration in peripheral blood neutrophil function during parturition (Cai et al., 1994). It has been stated that the decrease in prepartum neutrophil profiles is associated with diseases seen at the beginning of lactation (Cai et al., 1994; Kimura et al., 2002a; Hammon et al., 2006). Another study found that in vitro proliferative responses of peripheral blood mononuclear cells obtained from parturient cows predicted the incidence of subsequent diseases. In this study, cows with low-responding mononuclear cells experienced clinical mastitis, metritis or interdigital dermatitis within the first 60 days of lactation (Catalani et al., 2013). However, according to some findings, the cause of parity immune disorders is more complex. The effects on immunity and disease severity have been investigated by re-establishing the severe negative energy balance observed at parturition in mid-lactation cows using a food restriction method. However, to date, these studies have not been successful. For example, mid-lactation cows subjected to a 5-day food restriction showed minimal changes in immune function after mastitis induction (Moyes et al ., 2009). It has been said that the effect of experimentally induced negative energy balance on leukocyte adhesion or antigen-presenting molecules in cattle is minimal (Perkins et al., 2001). In addition, it has been reported that artificially induced negative energy balance in a mid-lactation cow has no effect on clinical symptoms following

acute endotoxin-induced mastitis (Perkins et al., 2002). These results suggest that negative energy balance and fat mobilization are important immune system disorders at the time of birth, and that nutritional deficiencies alone are not sufficient to create nutritional and metabolic imbalances at the time of birth.

A balanced diet is very important for production efficiency and immunity in dairy cows in early lactation. Deficiency of certain vitamins and trace minerals seen in the prepartum period is associated with diseases such as retained placenta, mastitis and metritis, etc. (Zhao et al., 2015). Micronutrients support the immune system and affect the incidence of disease in dairy cows (Wilde, 2006; Andrieu, 2008; Spears and Weiss, 2008; Sordillo and Aitken, 2009).

ANTIOXIDANTS

Micronutrients optimize the immune system with antioxidants. Antioxidants delay, prevent or eliminate oxidative damage to target macromolecules (Valko et al., 2007; Sordillo and Aitken, 2009). Oxidative stress is tissue damage caused by loss of redox homeostasis and excessive accumulation of reactive oxygen (ROS) or depletion of antioxidant defenses. In some studies, it has been observed that oxidative stress causes functional impairment in cows under metabolic stress due to imbalances in ROS levels and the presence of antioxidant defenses in the prepartum period (Sordillo and Aitken, 2009). The source of reactive oxygen (ROS) in transition cows is peroxisomal β-oxidation, which occurs in the liver during the conversion of consumed nutrients into energy sources by cellular respiration for milk synthesis and fatty acid metabolism (Valko et al., 2007) (Drackley, 1999; Grum et al., 2002). Reactive oxygen in healthy tissues results from increased cellular metabolism, mitochondrial energy production and various oxidative enzyme pathways associated with inflammatory responses. Reactive oxygen produced from these various sources is vital for the regulation of normal cellular processes controlling immunity and inflammatory responses. Therefore, the amount of micronutrients to be supplemented in the dairy cattle diet should both increase production efficiency and prevent oxidative stress and optimize immune cell functions (Sordillo, 2016).

Health problems occur due to inadequate antioxidant defense mechanisms and excessive accumulation of reactive oxygen species. Tissue

macromolecules such as DNA, proteins and lipids are damaged. The main target of reactive oxygen species damage is lipids and studies have shown that excessive lipid peroxidation occurs in dairy cattle during the transition period (Bernabucci et al., 2005; Sordillo et al., 2007; Sordillo and Aitken, 2009). Lipid peroxidation is a chain reaction that begins with reactive oxygen species taking electrons from lipids in cellular membranes. Electron withdrawal from fatty acids leads to the formation of lipid peroxyl radicals, which initiate an autonomous chain reaction in the plasma membrane by withdrawing additional electrons from neighboring fatty acids. Lipid peroxidation products damage cellular membranes and organelles, altering cellular functions and signaling (Sordillo, 2016). Studies using bovine endothelial cell cultures have shown that under oxidative stress conditions, lipid hydroperoxide production increases, and inflammatory responses and dysfunction in the vascular endothelium worsen (Sordillo et al., 2005, 2008). The capacity of host tissues to regulate reactive oxygen accumulation to nontoxic levels is critical for immunity optimization (Sordillo, 2016).

Antioxidant mechanisms involve a complex network of factors capable of neutralizing harmful reactive oxygen species produced during normal cellular activities and during immune cell activation against pathogens. Several vitamins and trace minerals are critical components of the antioxidant defense system. Deficiencies in these micronutrients are associated with impaired immunity in dairy cattle (Spears and Weiss, 2008). Vitamins and trace minerals directly neutralize oxidants or act as antioxidants as part of enzymatic redox couples that convert reactive oxygen species to less reactive metabolites.

Tocopherols, ascorbic acid, carotenoids and glutathione are examples of radical scavengers associated or derived from micronutrients. Catalase, superoxide dismutase and selenium-dependent enzyme systems are important reactive oxygen detoxifying enzyme systems associated with micronutrients (Sordillo and Aitken, 2009). In dairy cattle, selenium-dependent enzyme systems such as cytosolic glutathione peroxidase (GPX1) and thioredoxin reductase (TRX1), which have selenocysteine residues in their active sites, are among the antioxidant defense mechanisms. These mechanisms may not reduce H2O2 and fatty acid hydroperoxides to less reactive water and alcohol, respectively. For example, GPX1 reduces a broad spectrum of hydroperoxides for the redox couple glutathione. In addition, oxidized cysteine groups in

proteins can be reduced via the redox-active center of thioredoxin and disulfide bonds can be formed, which are reduced by TRX1 and NADPH (Sordillo and Aitken, 2009). Iron-containing catalase, cytosolic superoxide dismutase (copper and zinc) and mitochondrial superoxide dismutase (manganese and zinc) enzyme systems also play a role in preventing the formation of oxygen radicals (Sordillo, 2016).

Antioxidants act as free radical scavengers when they stabilize reactive oxygen species and donate electrons, thus becoming a more stable radical despite being oxidized. For example, α-tocopherol (vitamin E) becomes a tocopherol radical by donating hydrogen ions to hunt lipid peroxyl radicals. The tocopherol radical can be reduced again by ascorbic acid (vitamin C). In other words, ascorbic acid acts as both a free radical scavenger for oxidized biomolecules and maintains the redox balance of cells (Sordillo and Aitken, 2009).

β-Carotene is also an important free radical scavenger. Carotenoids are extremely important in neutralizing singlet oxygen and also have the ability to prevent the formation of secondary reactive oxygen species (Sordillo, 2016).

concentrations are generally reduced in dairy cattle at parturition . The decrease in serum-based micronutrients is thought to be due to the decrease in BMT and the increased utilization of micronutrients due to metabolic stress during the transition period (Spears and Weiss, 2008; Sordillo and Mavangira, 2014). The amount of vitamins and minerals to be supplemented in the dairy cattle diet should maximize productivity, prevent oxidative stress, and be sufficient to optimize immune cell functions (Sordillo, 2016).

MINERAL

Many micronutrients have been shown to affect different aspects of the immune system in cattle. The interactions between nutritional status, immune responses and disease resistance are complex. Although some micronutrients play a role in the immune system, their deficiencies do not always increase the susceptibility of animals to natural or experimental infections.

Considerable research has shown that chromium (Cr) can have an impact on the immune response and disease resistance of cattle. However, responses to the effects of added chromium are highly variable. Chromium is present in very low concentrations in the body and unfortunately there is currently no

method to reliably measure chromium status. Chromium concentrations in control diets have not been reported in many studies and there is also little information on the bioavailability of chromium from various feeds.

In one study, the effect of chromium (Cr) added to the diet on the ability of isolated peripheral lymphocytes to proliferate in response to mitogen stimulation was examined and it was observed that it increased blastogenic stimulation, i.e., positive effects on cellular immunity and humoral immunity (Burton et al., 1993). In another study, no effect on udder health was found in dairy cows supplemented with Cr-amino acid chelate (Chang et al., 1996).

Stress causes elevated cortisol levels, which suppress immune functions. In some studies, chromium (Cr) supplementation of cattle reduced serum cortisol levels (Chang and Mowat, 1992; Mossie-Shageer and Mowat, 1993; Kegley et al., 1996), but in other studies, no effect of chromium on cortisol was observed (Kegley and Spears, 1995; Kegley et al., 1997; Lindell et al., 1994).

In one study, it was reported that neutrophils from a Cu-deficient dairy cow had a reduced ability to inhibit S. aureus (Torre et al., 1996). Limited research with Cu has shown that dietary copper (Cu) can affect cytokine production in cattle.

Mononuclear cells from dairy cows with marginal Cu levels (6–7 mg/kg diet) produced less IFN-γ when stimulated with Con A compared to dairy cows receiving sufficient Cu, but Cu had no effect on IL-2 production by mononuclear cells (Torre et al., 1995). In another study, TNF-α and IL-1 production by isolated peripheral blood monocytes stimulated with E. coli lipopolysaccharide was not affected by Cu or Mo intake (Gengelbach and Spears, 1998).

In one study, animals were inoculated with pathogens to measure the effect of Cu deficiency on immunity. After inoculation with IBRV and P. haemolytica, it was observed that both groups of calves showed similar signs of respiratory disease. Before disease challenge, calves in the Cu deficient group were fed a diet containing 1.5 mg Cu/kg for 150 days, during which time plasma Cu levels decreased to approximately 0.2 mg/l (Stabel et al., 1993).

In another study, rectal temperature response in heifers inoculated intranasally with Bovine herpesvirus-1 was similar between those fed a diet supplemented with adequate Cu and those fed a diet supplemented with Mo. However, after viral immunity, plasma fibrinogen levels (acute phase protein)

increased in heifers fed a diet high in Mo, but no increase was observed in heifers receiving adequate Cu (Arthington et al, 1996).

In a study conducted by Gengelbach et al. (1997), the effects of dietary Cu, Fe and Mo on the immune response to IBRV and subsequently P. haemolytica in newly weaned calves were investigated. A control group containing 4.5 mg Cu/kg and a control group supplemented with 5 mg Mo, 600 mg Fe or 10 mg Cu/kg were formed for calves born from control heifers. Severe Cu deficiency was observed in the calves in the control, Fe and Mo treatment groups according to plasma Cu concentrations (<0.20 mg/l) and ceruloplasmin activities before weaning. In calves with sufficient Cu in their diets and those fed with high Fe, rectal temperatures were higher after the disease occurred than in the control and calves fed with Mo. It was observed that the calves had higher body temperatures after the disease and lower feed intake compared to those fed with Fe and Cu. Plasma TNF-α concentrations were observed to be higher in calves with adequate Cu in their diets, and this was sufficient to explain the higher body temperature and lower feed intake in animals with Cu supplemented diets. It is still unknown whether body temperature and feed intake in calves with Fe supplemented diets differ from the control and Mo supplemented groups (Spears, 2000)

Zinc

Studies conducted on various human and laboratory animals , it is said that zinc (Zn) deficiency reduces immune responses and disease resistance (Cheters, 1997). Controlled studies conducted on cattle indicate that Zn deficiency does not affect cellular or humoral immune responses (Spears, 2000). This finding is in contrast to the findings in studies conducted on mice and humans that Zn deficiency reduces immune responses (Fraker et al., 1984) .

Severe Zn deficiency has been observed in Holstein and Shorthorn calves due to a genetic defect affecting Zn absorption (lethal trait A46); these calves exhibited thymus atrophy and reduced lymphocyte responsiveness to mitogen stimulation (Perryman et al., 1989). In young calves, supplementation of 150 or 300 mg Zn/kg to a control diet containing 65 mg Zn/kg did not affect mitogen-induced blastogenesis, cytotoxicity, IL-2 production by lymphocytes, or phagocytic and bactericidal activities of isolated neutrophils (Kincaid et al.,

1997). Calves receiving 17 mg Zn/kg of the diet had a reduced induration response to intradermal PHA injection compared to those receiving Zn supplementation (Engle et al., 1997).

Although Zn deficiency in ruminants has no significant effect on immunity, some studies suggest that dietary Zn supplementation may affect disease resistance. Increasing the amount of Zn supplemented from 30 to 100 mg/kg has been observed to slightly reduce morbidity from respiratory diseases in newly weaned calves that are transferred (Galyean et al., 1995).

Cobalt

Limited studies on cobalt suggest that cobalt (Co) deficiency may negatively affect neutrophil function and resistance to parasitic infections . It has been observed that neutrophils obtained from Co-deficient calves have reduced ability to kill C. albicans (MacPherson et al., 1987; Paterson and MacPherson, 1990). In Co-deficient calves, it has been observed that the prepatent period after infection with Ostertagia ostertagi inoculation is shortened and the number of eggs excreted in the feces is increased (MacPherson et al., 1987).

Selenium

Some studies have shown that Se deficiency reduces the ability of cattle blood and milk neutrophils to kill yeast and bacteria (Boyne and Arthur, 1979; Grasso et al., 1990). In a study, it was observed that bovine mammary endothelial cells grown in Se-deficient cell culture medium increased neutrophil adhesion when stimulated with TNF-α and IL-2 (Maddox et al., 1999). Thus, migration of neutrophils to tissues and subsequent inflammation may be affected (Spears, 2000).

Low dietary selenium intake is reported not to consistently affect cellular immune responses in ruminants (Stabel and Spears, 1993).

It was reported that Se deficiency in dairy cows reduced the response of isolated peripheral blood lymphocytes to mitogen stimulation with Con A (Cao et al., 1992).

It has been suggested that the decreased response in lymphocytes of Se-deficient cows may be related to altered arachidonic acid oxidation by the 5-lipoxygenase pathway. Lymphocytes of Se-deficient cows produce less

arachidonic acid oxidation products (5-hydroxyeicosatetraenoic acid and leukotriene B4) when stimulated (Spears, 2000).

Mastitis induced by intramammary inoculation with E. coli was more severe and prolonged in cows receiving 0.04 mg selenium/kg than in cows receiving 0.14 mg selenium/kg (Erskine et al., 1989). However, in mastitis induced by intramammary inoculation with S. aureus, the severity and duration of infection were not affected by Se deficiency (Erskine et al., 1990).

VITAMIN

Selenium and vitamin E play a role together in the cellular antioxidant defense system. Vitamin E acts as an antioxidant by neutralizing free radicals and preventing lipid peroxidation (Bendich, 1993). Selenium is an important component of the GSH peroxidase enzyme, which destroys H2O2 and lipid hydroperoxides (Rotruck et al., 1973). There is evidence that taking these two components together increases the immune response (Stabel and Spears, 1993).

According to the study by Hogan et al. (1990), vitamin E supplementation to dairy cows and the bactericidal activity of blood neutrophils in calves fed milk replacer increased according to the study by Eicher et al. (1994).

It was observed that the decrease in postpartum neutrophil superoxide anion production and IL-1 production was prevented in dairy cows supplemented with 3000 mg vitamin E per day for 4 weeks before and 8 weeks after parturition, compared to cows not receiving vitamin E supplementation (Politis et al., 1995).

Vitamin E supplementation is said to increase blood lymphocyte responsiveness to PHA in young calves (Reddy et al., 1986).

Antibody responses have been reported to be most consistent with dietary supplementation of both Se and vitamin E (Stabel and Spears, 1993; Finch and Turner, 1996).

The vitamin E and Se status of dairy cows affects resistance to intramammary infections . In one study, it was observed that in cases where there was insufficient Se and vitamin E in the dry period diet, supplementation with 740 mg of vitamin E per day reduced the incidence of clinical mastitis at the time of parturition by 37%. In this study, it was found that injection of 1 mg Se per body weight 21 days before parturition did not affect the incidence of

clinical mastitis, but reduced the duration of clinical signs by 46%. Cows supplemented with both vitamin E and Se had shorter clinical mastitis symptoms than those supplemented with only one nutrient (Smith et al., 1984).

Vitamin E and Se status is said to be associated with the incidence of clinical mastitis and milk somatic cell count. High serum Se levels have been associated with reduced rates of mastitis and low milk somatic cell counts; high blood vitamin E levels have been associated with reduced rates of clinical mastitis (Weiss et al., 1990). In a study conducted by Weiss et al. (1997) on dairy cows fed Se-deficient diets (0.1 mg/kg diet), cows receiving high levels of vitamin E supplementation were less likely to develop clinical mastitis during the first 7 days of lactation. Cows in the high vitamin E group received 1000 mg/day during the first 46 days of the dry period, 4000 mg/day during the last 14 days of the dry period, and 2000 mg/day during lactation. Cows in the low vitamin E group received 100 mg/day during the dry period and lactation, while cows in the medium vitamin E group received 1000 mg/day during the dry period and 500 mg/day during lactation.

Vitamin A and β-Carotene

β-Carotene is a precursor to vitamin A, which occurs naturally in foods. Studies suggest that β-carotene may have effects on immune function. β-carotene may function as an antioxidant, whereas vitamin A is not a significant antioxidant. Animals with vitamin A deficiency are more susceptible to bacterial, viral, and parasitic infections (Chew, 1987). Supplementation of β-carotene to dry cows has been shown to reduce the incidence of mastitis in the early dry period (Chew, 1993). However, in dairy cows supplemented with 17.2 mg retinyl acetate/day, increasing vitamin A supplementation or supplementing with 300 mg β-carotene/day did not affect the incidence of clinical mastitis or new intramammary infections (Oldham et al., 1991).

In a study by Michal et al. (1994), the effects of β-carotene and vitamin A on immune function and the incidence of placental retention and metritis were evaluated. Control cows were not given vitamin A. Cows supplemented with 300 or 600 mg β-carotene for 4 weeks before calving had lower rates of placental retention and metritis than controls. The incidence of placental retention was similar in cows supplemented with β-carotene and those receiving 66 mg retinyl palmitate/day, while the incidence of metritis was lower in cows

supplemented with β-carotene. Furthermore, β-carotene has been found to enhance lymphocyte proliferation induced by Con A, PHA and PWM before and after birth (Michal et al., 1994). Addition of low concentrations of β-carotene to bovine lymphocyte cultures has been found to stimulate mitogen-induced blastogenesis (Daniel et al., 1991) . However, another study has reported that β-carotene supplementation did not consistently affect neutrophil bactericidal activity (Michal et al., 1994).

Choline

Choline is known as a critical nutrient in the nutrition of monogastric animals; It is a precursor of the synthesis of basement membrane phospholipids, functions in 1-carbon metabolism and acetylcholine synthesis (Zeisel and Da Costa, 2009). Choline has great importance in lipid intermediary metabolism. Choline deficiency causes the development of fatty liver disease as a result of decreased phosphatidylcholine biosynthesis. This prevents the excretion of liver triacylglycerols as very low density lipoprotein (VLDL), causing liver steatosis (Yao and Vance, 1988).

In ruminants, almost all of the choline taken in the diet is metabolized by rumen microorganisms (Sharma and Erdman, 1989a). Therefore, the choline to be supplemented to ruminants should be in a rumen-protective form. It is quite common for dairy cows to develop negative nutritional balance in the last weeks of pregnancy and the first few weeks of lactation. Most of the fat stores are catabolized and on average half of the cows have moderate or severe fatty liver (Bobe et al., 2004). This has been said to be related to increased fatty acid uptake by the liver and a slow lipoprotein excretion rate (Drackley, 1999). One study showed an increase in fatty acid uptake by the liver of approximately 2.28 mol/day between 19 days before and 11 days after parturition. This increase reflected the change in lipolysis at the beginning of lactation, placing cows at risk of fatty liver (Reynolds et al., 2003).

Many studies have been conducted to determine the effects of different forms and doses of choline supplementation on postpartum performance (Hartwell et al., 2000; Zhou et al., 2016; Zenobi et al., 2018a). In general, it has been observed that supplementation of protected choline to transition cows increases the yields of milk, fat and energy-conserving matrix (Baldi and

Pinotti, 2006; Sun et al., 2016; Zenobi et al., 2018a; but the accuracy of the results has not been fully determined (Hartwell et al., 2000; Zhou et al., 2016).

Dietary supplementation with protected choline (RPC) provides positive effects by increasing dry matter consumption, milk yield and metabolism (Humer et al., 2019; Arshad et al., 2020). However, since health outcomes may vary; in some studies, fetal membrane expulsion and mastitis events were reduced with choline supplementation, but choline had no effect on hypocalcemia and ketosis (Lima et al., 2012).

In another study, supplementation of protected choline (RPC) to the diet of transitional dairy cows increased milk yield and reduced the risk of retention of foal membranes and mastitis. However, metabolized methionine consumed after parturition affected the function of choline and caused changes in yield. As the methionine rate increased, the response to choline decreased, indicating that there may be an interaction between the two nutrients. Due to the linearly related responses of choline, the optimum amount of choline that should be supplemented to transitional cows is unknown. Most studies indicate that it may be more than 12.9 g per day (Arshad et al., 2020).

Choline is a vital nutrient for dairy cows and plays a critical role in various physiological functions such as lipid metabolism, milk production, and liver metabolism (Caprarulo et al., 2020).

Rumen-protective choline supplementation increases antioxidant ability during the transition period and improves immunity and inflammation in dairy cows. Therefore, adequate rumen-protective choline supplementation is recommended to protect animals from diseases and increase productivity (Khan et al., 2023).

In a study, choline was given to cows in late pregnancy and the incidence of subclinical ketosis decreased, lactation performance increased, the immune system was strengthened and resistance to uterine diseases increased by changing the vaginal discharge microbiome (Marques et al., 2024).

