HEALTH AND LIFE WITH DIFFERENT ASPECTS

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(The Licence Number of Publicator: 2014/31220)
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E mail: iksadyayinevi@gmail.com
www.iksadyayinevi.com

ISBN: 978-625-367-616-2
Cover Design: Dicle ÖZAVCI
December / 2023
Ankara / Türkiye
Size= 16x24 cm

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PREFACE

The continuous updating of information and the ease of communication, transport and access to information over time have caused changes in the approach models of scientific studies on human health in today's world. With the emergence of both national and international COVID-19 pandemic, in order to protect human health; It has also brought the protection of plant, environment, food and animal health to the agenda. The emergence of new conditions has necessitated the creation of common perspectives by bringing together multidisciplinary studies.

The aim of the preparation of this book considering the current changes experienced globally, is to bring together the scientific researches of scientists in different fields and to disseminate multidisciplinary approaches. Each chapter of this book has been accepted and reviewed by blind reviewers; we hope that this book will have a wide readership interested in medical, veterinary, agricultural and health studies and we hope that it will provide readers with a multi-disciplinary health perspective.

Assoc. Prof. Dr. Memiş BOLACALI
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CHAPTER 1

THE PLACE OF ADDITIVE MANUFACTURING IN DENTAL PROSTHESES

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DOI: https://dx.doi.org/10.5281/zenodo.10458452

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1. INTRODUCTION

1.1 Computer aided production methods

The impact of rapidly developing technology in our age is great in the dentistry sector, as in other sectors. With computer systems, the design and production of dental restorations can be carried out in a much shorter time.

The 3D design of the restoration to be produced is made using Computer Aided Design (CAD - Computer Aided Design) and Computer Aided Manufacturing (CAM - Computer Aided Manufacturing) systems, and the design can be produced with computer systems.(Jedynakiewicz& Martin, 2001)

CAD/CAM systems have been used for industrial production since 1950. This technology entered the dental sector in 1970, and the first CAD/CAM systems that could take digital measurements were launched by Francois Duret under the name "SophaBioconcept". (Liu & Essig, 2008)

After the first system, which was costly and very complicated to use, Werner Mörmann and Marco Brandesti, who further developed this system, presented the first scientifically accepted and usable CAD/CAM system to the dental industry under the name "Cerec". (Mörmann, 2006)

Today, CAD/CAM technology is used in the production of many restorations and materials, including fixed prostheses, implant abutments, removable partial denture frames, complete dentures, surgical stents and maxillofacial prostheses. (Ucar et al., 2009)

CAD/CAM systems consist of 3 basic parts.

- Three-dimensional surface scanner: It enables the transfer of image data scanned from inside the mouth or from the model to the computer system.
- The software system in which the design is made**:** It is the software system that enables the design for the restoration to be produced by processing the data received from the scanner.
- Hardware system where the design is produced**:** It is the unit where the restoration is produced in line with the processed data, the design and the selected material. (Beuer et al., 2008; Ucar et al., 2009)

Advantages of using CAD/CAM system;

- No need for traditional impression taking by using an intraoral scanner
- Faster production of restorations
- Less potential for error during production
- Producing restorations more harmoniously
- Ability to control parameters
- Better quality use of production materials
- Saving labor and time by eliminating multi-stage and technical precision-requiring processes such as casting. (Mehl et al., 2013; Miyazaki et al., 2009)

Disadvantages of using CAD/CAM system;

- Owning the system requires serious financial investment
- Production is quite costly compared to traditional methodsinability to fully transfer the information of the teeth to be restored to the computer system because of the scanner's insufficient data acquisition in inappropriate intraoral conditions.
- Requirement of trained experts to use the system. (Çetindağ et al., 2016; Christensen, 2001)

Production in CAD/CAM systems is carried out by two basic methods;

- Subtractive manufacturing method
- Adding additive manufacturing methods

1.2 Subtractive manufacturing method

In this method, restorations; Production is carried out by abrading blocks produced for dental use such as metal, zirconium, polymethylmethacrylate and ceramics with diamond burs or discs on a computer-aided numerical control device. (Beuer et al., 2008; Uzun, 2008)

It is possible to produce restorations with complex structures, which are difficult to obtain with traditional methods, with much more precision and in a shorter time with the milling system. (Van Noort, 2012)

With the scraping process, after the production of the restorations is completed, the remaining unusable block parts are wasted, which creates a great disadvantage by increasing the production cost. (Witkowski, 2005)

1.3 Additive Manufacturing Methods

Industry 4.0, the last stage of the industrial revolution, combines information technologies and production systems. Additive manufacturing is a crucial component of these developments.

Additive manufacturing, also known as 3D printing; It is a method of combining raw materials by creating layer upon layer to produce parts in line with the data of the 3D model. Allows parts with high design complexity to be created precisely and quickly. (Dilberoglu et al., 2017)

Additive manufacturing methods are classified and named according to the ISO/ASTM 52900 standard published by the joint work of