- **Conclusion**

Dairy cows are prone to metabolic and infectious diseases during the transition period due to changes in the immune system. Conditions such as mastitis, metritis and ketosis not only affect cow health, but also reduce milk production, affecting farm profitability. During early lactation, metabolic and

immune disorders are interconnected, and immune dysfunction is a major concern. Hormonal fluctuations and increased metabolic demands contribute to immune suppression, while malnutrition further exacerbates health risks.

Appropriate nutrition is essential for optimal immunity and lactation performance. Deficiencies in vitamins and trace minerals can lead to conditions such as retained placenta and mastitis. Metabolic stress and nutritional imbalances (overfeeding or underfeeding) impair immune function, and poor body condition or negative energy balance (NEB) increase disease risk. However, nutritional deficiencies alone do not fully explain immune dysfunction; balanced diets are critical.

During the transition period, oxidative stress from increased reactive oxygen species (ROS) production can damage cells, weaken immunity and exacerbate inflammation. Antioxidants such as vitamins E and C and β-carotene play a vital role in neutralizing ROS and supporting immune function. Transition cows often experience decreased serum micronutrient levels, which weakens antioxidant capacity and immune defenses.

Minerals such as chromium, copper, zinc, cobalt, and selenium play important roles in immune function, but the effects of deficiencies are often inconsistent. Selenium and vitamin E work together in the antioxidant defense system to improve immune function. Supplementation can reduce the incidence and severity of clinical mastitis. β-Carotene supports immune function and reduces the incidence of mastitis, while vitamin A deficiency increases susceptibility to infection.

Choline is vital for lipid metabolism and liver function. Supplementation prevents fatty liver disease, supports milk production, and enhances immune function, especially during the transition period. Vitamins and minerals such as selenium, vitamin E, vitamin A, β-carotene, and choline are essential for the health, immune function, and productivity of dairy cows. Adequate supplementation during the transition period can reduce disease risk, improve immune responses and enhance overall performance. Personalized nutrition plans and proactive health management are essential to support dairy cow health and maximize farm profitability.

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CHAPTER 8

STRUCTURE, FUNCTION AND RECEPTORS OF GALANIN

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INTRODUCTION

Galanin, a neuropeptide 29 amino acids long (30 in humans) encoded by the galanin gene in mammals, is involved in physiological and pathological functions, including neuronal regeneration, control of food intake, reproduction, energy expenditure, various neuroendocrine functions, osmotic regulation, and water intake (Fang et al., 2012; Palus et al., 2019; Demsie et al., 2020). Discovered in 1983 in pig intestinal extract, galanin (Tatemoto et al, 1983) molecular formula $C_{146}H_{213}N_{43}O_{40}$, molecular weight 3210.5 g/mol (Anonymous, 2024) and amino acid sequence was reported as Gly-Trp-Thr-Leu-Asn-Ser-Ala-Gly-Tyr-Leu-Leu-Gly-Pro-His-Ala-Ile-Asp-Asn-His-Arg-Ser-Phe-His-Asp-Lys-Tyr-Gly-Leu-Ala-NH_2 (Figure 1) (Tatemoto et al., 1983).

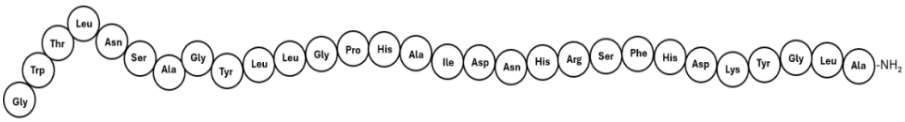

Figure 1. Amino acid sequence of porcine galanin

Today, the galanin family consists of galanin-like peptide (GALP), galanin message-associated peptide (GMAP) and alarin (Fang et al., 2012). In 1999, GALP was obtained from porcine hypothalamus. The GALP molecule, which shows similar sequence homology with galanin, is produced from a polypeptide precursor containing 115-120 amino acids. In humans, it is a peptide of 60 amino acids (Ohtaki et al., 1999). Amino acids 1-13 of galanin at positions 9 and 21 of this amino acid chain show a fixed sequence (Lawrence, 2009). GALP has been shown to increase luteinizing hormone release and food interest in mice (Matsumoto et al., 2001). Thus, GALP has an orexigenic (appetite stimulating) effect on feeding, similar to galanin. On the other hand, it causes anorexic (appetite inhibitory) effects over a certain period of time, causing two opposite reactions on energy homeostasis (Seth et al., 2003). GMAP, a 59 amino acid peptide, was isolated from porcine adrenal medulla. Most neurons in the peripheral and central nervous system express both GMAP and galanin-immunoreactivity (Fang et al., 2015). Alarin, a variant of GALP, (Fang et al., 2012) is a 25 amino acid neuropeptide (Webling et al., 2012). It was first isolated from gangliocytes of human neuroblastic tumours in 2006

(Santic et al., 2006) and named after N-terminal alanine and C-terminal serine amino acids (Webling et al., 2012).

Galanin was first isolated from the myenteric plexus and mucous and submucous plexuses in the gastrointestinal tract. Later, it was found in the central and peripheral nervous systems and the endocrine system. In the spinal cord, galanin was detected in spinal cord-specific nerve cell bodies, intermediate dorsal laminae and lower motor neurons (Kerr et al., 2015, Brzozowska and Calka, 2021). In addition, galanin has been found to prevent pain by causing the release of acetyl choline, serotonin, glutamate, γ-amino butyric acid (GABA), noradrenaline and dopamine from the hypothalamo-pituitary axis in the central nervous system, and to affect homeostasis in the sleep/wake cycle (Rattan, 1991; Webling et al., 2012). In this book chapter, information about the structure, function and receptors of galanin is given.

1. STRUCTURAL PROPERTIES OF GALANIN

Galanin is a 29 amino acid structure formed by amidation of the peptide at the C-terminal end. This structure is common to most species except humans. In humans, galanin consists of a 30 amino acid molecular structure that does not include the C-terminal end amidation. In all species, the sequence of 1-15 amino acids at the N-terminal is identical, whereas there are differences in amino acid subtypes at the C-terminal between species. The receptor-ligand interaction occurring on galanin frequently takes place at the N-terminal part. Receptor-ligand binding occurs by cross-identification reaction between different species. This cross-labelling has been clearly demonstrated in autoradiographic studies of pig, mouse, and human galanin (Köhler et al., 1989; Hulting et al., 1993). Galanin is derived from the cloning of GMAP structures and ‘preprogalanin’, a single-copy gene resulting from proteolysis of a 123- (pig, human) or 124- (mouse and other species) amino acid precursor pro-peptide (Kofler et al., 1996; Lang et al., 2007; Lang et al., 2015).

2. FUNCTIONS OF GALANIN

The structural properties of galanin allow the molecule to play a role in a wide range of physiological processes (Mills et al., 2021). In relation to food intake, in addition to increasing appetite and promoting energy intake (Edwards et al., 1999), it protects nerve cells from damage and promotes repair, thus

providing neuronal regeneration and protection (Hevesi et al., 2024). It also affects energy expenditure by regulating metabolism (Fang et al., 2015).

Galanin also affects the secretion of various hormones. For example, it stimulates the secretion of growth hormone, prolactin and corticotropin, while inhibiting insulin secretion (Fang et al., 2015; Zhu et al., 2022). In addition, it affects reproductive functions by regulating the secretion of reproductive hormones (Gundlach, 2002).

Galanin is an important molecule that shows analgesic effect by regulating pain perception. It is synthesised in neurons located in the spinal cord and pain transmission pathways in the brain and shows pain inhibitory effects (Fonseca-Rodrigues et al., 2022). It can also reduce the severity of these conditions by modulating the stress response (Picciotto et al., 2010; Corradi et al., 2022).

Having neuroprotective properties that protect nerve cells from damage, galanin may delay or prevent the death of nerve cells in brain damage or neurodegenerative diseases (Jiang and Zheng, 2022). Galanin inhibits gastric acid secretion in the digestive system, slows bowel movements and increases food intake (Brzozowska and Calka, 2021). It is thought that galanin metabolism is impaired in metabolic diseases such as obesity (Gallagher et al., 2024).

3. GALANIN RECEPTORS

Galanin peptide has three receptor subtypes. These are; galanin receptor 1 (GALR1), galanin receptor 2 (GALR2) and galanin receptor 3 (GALR3) (Webling et al., 2012).

Galanin Receptor 1 (GALR1): GALR1 was identified as the first functional galanin receptor. This was isolated from human melanoma cells in the skin. Activation of GALR1 is mediated by multiple transduction involving G-protein mediated suppression of the cyclic AMP/protein kinase A pathway and activation of phospholipase C. GALR1 is frequently found in central nervous system tissues such as thalamus, hypothalamus, amygdala, olfactory system, pons, medulla and spinal cord (Shimosegawa et al., 1992). In the central nervous system it is involved in the control of neuropathic pain and in the hypothalamus it regulates the response to thermal stimuli. GALR1 has been found to mediate antinociception in cases where nerve damage occurs (Zhang

et al., 2019). In addition, GALR1 regulates secretion increase and motility in the intestine by stimulating intestinal hormones through peripheral nerves in the gastrointestinal tract (Brzozowska and Calka, 2021; Kiezun et al., 2022). It also controls glucose metabolism by suppressing insulin secretion from small intestinal cells (Fang et al., 2020).

Galanin Receptor 2 (GALR2): GALR2 was isolated from cDNA identified in mouse tissue (Lundström et al., 2005). Its human receptor consists of 387 amino acids (Smith et al., 1997). GALR2 stimulates different pathways within the cell through G protein subtypes. Galanin peptides, which are detected as residues at the N-terminal ends of cells, also take part in various functions through the interaction of seven different membrane receptors specific to G protein. The stimuli generated in this way lead to the activation of phospholipase C in intercellular pathways. As a result of this activation, calcium-dependent chloride channels located in the cytoplasm open and calcium stored in the cell is released and provides hydrolysis of inositol phosphate (Webling et al., 2012; Sipkova et al., 2017).

Galanin Receptor 3 (GALR3): GALR3 was first isolated from cDNA in the mouse hypothalamus. It consists of 386 amino acid sequences. 36% of this amino acid sequence is similar to human GALR1 and 58% is similar to GALR2. GALR3 is found 90% in mice (Lang et al., 2007; Webling et al., 2012).

4. CONCLUSION

The involvement of galanin in many physiological and pathological processes has increased the interest in this neuropeptide in recent years. Further research on the effect of galanin in the organism will help to understand many physiological and pathological processes as well as to develop the right strategies for these processes.

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CHAPTER 9

ARTIFICIAL INTELLIGENCE AND LIVESTOCK

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1. Introduction

Every year, the world's nutritional needs increase due to the increase in the world's population. Today, the planet's population has exceeded 8 billion, and is estimated to reach 8.5 billion by 2030 and approximately 10 billion by 2050 (FAO, 2024). With the population set to increase by approximately 2 billion, the agricultural sector is under more stress than ever. In other words, simply expanding cropland or increasing animal herds may not be an option to meet the projected demand (Melak et al., 2024).

Today's livestock industry has become more innovative in its approaches to improving production activities as a result of various factors such as climate change, food security concerns, changes in production systems, and adaptation efforts (Rojas-Downing et al., 2017).

Despite the continuous growth of the herds in production, the difficulty of finding personnel to work in these herds and the lack of interest of young people in entering production lead producers to use newer and more complex methods (Aydan, 2019; Bouhali et al., 2022; Congdon et al., 2022). Producers will be able to obtain more output with less input by increasing efficiency in production.

Existing and new technologies such as the Internet of Things (IoT), artificial intelligence, robotics and big data can contribute to making processes more efficient and help create new products and services. Digital technologies such as smartphones, tablets, in-field sensors, drones and satellites are used by many producers. These technologies can provide various agricultural solutions such as remote measurement of soil conditions, better water management, monitoring of livestock activities and crops. The collected data can be analyzed to gain insight into possible future crop models or animal health and welfare. This allows producers to plan more effectively and be more productive (EIP-AGRI Service Point, 2017).

In terms of increasing efficiency, technologies based on artificial intelligence are used in farms for production planning, herd management, sustainable resource use and disease management (Fuentes et al., 2022).

Artificial intelligence (AI) is a branch of computer science that aims to provide computers with the ability to learn by imitating living organisms and

to make past-based evaluations. The term “Artificial Intelligence” can be used both to refer to a specific branch of technology in this field and, in a broader sense, to refer to various technologies in this field, such as Machine Learning, Deep Learning, etc. In most cases, it exhibits much higher accuracy compared to traditional methods (Naddaf-Sh et al., 2018).

Artificial intelligence is an individual-specific electronic device that is expected to be applied immediately in cattle production. Artificial intelligence helps producers collect and analyze files to accurately predict service performance by performing purchasing patterns and trend analysis. Artificial intelligence applications increase efficiency in current and future agricultural systems to increase production and reduce waste while minimizing environmental impact. Artificial intelligence is an intelligent monitoring system that can suggest solutions that can help producers use resources more sustainably, obtain more, healthier products from animal breeds, control diseases, monitor movements and reduce workload, thus steadily emerging as part of the technological evolution of the sector (Morota et al., 2018; Smith et al., 2006; Rotaru et al., 2021; Watts, 1998; Zuraw and Aeffner, 2022).

This study aims to reveal the potential effects and importance of artificial intelligence technology in the livestock sector.

2. What is Artificial Intelligence?

According to the OECD recommendation, the updated definition of an artificial intelligence system is "machine-based systems that infer how outputs such as predictions, content, suggestions or decisions that may affect physical or virtual environments can be produced from the inputs they receive for explicit or implicit goals (OECD, 2024). In the Turkish Language Association dictionary; artificial intelligence, which is expressed as the ability of a computer, a robot under computer control or a programmable device to exhibit functions similar to humans such as perception, learning, reasoning, decision-making, problem-solving, communication, etc., can be defined briefly as automatic systems that can produce algorithms (Köroğlu, 2017; TDK, 2024).

Artificial intelligence has benefits that can be used in many areas today. They are systems that make life easier by allowing some tasks that a human can do to be done much faster with more data. In daily life, there are artificial

intelligence applications used in many areas such as image processing (face recognition, security and surveillance, photo tagging on social networks ...), sound processing (music recognition, voice response and password, speech-to-text synthesis ...), text processing (translation services, online chat and assistant, personalized spelling correction and suggestions ...), data processing (weather, traffic density route planning, recruitment and performance evaluation systems ...), health data analysis and treatment planning (applications that assist doctors in the diagnosis and treatment planning process), unmanned artificial intelligence-supported driving systems, insurance and finance, big data analytics, smart applications in agriculture and livestock (precision animal production), cyber security (Presidency of the Republic of Turkiye Digital Transformation Office (CBDDO), 2024).

3.History of Artificial Intelligence

George Boole was the first to define a formal language for logical reasoning in 1847. Later, in the history of artificial intelligence, the definition of the Turing machine by Alan M. Turing in 1936 was reported as a turning point. It was stated that Warren McCulloch and Walter Pitts created a model of artificial neurons in 1943, and in 1944, J. Neumann and O. Morgenstern determined the decision theory to determine agent preferences. It was stated by Donald Hebb in 1949 that a rule was put forward that changed the value for the connections of artificial neurons that provided a chance to learn, and in 1951, Marvin Minsky and Dean Edmonds created the first neural computer (Benko and Lányi, 2009). It was reported that the term artificial intelligence (AI) was first used by John McCarthy in 1955 (Hamet and Tremblay, 2017). The Datmouth conference, pioneered by McCarth, became a turning point for the subject of artificial intelligence and attracted the attention of many researchers. McCarty, who is considered the father of artificial intelligence, developed the LISP language to create artificial intelligence software and is still used regularly today (Frpnet, 2024).

In 1965, Herbert Simon predicted that machines would be capable of doing any job a human being can do in twenty years. In the same year, Lotfi

A. Zadeh published the Fuzzy Sets article, which introduced fuzzy logic (Artificial İntellegence, 2024).

Perceptrons were published in 1968 by Marvin Minsky and Seymour Papert as a demonstration of the limits of simple neural networks. PROLOG, a new programming language for creating artificial intelligence systems, was developed by Alain Colmerauer in 1972. In 1983, Johnson Laird, Paul Rosenbloom, and Allen Newell completed their CMU theses on SOAR (Benko A. and Lányi C. S., 2009).

Since the late 1980s, AI researchers have deepened their work in subfields such as expert systems, natural language understanding and translation, case-based reasoning, machine learning and robotics, virtual reality, and games. Since the early 2000s, with the spread of the Internet and the increase in large data sets, AI research has become widespread. In particular, technology giants such as Google, Facebook, and Amazon have begun to make major investments in the field of AI and machine learning (Yapayzeka, 2024).

Since the 2010s, a subfield called deep learning has become a focus of great interest in the field of artificial intelligence. Deep learning is a technique that increases the ability to recognize and learn complex patterns by using multi-layered structures of artificial neural networks. In particular, deep learning methods have achieved great success in areas such as image recognition, speech recognition, and natural language processing (Kaya and Ata, 2019).

When we evaluate the developments in the field of artificial intelligence in 3 phases; in the first phase, the focus was on studies on learning, which were limited in scope and aimed to solve workshop-level problems that excited people. In the second phase, emphasis was placed on machine learning by imitating human learning mechanisms. In this phase, many artificial intelligence algorithms, especially artificial neural networks, and deep learning methods were developed and valuable studies were carried out at the automation level. In the third phase, studies are being carried out on the development of artificial intelligence technologies that can learn much faster, imitate humans at the cognitive level, make autonomous decisions and socialize. (Dereli, 2020).

Artificial intelligence has sub-branches such as machine learning, artificial neural networks, natural language processing, evolutionary computing, expert systems, vision, robotics. To briefly provide information about these sub-branches; expert systems are computer systems that mimic a human's decision-making ability. Rather than traditional codes, they were originally developed to solve complex problems by reasoning through If-then rules. Machine learning allows algorithms to learn using data to perform a specific task and automates analytical model creation. It uses neural networks, statistics, operations research, and physics methods to find hidden information in data without being explicitly programmed on where to look or what to search for. When it comes to robotics, structures that produce machines that can think to make human life easier using artificial intelligence technology come to mind. It is possible to transfer human characteristics to machines through various sensors used. Natural language processing is the ability of computers to analyze human language, including speech, understand and produce. Artificial neural networks are among the most commonly used structures in artificial intelligence technology. Image recognition and speech recognition applications can be given as examples of deep learning applications (Sargın, 2023).

4. Developments in Artificial Intelligence in the World

The United States and China are the leading countries in the international arena when it comes to investing in artificial intelligence technologies. As in these leading countries, the importance of this field in achieving political and strategic goals, economic growth and development is increasing in other countries around the world. (Yıldız, 2023). As of 2022, the main countries that have published a national artificial intelligence strategy document are Algeria, Argentina, Austria, Australia, Bangladesh, Botswana, Brazil, Bulgaria, Canada, Chile, China, Colombia, Croatia, Cyprus, Czechia, Denmark, Egypt, Estonia, Finland, France, Germany, Greece, Hungary, India, Indonesia, Ireland, Italy, Japan, Kenya, Latvia, Lithuania, Luxemburg, Malta, Mauritius, Mexico, Netherlands, Norway, Peru, Philippines, Poland, Portugal, Qatar, Romania, Russia, Saudi Arabia, Serbia, Singapore, Sierra Leone, Slovenia, South Korea, Spain, Sweden, Switzerland, Thailand, Tunisia,

Türkiye, Ukraine, UAE, United Kingdom, USA, Uruguay and Vietnam. It is also seen that many countries are in the process of preparing national artificial intelligence strategic documents (Our World in Data, 2023).

National AI strategies and policies prepared in many countries tend to prioritize many sectors such as agriculture and food, cybersecurity, defense/security, education, energy, environment, finance, healthcare, productivity, aviation/space and telecommunications. Countries such as Australia, Denmark, Hungary, Japan, Korea, Lithuania, Netherlands, Poland Türkiye, the United States, China and India are among the countries that prioritize agriculture and food sectors (Galindo et al., 2021).

5. Developments in Artificial Intelligence in Türkiye

In our country, the "National Artificial Intelligence Strategy 2021-2025" was published in the Official Gazette dated 20/08/2021 and numbered 31574 and entered into force. The strategy has 6 strategic priorities, and these are; It is designed to train artificial intelligence experts and increase employment in the field, support research, entrepreneurship and innovation, expand access to quality data and technical infrastructure, make arrangements to accelerate socioeconomic harmony, strengthen international cooperation and accelerate structural and workforce transformation (Digital Transformation Office of the Presidency of the Republic of Türkiye, 2024).

In our country, within the scope of the TÜBİTAK 2024-2025 Priority R&D and Innovation Topics, the Scientific and Technological Research Council of Türkiye (TÜBİTAK), one of the "related" institutions of the Ministry of Industry and Technology, includes multi-purpose autonomous robots and quality and efficiency in agriculture and animal husbandry among the priority sectoral applications. It has been reported that innovative projects will be supported on the subjects of feeding and caring for each animal within the framework of its own needs, monitoring its health and increasing productivity per unit animal, collecting more healthy data quickly, ensuring that possible problems are quickly detected and necessary decisions are taken as a result of processing, preventing human-related errors and ensuring employee safety in terms of artificial intelligence - multi-purpose autonomous robots. Artificial intelligence - includes support for innovative projects

focusing on developing and using artificial intelligence solutions in addition to traditional methods in increasing quality and efficiency in agriculture and animal husbandry, instant monitoring of parameters related to animal health and productivity through artificial intelligence-based software and systems, and development of decision support systems and their implementation in enterprises with high digital maturity (TÜBİTAK, 2024).

6. Uses of Artificial Intelligence in Livestock

Livestock has many economic functions such as providing raw materials for the industry, increasing national income and developing foreign trade, creating new employment areas for the industry and services sector, developing employment in rural areas, ensuring social and economic development between regions, and ensuring that balanced development between sectors takes place in a stable manner (Cevger, Y., 2019).

The importance of the food, agriculture and livestock sector, which is undoubtedly a strategic sector, has gained even more importance especially after the pandemic that affected the whole world. In the face of situations such as epidemics, wars and natural disasters, it is necessary to prevent price instability due to disruptions in the supply-demand balance, to meet the needs of the ever-increasing population and demand, and to increase productivity in order not to negatively affect the quality of life. In this context, supporting self-sufficiency policies, especially in agriculture and livestock, without external dependency is of great importance in overcoming possible problems with less damage.

In addition, developments in technological fields cause changes in the livestock as well as in the industry and services sectors. The use of developing technology in all stages from production to marketing in animal husbandry is becoming widespread and is actively used especially in management, health and many other areas. With this digitalization, it is aimed to reduce possible human errors and increase service quality, efficiency and profitability. However, unfortunately, these developments cannot be effectively utilized in our country. It is thought that many structural problems and deficiencies that are intertwined such as the density of small, scattered and irrational animal

husbandry enterprises, the education and awareness levels of producers, and production costs may be effective in the formation of this situation.

In fact, in a study, significant investments were made with the aim of reducing bureaucratic time and costs and improving service quality by providing public services in digital format. It has been reported that it is likely that the use of information technologies will contribute greatly to many areas such as increasing food service quality, reducing costs, preventing confusion, providing savings and increasing institutional efficiency. It has been reported that factors such as education level and social environment create differences in the functional use of the internet environment among citizens. It has been stated that those who carry out agricultural and animal husbandry activities cannot effectively benefit from digital tools, and it has been reported that great efficiency can be achieved if citizens in this production area effectively benefit from the digital environment (Tunç et al., 2017).

Literature examples on artificial intelligence applications in the field of livestock are given below.

For profitable and efficient animal husbandry, a large amount of data obtained must be evaluated precisely and forward-looking predictions must be made. In order to solve complex problems in animal husbandry, it is of great importance to systematically collect and analyze big data. Artificial intelligence technologies such as sensors, big data, cloud computing, and machine learning algorithms are needed to store, preserve, and process large volumes of data sets consisting of different texts, sounds, and videos, such as weather conditions and quality, animal sound signals, and visual animal behaviors collected during the production process. With the use of these technologies, it will be possible to make early diagnosis of various diseases by observing unbalanced body movements and decreased activity in animals. A profitable and sustainable animal husbandry activity will be possible with the increase in animal productivity and the preservation of animal health (Kopuzlu, 2023).

The aim of the study was to detect subclinical mastitis in Holstein cattle using artificial intelligence methods in a farm with an automatic milking system. Data such as lactation order, milk yield, electrical conductivity, average milking time and control season were used as input data, and somatic

cell count calculated from milk samples collected for 15 months was used as output data. In the study, artificial intelligence methods such as fuzzy logic (FL), artificial neural networks (ANN), artificial neural networks with fuzzy interface (ANFIS) and support vector machines (SVM) were used to detect subclinical mastitis in the early period. According to the study, it was reported that the most suitable model for the prediction of subclinical mastitis was SVM due to its high sensitivity and specificity and low error (Memmedova, 2012).

As a result of a review made about fuzzy logic and neural networks in dairy cattle farming, it was stated that among the methods introduced for dairy cattle farming, fuzzy logic was recommended for decision support systems and artificial neural networks were recommended for estimation purposes (Akıllı and Atıl, 2014).

In a study conducted on raw milk quality assessment with the fuzzy logic approach, 80 percent success was achieved through a fuzzy logic-based decision support system in which the number of somatic cells, total bacterial count and protein amount were designed as input variables and the raw milk quality assessment as output variable, and it was reported that it was effective in raw milk quality assessment (Akıllı et al., 2014).

In a study conducted by Firk et al. (2003) on a commercial dairy farm, the data were analyzed using a fuzzy logic model using activity data obtained from pedometers attached to the left foreleg of cows and the time elapsed since the last estrus. It was reported that automatic estrus detection could be improved in cows with information about previous estrus events (Firk et al., 2003).

In a review on robotic systems and artificial intelligence applications in livestock farming; It has been reported that the desire to minimize human errors and benefit from animals at the optimum level can be possible with the capabilities of artificial intelligence such as image processing, decision-making, classification, prediction and herd management systems. It was also stated that disease detection, optimum feeding, determination of animal behavior, and analysis of animal health could be carried out with artificial intelligence tools (Isık et al., 2021).

In a study conducted by Morag I. et al. (2001) on individual feeding allocation decision support systems for dairy farms, a decision support system was developed that allows the determination of feed amount using parameters such as body weight and milk yield through fuzzy logic. It is a dynamic program that aims to save unnecessary feed costs and maximize milk yield by creating the right feeding program for each cow, not according to herd value. It has been reported that herd management programs can work in an integrated manner with this model (Morag et al., 2001).

In the study conducted to develop a fuzzy logic model for the classification and control of lameness and mastitis in dairy cows, a data set consisting of approximately 13500 records from 125 cows was used. The most adequate accuracy in lameness detection was obtained using variables such as dry matter intake, feeding time, number of feeding visits, activity and pre-lameness status in lactation. The best mastitis detection model was obtained by combining parameters such as milk yield, water intake, dry matter intake, feeding time, number of feeding visits and pre-mastitis cases in current and previous lactations. As a result of the study, it was reported that automation of lameness or mastitis detection with fuzzy logic models using features related to performance (milk yield, water and dry matter intake) or behavior (feeding behavior, activity) did not perform well enough for practical use (Kramer et al., 2009).

In a study where CNN-LSTM (Convolutional Neural Network-Long Short-Term Memory Model) network was used for lameness detection based on pose estimation in broilers, a model for lameness detection was developed by analyzing video images. It has been reported that the pose estimation-based model, as an automatic and non-invasive lameness assessment tool, can be applied in poultry farms in terms of efficient management practices (Nasiri et al., 2022).

A study on developing a new intelligent electronic nose system to measure and analyze odors in livestock and poultry farms appears to benefit from advanced artificial intelligence technologies (Pan and Yang, 2007).

When the literature reviewed above is evaluated; It is seen that artificial intelligence technologies are widely used in many areas such as herd management, early diagnosis of some diseases, determination of raw milk

quality, detection of animal behavior and development of decision support systems in the livestock sector as well as in many sectors such as education, industry, energy, environment and finance. It is estimated that the use of artificial intelligence-based systems in livestock enterprises may be an important turning point in terms of the efficiency and profitability of enterprises.

7. Conclusion

It is possible to talk about the effects of increasing efficiency and quality and reducing costs by offering artificial intelligence-based applications that are designed and constructed correctly by taking ethical issues into account and put them into service for people. In order to effectively benefit from the possible benefits of artificial intelligence in public institutions and private sector enterprises and to compete with developed countries in this regard, it will be possible to the extent that personnel training is provided, access to a registered and reliable data set and the development of existing and new investments that can benefit humanity by using this data.

Considering the investments made by developed countries on artificial intelligence, in order to become an independent country that is self-sufficient in terms of agriculture and animal husbandry, the current support policies on this issue should be given due importance and new support packages should be opened. Initiatives should be taken to encourage public and private sector enterprises working in this field.

In order to effectively benefit from artificial intelligence technologies, reliable recorded data is needed. Awareness studies should be conducted on how important issues such as data quality and data fluidity are. In order to benefit from these technological developments in livestock enterprises, producers should be given the habit of keeping records and the necessary information about its importance. In this way, the welfare level of breeders operating in this production area, which requires difficult working conditions requiring 24/7 maintenance, will increase and they will have the opportunity to produce more efficiently and profitably.

Considering the contributions that these artificial intelligence-based technological systems, which can be used in many areas in terms of animal husbandry such as minimizing human-induced errors and achieving more efficient and profitable production, diagnosing some diseases at an early stage, detecting variables that may negatively affect product quality, and determining and removing animals that reduce the average yield level in herds and therefore the profitability of the enterprise, to the national economy and businesses, and the investments made by developed countries in this field, it is possible to say that this issue will become even more important in the future. In order to gain competence in this issue and reach the level of developed countries, public and private sector institutions, especially universities, should work together and awareness should be created about the cooperation of different disciplines.

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CHAPTER 10

THE ROLE AND POTENTIAL APPLICATIONS OF CIRCULAR RNA IN ANIMAL BREEDING

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1. INTRODUCTION

Circular RNA, or circRNA, is a diverse and abundant class of endogenous noncoding RNA forms. CircRNA is an old topic in molecular biology. However, with the development of sequencing and bioinformatics technology, the regulation of circRNA in gene expression has become a growing concern. CircRNA is a single-strand RNA with covalently closed loop structures. In comparison with linear RNA, circRNA lacks the classic 5' to 3' polarity and a polyadenylated tail at the 3' end. The past few years have seen a surge in circRNA research, from recognizing and annotating the circRNA transcriptome to elucidating the function and biogenesis of circRNA. The large number of stable circRNAs in the cells has attracted the attention of scientists and has been confirmed to be involved in various biological processes, including the regulation of gene expression. CircRNA molecules exist in many animal species and tissues and play a significant regulatory role in the physiological and biochemical processes of various animals. In animals, circRNA is expressed in different tissues or stages. Moreover, the expression of circRNAs has obvious tissue or age specificity. In addition, compared with linear RNA, circRNA is more resistant to exonucleases, which makes circRNA more stable. Recent studies have found that circRNAs have a wide range of regulatory effects in mammals and widely participate in the regulation of many genes associated with fertility, reproduction, and growth. Therefore, circRNA may become an important research topic in the fields of animal research and breeding after miRNA and lncRNA. In this part, we summarize the formation, function, and research progress of circRNA, and we also discuss the potential future applications and prospective directions of circRNA in the genetic selection of livestock and poultry (Lin et al., 2021; J. Zhang et al., 2022; W.-Y. Zhou et al., 2020; Zucko & Boris-Lawrie, 2020).

2. BIOGENESİS AND CHARACTERISTICS OF CIRCULAR RNA

In animals, the biogenesis of circRNA has been confirmed to be less complicated and has attracted a great amount of attention. The mechanism responsible for circRNA production has been well investigated. The biogenesis of circRNA is associated with the back-splicing of pre-mRNA. Pre-mRNA exons, which are identified and cut off from a pre-mRNA, are rearranged and

then reconnected, which results in a covalently closed loop. The disintegration factor, which is an upstream 3' splice acceptor site and a downstream 5' splice donor site, contains reverse splicing boundaries. The back-splicing pattern determines the features of circRNA, which is characterized by a covalently closed loop, exonuclease resistance, stability, and can be retained in animal cells with special splicing motifs (Shen et al., 2022; M.-S. Xiao et al., 2020).

It has been certified in nature that circRNA is abundant in various cells, preserved tissues, and biological fluids. CircRNAs are typically stably expressed in biological samples when compared to linear RNAs, especially under RNA exonuclease digestion. Studies indicate that circRNAs are conserved across different species and are frequently expressed more in higher vertebrates than in others. There is a close relationship between the expression levels of circRNA and linear mRNA not only among different individuals but also among diverse tissues in the same individual. CircRNA can form various internal or material structures with various functions depending on different sizes, sequences, and expressions of circRNA species. Some circRNAs, only in small amounts, may play special functions because they contain only 1–3 exons. It is supposed that circRNA functionality is associated with the expression level of circRNA and linear mRNA. As a competitive endogenous RNA molecule, for instance, circRNA's function and the degradation of linear mRNA are consistent. Provided the high stability and diversity of circRNA, it can be an especially good candidate and exhibit unique expression and biological potential for the RNA-animal multi-level study in various cell and tissue types. Also, numerous studies have indicated that circRNAs play very important roles in various biological processes and diseases by acting as miRNA sponges, RBPs, or serving as protein translation templates and participating in extensive biological processes(Raza et al., 2022; Rbbani et al., 2021).

3. FUNCTIONS AND MECHANISMS OF ACTION OF CIRCULAR RNA

Alternative splicing or polyadenylation generates circRNA. The most studied function of circRNAs is their role as microRNA sponges and participants in the microRNA regulation process. In addition, circRNAs have been reported to interact with RNA-binding proteins (RBP) directly or to act as

RBP sponges that inhibit RBP function. There was also a report indicating the reversal transcription of circRNA to regulate parental gene expression. Some circRNAs translocate from the cytoplasm to the nucleus and can bind to their parental genes to regulate gene expression at the transcriptional level. Furthermore, studies suggest that circRNA interacting with proteins or other RNAs may directly or indirectly affect the biological activity of the circRNA-associated protein or RNA(J. Xiao et al., 2022; J. Zhou et al., 2021).

These studies suggest that circRNA is involved in the regulation of gene expression at multiple levels under different physiological and disease conditions. Some functions are unique to circRNAs, while others may be combined with mRNAs, such as mRNA isoform cancellation events or colocalization with mRNAs. Some functions are directly associated with the properties of circular transcripts, such as those involving exon skipping, acting as microRNA sponges, or cap-independent mechanisms of translation. Moreover, most circRNAs demonstrate tissue-specific expression during embryo or fetal development, as well as spatiotemporal expression regarding tissue development or regeneration in adult individuals. The circRNAs identified in the skeletal muscles of farmed animals indicate that numerous circRNAs are closely related to meat quality traits in pigs or cows based on their circRNA/mRNA co-expression networks and enrichment. Understanding the genotype-phenotype mapping of the features of circRNA has significance for animal breeding (Hou et al., 2021; Yan et al., 2023).

4. TECHNIQUES FOR DETECTING AND ANALYZING CIRCULAR RNA

Various methods are applied for detecting circular RNA (circRNA) in scientific research. High-throughput sequencing has become popular, and RNA-Seq protocols perform important roles in circRNA identification. In silico prediction is currently a powerful method that is combined with RNA-Seq data to enhance the accuracy of circRNA detection. On the other hand, circRNA quantification is performed in large-scale studies that explore its potential regulatory functions in different species. Differentially expressed circRNAs are sometimes validated using reverse transcription polymerase chain reaction or Northern blotting techniques. Notably, there are several RT-PCR protocols, such as divergent primers, circular selective primers, loop primers, internal and

external primers, RT-qPCR quantification, and predigestion of linear RNA. Meanwhile, Northern blotting requires a large amount of starting RNA, making it difficult to perform circRNA detection on a large scale. Bioinformatics analysis contributes to characterizing circRNAs in sequencing data and is used to prioritize candidate circRNAs for validation. Several grouping criteria apply to bioinformatics analysis, such as circRNA data integration, co-expression networks, circRNA target prediction and miRNA associations, and coding and noncoding parental genes (Drula et al., 2024; Nguyen et al., 2022).

A proper circRNA detection works effectively in identifying and exploring circRNA roles in research. The recently developed circRNA detection methods optimize circular-to-linear conversion and sequencing data validation for circRNA quantification. Divergent primer amplification was used to confirm circular-to-linear RNA conversion using RT-PCR. The study performed a putative fetal dataset to compare the expression level of the most differentially expressed circRNA with the RT-PCR results. Their study confirmed the circRNA presence in the eight main animal tissue database groups and identified different circRNA expression in all tissues of the investigated species. Therefore, this developed protocol provides RT-PCR sensitivity that supports the expression level validation constrained by pre-amplification by divergent primers (Chen et al., 2022).

5. CIRCULAR RNA IN ANIMAL REPRODUCTION

This special issue aims to provide an overview of the latest advances in circRNA research related to the reproductive system and explore the potential application of circRNA as a biomarker of reproductive performance. Although the role of the reproductive system's circRNAs is less discussed than microRNAs, it has been shown to be affected by gonadotropin and prolactin, which play a role in the steroid hormone pathway in the hypothalamic–pituitary–ovary axis. With microarrays and high-throughput sequencing technology, circACAP2 is differentially expressed in the ovary between high-fertility and low-fertility Holstein cows, but our understanding of circRNA has not yet been established (Huang et al., 2020; Raza et al., 2022).

The understanding of circRNA roles in reproduction is relatively poor in various species. At least, circRNAs are involved in oocyte maturation in

humans, mice, and pigs, steroidogenesis in Leydig cells, monocygous/father genes, and sperm quality in pigs. Significantly different circRNA of the testis and epididymis has been found in Drosophila, suggesting its importance in sperm maturation. However, research on the potential roles and mechanisms of circRNA in animal reproduction, especially circRNA in livestock reproduction, is very limited. Further, no studies have investigated circRNA's potential as a diagnostic tool in animal reproduction. Given these situations, the goal of this special issue is to collect work on circRNA related to animal reproduction that is still at the early stage. We also welcome additional work that contributes to the knowledge of potential roles and applications of circRNA in animal reproduction (Huang et al., 2020; Raza et al., 2022).

6. CIRCULAR RNA IN IMPROVING ANIMAL HEALTH AND DISEASE RESISTANCE

The understanding of the role of circRNAs in animal health has aroused the curiosity of animal breeders and researchers worldwide. The modulation of circRNA expression may be a means to improve animal health and its potential to develop resistance to diseases. Several studies have suggested the potential involvement of circRNAs in disease resistance through the modulation of immune responses. A growing number of reports have identified the connection between circRNA and diseases, evidenced by the altered circRNA expression profile in diseased conditions. Some specific circRNA molecules have been related to multifactorial infectious diseases, even in productive animals. These circRNAs might play a role in the pathophysiological mechanisms involved in resistance or susceptibility to certain infective agents and can be used as potential novel diagnostic or therapeutic biomarkers. The number of studies on circRNA has increased in the past 10 years, providing an avenue for discussion regarding new dimensions in holistic animal health management to improve animal resistance to diseases and pests in breeding programs. Specific circRNAs may serve as ideal therapeutic targets to develop sustainable animal breeding goals directed at animal health and resistance, based on the pathogenic agents in the rearing environment of the animals. However, little information exists on the role of circRNA in responding to animal health. Only a few studies have investigated the relationship between circRNA and the stress response at the production level, such as heat stress and lipopolysaccharide challenges in

animals. These physiological stressors may result in increased susceptibility to pathogen infections and diseases due to decreased immune function and changes in the structure of gut microflora. Hence, it is of paramount importance to uncover the role of circRNA in farm animal health management. Environmental stress factors can contribute to livestock production limitations and potential economic costs, and circRNA may be a candidate biomarker for stress susceptibility in diseases, which is an emerging issue in large-scale animal farming. The reason for the payoff of such research is that it paves the way for a deeper and comprehensive understanding to discover gene circuits associated with desired health and immune status quantitative traits for utilization in applied molecular breeding or animal rearing management (Kirgiafini et al., 2024).

7. CIRCULAR RNA AS BIOMARKERS IN ANIMAL BREEDING PROGRAMS

Biomarkers are biological molecules used to investigate a particular biological status, pathological condition, or as a therapeutic target. They can be discovered through a range of transcriptomic, proteomic, and metabolomic techniques. Circular RNAs have demonstrated potential as useful biomarkers in a range of human diseases and some agricultural applications. Animal breeding programs aim to improve the genetic merit of the population through the selection of superior individuals. The discovery of valuable biomarkers such as circRNA holds potential to enhance this process. Recently, a growing number of studies have identified circRNAs that are associated with various phenotypic traits, including growth rate, meat quality, disease conditions, and age of puberty. Circular RNAs are thought to be valuable biomarkers due to their unique properties. CircRNAs are more conserved across tissue and species than their linear counterparts. CircRNAs are also more stable across different storage conditions compared with linear mRNA in biological samples. One report also highlighted the potential of using circRNAs as tissue or disease biomarkers due to their tissue-specific expression. Integrating circRNA biomarkers into genetic selection strategies could reduce the generation interval length and ease the performance testing requirements of breeding animals. While the majority of circRNA studies focus on human disease, a few reports have demonstrated the potential utility in livestock species. Data tables share

some examples of putative circRNA biomarkers identified in livestock (Li et al., 2020; Rbbani et al., 2021).

8. POTENTIAL APPLICATIONS OF CIRCULAR RNA IN ANIMAL BREEDING

Circular RNA (circRNA), an ancient RNA molecular group, has various physiological functions and may serve as a novel research hotspot. In the field of animal breeding, circRNA has many characteristics, such as operational convenience, stability, conservation, and tissue specificity, that make this subdiscipline popular and well received. CircRNA may be beneficial to animal communication, embryonic development, breeding and reproduction, disease resistance, and other areas. In summary, circRNA plays a key role in the course of breeding and other physiological processes and has a broad application prospect in livestock breeding programs. Animals usually come into contact with the external environment through breathing, eating, and other behaviors. However, the quality of external breeding for them and the efficiency of their reproductive performance can also be improved from the internal genetic level. At present, the main strategy for livestock breeding is to improve or control a variety of optimal economic traits, such as the growth rate, production performance, resistance, and reproductive efficiency of animals. This can be achieved using traditional breeding methods, which cause the results of selection and breeding to come from the continuous and slow accumulation of the genetic background. Interestingly, circRNA has the potential to be used in future breeding processes in various innovative ways. Most importantly, the levels of circRNA can be upregulated or downregulated in animals by genetic engineering technology and through new technological methods for the combination of gene editing with reproductive technologies for in vitro fertilization. These processes can also be used to promote livestock breeding, even if the process is slow and controversial. In addition, the application of small interfering RNA, short hairpin RNA, and other gene knockout or editing techniques may subsequently drive the availability of methods to regulate circRNA levels in agriculture, thereby promoting progress toward precision agriculture. Based on the above research advances in circRNA, our research conclusions are that the regulatory mechanism of circRNA in livestock breeding is mainly involved in many aspects. First, circRNA can regulate

growth performance, including growth rate and muscle development. Moreover, in theory and some experimental results, circRNA can regulate the fertility of animals, which may be through the regulation of male infertility-related mRNAs, which is more in-depth than regulating breeding performance. It has a positive effect, provided that circRNA's impact on fertility is magnified. Second, circRNA can regulate the growth rate or meat quality and facilitate the breeding process. Studies of the function of circRNA indicate that circRNA in diseases and tumors, plant abiotic stress, and other disciplines mainly plays the role of repressing translation. In-depth studies will eventually affect the breeding process. In conclusion, if successfully using the properties of circRNA to intervene to improve animal breeding, particularly in terms of growth and development, it will be a more powerful approach. The possible applications of circRNA in animal breeding are listed, showing efforts already underway. In addition, acceptables have been provided. The value of the mainstream breeding method was also presented in detail, with potential changes to include circRNA, in vitro embryo systems, and somatic cell nuclear transfer. In terms of whether it can be applied in animal breeding, the development and promotion of these emerging technologies require the improvement of multidisciplinary collaboration and the resolution of a series of ethical controversies (Bao et al., 2022; He et al., 2021; Zhu et al., 2023).

9. CHALLENGES AND FUTURE DIRECTIONS IN HARNESSING CIRCULAR RNA FOR ANIMAL BREEDING

9a. Challenges and Perspectives in Research on circRNA

The functional roles of circRNA in animal breeding programs are largely unknown. Most circRNAs are uncommonly abundant and have tissue- and development-specific expressions, making them complex and challenging to study. These characteristics increase the difficulty and cost of circRNA detection and data analysis, leading to high error rates in RNA-Seq analysis and low repeatability of data. Gaining insight into the comprehensive analysis of the function of circRNAs in different species will promote meeting the challenges mentioned above to some extent. To address gaps in this field of study, further in-depth studies should be conducted to understand the biological and physiological functions and to validate the mechanisms involved in circRNA production and actions based on the evolutionary relationships and

genetic backgrounds of the key roles of extremely diverse circRNAs from different species. This knowledge will also be useful in standardizing the methodologies and conditions used to confirm the mechanisms resulting in the formation of circRNAs, leading to the proposal and development of standardized protocols suitable for study in different species. With the systematic consultation of researchers, industry stakeholders, and regulatory bodies, it will be important to discuss the pros and cons of the putative applications of circRNAs and to provide ethical guidelines for circRNA manipulation in breeding (Huang et al., 2020; Sun et al., 2023).

9b. CircRNA Application in Genetic Improvement

Genetic improvement is one of the long-term goals in animal breeding. Exploring enough circRNA differentiation that is closely related to the target traits in multiple breeds will facilitate the use of these traits to achieve the effects of indirect or direct genetic improvement of animals. Some circRNAs have been identified as potential effectors of meat quality in pigs and were differentially expressed in distinctive meat tissues. As "molecular markers," these might facilitate the molecular selection of these traits, but the effects of circRNA expression variation on milk production traits have rarely been studied and require elucidation (Cheng et al., 2021; Hou et al., 2021).

9c. CircRNA Application in Animal Welfare

Stress leads to the activation of the body's HPA axis, which can stimulate the release of cortisol. The prolonged activation of the HPA axis causes the body to stay in a state of chronic negative feedback. At work, the excitability of the adrenal corticosteroid receptor is decreased, negative feedback fails, and glucocorticoid levels in the blood increase, causing muscle cells to consume a lot of protein, thereby degrading muscle fiber proteins. CircRNA can function as endogenous RNA to regulate the expression of genes that are related to stress and nutrient metabolism in muscles. In addition, stress can also regulate energy metabolism and the immune system in the body, and the production of some secretory factors, which will affect the body's levels of free fatty acids, glucose, lymphocytes, and other substances. Studies have shown that some circRNAs are closely related to the regulation of immunity and nutrient absorption in animal muscles after stress and are involved in meat quality traits (Y. Zhang et al., 2021; T. Zhou et al., 2021).

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CHAPTER 11

NUTRITION GUIDE FOR BEEF CATTLE

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1.INTRODUCTION

Names of Cattle: *Calf*, male and female cattle up to 6 months of age (up to 1 year old in the USA). *Young Heifer, Heifer Calf*, 6-12 months old female cattle. *Young Bull, Young Steer (castrated)*, 6-12 months old male cattle. *Veal Calf*, calves fed with liquid feed up to a maximum weight of 250 kg BW. *Yearling Steer or Yearling Bull*, 1-2 year old male cattle. *Heifer*, non-calving cattle. *Cow*, cattle that has given birth. *Bull*, cattle that can be used for breeding at the age of 2 years and above. *Ox*, male cattle aged 4 years and above that are not used for breeding (castrated or not).

Beef Cattle Breeds: Angus, Hereford, Simmental, Limousine, Charolais, Belgian Blue, Danish Black and White, etc.

Fattening: The fast or slow muscle building or fleshing of animals (not fattening) within the desired period is called fattening. If there is fattening, this is not "fattening" but "feeding". Meat breeds such as Angus, Hereford, Limousin, Charolais with a meat yield of 65% should be preferred for fattening. If breeds such as Holstein, Simmental, Montafon are used, the yield is 10% lower.

Body Weight (BW): Live weight of the animal to be fattened, kg.

Shrunk Body Weight (SBW): The weight of the ruminant animal to be fattened after it has been fasted for 14-16 hours. BW x 0.96. For example, SBW of a ruminant with a live weight of 400 kg = 400 x 0.96 = 384 kg. SBW is used in theoretical calculations, in practice the animal is not starved and weighed.

Empty Body Weight (EBW): It is an assessment made by assuming that the digestive tract of the male or female (fetal weight is subtracted) animal to be fattened is completely empty. EBW = EBW x 0.91. For example, the EBW of a ruminant with a live weight of 400 kg = 400 x 0.96 = 384 kg and the EBW = 384 x 0.91 = 349.44 kg.

Initial Shrunk Body Weight: It is the SBW weight of the ruminant that will start fattening. In other words, it is 0.96 of the Initial Shrunk Body Weight.

Fattening Finishing (Slaughtering) Weight (Final Shrunk Body Weight): It is the ACA weight that the feed is targeted for or will be finished (slaughtered). In other words, it is 0.96 of the Final Shrunk Body Weight.

Average Daily Gain (ADG) kg/day: The breeder determines this as he/she wishes, according to the characteristics of the animal, the amount or type of feed, market conditions, etc.

Calculation of ADG= Fattening Finishing Weight - Fattening Starting Weight/Number of Days

Example: 350-250/100 days= 1 kg/day ADG is found.

Fattening Period: It is the period starting from the 4th month until the day of slaughter. The fattening period is extended or shortened according to the desired ADG.

Ideal Slaughter Age or Ideal Slaughter Live Weight: It is the age or live weight at the beginning of the period when the growth hormone (Growth Hormone) decreases and sex hormones increase in farm animals, fattening begins, ADG decreases or FCR increases. For example, it is the 42nd day in broiler chickens.

Dry Matter Intake (DMI), kg/day = The amount of dry matter in the ration consumed by the animal in 1 day.

For Beef Cattle;

DMI, kg/day = $(0.084 \times BW^{0.75} / 2.13) + (ADG^{1.097} \times 0.0557 \times BW^{0.75} / 1.45)$ (NRC,1984)

Feed Conversion Rate (FCR): It is the conversion rate of 1 kg ration DM into meat, milk, eggs, etc. in the animal.

Feed Conversion Rate for Beef Cattle = Total Daily Feed Dry Matter Eaten / ADG

It is desired to be low. The FCR rate in beef cattle should ideally be 6-8. For example, if a beef cattle consumes 7 kg DM per day and ADG is 1 kg/day; FCR=7/1. In the 1950s, while the FCR in beef cattle (Beef Cattle) was 10 kg/day DM feed: 1 kg ADG, this ratio decreased to 6:1 in 2016 (Nursoy 2024).

2. FACTORS AFFECTING FATTENING

2.1- Breed and Origin: The efficiency of meat breeds is 65%, it is high. An animal with good parents or high breeding value is also good itself. In our country, birth weight, ADG, slaughter weight are low in native breeds, while they are high in culture breeds and crossbreeds. Bones are thick and short in meat breeds. Humidity needs of dairy breeds such as Holstein are 10-12% higher than meat breeds, but their muscling is lower than meat breeds. For example, in the Belgian Blue breed, live weight in males is 1047 kg, and live weight in females is 816 kg.

2.2-Gender: Birth weight and ADG are 10% higher in male animals. The meat yield of male animals is higher than females in the same BW. Because males have less adipose tissue, lower fat ratios and higher muscle ratios. There is a 20% difference in carcass meat between females and males of the same live weight. This is a very large ratio. Female animals are less productive because they cannot take the necessary feed with the required appetite during estrus. FCR is lower in males. Their meat holding capacity is higher due to testosterone and other androgen hormones. Females are expensive and are not fattened.

2.3-Age: The ideal age is from 4 months to 15 months in Holsteins and 18 months in Simmental and Brown Swiss. Fattening should not be done afterwards, the animal should be slaughtered. Beef cattle reach physical and sexual maturity (breeding age or adjective age) at the age of 1.5-2 years, sex hormones increase instead of growth hormone. After this, they get fat, and ADG increases. Since the survival rate is low in young animals, less feed is spent, and feeding is more economical. The slaughter age is determined for each breed. For example, FCR increases after Holsteins exceed 450 kg. There is a growth of 100% in the first year, 70% in the second year and 50% in the third year. While the need for CP in the ration is 16% in young animals at 150 kg, it decreases to 10% in 400 kg. This protein rate can be met by grains alone.

2.4-Care, Feeding, Environment, Shelter, Diseases, Health (Especially Internal-External Parasites): Barley is also used instead of corn in finishing rations. For fattening cattle, it is sufficient for the corn in the concentrated feed to be cracked. Corn silage is both a source of energy and cellulose and should be given to fattening cattle. Compared to the summer months, the feed intake of animals under cold stress in November, December and January is 8% more.

2.5-Constitution and Metabolic Factors: The general structure of the animal, the brightness of its skin, its liveliness, being weak or skinny, etc. These factors affect fattening performance by 5-10% feed intake, 10-30% ADG and 15-40% FCR.

2.6-Body Condition Score (BCS) and Body Composition (Capacity of the Animal to Hold Muscle and Fat on It): A Body Condition Score (BCS) of 4 and above is not desired in beef cattle. In meat quality, it is desired for the fat to be distributed among the meat or to become marbled. Therefore, early

slaughter is not done. Vitamin E and antioxidant substances extend the shelf life of the meat (Nursoy 2024).

3. FEEDLOT (OPEN-FEEDLOT) FATTENING

Feedlot: Fattening of cattle with concentrated feeds in limited paddocks, in open shelters in summer and winter, without grazing in pastures. Water and mineral salts are given ad libitum. Feeds are selected from those with high energy but low starch. The FCR should be 5-8/1 in the feedlot. DM requirement should be 2.5-3% of CA, 9-14% CP, 60-85% TDN, 0.3-0.6% Ca, 0.2-0.4% P and 2/1 Ca/P ratio (to prevent urinary stones). 97% of beef in the USA is produced by feedlot fattening and the remaining 3% is produced by pasture-based fattening.

Two periods are applied in feedlot fattening for male calves, young bulls and bulls for meat purposes:

3.1. Backgrounding Period

This period for male of beef – dairy cattle from weaning until approximately 300 kg BW or 1 year of age, backgrounding diet containing 40-70% quality roughage are offered to young cattle to be fattened. DM content of the diets should not fall below 75%, DM should contain 13-14% CP (urea is very low and can be used after 6 months) and at least 2600 kcal/kg ME energy. These cattle can be given silage starting from the age of 6 months. During this period, DMI is approximately 3% of BW. Depending on the desired ADG, the roughage ratio in the diet can be reduced up to 40%. During this period, silage or quality hay is given ad libitum, and an additional 3-6 kg of concentrated feed is fed per animal. In general, quality forages are given at 2.5% of BW (as DM) (Hinton, 2007; Nursoy, 2022).

3.2. Finishing Period

Finishing diets containing 70-85% concentrated feed are given to young bulls weighing approximately 300 kg or after 1 year of age until slaughter, up to approximately 450-550 kg (approximately 250-300 kg carcass is obtained). There should be 11% CP and at least 2900 ME/kg energy in the DM of the diet. In this period, DMI is 2.5% of BW. Depending on the desired ADG, the roughage ratio in the diet can be reduced to 10% (Nursoy, 2022). When one-year-old male cattle with an average live weight of 374 kg were fed with wheat-

based concentrated feeds and forages, ad libitum feeding and restricted feeding of 15% of the ad libitum feeding, the FCR value was 6.82 and 6.16 kg DM/kg BW, respectively (Hicks et al., 1990). Cattle to be fattened can be given roughage + concentrated feed ad libitum from weaning up to 200 kg BW, daily feed or DM consumption can be made 5-20% less ad libitum, starting from 200 kg until slaughter. Or roughages can be given ad libitum, while concentrates can be restricted to 1% of the BW.

Table 1. Practical feeding of male beef-dairy cattle from three months to slaughter (Nursoy, 2022)

Age	*Feeds*	*Ratios and methods of feeding*
90th Day - 1 Year Old, Male Calves and Male Calves (For Fattening Purposes)	Calf Concentrate Feed (CP: 16-20%, ME: 2600-3000 kcal/kg and TDN: 65-70%, at DM)	1-1.5% of BW daily
	Quality Roughage (It is fresh or dry meadow-pasture grasses and other dry grasses and silages with low lignin content. Dry grass / Silage ratio (Normal): 50 / 50 and in this mixture, at least 12-16% CP in DM and It should be 1400-1800 kcal/kg ME. Fresh alfalfa, clover and sorghum cannot be given. Wheat straw can be given at most 1 kg/head/day during this period)	Ad Libitum
	Water	Ad libitum
Yearling bull and 18-24 months of bull (For Fattening Purposes)	Yearling Bull Concentrate Feed (CP:	Up to 2% of BW daily

	% 14-16, ME: 2600-3000 kcal/kg and TDN: % 60, in DM)	
	Quality Roughage (It is fresh or dry meadow-pasture grasses and other dry grasses and silages with low lignin content. Dry grass / Silage ratio (Normal): 50 / 50 and in this mixture, at least 12-16% CP in DM and It should be 1400-1800 kcal/kg ME. Fresh alfalfa, clover and sorghum cannot be given. Wheat straw can be given at most 1 kg/head/day during this period)	Ad Libitum
	Water	Ad Libitum
Bulls 2 Years Old and Above (Breeding Purposes)	Bulls Concentrated Feed (at DM, CP: 12%, ME 2400-2600 kcal/kg and TDN: 60%)	0.5-1% of BW daily
	Quality Roughage (It is fresh or dry meadow-pasture grasses and other dry grasses and silages with low lignin content. Dry grass / Silage ratio (Normal): 50 / 50 and in this mixture, at least 12-16% CP in DM. It should be 1400-1800 kcal/kg ME. Fresh alfalfa, clover and sorghum cannot be	Ad Libitum

	given. Wheat straw can be given at most 1 kg/head/day during this period)	
	Water	Ad libitum

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CHAPTER 12

ANIMAL RESCUE EQUIPMENTS IN ŞANLIURFA FIRE DEPARTMENT

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INTRODUCTION

Animal rescue means transferring an animal from a place of danger to a safe place by the most appropriate method, for both rescuers and the victims. Animal rescue is a partnership between the rescuer and the Veterinarian. In this review; animal rescue capacity of Şanlıurfa fire service has been discussed.

Modern animal rescue techniques in Türkiye began in 2009 with the "Capacity Development Project on Reducing Animal Casualties in Floods in Şanlıurfa" financed by the EU within the scope of the "Grant Program for Reducing Flood Risk in Flooded Areas in the GAP Region (GAPSEL)" carried out jointly by the Şanlıurfa Provincial Civil Defense Directorate and the Southeastern Anatolia Project, Culture Research and Development Association (GAPDER). Within the scope of the project, veterinarians, emergency personnel, geographers, and young volunteers were trained to establish animal rescue teams; the 'First International Animal Rescue Conference and Animal Rescue Drill' was organized in Şanlıurfa together with Harran University, Faculty of Veterinary Medicine, Royal Veterinary College of UK, Hampshire and Şanlıurfa Municipalities Fire Department. Rescue equipments (Fully articulated manakin horse for training purposes, rescue glides, slings for both small and large animals, mud lance and path, nikopoulos needle, strop guide, basic rescue kit) was purchased from UK within the scope of the project and donated to Şanlıurfa Fire Department. Today, the Şanlıurfa Fire Department successfully intervenes in cases using these equipments below. These equipments were bought from UK in collaboration with two experts of British Animal Rescue and Trauma care Association (BARTA). Jim Green of Hampshire Fire and Rescue Service as a firefighter, director of BARTA, is internationally recognised and has been keynote speaker or coordinator of conferences in the UK, Turkey, USA, Australia and Europe. Prof. Dr. Josh Slater co-founded BARTA and has been a veterinary lead in the large animal rescue initiative in the UK.

Current Animal rescue equipments in Şanlıurfa Fire Department

1 x Manakin horse

1x medical suspension harness

1x Rescue sling set (complete)

2x Strop guides, regular

3x 5m strops, 3” wide

2x 9m strops, 3” wide

2x 12m (trailer righting) strops, 6” wide

2x 3m continuous loop strops

2 Crooks- heavy limb

6 halters (heavy improvised)

1 pair of Hobbles (restraining and lifting)

2x small carry sheets

1x Mud lance complete

4x Jameson extension poles with attachments

1x skaff pack

Fig. 1: Şanlıurfa Fire Service Personnel with Manakin horse and other animal rescue equipments

Fig. 2: Manakin horse for trainings

About manakin horse: Rescues involving large animals like horses and ponies are dangerous and can result in the death of the equine and injury to rescue team members, so it's important for firefighters to rehearse practical, realistic scenarios and to share that knowledge. Manakin could be used to demonstrate the safest way to recover larger animals from dangerous situations. It allows rescuers to train with a life-size model and put ourselves in realistic situations. Horse manakin for ethical handling of equines can be used to demonstrate the safest way to recover large animals from trenches, ditches and cliffs, and from water and mud entrapment. This manakin was produced by specialist UK company Resquip and fully operable with realistic joint articulation and muscle definition. In conclusion, it is stressed that the manakin horse is an essential tool for training and without it the animal rescue courses impact will be limited.

Fig. 3: Pair of Hobbles (restraining and lifting)

Fig. 4: Small animal lifting equipment

Fig. 5: Towing hooks

Fig. 6: Personnel protective equipment

Fig. 7: Lifting equipment for horses

Fig. 8: Rescue sling for cows

Fig. 9: Stretcher carrying apparatus

Fig. 10: Strop guide

Fig. 11: Mud lance complete

Fig. 12: Material needed to reach animals stranded in high places such as trees

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CHAPTER 13

TREATMENT OPTIONS FOR COXOFEMORAL LUXATION

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1. INTRODUCTION

In animals, joints are structures that form the skeletal system and provide functional connections between bones. The coxofemoral joint, also known as the hip joint (articulatio coxae), is a spheroid (rotating along its axis) group joint structure formed by the placement of the femoral head (caput femoris) into the acetabulum. The acetabulum is formed as a result of a deep depression formed at the junction of the three bones of the pelvis, namely the ilium, ischium and pubis (Arıcan, 2020; König and Bragulla, 2007; Sharma, 2024). The deep fossa within the acetabulum is non-articular and serves as an attachment point for the ligamentum teres, which connects the femoral head to the acetabulum. It provides stability by creating a good configuration for the acetabular fossa, where the femoral head fits into the socket, allowing a wide range of joint motion, including flexion, extension, adduction, abduction, and rotation. Factors contributing to the stability of the joint include the acetabular fossa, joint capsule, ligamentum teres, and surrounding muscles, forming a harmonious structure (Sharma, 2024).

Interruption of normal joint mechanics and stabilization leads to painful osteoarthritis and physical disability, which reduces the animal's quality of life. Acetabular fracture, hip dislocation, femoral head physis fracture, femoral head and neck fracture, hip dysplasia disease, leg perthe calve disease and conditions related to the hip joint (Prasad et al., 2015).

It is an orthopedic problem that occurs when the caput femors are dislocated from the acetabulum due to trauma or certain diseases (hip dysplasia, Legg Calve-perthes). It is seen in all genders, ages and breeds, but is more common in dogs older than 1 year. In a study conducted on a total of 575 coxofemoral joint disorder cases, a higher rate of coxofemoral joint disorder was reported in larger breeds such as German Shepherd Dog (23%) and Labrador Retriever (34%), followed by Spitz (11%), German Shepherd Dog (6%), Rottweiler (4%) and unidentified breeds (8%). (Prasad, 2009).

It is reported that coxofemoral dislocation (separation of the caput femoris from the acetabulum and displacement in different degrees and directions) that occurs especially as a result of high-energy traumas, consists of 90% of all dislocations seen in cats and dogs, coxofemoral joint dislocations and usually occurs together with other simultaneous orthopedic problems (Ergın et al., 2016; LeFloch and Coronado, 2022; Pratesi et al., 2012).

Most coxofemoral dislocations (79-83%) caused by vehicular trauma; however, other causes include falls from height, hip dysplasia, and dislocations of unknown cause (Harasen, 2005). Dislocation means damage to the joint capsule and should be repaired if possible due to the deterioration of the structure that provides stability to the joint. The teres ligament is also usually torn and detailed clinical examination and radiographs will help determine if there is any damage or fracture to the dorsal acetabular rim and will contribute to the selection of treatment protocols as this also plays a role in hip stability. It is very important to provide early reduction without delay for all hip dislocations (Arıcan, 2020; Bone et al., 1984).

2. PHYSICAL EXAMINATION

The most important symptoms of hip joint dislocation are unilateral lameness and inability to bear weight on the affected extremity. Clinical examination findings include pain, deformity, crepitation, and limited or abnormal movement in the leg. In extension of both hind limbs, the affected extremity is seen to be shorter compared to the healthy leg. In femur adduction, the A. genu rotates outward, while the tarsal joint rotates inward (Arıcan, 2020).

3. RADIOLOGICAL EXAMINATION

Although clinical symptoms are usually sufficient for diagnosis, radiography is used for diagnosis. It is necessary to distinguish between acetabulum fracture, physis of the caput femoris, fracture of the collar and caput femoris, which show similar clinical symptoms, and these pathologies. Again, the presence of hip dysplasia or Legg Calve-Perthes disease will prevent the joint from remaining stable after reduction. Therefore, radiography should be used as an additional auxiliary examination method to avoid confusion with such diseases and to determine the diagnosis (Arıcan, 2020; El-Seddawy et al., 2022).

4. TREATMENT OPTIONS

There are various approaches for surgical treatment of coxofemoral dislocations in companion animals. Treatment options include closed reduction (exercise restriction, analgesic agents), open reduction and stabilization (toggle suture, iliofemoral suture, and transarticular pin), femoral head and neck excision (FHNE), and total hip replacement (THR) (Espinel Rupérez et al.,

2021; Scott et al., 2007). In general, we can classify reduction options in two ways: closed and open (Arıcan, 2020).

4.1. Closed Reduction

Although there is general agreement that reduction should be attempted as the first treatment option in the majority of cases to achieve coxofemoral dislocation, published success rates associated with closed reduction vary widely, ranging from 12% to 71% (Harasen, 2005).

Performing closed reduction within the first 48 hours of the formation of the luxation increases both the ease of application and the success rate. It is thought that as time passes, there will be more difficulties in reduction and more reluxations will occur. The acetabular cavity, which is one of the structures that form the joint, will fill with the remnants of soft tissue and ligament teres breaks over time, which will cause difficulties in reduction (Arıcan, 2020).

Contraindications to closed reduction for the treatment of these dislocations include avulsion fractures at the insertion of the teres ligament on the femoral head, moderate to severe hip dysplasia, and injuries to the contralateral leg that impair the patient's ability to bear weight (Denny and Butterworth, 2008; Harasen, 2005). Avulsion fractures involving the ligament are usually the most important factor causing reluxation of the joint within hours or days of the occurrence of dislocation. In addition, the presence of any fracture fragment within the joint accelerates degenerative joint disease. Remnants of the teres ligament rupture may attach to the gluteal muscles, making mobilization of the femoral head difficult (Fox, 1993; Harasen, 2005). The biggest obstacle that makes it difficult to achieve successful closed reduction of long-term coxofemoral dislocations, especially in large breed dogs, is muscle contractions. Contraction of the gluteal muscles may require some force to move the femoral head and reduce the dislocation (BONEet al., 1984; Denny and Butterworth, 2008). In addition, analgesia and general anesthesia can be very helpful in achieving reduction, providing sufficient muscle relaxation and muscle fatigue. It is argued that there is no contraindication for closed reduction in long-term coxofemoral luxations, because it is reported that even in this case, the chance of success and the results of surgical treatment after unsuccessful closed reduction do not have a negative effect (Bone et al., 1984).

However, it has recently been advocated that the chance of success is low in cases where closed reduction is not achieved within the first 48-72 hours as a treatment option. There are two main techniques that are most ideal for closed reduction of coxofemoral dislocations. In the case of craniodorsal dislocation, one hand is grasped by the heel or mid-tibial region to medially rotate and abduct the extremity, while the other hand is used to push the trochanter major over the cranial acetabular rim, resulting in distal and caudal traction. While performing this procedure, it is usually useful for an assistant to provide opposing traction by pulling steadily from the opposite side on a piece of rope hooked around the femur and toward the groin, so that the patient does not progress. Alternatively, closed reduction can be performed by grasping the knee, rotating the femur outward, and then applying traction in a caudal-distal direction, as previously described. External rotation of the femur rotates its head over the acetabular rim, so that it can be placed in the acetabulum. Once the closed reduction is complete, the joint is placed through full range of motion by applying lateral and medial pressure to the greater trochanter. This forces hematomas, portions of the joint capsule, and fibrous tissue out of the acetabulum and allows the femoral head to settle more deeply (Pozzi, 2012).

Closed reduction is the preferred first treatment option, but is contraindicated in cases where there are pathological problems in the coxofemoral joint, although a reluxation rate of 43-47% has been reported (Harasen, 2005).

In a study conducted by Schlang et al. (2019), according to the results of treatment with closed reduction and Ehmer bandage for coxofemoral joint dislocation in 92 dogs; 42 of 92 dogs (43.5%) reported that there was reluxation in the affected hip joint during removal of the sling. In this study, 46 (50%) dogs reported mild soft tissue injuries as a result of sling use, 17 of these cases had serious soft tissue injuries and one dog had an extremity amputation. The study results reported that closed reduction and Ehmer bandage application of craniodorsal hip dislocation resulted in a low success rate and a high complication rate (Schlag et al., 2019).

4.2. OPEN REDUCTION

Although there are many different techniques reported for open reduction and stabilization, the most commonly applied methods are toggle rod suture,

iliofemoral suture and transarticular pin techniques, and these techniques are defined as primary treatment options (LeFloch and Coronado, 2022).

The toggle wrench, essentially a metal rod with a central eyelet for passing the suture, is passed through the acetabular tunnel and then swung so that the toggle wrench is adjacent to the medial acetabular wall. The suture used can be a 50 lb nylon artificial ligamentum teres substitute, or two strands of 4M nylon or polydioxanone can be used in the drilled line. A second hole (usually 2 mm in size) is then drilled through the femoral head and neck, required to reattach the femoral head to the acetabulum (Figure 1). A study of 14 cats with coxofemoral luxation treated with toggle stabilization reported an 86% success rate and an owner-rated outcome of good to excellent (Pratesi et al., 2012).

Torstel and Fox (2020) published a retrospective study to determine the complications and outcomes of open reduction and internal fixation of coxofemoral luxation using toggle suture stabilization in a total of 58 dogs and found that 25 out of 58 dogs (43%) developed postoperative complications and 9 cases (15.5%) had major complications after being treated using commercial toggle rods and different suture materials (OrthoFiber, FiberWire or monofilament nylon). They noted that the most common major complication was reluxation, which was seen in 6 dogs (10%) and that monofilament sutures were applied in 5 of the 6 dogs with reluxation, but there was no statistically significant relationship between suture type and reluxation. (Trostel and Fox, 2020).

Gomma et al. compared three different suture materials for the toggle pin technique in 12 dogs (monofilament polypropylene 2 USP, multifilament polyester 5 USP and 0.5 mm stainless steel wire). According to the results of this study, monofilament polypropylene suture material 2USP is the best material for sutures in the toggle pin technique, followed by multifilament polyester 5 USP and finally 0.5 mm stainless steel wire (Gomaa et al., 2023).

In a study on the use of nylon monofilament sutures to be used in toggle pin application, it was recommended that the tunnel size created in the femur be $\leq$ 20% smaller than the width of the caput femoris or a tunnel that is a maximum of 1/3 of the bone diameter (Kieves et al., 2014).

Bone et al. (1984) reported in their study that long-term follow-up of coxofemoral luxation treatments in 171 traumatic dogs was evaluated radiographically and through patient relatives' questionnaires, and emphasized

that radiographs revealed varying levels of degenerative joint disease 1 year after successful reduction; however, the changes observed on radiographs were not always associated with clinical signs (Bone et al., 1984).

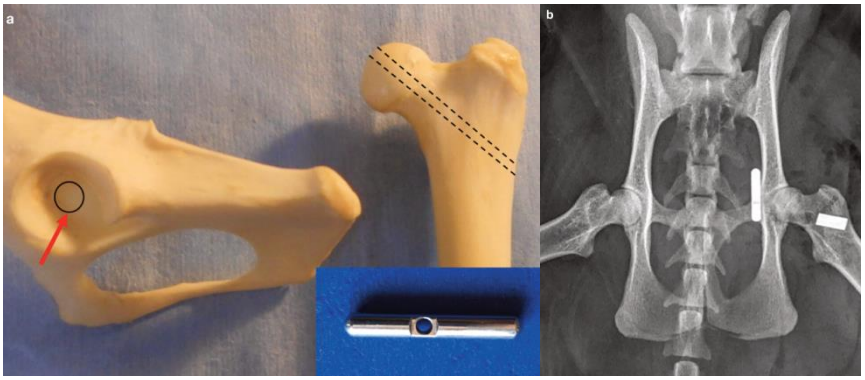

Figure 1. Application of the articulating pin a) acetabular part b) postoperative application (Meeson and Strickland, 2021).

4.2.1. Iliofemoral Suture

The iliofemoral suture is the most commonly used method to hold the femur in internal rotation and adduction similar to the Ehmer sling but is used only in cases where capsulorrhaphy can be performed. A lateral-medial tunnel is opened in the ilial body in the ventral ilum, just cranial to the acetabulum (Figure 2). A second bone tunnel is then opened in the femur, just distal to the greater trochanter, in a caudocranial direction (Meeson and Strickland, 2021).

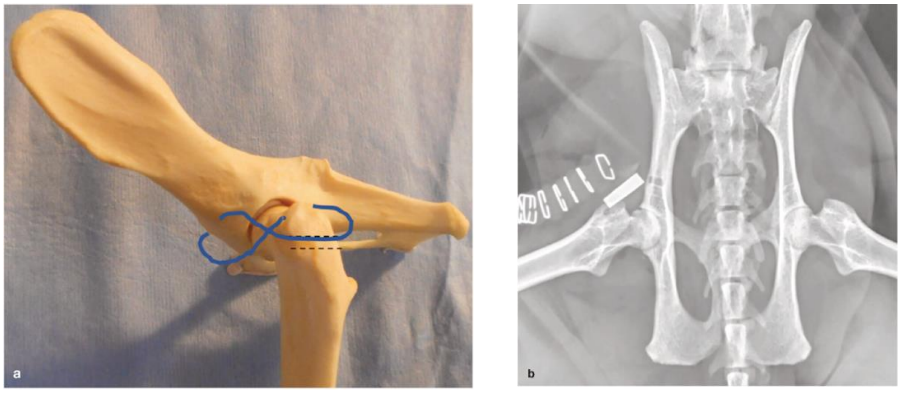

Figure 2. a) Application method b) postoperatif radyografi (Meeson and Strickland, 2021)

4.2.2. Transarticular Pinning

Transarticular pinning is usually performed as an open technique, as well as a closed technique. It is a method of placing a K-wire from the trochanter tertius to the caput femoris, exiting from the insertion of the ligamentum teres femoris, to provide joint stability. A study was performed on 20 cats and 77% success was achieved, while 19 of the pins applied to 20 cats were appropriately removed an average of 3.5 weeks after surgery (Figure 3) (Sissener et al., 2009).

The pin must be removed in approximately 3-4 weeks through a small incision over its tip. Proper placement of the pin is critical, as suboptimal placement can damage the articular surfaces of the hip joint and predispose to pin fracture (Meeson and Strickland, 2021).

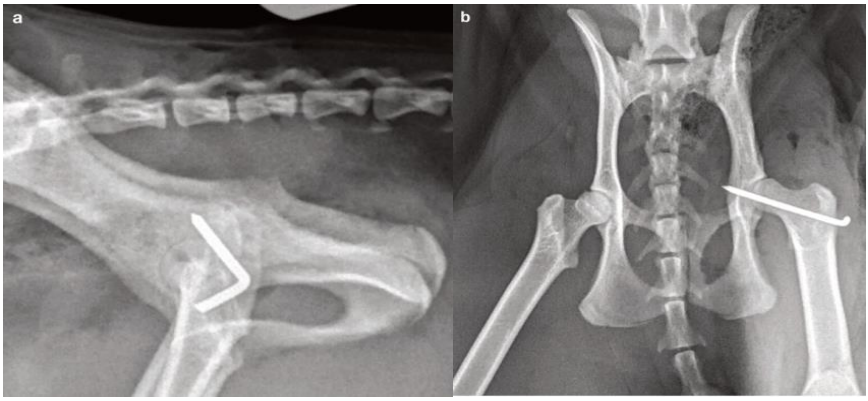

Figure 3. a) Postoperative L/L imagine b) Postoperative V/D imagine (Meeson and Strickland, 2021).

4.2.3. Recovery Procedures

In cases where closed and open reduction cannot be applied or have failed, other methods known as salvage procedures can be applied. They are also indicated options in cases of pre-existing diseases such as hip dysplasia or severe osteoarthritis changes (Meeson and Strickland, 2021).

4.2.3.1. Femoral Head and Neck Excision

Femoral head and neck excision (FHNE) is a procedure performed to surgically remove a weak or dysfunctional joint and restore its function without pain (Figure 4). For a successful FHNE, it is recommended to apply physical therapy procedures after the operation to increase the muscle strength of the

operated part and the long extremity to create an ideal osteotomy and false joint. The results of the operation depend on the absence of complications during the operation and postoperative care and may also vary depending on the performance expected from the case. Although it is known that cats tolerate the period after FHNE operation well according to many studies, it is recommended to reduce bone-to-bone contact and remove the femoral neck from that area to ensure a complication-free process in the postoperative period. Creating a smoother bone piece in the cut sections with an oscillating saw is possible and checking that there is no rough structure in the area by palpation by hand, if there is, removing it with a rasp or rongeur will accelerate healing and reduce complications. At the end of the operation, it is necessary to appropriately suture the cut or damaged parts and close the tissue according to the operative method used to reach the area. If it can be done in the first two weeks after the operation, cats should be given exercise to play with them and passive range of motion if tolerated, and the atrophied muscles should be strengthened in the home environment. The most reported complications after the operation include permanent lameness, patellar dislocation and sciatic nerve damage. In the study where the patient relative survey results were compared with the veterinarian controls after FHNE applied to a total of 15 cats, it was determined that 7 out of 15 cats had muscle atrophy and 4 out of 15 cats were sensitive to pain in the observation of passive range of motion, while the patient relatives' results determined that all cats had satisfactory results. (Off and Matis, 2010).

In a recent study of 18 cats, the family members of the patients reported that 14 of the cats were running, climbing, jumping, walking, playing, and grooming themselves normally, with excellent to good results at both medium and long-term follow-ups (Yap et al., 2015).

It was reported that all 7 cases treated with FHNE were observed to start touching their toes one week after the FHNE operation. After 3 weeks, they started weight bearing with painless limping and after 5 weeks, a slight painless limping was recorded with weight bearing. It was emphasized that at week 12, no limping was observed, although a slightly abnormal gait continued (El-Seddawy et al., 2022).

The average recovery period for cats and small dogs is reported to be 4-6 weeks, and for dogs over 15 kg it is 7-9 weeks (Off and Matis, 2010) . The study conducted by (Off and Matis 2010), according to the long-term follow-

up results of 7-10 months in 66 dogs and 15 cats of 81 patients according to the patient relatives' survey and radiographic and treadmill results in 132 dogs and 51 cats; they stated that there were few dogs weighing more than 15 kg and no significant difference was found between body weight and functional outcomes, and the kinematic amplitudes of the hip, knee and tarsal joints changed significantly. According to the study results, they stated that there was no correlation between patient age and recovery, although the study was conducted in limited cases (Off and Matis, 2010).

Ober et al. (2018) evaluated the unsatisfactory long-term results after FHNE in four large breed dogs according to clinical and computed tomography findings; they observed extensive remodeling and significant bone proliferation involving the femoral neck and acetabulum in three cases, and FHNE failure in all four dogs, as reported by their owners, was an unsatisfactory limitation in function with lameness, pain, muscle atrophy and restriction in range of motion observed on clinical examination. They stated that deep gluteal muscle interposition was performed in one case, but no improvement was observed 3 months after surgery. According to the study findings, they argued that it is necessary to explain to all dog owners that the surgical outcome is unpredictable, especially when FHNE is recommended in large breed dogs. In the radiographic images presented in the same study, it is seen that the pelvic structure has changed and the structure between the acetabulum and the collum femoris has deteriorated. However, the authors ultimately state that the reason for this is that the operation was not performed in accordance with its procedures. (Ober et al., 2018).

Engstig et al. (2022) published the results of a study consisting of orthopedic examination, anatomical measurements, pressure-sensitive gait analysis and a patient relative survey to evaluate the long-term effect of FHNE on functional pelvic position and movement in dogs. The study reported a decrease in extension in the coxofemoral joint ($p = 0.003$), less static weight bearing of the FHNE leg ($p = 0.003$) and muscle atrophy ($p = 0.005$) according to the results of 2.5-year follow-up of 10 dogs that underwent unilateral FHNE. It stated that there was no noticeable trend in the inclination or position of the pelvis ($p = 0.39$) when measuring the height of the ischial tuber. They noted that there was no difference in stance time, swing time or peak pressure between the FHNE and non-FHNE limbs at trot ($p = 0.70$, $p = 0.26$ and $p = 0.91$,

respectively) and that in the long term, there was muscle atrophy, decreased coxofemoral extension and decreased static weight bearing in the FHNE limb. Questionnaires filled out by the dog's relatives reported that the outcome of the surgery was good or excellent (Engstig et al., 2022).

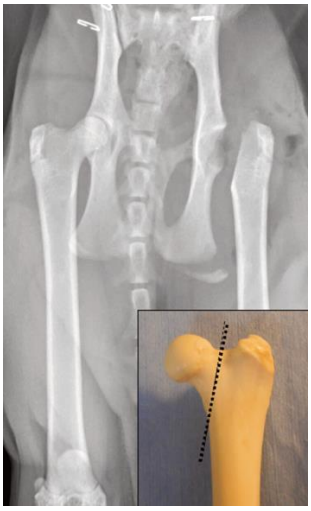

Figure 4. FHNE application site and postoperative image (Meeson and Strickland, 2021).

4.2.3.2. Total Hip Replacement

Total hip replacement (THR) has become a potential alternative salvage procedure with the advent of smaller THR prostheses over the last 15 years (Figure 5). THR may result in improved quality of life due to more normal biomechanical function as an alternative to FHNE. In dogs, the procedure is associated with a good success rate and a low complication rate, but there is considerably more experience performing canine THR. Several case series ranging in size from five to 15 cats are available in the literature, all reporting generally favorable outcomes of THR, although complications such as patellar dislocation and prosthesis dislocation have occurred in a small number of patients (Guthrie and Fitzpatrick, 2017).

Figure 5. postoperative images (Meeson and Strickland, 2021).

5. CONCLUSION

Coxofemoral dislocations are a very important orthopedic problem that is frequently encountered especially in pets. It is encountered especially as a result of high-energy traumas caused by traffic accidents, but it can also occur after diseases such as hip dysplasia and legg calve-perthe. Although there are many studies in the relevant field, all treatments have advantages and disadvantages. Choosing the treatment method with a multifactorial approach, which should be selected according to the time of the dislocation, its shape, the age of the case, the live weight and the financial situation of the patient's relative, can be counted among the indications that increase success. Although the closed method is recommended to be tried first, it is known that reluxation rates are high.

However, when considered as the first option, it allows open reduction to be performed in cases where it is unsuccessful or reluxations are repeated frequently. In cases where open fractures are indicated, the method chosen among the primary treatment options varies according to the experience of the operator, but choosing all treatment options considering their advantages and disadvantages will increase the success of the treatment.

Secondary treatment methods should be evaluated as a suitable method because it requires considerable experience in the application of total hip prosthesis and the evaluation of the patient's financial resources.

Femoral head and neck excision is a cheap and practical method that has been used for many years, and since it is an irreversible salvage operation, it remains up to date as a very effective method when evaluated in appropriate cases.

Therefore, all closed or open methods are very effective treatment methods when used in appropriate cases in hip dislocation.

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CHAPTER 14

REVIVING OF FARRIERY; DISAPPEARING PROFESSION

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INTRODUCTION

The welfare of working equine is a nationwide problem that requires the attention and involvement of veterinary experts and related institutions. The occupation of farriery which is currently being forgotten was popular occupation among society when animals were being used for transportation and when there weren't many motor vehicles. The occupation's being lost is because of animals' not being used for transportation any more. Normal hoof is essential for the equine health. Horse activity is significantly influenced by hoof welfare. The importance of hoof management in horses should be understood detailed. The saying; "No Foot, No Horse" is the most elucidatory word in this subject.

Working horses and donkeys are an essential resource in rural and remote communities throughout the world. Commonly used as an affordable means of transport, for pulling carts, ploughing and cultivating land. There are an estimated 90 million equines (horses, donkeys and mules) in developing countries, and many factors affect their health and welfare. Frequently, these include their owners' lack of knowledge and skills, affordability and accessibility of veterinary services and direct welfare implications arising from emergency situations. Experience and training enables the management of operational risks. For this, training courses are needed for vets, vet technicians and volunteers.

Although farriery is disappearing profession, it's still important occupation in many countries of Europe and Asia. Horse power is preferred in agriculturing vineyard and gardens, as tractor is harmful to branch of plants. Thus, horse and donkeys are commonly used by farmers as they are less harmful. The usage of horses are also getting more and more common and popular because of touristic attractions. Many enterprises are tended to feed different kinds of horses such as working, racing or ponies for adults and children.

Claw diseases of large animals such as cow and horses are a global matter of veterinary society and there is a current need and demand for effective models of prevention and combatting against such conditions. More emphasis on common risk factors, simple and easy ways of prevention and team work is needed.

The primary added value of the teaching activities of the project is being about farriery and prevention of working equine and cattle claw abnormalities. It is clear that improvements in science and research, advances in technology and better understanding of the causes of claw disturbances and the risk factors of many chronic conditions lead to improve animal welfare. Prevention is especially crucial for claw health. Thus, many reports highlight the importance of claw care through preventive measures in horse and cattle.

With regard to exchange of experience on farriery, and for further strategic collaboration, we have partnered with EU countries to contribute further development of farriery in Turkey. The partners provide education and training programs to improve equine welfare in developing world. We furthermore intend to expand our cooperation beyond the extent of the project activities through the establishment of a certified training program on farriery, where the partner institutions would be contributing to its academic, operational and training aspects on a regular basis.

ABOUT PROJECT

Current Erasmus + project is titled as "*Reviving of Farriery; Disappearing Profession*" under "Erasmus+ Vocational Education**",** ***Key Action 2: Cooperation for Innovation and Exchange of Good Practices program.*** The project coordinator association is Afyon Kocatepe University-Turkey. Ss. Cyril and Methodius University in Skopje and Latvia University of Life Sciences and Technologies (LLU) Veterinary faculties are partner institutions.

The main objective of the project is to improve working equine and cattle welfare using EU farriery practices and mutual exchange of experience between partners through strengthening the contacts. Specific objective is to encourage horse related professionals and poor families working with horse and donkeys, enterprises via organizing farriery training courses and dissemination activities in partner countries.

The project started with Kick off meeting via participation of members of Afyon Kocatepe University, Ss. Cyrill and Methodius University in Skopje, Faculty of Veterinary Medicine, Faculty of Veterinary Medicine, LLU and Afyonkarahisar governorship EU and foreign affairs office (as observing

association). Afyon Kocatepe University organized the meeting between the dates of 8-9 February 2019.

In the meeting; project text and budget, visibility rules of EU have been discussed and road map of the project have been drawn. With regard to the text, all sections have been comprehensively explained to the participants. Three planned farriery courses have been detailed discussed and decided to perform first course in Latvia, second and third in Macedonia. Courses contents have been consulted. **The** next transnational meetings were held in Macedonia and Latvia under organisations of Ss. Cyrill and Methodius and LLU Universities.

Planned activities were to determine course program, to arrange visual materials such as brochures in order to deliver the trainees, to prepare leaflets, to produce and deliver short basic farriery techniques films, to arrange tours to stud farms and to achieve farriery trainings as "***Short-term joint farriery training event*****"** in Latvia and Macedonia with participation of Veterinarians.

In **this scope;** the first farriery course were organized **under organization of Faculty of Veterinary Medicine, LLU in Jelgava, from 02 till 08th September 2019. Theoretical and practical sessions were about** "Basic trimming procedures in cattle and horses; **horse foot anatomy, horseshoeing, foot preventive measures, main foot medicaments,** hoof trimming techniques in cattle; horse and cattle handling, safety for hoof trimmer and farrier".

Latvia University of Life Sciences and Technologies Veterinary faculty 100th years anniversary, and conference "Research and Practice in Veterinary Medicine" has been held in Jelgava city, on November 22 nd and 23rd 2019. Project photo gallery has been exhibited during conference and poster titled as "Reviving of Farriery; Disappearing Profession" with contributon of project team was presented.

Furthermore, international farriery conference will be held in Turkey with the participation of local and foreign partners. A photo exhibition about the history of farriery was organized in Macedonia in order to raise awareness on the disappearing profession 'farriery'. Historical farriery museum establishment has been started in Turkey under leadership of Afyonkarahisar governorship. The museum includes formerly used antique farriery tools which is collected from older farriers.

Fig. 1: Project team visited Afyon Governor during kick off meeting

Fig. 2: Photo gallery in Turkey

Fig. 3: Photo gallery in Turkey. Antique equipments for agriculture

Fig. 4: Old farriery equipments

Fig. 5: Latvian farrier

Fig. 6: Macedonian farrier

Fig. 7: Turkish farrier

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CHAPTER 15

BEHAVIOR IN CATTLE

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1. INTRODUCTION

1.1. The Concept of Behavior

The voluntary or involuntary movements of living beings define the concept of behavior. The way an animal eats, drinks, or breathes gives insight into its inner world. In addition, behaviors of animals such as fighting, mating, showing signs of illness, and producing milk and eggs are also evaluated within this scope (Savaş and Yurtman, 2008; Akbaş, 2013). The science of animal behavior is often referred to as "ethology." However, some define this field as "behavioral biology." Recently, the term "applied ethology" has become more common. This term encompasses topics such as the social behaviors of farm animals, feeding habits, health conditions, stress responses, and behaviors during transportation. It also examines their behaviors within herds. This is an important area of research aimed at improving the quality of life of animals and better understanding their needs (Savaş and Yurtman, 2008; Çavuşoğlu and Akyürek, 2018).

The science of animal husbandry (zootechnics) is a discipline that focuses on the breeding and improvement of domestic animals. The science of animal behavior is a fundamental field for zootechnics. Understanding animal behavior helps us manage them better and improve their welfare. The methodology of ethology is used to observe and analyze the behavior of animals in their natural environments. This information enables us to better meet the needs of animals and reduce stress (Marchant-Forde, 2015; Çavuşoğlu and Akyürek, 2018).

1.2. The Importance of Understanding Animal Behavior

Understanding animal behavior not only goes beyond achieving maximum production but also allows us to derive greater benefits from animal husbandry. Different production methods vary according to the environment in which animals live and have evolved. The behaviors they have developed to survive in these complex environments can help optimize our breeding practices. This, in turn, makes it possible to enhance both animal welfare and productivity simultaneously (Lund et al., 2006; Mellor et al., 2009).

The science of animal behavior is of great importance in animal husbandry activities. This field of study aims to understand how animals behave

in natural conditions and why they exhibit certain behaviors by examining their actions (Canbolat, 2019).

1.3. The Science of Behavior and Its Historical Development

Animal behavior is of vital importance for individuals who are part of human society. Since prehistoric times, humanity has started observing animal behavior to deal with wild animals and engage in hunting. Scientists such as John Ray (who studied the instinctual behavior of birds), Charles Georges Leroy (who researched animal intelligence and adaptation and wrote books on the subject), and Douglas Spalding (who conducted experiments on instinct and experience and published numerous scientific papers) have studied animal behavior from a modern perspective. The most significant figure in the development of ethology as a modern science is Charles Darwin, the pioneer of the theory of evolution. Konrad Lorenz and Nikolaas Tinbergen played a key role in shaping the current meaning of ethology and are considered the founders of modern ethology. Tinbergen's fundamental questions form the basis of ethological research and include the following (Cengiz, 2013; Canbolat, 2019):

Causality (What is the cause of the behavior?): This question examines what stimuli trigger a behavior and which physiological mechanisms play a role in its formation.

Development (How does the ontogeny of behavior occur?): This question aims to study how behavior evolves under the influence of individual and environmental factors.

Evolution (How does the phylogeny of behavior occur?): This question generally aims to understand evolutionary processes by comparing behavioral traits among similar species (Canbolat, 2019).

The science of animal behavior is a crucial tool within the field of zootechnics, serving as a foundation for fundamental research and the exploration of unique inquiries. In such studies, the methodology of ethology (comparative behavior science) is frequently utilized. During the 19th century, the interpretation of animal behavior was dominated by anthropocentrism, a human-centered perspective asserting that animals think similarly to humans. One of the significant works of this period was Charles Darwin's (1809–1882) research, which explored the relationship between animal behavior and survival. Following Darwin, J. Henri Fabre (1823–1915) emphasized the

complexity of insect behavior through his observations of wild bees. C. Lloyd Morgan (1852–1936) proposed that animal behavior could be explained by a psychology distinct from human psychology.

The development of ethology as a scientific discipline occurred in the 20th century. I. P. Pavlov (1849–1936) pioneered the use of experimental methods in behavioral research by introducing the concept of "conditioned response." Konrad Lorenz (1982) described comparative behavior science as a discipline aimed at understanding animal and human behavior by incorporating methodologies and questions from various biological fields, building upon the work of Charles Darwin. Initially, the science of animal behavior was employed as a systematic approach to investigate phylogenetic relationships. Over time, however, ethology evolved beyond comparative analyses, becoming a field dedicated to examining all aspects of animal behavior and providing comprehensive explanations for these behaviors (Savaş and Yurtman, 2008; Canbolat, 2019).

The practice of animal husbandry began with the formation of agricultural societies. However, the scientific and systematic study of animal behavior is a relatively recent development in the scientific world. Local scientific resources are only beginning to engage with research on behavioral science, and it has been observed that the popularity of presentations on this subject at national conferences is steadily increasing. This field, previously considered overlooked but crucial for sustainability, requires greater attention. To foster this interest, it is essential to promote the field of animal behavior science (Hughes, 1988; Savaş and Yurtman, 2008).

1.4. Ethology and Applied Ethology

The observation and interpretation of animal behaviors are simpler than many other biological characteristics. In numerous scientific disciplines, data collection requires specialized techniques, necessitating expertise in these methods. However, the challenge of quantifying animal behaviors posed a significant obstacle to the development of ethology as a scientific discipline, compared to areas such as physiology or genetics, where data collection is more straightforward (Mench, 1998; MacDougall-Shackleton, 2011).

The acceptance of ethology as an applied science is a relatively recent development in the history of science. Nevertheless, animal behavior has long

been addressed as a technical detail in books on animal husbandry. For example, indicators of reproductive periods, the ideal height of feeding troughs, and barn dimensions have been discussed. Thus, even though it was not approached within a scientific framework, animal behavior has always been considered in studies related to animal husbandry (Savaş and Yurtman, 2008; Desire et. al., 2002).

Initial studies in applied ethology generally focused on creating behavior inventories (ethograms) for domestic animal species. These studies involved observing and defining species-specific behaviors to establish a "species-specific ethogram" (Smidt et al., 1995). During the same period, domestic animals became a subject of research in ethological theories, influenced by Tinbergen's contributions (Bessei, 1983, Sandilands, 2004).

Research aimed at identifying behaviors led to an evolutionary shift from qualitative to quantitative analyses, examining the frequency of behaviors based on time and spatial factors (Mench, 1998).

Behavioral sciences have contributed not only to understanding biological phenomena but also to improving animal husbandry conditions, identifying welfare issues, detecting adverse effects caused by individual and environmental factors, and advancing health protection practices (Fraser, 2008; Savaş and Yurtman, 2008).

Applied ethology focuses on studying the behavioral biology and behavioral needs of species living in environments largely shaped by humans. It also conducts research to define animal welfare using "biological indicators" and investigates how different housing and feeding conditions influence behaviors (Smidt et al., 1995; Linda, 2005).

Behavioral science cannot exist in isolation. Subfields of ethology have emerged alongside other biological disciplines. Some of these include:

- **Human ethology:** Examines the biological foundations of human behavior.
- **Eco-ethology:** Studies how the behaviors of organisms interact with their environment.
- **Behavioral physiology:** Investigates the physiological mechanisms underlying behavior.
- **Behavioral genetics:** Explores the hereditary aspects of behavior.
- **Neuroethology:** Examines how the brain controls behavior.

Findings from ethology have contributed to the development of new scientific fields, such as psychoneuroimmunology and sociobiology.

These fields and their subcategories, as their names suggest, attract researchers from diverse disciplines, including veterinarians, biologists, medical doctors, and sociologists (Savaş and Yurtman, 2008; Bels et al., 2022).

1.5. Factors Shaping Animal Behavior

1.5.1. Genetics

Animal behavior is closely related to the activities of nerve cells and muscles, with genetics playing a significant role in these processes. Genetic information controls the production of proteins that regulate the development of body tissues and chemical reactions within cells. This regulation can influence the physiological foundations of specific behaviors. Studies have shown that certain genes or groups of genes can affect animal behavior. However, it cannot be claimed that these behaviors are solely determined by genes. Environmental factors also significantly influence the development and expression of behavior. Animal behavior arises as an interaction between genetic and environmental factors (Uruk and Yenilmez, 2017; Barroeta and Kirchner, 2020).

1.5.2. Environmental Influence

Animals interact with and adapt to their environment to survive. Various environmental factors play a role in this adaptation process, including:

- **Environmental conditions:** Farm animals respond to temperature fluctuations by seeking the most suitable shelters.
- **Photoperiod (day length):** It affects the breeding periods of sheep, goats, and horses, as well as egg production in chickens.
- **Type of habitat:** Herd animals, such as cattle and sheep, tend to remain within a defined area, even in regions with vast open spaces.
- **Feed availability:** Grazing behavior depends on the quantity and type of roughage. During feed scarcity, the amount of available grazing space also influences behavior.
- **Social groups:** Grouping significantly impacts animal behavior. Age distribution and the gender of animals within a group influence intra-group interactions.

- **Human influence:** Animals generally respond positively to caretakers but react negatively to harsh treatment (Dellal and Cedden, 2002; Vogt, 2023).

1.5.3. Domestication

Commercial farm enterprises facilitate behavioral changes in animals by selecting those with traits of economic value. Modern farm animals typically live under comfortable conditions created by humans, achieving a good quality of life in these environments. Breeding programs often focus on traits such as docility and maternal behaviors, which become prominently expressed in the offspring of selected parents (Hughes, 1988; Mench, 1998; Stricklin, 2001).

1.5.4. Physiological Foundations

The nervous system perceives information from the external environment and determines how the body should respond. Meanwhile, the endocrine system regulates bodily functions and maintains internal balance through hormones. The collaboration between these two systems enables the emergence of complex behaviors essential for survival and reproduction (Özdin and Mundan, 2018).

1.5.5. Sensory Systems

Animals gather environmental information through their sensory systems, including vision, hearing, smell, taste, and touch, allowing them to adapt to their surroundings. The capacity of these sensory systems varies across species, with data from multiple senses often integrated to produce an appropriate response.

The positioning and angle of an animal's eyes influence their field of vision and behavior. Animals like horses, cattle, goats, and sheep have eyes positioned on the sides of their heads, providing a broad panoramic view and enabling them to see almost their entire surroundings. However, this placement results in a smaller binocular field of vision and reduced depth perception. In contrast, animals with forward-facing eyes possess a larger binocular field and greater depth perception. The color vision capabilities of farm animals remain incompletely understood (Wieckert, 1971; Aureli et al., 1995).

The sense of smell plays a minor role in feed selection for farm animals, yet it is critical in reproductive behaviors and recognizing offspring. In poultry

species, the olfactory capability is comparatively less developed (Cengiz, 2013; Anonymous, 2024).

All farm animals and poultry possess taste buds that influence their feed preferences. While taste responses in humans are categorized as sour, bitter, sweet, and salty, experimental studies indicate that animals and poultry classify feed tastes as unpleasant, sweet, or neutral. Many farm animals exhibit a well-developed sense of touch. For instance, horses can respond to subtle cues from a rider's reins or leg pressure to alter direction or gait. Some species, such as pigs, prefer close physical contact even during rest. Animals often engage in behaviors like rubbing against each other or scratching (Mench, 1998; Anonymous, 2024).

2. TYPES OF BEHAVIORS IN ANIMALS

Animals exhibit different behavior types depending on their species. Some behavioral systems are more developed in certain species compared to others. Additionally, feeding and sexual behaviors are the most commonly studied due to their economic and commercial significance (Sahu et. al., 2020; Gülay Yıldız and Gülay, 2022).

2.1. Feeding Behavior

Cattle, sheep, and goats are ruminant animals, meaning they swallow their food without chewing it first. Cattle spend 4-9 hours grazing daily, while sheep can graze for 9-11 hours and ruminate longer than cattle. Goats prefer fresh shoots from trees in addition to the plants favored by cattle and sheep (Dias-Silva and Abdalla, 2021; Gülay Yıldız and Gülay, 2022).

Horses cut, chew, and swallow grass, but they do not engage in rumination. They tend to graze over larger areas and often rub against the ground while eating. Pigs do not exhibit grazing behavior and prefer a diet based mainly on grains. They consume food frequently throughout the 24-hour day and feed on roots, seeds, nuts, and insects found in pastures and meadows. As a result, pigs' feeding behavior differs from that of ruminants (Wieckert, 1971; Gülay Yıldız and Gülay, 2022).

2.2. Defecation Behavior

Cattle, sheep, goats, and poultry excrete feces and urine at random times according to their needs. Horses tend to defecate near previous defecation sites

and, like other animals, excrete randomly throughout the day. Pigs typically attempt to excrete away from areas where sheep are present. However, this behavior may change when they are confined to a small space. Llamas use defecation to mark territory, and manure areas may be designated in specific locations (Ekesbo and Gunnarsson, 2018; Semantic Scholar, 2020).

2.3. Sexual Behavior

The learning of sexual behaviors is critical for developing effective breeding programs. Well-managed breeding programs are designed to highlight economically important traits in livestock. These behavioral patterns may change in animals raised in confined spaces. Observing and understanding these behaviors is necessary for livestock breeders to enhance productivity (Semantic Scholar, 2020; Anonymous, 2024).

While sexual behaviors vary among species, common behaviors across species include mating calls, dances, visual signals, and pheromones. These behaviors typically emerge instinctively and may have evolved for species survival. Hormones, particularly testosterone in males and estrogen in females, play a key role in sexual behavior. Male animals castrated at a young age display fewer sexual behaviors than those castrated later, suggesting that some sexual behaviors can be learned through observation over time (Phillips, 2008; Anitaş et al., 2015; Semantic Scholar, 2020).

In most mammals, a typical sign of sexual readiness is a change in external genital organs, such as vulvar swelling and mucous discharge at the onset of estrus. In some species, female urine and vaginal discharge may contain sexual attractants. Stallions, rams, and bulls have an organ in their nose capable of detecting pheromones in female urine and vaginal discharges. Some male animals also have special scent glands to attract females. Reproductive behavior in poultry varies by species. Some species are monogamous, choosing a mate for life and nesting together, while others are polygamous and mate with multiple partners (Katz and McDonald, 1992; Phillips, 2008)

2.4. Maternal and Offspring Behavior

Female farm animals typically lick their offspring immediately after birth to help them stand and nurse. This behavior is common in cows, sheep, and horses but is not observed in sows or llamas. Mothers recognize their offspring by appearance, scent, and sound and defend them against any perceived threats.

The distress calls of young animals alert their mothers. Workers in environments with newborns should remain vigilant against the protective behaviors of mothers (Wieckert, 1971; Chenoweth et. al., 2022; Anonymous, 2024).

In poultry farming, the increased use of incubators has led to a decrease in maternal instincts. Breeders use specific techniques to reduce brooding tendencies. Poultry raised using traditional methods continue to display natural maternal behaviors, such as calling loudly to alert their offspring, foraging for food, and providing protection (Phillips, 2008).

2.5. Aggressive Behavior

Aggressive behavior emerges as a result of dynamic interactions between animals and often involves elements of fighting, fleeing, and determining social rank. This behavior is especially observed during competition for dominance, particularly between genders and within groups. For example, among farm animals and poultry, aggressive behavior is common among males, especially those competing to attract females (Cengiz, 2008; Metin and Kaliber, 2011).

A typical example of aggressive behavior is observed between cattle. Cattle assume a threatening posture, lowering their heads, staring at their opponents, and pulling their hind legs forward. They dig at the ground with their front feet, bellow, and push their heads toward their opponents in a challenging manner (Canbolat, 2019; Anonymous, 2024).

Pigs exhibit aggressive behavior differently. When encountering a strange male pig, they adopt a defensive posture to protect themselves. They puff up their fur along their backs, move in circles to monitor the opponent's movements, and prick their ears. During this time, they make growling sounds and use their teeth to bite and injure their opponent (Cengiz, 2008; Ekesbo and Gunnarsson, 2018).

Factors contributing to aggressive behavior include competition for scarce resources, such as feed and feeding space, which can increase tension within the group and encourage aggression. However, this behavior is typically limited to individuals of the same species, and castrated males generally display less aggression (Tölü and Savaş, 2006; Phillips, 2008).

The establishment of social hierarchies is common among farm animals and poultry. Individuals within a group often have a ranking system based on

access to resources, food, and reproduction. The term "pecking order" is sometimes used to describe this, especially in poultry, where it relates to pecking behavior. Typically, older males dominate younger males, and similarly, in female groups, dominance is clear. In mixed-gender groups, males tend to dominate females, and older individuals typically hold rank over younger ones. Once the social hierarchy is established, fighting behavior tends to decrease within the group (Tölü and Savaş, 2006; Titterington et. Al., 2022; Anonymous, 2024).

2.6. Allelomimetic (Mimicking) and Herd Behavior

Farm animals and poultry tend to follow each other in herds, a behavior that serves protective functions against predators and helps preserve their living space. This herd instinct, observed during grazing, is an innate response in animals (Rath et al., 2015).

Some species tend to graze in large groups, while others prefer to break into smaller subgroups. This behavior allows for more effective management of animal groups. Within a group, there is a tendency to follow or mimic the behavior of a leader, a phenomenon known as “allelomimetic behavior”. For example, a cow grazing in a field may begin to move toward the barn, prompting other cows to follow suit. The leader is often the strongest or oldest animal, though this can vary by species (Wieckert, 1971; Rath et al., 2015).

Herd behavior models help animals survive by working together and maximizing their interaction with the environment. This behavior strengthens communication among animals and ensures balance within the group (Anonymous, 2024).

2.7. Shelter-Seeking Behavior

Farm animals seek shelter to maintain homeostasis (internal balance) in response to changing environmental conditions. This behavior manifests differently in hot and cold weather.

Seeking Shade: In hot weather, animals search for shade to protect themselves from the sun. Sheep and cattle, in particular, gather in shaded areas. Pigs use mud baths to cool down or seek cooler shaded spaces. Cattle pant, produce sounds, sweat, and drink more water when overheated. Ruminants reduce their grass intake when temperatures rise (Wieckert, 1971; Canbolat, 2019).

Grazing Timing: Animals prefer to graze during cooler times of the day, such as evenings or nights.

Heat Protection in Cold Weather: In cold weather, animals huddle together to conserve warmth. Pigs lie close to each other, while cattle, sheep, and horses face away from the wind for protection. Fur, coats, and fat layers provide insulation against the cold. Compared to other farm animals, poultry seek shelter less frequently. Chicks huddle together to warm up when temperatures drop, although this sometimes leads to unfortunate outcomes. In cold, snowy weather, turkeys rarely seek shelter, which can lead to serious losses. Shelter-seeking behavior is an essential adaptation for farm animals to adjust to changing environmental conditions. Understanding this behavior is crucial for optimizing animal welfare (Cengiz, 2008; Metin and Kaliber, 2011).

2.8. Curiosity (Exploratory) Behavior

Exploratory behavior is categorized as follows: (1) extrinsic exploration, where the animal seeks information related to conventional reinforcers like food, and (2) intrinsic exploration, which focuses on stimuli that may lack biological significance and is further divided into inspective and inquisitive exploration. All animal species exhibit curiosity about their environment. This curiosity, referred to as "exploratory behavior," varies among species and according to the animals' age. Exploratory behavior involves actively exploring the environment and seeking information through various sensory cues (Wood-Gush and Vestergaard, 1989; Ekesbo and Gunnarsson, 2018).

Some species exhibit more exploratory behavior than others. For example, cattle, pigs, and horses are more willing to explore their surroundings when introduced to a new environment. Sheep and poultry tend to be more timid and prefer staying in familiar environments rather than exploring (Creamer and Horback, 2024; Anonymous, 2024).

Generally, younger members of a species display more curiosity than adults. Young animals are more motivated to explore and learn about their surroundings. Exploratory behavior is a natural inclination observed in all animal species and plays a vital role in their survival and development (Ekesbo and Gunnarsson, 2018).

3. BEHAVIOR IN CATTLE

Cattle have played a significant role in human development since prehistoric times, providing meat, milk, clothing materials, various tools, and labor. They have also been used as a means of trade in some regions and hold religious significance in certain cultures. Additionally, cattle have been utilized for sporting purposes. For the past century, cattle have been raised within modern livestock farming practices for meat and milk production (Arave and Albright, 1981; Demirtaş, 2022).

3.1. Behavioral Description

The reaction of an organism to a stimulus is referred to as behavior. In other words, behavior is the way an organism responds to a specific situation, condition, or stimulus. Stimuli can be either internal (endogenous) or external (exogenous). An organism's response to a stimulus may vary based on the spatial and temporal context. Additionally, behavior can differ depending on the system involved or the organism's function. For instance, the behavioral tendencies associated with bird migration are termed migratory behavior, while actions aiding thermoregulation in mammals are known as thermoregulatory behavior. Certain behavioral responses, such as foraging, locomotion, and reproduction, are obligatory, whereas others, like mimicry or predator escape, are facultative. The study of animal behavior falls under the discipline of "ethology." As one of the most fascinating fields within zoology and animal science, ethology focuses on the systematic and scientific investigation of animal behavior (Sahu et. al., 2020).

Cattle are large-bodied animals that live in herds in vast pastures, meadows, and grazing lands. In natural environments, females typically separate from the herd to choose a place for calving, and calves are often separated from their mothers during the first few weeks. Therefore, unlike sheep herds, cattle tend to remain quite distant from other animals while grazing. In smaller herds, social dominance hierarchies are observed and persist for extended periods. In the wild, cattle's grazing behavior follows a distinct rhythm. They generally rest during the hot hours of the day and intensify grazing activities in the evening, at midnight, and at dawn. During these times, grazing is less frequent to avoid predators (Cengiz, 2013).

Mating can occur year-round but typically intensifies during specific seasons, usually from late spring to early summer. These periods coincide with the availability of abundant pasture and the suitable time for calving. The gestation period is approximately nine months, and signs of estrus in females are rarely observed during intense lactation periods (Cengiz, 2013).

After weaning, young cattle remain within the herd, while males leave the herd at around two years of age to form their own subgroups. Within these groups, they compete for the position of "dominant bull." To detect females in estrus, bulls move towards areas where females are located and initiate mating behavior (Anonymous, 2024).

3.2. Feeding Behavior

The most important tool for cattle in the feed intake process is their tongue. Due to the limited mobility of their lips, their tongues play a dominant role in gathering food. With their sharp teeth, long and coarse tongues efficiently cut long grasses. Short grasses, on the other hand, are gathered with the help of lips and incisors. In the case of grain feeds, both the tongue and lips are involved (Albright, 1993; Canbolat, 2019).

Feeding behavior varies depending on the chewing time. Chewing occurs with the movement of the lower jaw and allows for the mechanical breakdown of food in the mouth. During this process, food is divided into small pieces, which increases its solubility and allows it to mix with digestive tract secretions. Additionally, food is mixed with saliva during chewing. The chewing duration varies with age, with younger animals chewing for longer periods than older ones. Feeding behavior is also influenced by various external factors, such as environmental conditions, dental health, the age of the animal, and the type of food consumed. Generally, food intake decreases with increasing environmental heat. Cattle feeding behaviors include activities such as finding feed, consumption, rumination, drinking water, and other related feeding practices (Smid et al., 2020).

3.2.1. Grazing Behavior of Cattle

The term grazing is generally described as the repetitive and nonplanned consumption of small amounts of food, not in response to hunger/satiety sensations. Cattle primarily use their sense of smell to distinguish plants during grazing. They are capable of recognizing the growth stages of plants and, based

on this information, selecting their grazing preferences. This ability is known as "selective grazing." Selectivity increases with age, with young cattle preferring leaves, while adult cattle consume the whole plant. Taste is a determining sense in food preference, and cattle choose or reject different foods based on their taste. Smell and sight are secondary senses, coming after taste. The smell of plants or areas contaminated with feces can influence cattle's grazing choices, and typically, grass contaminated with feces is avoided. Moreover, the sense of touch also plays a significant role in feed selection. In narrow pastures, cattle have been observed eating irritating plants like nettles, processing them in a way that reduces their irritating effects before consumption. Cattle spend an average of 4 to 9 hours grazing daily, with an additional 2 to 3 hours spent searching for food. Grazing times may vary each day (Cengiz, 2008; Endres, 2021).

Cattle usually follow the same feeding routine while grazing. They move slowly, using their long tongues to gather grass, which they pinch and break off with their incisors between their upper palate. They swallow the gathered grass without chewing it thoroughly. While standing, they increase their head and jaw movements to eat selected plants. However, they may find it difficult to gather short grass, as the tongue's gathering and the palate's pinching methods are better suited for longer plants. In the case of cut grass, the tongue and lips remain the primary tools. The distance traveled during grazing averages around 4-6 kilometers per day, which varies depending on daylight and grass quality. Cattle grazing on lower-quality grasslands typically travel twice the distance compared to those on higher-quality pastures. It has been observed that cattle usually graze in an easterly direction. Grazing periods typically occur before the first light of dawn, just before noon, late afternoon, and around sunset. Morning and late afternoon grazing periods are the longest, and these times vary with the seasons. Studies have shown that dairy cows spend 8-10 hours a day grazing to suppress their appetite. However, this may vary depending on factors such as climate, pasture productivity and quality, nutritional requirements, estrus, pregnancy, and health (Cengiz, 2013; Demirtaş, 2022).

For cows that have recently calved, the nutritional balance is usually negative. If the animal's energy requirements exceed its intake, it depletes body reserves. Furthermore, in pregnant animals, the rumen capacity is reduced due to the enlargement of the uterus. All these factors influence grazing time.

Research has shown that the maximum grazing duration is 12 hours, although in pastures that are extremely insufficient, this period may extend to 13 hours (Hafez and Bouissou, 1975).

Studies suggest that grazing time has less of an impact on chewing frequency compared to feed intake behavior. The number of chews can vary depending on the ease of taking and tearing grass and the quality of the pasture. During an 8-12 hour period of rumination and grazing, it has been observed that the number of chews ranges from 45,000 to 75,000 (Demirtaş, 2022).

The early stages of lactation in dairy cows are the most stressful period. One significant stressor is the separation of the calf from the cow, while milking represents another important stress factor. Additionally, the animal's high nutritional requirements during this period act as another stressor. It has been reported that this last factor (high nutritional needs) has a significant impact on grazing duration (Canbolat, 2019; Endres, 2021).

In terms of management practices, attention must be paid to the social order of grazing behavior. After the resting and rumination periods, animals in the herd often copy the behavior of the first cow to begin grazing. Therefore, whether the first grazing cow is high, or low yielding can affect the herd's milk production. A practical solution to these issues could be grouping animals based on their productivity levels. As such, it can be stated that cattle generally prefer to graze during cooler hours. Considering this, providing their feed during cooler hours could increase feed intake and contribute to higher productivity (Phillips, 2008; Ekesbo and Gunnarsson, 2018).

The time cattle spend in pastures varies depending on external factors such as climate conditions, grass quality, and their own nutritional needs. Grazing patterns may be disrupted during biological processes such as reproduction. An increase in temperature leads to wider individual distances within the herd (Canbolat, 2019).

3.2.2. Stimulation of Grazing Behavior

Cattle, in addition to distinguishing between plant species during grazing, are also capable of recognizing different growth stages of plants and making grazing decisions based on this knowledge. A characteristic feature of cattle's grazing behavior is their marked preference for areas rich in protein, such as those containing legumes. This selectivity is inversely proportional to

their age; young calves prefer leaves that they carefully separate from the stems of plants, while adult cattle eagerly consume the entire plant (Wieckert 1971, Cengiz 2013; Diaz Falu et al., 2014).

The sense of taste plays a decisive role in cattle's food preferences. While they eat some grasses with great pleasure, they consume others with less appetite and reject some grasses completely. The senses of smell and sight are less important than the sense of taste. During grazing, cattle constantly smell the grass and can decide whether to accept or reject the grasses just by smelling them, which is a very interesting behavior (Phillips, 2008). The smell of aromatic grasses or an area contaminated with feces can directly affect their grazing choices. They usually reject grasses contaminated with feces; however, if the entire pasture is contaminated, they may have difficulty grazing but may still continue to graze. It is reported that the desire to graze is triggered by the hypothalamus with the decrease in blood glucose and the molar concentrations of volatile fatty acids produced in the rumen (Diaz Falu et al., 2014; Anonymous, 2024)

3.2.3. Factors Influencing Grazing Behavior

The grazing habits of cattle are influenced by factors such as genetic diversity, climate adaptation capacity, and the size of the digestive tract. Dairy cattle, in particular, tend to graze immediately after morning milking, with grazing intensity gradually decreasing until the afternoon milking. Ambient temperature significantly impacts social spacing within the herd; high temperatures increase the distance between individuals and negatively affect forage intake. The quality of pasture forage also influences grazing behavior; cattle tend to stand closer in high-quality forage areas and farther apart in low-quality areas. Additionally, cattle find it difficult to graze on grass shorter than 1 cm (Diaz Falu et al., 2014; Endres, 2021).

3.2.4. Stimuli Facilitating Grazing Behavior

Cattle primarily use their sense of smell to distinguish plants during grazing. They can recognize the growth stages of plants and select forage accordingly, a skill known as "selective grazing." Selectivity increases with age; calves primarily consume leaves, whereas adult cattle consume the entire plant. Taste plays a key role in food preferences, with cattle selecting or rejecting foods based on their flavors. Smell and sight serve as secondary senses

after taste. For instance, the smell of plants or areas contaminated with feces influences grazing choices, as cattle generally avoid forage contaminated with feces. Touch is another important factor in feed selection. On restricted pastures, cattle have been observed to consume irritant plants like nettles, processing them in ways that mitigate their irritating effects. Cattle allocate an average of 4 to 9 hours per day to grazing and an additional 2 to 3 hours searching for feed. Grazing times can vary daily (Cengiz, 2008; Endres, 2021).

3.2.5. Feeding Behavior in Modern Farms

In modern cattle farming, grazing has almost been eliminated. Feed is prepared using specialized feed mixers and provided to cattle at least twice daily. While consuming feed, cattle use their tongues effectively as their primary feeding tool. The tongue wraps around forage, and the lower incisors, in conjunction with the hard palate of the upper jaw, cut the forage before it is ingested. For mixed feed, cattle use both their tongues and lips to gather feed. The chewing duration depends on the type of feed. Chewing involves the movement of the lower jaw against the stationary upper jaw to break down feed in the mouth. The primary purpose of chewing is to reduce feed into smaller particles to increase the surface area for digestion. Chewing lasts approximately 6-8 hours daily, with longer durations observed in younger cattle. Feed consumption in modern farms varies depending on environmental temperature, dental condition, cattle age, and the type of feed. High environmental temperatures reduce feed intake (Canbolat, 2019; Endres, 2021).

As the roughage ratio in the diet increases, feeding speed decreases. Cattle typically make 70-80 jaw movements per minute when consuming feed. Reports indicate that cattle take approximately 15-18 minutes to consume 1 kg of dry hay and 3-5 minutes for silage or other moist feeds (Demirtaş, 2022; Anonymous, 2024). Maximum feed intake occurs when sufficient feed is available, adequate feeding time is provided, and cattle have access to water. Access to feed is more critical than feed quality, as cattle can adjust their intake to meet their nutritional needs. Providing concentrated feed for 5 hours daily can increase 24-hour feed intake by 15-30%. In beef cattle nutrition, providing constant free access to feed or implementing a three-day cycle where feed is offered at 110% and 90% of the normal amount affects rumen pH, growth rates, and feeding habits (Göncü and Bozkurt, 2019).

3.3. Drinking Behavior in Cattle

In dairy cows, an adequate water supply is necessary for optimal feed consumption, productivity, health, and animal welfare. Cattle use their lips and tongues while drinking, though the tongue plays a minor role in this process. When drinking, cattle submerge their mouths approximately 3-4 cm into the water at a 60° angle and draw water in through suction without lifting their heads. Cattle drink water several times daily while grazing. Water consumption varies depending on cattle species, age, dry matter intake, ambient temperature, feed composition, salt content, pregnancy status, and milk yield. Grazing cattle typically prefer to drink water before noon, in the afternoon, and evening, with minimal water intake during the night or early morning. Dairy cows often feel thirsty after milking (Canbolat, 2019; Demirtaş, 2022; Burkhardt et. al., 2022).

Unlimited access to clean, fresh drinking water is as essential as access to fresh feed. Insufficient water intake reduces dry matter consumption, adversely affecting digestive health and productivity. Cattle prefer drinking from wide, low water surfaces. They require calm, stable, and safe environments for drinking. Given the choice, cattle will always select the cleanest and freshest water available (Phillips, 2008; Ekesbo and Gunnarsson, 2018; Canbolat, 2019; Demirtaş, 2022).

Cattle prioritize feeding over drinking at the start of the day and usually follow a consistent pattern when drinking water. After milking, their desire for water increases. Thus, adequate water troughs should be placed in barns, positioned away from feeding areas. Contaminants at the bottom of water troughs impart undesirable flavors to the water. Warm weather promotes bacterial growth in such troughs, causing odors and health risks. Troughs should be emptied daily and thoroughly cleaned at least once a week (Yaylak and Yavuz, 2016; Canbolat, 2019; Demirtaş, 2022).

The process of cattle drinking water occurs in three stages:

1. The cow touches the water with its tongue to taste it.
2. After tasting, the cow keeps its tongue in the water for a prolonged taste.
3. Finally, the cow submerges its head and swallows the water.

In winter, if the water is too cold, cattle may lick the water instead of drinking it. Warming the water encourages cattle to drink more, leading to increased feed intake. Observations indicate that cattle avoid drinking water

while grazing but drink primarily in barns, especially after milking (Canbolat, 2019; Endres, 2021).

3.4. Rumination Behavior in Cattle

Ruminants regurgitate previously ingested feed for further chewing to enhance digestion, a process known as rumination. After feeding, ruminants retreat to a safe location to ruminate. Partially digested feed material from the rumen is brought back to the mouth, chewed into smaller particles, and swallowed again (Phillips, 2008; Schirmann et al., 2013; Anonymous, 2024).

Observations indicate that 65-80% of rumination occurs while lying down. However, cattle may ruminate while standing in wet or rainy conditions. Ruminants can ruminate in various positions: lying down, standing, or slowly moving. Calves begin rumination at around three weeks old, but the frequency and duration of rumination do not fully develop until they are 6-8 months old. The duration of rumination depends on the type of feed and may occur 15-20 times per day. Each rumination period can last from two minutes to an hour. Young cattle are sensitive, and minor discomforts can interrupt rumination. Factors such as hunger, stress, pain, anxiety, or reproductive phases can disrupt rumination. For example, cows may stop ruminating when separated from their calves, during estrus, or in the presence of illness or stress. However, rumination typically resumes after such interruptions (Lidfors et al., 1994; Schirmann et al., 2013; Ekesbo and Gunnarsson, 2018).

High-yielding dairy cattle require sufficient crude fiber (≥18% crude cellulose) in their diet to prevent acidosis. Adequate cellulose promotes rumination, increases saliva production, and facilitates the rapid absorption of volatile fatty acids produced in the rumen through the rumen wall. The particle size of the fiber, ranging between 0.6 cm and 4 cm, is also critical for stimulating rumination. Healthy herds are expected to spend 6-9 hours per day ruminating. At any given time, 50% of lying cattle should be ruminating, with this figure rising to 90% within two hours of feeding. During rumination, each bolus (rumen content) is typically chewed 50-70 times. Diets rich in cellulose (e.g., straw) increase the number of chews per bolus. If a bolus is chewed fewer than 50 times, the fiber content of the diet is considered insufficient. Rumination, including chewing, generates approximately 200-300 liters of saliva daily, most of which is produced during rumination. This saliva helps

maintain rumen health and prevents metabolic disorders (Savaş and Yurtman, 2008; Canbolat, 2019).

3.5. Suckling Behavior

A newborn calf typically begins suckling within 2 to 5 hours after birth. Its initial movements involve standing up, orienting itself toward its mother, and exploring its surroundings by rubbing its nose. The calf may attempt to suckle various protrusions on the mother's body until it locates the teat. The mother assists the calf in finding the teat. The calf usually positions itself on the side, near the rear, or standing at an angle to the mother. Rarely, the calf approaches its mother from a perpendicular position. The mother remains standing during nursing, as nursing from a long distance often fails (Cengiz 2013; Demirtaş, 2022).

3.6. Sexual Behavior in Cattle

3.6.1. Sexual Behavior of Male Cattle

In wild cattle herds, cows and calves generally remain together in the same herd for approximately two years. However, male calves begin to assert dominance over their mothers around 18 months of age and soon join groups of other males, separating from the main herd. These males engage in competitive behaviors to climb the social hierarchy, aiming to become dominant bulls and gain mating rights (Phillips, 2008; Canbolat, 2019; Demirtaş, 2022).

As animals reach two years of age, they become increasingly aggressive and attack other animals. While young, calves engage in playful behaviors, but these diminish by maturity. Starting around one year of age, animals learn mounting behaviors. Bulls establish territories in pastures by four years of age and exclude other animals from these areas, leading to frequent fights. Evidence of these fights includes hoof marks and impact depressions from head-butting. After such conflicts, bulls retreat to their respective territories and resume daily activities. Bulls communicate various signals through head and body positions (Ekesbo and Gunnarsson 2018; Cabolat, 2019).

A bull's aggressive posture includes nodding its head while pulling its shoulders together, creating a tense body stance. If the opposing bull or other individual retreats approximately 6 meters, the aggressor returns to a neutral stance. Otherwise, the bull adopts a coiled posture and begins circling,

ultimately head-butting the opponent's body or head (Barroso et al., 2000; Demirtaş, 2022).

Before mating, bulls exhibit behaviors such as scraping the ground with their horns, throwing mud backward, and rubbing their necks. Animals other than pigs display a behavior called "lip curl," where males stand upright, raise their heads, curl their upper lips, and gently shake their heads. This behavior, lasting 10 to 30 seconds, is triggered by the scent of a female's urine or the male sniffing the female's genital area. Copulation in cattle and sheep involves the male mounting the female's pelvis, with variations influenced by species traits, nutritional status, age, and climatic conditions (Houpt et al., 1989; Demirtaş, 2022).

Environmental factors significantly impact sexual behaviors in animals. Sudden changes in habitat, such as relocation or changes in caretakers, can stress males, reducing sexual activity. Nutritional deficiencies, including Vitamin A, protein, or phosphorus deficits, or toxicities like molybdenum poisoning, also suppress sexual behaviors. Illnesses reduce sexual interest, and social upbringing affects the development of sexual behaviors; group-raised animals exhibit more advanced sexual activity compared to those raised in isolation (Canbolat, 2019; Demirtaş, 2022).

Dominance behavior in bulls is also influenced by age. Young bulls (under 2.5 years) show regular grazing cycles and amicable social interactions within their groups. Between 3.5 and 4.5 years, dominance struggles intensify, and by 5.5 to 6.5 years, bulls become largely independent and establish individual territories. Attempting to relocate these bulls can be dangerous. Territorial disputes arise when bulls are grouped together for transportation or management purposes (Hradecky et al., 1983; Tölü and Savaş, 2006).

3.6.2. Sexual Behavior of Female Cattle

Female cattle reach sexual maturity between 8 and 12 months, although some dairy breeds show estrus as early as 6 months. The estrous cycle lasts 18 to 23 days, with the estrus phase lasting 6 to 23 hours, influenced by season, diet, and the presence of other animals. Herd size significantly affects estrus duration; larger herds exhibit shorter estrus cycles (Senger, 2003; Uygur, 2004).

Three types of estrus behaviors are identified:

- **Male-like Mounting Behavior:** Females in estrus mount other females, mimicking male mounting behaviors. This is common among cows.
- **Increased Activity (Hyperactivity):** Estrus females become restless, impatient, and frequently move around.
- **Mating Response:** Females exhibit positive reactions to male approaches, such as standing still, raising their tails, and presenting their genitalia (Lewis, 1978; Anonymous 2024).

Factors influencing estrus behaviors include genetic predispositions, environmental conditions, the presence of males, and interactions with other estrus females (Phillips, 2008; Canbolat, 2019).

Signs of impending estrus include restlessness, frequent urination, tail lifting, and swelling of the vulva. During estrus, cows stand still for mounting, vocalize loudly, and exhibit clear mucus discharge from the vulva (Nebel, 2003; Senger, 2003). Observing estrus in confined spaces is challenging, as most cows show estrus behaviors at night. In open environments, estrus detection improves during activities such as watering or feeding (Uygur, 2004; Reith and Hoy, 2018).

3.6.3. Courtship and Mating Behavior

Both males and females exhibit signals indicating their readiness for mating, regardless of whether other potential mating-ready males are present. Among these signals, olfactory stimulation is the most significant. Additional signals include restlessness and vocalizations. While vocalization is one of the most important cues emitted by estrous cows, it is often overlooked by caretakers (Reinhardt, 1983, Schofield, 1988).

Olfaction is another critical factor in determining sexuality. The cow's scent provides significant information about the animal's gender, the approach of estrus, or the state of being in estrus. For instance, it is common for bulls to sniff the vulva region of a cow, a behavior that helps them detect whether the cow is in estrus (Phillips, 2008).

When an estrous cow is sniffed, it may urinate. The bull may lick the urine to analyze its components and thus determine if the cow is ready for mating. Such courtship behaviors are not limited to bull-to-cow interactions but can also occur between bulls or between cows. After the male and female come

together as described, synchronization stimuli allow these animals to coordinate before mating. During this period, the bull mounts the cow, resting its chest on the cow's rump in a balanced position. Before the actual mating occurs, partial mounting movements may be observed (Hradecky et al., 1983; Danvic et al., 2015).

Finally, actual copulation and mating take place. At this stage, the bull clasps the cow with its forelegs and makes a thrusting movement, leveraging ground force during ejaculation. After mating, a resting period follows, during which the bull regains energy, and interest in a second mating attempt can emerge afterward (Canbolat, 2019, Anonymous, 2024).

3.7. Social Behaviors in Cattle

Cattle are inherently social animals, characterized by complex communication channels and allelomimetic behaviors exhibited in many activities. To fully understand the social behaviors of domestic cattle, it is necessary to comprehend the reasons underlying the cohabitation of non-domesticated cattle. Forming a herd reduces the risk of predation by leaving large sections of grazing areas unoccupied, thereby lowering the likelihood of predators hunting or tracking the animals. Moreover, the random, rapid dispersal of numerous animals can confuse predators, reducing their hunting success. Surveillance activities are more effective in a herd, although in situations of insufficient pasture, animals increase the distance between themselves to utilize the entire area more effectively. Additionally, through social learning, herd members can better acquire survival tactics (Dumont and Boissy, 1999).

Animals living collectively in a herd cannot survive alone in nature. The behavior of each individual within a herd is strongly influenced by the presence of other members. Each group organizes itself easily, but social structures vary depending on the species (Tölü and Savaş, 2006; Phillips, 2008).

The Bovidae family is mostly matriarchal. A matriarchal herd consists of an older female, her adult daughters, and their offspring. Adult males are generally solitary or form bachelor groups and join the females only during the mating season (Hafez and Bouissou, 1975; Uygur, 2004).

3.7.1. Social Organization Among Cattle

Cattle are highly social animals that exhibit complex social structures within their herds. These social organizations are typically hierarchical, with dominant and subordinate relationships that influence access to resources such as food, water, and resting areas. The hierarchy is often established through physical interactions, age, size, or prior social experiences, and it plays a crucial role in maintaining order and reducing conflict within the group.

Within the herd, cattle also demonstrate affiliative behaviors, such as grooming and close physical proximity, which strengthen social bonds and contribute to group cohesion. Understanding these social dynamics is not only important for managing cattle in agricultural systems but also for ensuring their welfare and reducing stress in domesticated environments. Studies indicate that disruptions to these social structures, such as the introduction of new members or isolation, can significantly impact the well-being and productivity of cattle (Bouissou et al., 2001; Reinhardt and Reinhardt, 1981).

Feeding influences social development in cattle. Older cows dominate younger ones, and heavier animals tend to exhibit dominance. However, among cows of similar age raised together, smaller and more aggressive individuals often become dominant. Similarly, older cows in the group are more likely to occupy higher social ranks. Aggression in cows is often displayed through actions like bumping, approaching, sniffing, and physical contact, which become habitual (Hafez and Bouissou, 1975).

Age plays a significant role in the organization of cattle herds. Older cattle, particularly older cows, often occupy higher positions in the social hierarchy. Studies indicate that senior cows tend to guide herd movement, especially when searching for resources such as food and water (Fraser and Broom, 1990; Broom, 2022). Younger animals, on the other hand, tend to form subgroups within the herd, engaging in playful and exploratory behavior that facilitates social bonding and skill development. Limited studies indicate a strong correlation between the social status, rank, or social distance of cattle. Higher-ranked cows are expected to be in proximity to other herd members, as dominant cows tend to exhibit more exclusionary approaches compared to non-dominant ones. In large, mixed-sex cattle herds, the hierarchy is well-defined: adult females, adult males, and juveniles. Adult males dominate adult females, and this dominance extends to juveniles. However, around 1.5 years of age,

young males begin challenging adult females, and by 2.5 years, they dominate all females, achieving the status of adult males. When grouped together in the wild, bulls remain solitary and are not socially organized (Bouissou et al., 2001; Anonymous, 2024).

In dairy herds, cows move in a consistent leadership order when going to pastures, paddocks, or milking parlors. Social hierarchies are established through competitive interactions, often involving displays of strength such as pushing or head-butting. Dominance is typically linked to factors such as physical size, age, and health. Once the hierarchy is established, aggressive interactions decrease, resulting in a more stable social structure. Dominant cows tend to lead the group, while younger heifers usually stay at the back. In most herds, there is a specific order in which cows enter the barn. Middle-aged, moderately dominant cows are the first to be milked, followed by older cows (Phillips and Rind, 2002; Phillips, 2008; Sarova et al., 2010).

3.7.2. Social Relations Between Young Animals

Social communication patterns in young animals are similar to those in older animals. In the first months of life, licking is frequently observed as a friendly interaction. This behavior is considered a necessity, particularly in artificially fed calves, due to the lack of suckling behavior. Homosexual behaviors, such as mounting, are observed in early life. Head-butting is generally regarded as a hostile behavior. Threatening behaviors are rarely seen before six months of age. Playful fights occur during the third month but do not resemble adult fights and are purely for play rather than hostility. These playful fights rarely occur after the fourth month and are referred to in ethology literature as play behaviors (Cengiz, 2013; Endres, 2021).

3.8. Curiosity (Investigation) Behavior in Cattle

The investigation behaviors of cattle are related to their sensory perception abilities. Initial interest in an object typically arises through visual or auditory perception. If the situation does not induce fear, cattle cautiously approach with their heads parallel to the ground, ears erect, and eyes fixed on the object. The exploratory posture resembles a general alert stance, but during exploration, cattle sniff the object with moving nostrils. Upon reaching the object, sniffing transitions to licking. If the object is small or soft, the animal may take it into its mouth, chew it, or even swallow it. During this process, the

senses of smell, taste, and touch play a role. Stimuli that attract interest must have two main characteristics: they should not induce fear and must be unfamiliar, such as a new object in a familiar place or a familiar object in a new location (Phillips, 2008; Cengiz, 2013; Anonymous, 2024).

Exploratory behavior is inversely related to age. This inverse relationship is due to factors such as older animals recognizing many objects in their environment, reducing the stimulus effect of exploration. Additionally, the degree of exploratory behavior tends to decrease in all older animals due to the effects of growth and learning, making exploration mechanisms less pronounced (Cook and Nordlund, 2009; Ekesbo and Gunnasson, 2018).

3.9. Aggressive Behaviors in Cattle

Young cattle rarely fight and typically move together. However, the gathering of sexually mature unfamiliar males almost always results in fights. The intensity of these fights depends on the persistence of the participants. Although very few fights result in death, they usually continue until one party withdraws. In grazing conditions, it is common for multiple bulls to roam with a cow herd. Despite the presence of bulls of varying ages in the herd, fights are rare. Outside of mating seasons, such as autumn, it is uncommon to see bulls grouped away from the cow herd on pastures (Phillips, 2008; Endres, 2021).

The most common conflict-related behavior patterns include passive avoidance behaviors. Withdrawal is often interpreted as a submissive behavior. In this situation, the animal extends its neck, lowers its head, and aligns its forehead parallel to the ground. Self-withdrawal may sometimes trigger aggression from other group members. When animals are within approximately 1.5 meters of each other, one or both may exhibit threatening behaviors (Tölü and Savaş, 2006; Cengiz, 2013).

The body posture of females during threats resembles the posture of males during fights. The head is lowered, and the eyes are directed straight at the opposing animal. Other threatening behaviors include pawing the ground, rubbing the neck against the ground, and digging the ground with horns (Fraser and Broom, 1997; Cengiz, 2013).

Fighting among cattle usually involves head-to-head combat, a common behavior in all ruminants. Animals attack each other with their foreheads or horns. Attacks may occur head-on or from the side. Although young bulls raised

together rarely fight, a group of bulls may single out an individual from the herd and mount it to the point of fatality. Such bulls must be removed from the herd (Barroso et al., 2000).

3.10. Resting and Sleeping Behavior in Cattle

Lying down is essential for recovery, and cattle are highly motivated to exhibit this behavior. When deprived of the ability to lie down, signs of stress and physical fatigue become apparent (Munksgaard and Simonsen, 1996; Munksgaard et al., 1999). Lying down serves multiple purposes, including rest, avoidance of predators, and social bonding. Avoidance of predators is particularly evident in young calves that prefer hiding in tall grass (Langbein and Raasch, 2000). Determining the preferred lying time for cattle is challenging due to the influence of various factors, but it is estimated to be approximately 10 hours per day. Most adult cattle lie in a sternal recumbent position, meaning they rest on their sternum. Occasionally, they lie laterally (lying flat on their sides), but this is often constrained in stalls due to limited space (Balch, 1955; Osterman and Redbo, 2001).

Sleep in cattle is characterized by a temporary period of immobility and an elevated response threshold. It occurs in a distinct daily rhythm and is accompanied by a specific posture that allows relaxation of the neck muscles. Adult cattle are generally considered to sleep very little. The typical sleeping position involves the animal tucking its four legs under its body with the sternum resting on the ground. The head is turned sideways, allowing it to rest on the ground or curled and placed on the thorax (Fraser and Broom, 1997).

Sleep duration in cattle remains largely unchanged until about 24 hours before calving. During the pre-calving period, sleep becomes fragmented. Calving or moving grazing cattle to indoor housing can negatively affect their sleep patterns, requiring 3-6 days to restore normal sleep. In housing conditions, achieving regular sleep behaviors may take up to 3-6 weeks. When cattle are disturbed at night, they compensate by sleeping more during the day (Canbolat, 2019).

Disruptions such as transferring cows to different housing, altering daily routines, or confining them to uncomfortable stalls are factors that adversely affect sleep patterns and, consequently, productivity. Under appropriate

conditions, cows are expected to sleep for approximately 7-9 hours daily (Ellingson, 1972; Phillips, 2008).

3.11. Pre-Calving Behavior in Cattle

Prior to calving, cattle exhibit distinct behavioral changes, including restlessness, increased isolation from the herd, and frequent lying and standing. These behaviors are driven by hormonal changes, particularly the rise in estrogen and the drop in progesterone, which prepare the animal for parturition (Huzzey et al., 2005). Additionally, cows may vocalize more and show a preference for secluded areas as part of their natural instinct to ensure a safe calving environment (Proudfoot et al., 2013).

Cows approaching calving tend to move away from the herd center towards the periphery and become notably docile during this period. Additionally, they avoid conflicts, even with animals of lower social rank. A calving cow usually seeks a quiet and secluded area, often covered, to give birth away from humans and other cows. This natural preference for calving locations has been adapted in modern farming as calving pens. These pens should provide a calm and undisturbed environment for the cow to deliver. During calving, observation should be conducted from a distance, and intervention should only occur if complications arise. Disturbing the cow during calving can delay the process from a few minutes to several hours (Hafez and Bouissou, 1975; Lidfors et al., 1994).

It is crucial not to disturb a cow during calving. However, cows calving indoors are sometimes disturbed by caretakers. For instance, caretakers often feed cows expected to calve between 9:00 and 11:00 in the morning, which can disrupt the cows and delay calving until the following morning or induce nighttime calving. (Sepulveda-Varas et al., 2013).

3.12. Calving Behavior in Cattle

Numerous reliable signs indicate that calving is imminent in cows. These include restlessness, milk letdown, loosening of pelvic ligaments around the tail, vulvar swelling, and mucus discharge from the vagina (Ekesbo and Gunnarson, 2018; Endres 2021).

Observations indicate that labor begins approximately two hours before calving, characterized by contractions that push the calf through the birth canal. These contractions forcefully propel the calf into the birth pathway, after which

the cow often stands up. The calf then falls to the ground or surface, but the cow does not always stand during calving. Afterward, the cow turns to lick the calf. A healthy calf shivers immediately after birth, clears its head of membranes, and begins breathing (Schilling and Hartwig, 1984).

In cases of prolonged or difficult labor (dystocia caused by a narrow birth canal) or when the cow is unwell (e.g., milk fever, ketosis), the mother may be too weak or exhausted to lick the calf. In such situations, assistance is required, and injuries or conditions such as uterine tears or temporary paralysis may occur. These cows may also fail to bond adequately with their calves, sometimes rejecting or even harming them. Examples of difficult births and abnormal presentations are documented (Canbolat 2019).

The bond between a cow and her calf forms within just a few minutes. If a foreign calf is introduced to the cow after this period, the cow will reject and chase it away. This makes efforts to foster a cow with multiple calves challenging. It is often reported that separating the calf from the mother immediately after birth facilitates milk production and eases milking practices (Endres, 2021).

3.13. Postpartum Maternal Behavior Towards The Calf

Cows typically lick their newborn calves immediately after birth. During this licking process, the calf may fall and rise several times. An experienced dam stands motionless while the calf moves from front to back to suckle. Subsequently, the cow licks the calf's tail and hindquarters as the calf searches for the udder, facilitating the calf's ability to locate the teats (Fraser and Broom, 1997; Canbolat, 2019).

Primiparous cows may display anxiety and move away when the calf approaches them. These cows may turn to face the calf, preventing it from reaching the udder by obstructing its path. Some cows may even attack their own calf, a behavior that intensifies if unfamiliar animals, such as humans or dogs, are present. Cows giving birth outdoors may experience social stress within the herd due to other cows approaching and sniffing or interacting with their calves. However, instances of cows stealing and adopting another cow's calf are rare (Hafez and Bouissou, 1975).

As observed, the cow's licking behavior serves to clean the calf, allowing the dam to recognize her own offspring and reject foreign calves. Additionally,

licking reduces the odor of birth fluids, thereby lowering the risk of predator attacks. In most ruminants, consuming the placenta or fetal membranes is a normal behavior. While some farmers discourage this practice due to potential risks of choking or obstruction, others encourage it, citing the placenta's high hormone and protein content. In modern livestock management, the consumption of placental membranes is generally not permitted (Ekesbo and Gunnarsson, 2018).

3.14. Calf Behavior Towards The Mother

Once the newborn calf can stand, it approaches the cow and attempts to locate the teats through trial and error. During this process, the calf explores the cow's front, abdominal, and udder regions, as well as the hind legs. Observational studies indicate that calves typically locate and suckle from the front teats first (97%) (Cengiz, 2008; Canbolat, 2019).

The cow's stationary posture while standing, combined with licking and sniffing the calf's hindquarters, assists the calf in locating the teats. Initially, the calf typically accesses the front teats to receive its first feed. However, in cows with abnormally large or pendulous udders, the teats may take longer to locate. Calves may require over two hours to latch onto the teats of such cows, leading to fatigue and exhaustion. Farmers should intervene in these cases to ensure calves receive colostrum within the first six hours post-birth (Phillips, 2008).

Most calves consume their first meal within six hours of birth. On the first day, calves nurse approximately every five hours. In subsequent days, the interval reduces to every three hours. Calves generally nurse from both sides of the cow but may occasionally suckle from the rear. During the early suckling period, feeding sessions lasting 10–15 minutes are sufficient to satiate the calf. During the first few days, calves spend 18–20 hours of the day resting or sleeping (Lidfors et al., 1994; Fraser and Broom, 1997).

3.15. Daily Maternal-Offspring Relationship in Cattle

In herds grazing on pastures and spending nights outdoors, cows and calves are observed resting in groups at dawn. When cows rise, calves take their first feeding and then separate from their mothers to rest again. The cows begin grazing, and when calves start vocalizing, the cows grazing at the periphery of the group are the first to return to their calves. Cows show interest only in their

own calves rather than the entire group. As the morning progresses, one or two calves may call for their mothers, triggering a social response among the cows. This leads to a collective nursing period where all mothers feed their calves. Such mass nursing events also occur in the late afternoon and evening. After sunset, cows and calves regroup to spend the night together (Canbolat, 2019).

The tendency of calves to rest together in groups can make it challenging for cows to locate their calves, unlike sheep and lambs. However, after 2–3 weeks, calves begin spending more time with their mothers and become increasingly active. Any interventions, such as tagging or dehorning, should be performed within the first 2–3 weeks to avoid difficulties. Calves raised under farmer supervision are allowed to nurse freely for 1–3 days postpartum to receive adequate colostrum and nutrients. In later stages, 2–3 nursing sessions per day are typically sufficient for the calves (Sainsbury and Sainsbury 1979; Lidfors, 1994).

4. CONCLUSION

Understanding animal behavior not only improves animal welfare but also significantly impacts the sustainability of economically important sectors such as cattle farming. Comprehending animal behavior has evolved from being merely an ethical obligation to an economic necessity. Healthy and well-cared-for cattle provide tangible benefits for farms, such as reduced veterinary costs and higher reproductive rates. These factors lead to improved meat and milk productivity, directly enhancing the profitability of operations.

From an economic perspective, raising healthy and well-cared-for cattle is critical for the long-term success of cattle farming enterprises. Healthy and content animals result in higher reproductive rates and better meat and milk yields.

Technological advancements are revolutionizing the study of animal behavior. Sensors, cameras, and artificial intelligence-based systems enable continuous monitoring and analysis of cattle behavior. These systems facilitate the early detection of abnormal behaviors and prompt intervention. For example, if a cow exhibit reduced movement or feeding behavior, it could indicate a potential health issue requiring veterinary attention. Such technological innovations offer significant advantages in maintaining animal health and reducing operational costs.

The study of cattle behavior also plays a crucial role in training and management processes. As farmers and agricultural workers gain deeper insights into animal behavior, they can interact with livestock more effectively and efficiently. Moreover, meeting the increasing consumer demand for well-cared-for animals and ensuring their welfare is essential for marketing and brand value. Thus, understanding animal behavior-especially focusing on birthing and calf behavior-is critical for improving both welfare and economic success in the cattle farming sector.

In conclusion, understanding animal behavior and applying this knowledge in the cattle farming industry can enhance not only animal productivity and welfare but also the economic performance of the sector. Future research should focus more extensively on this field, and widespread adoption of this knowledge in the industry is essential.

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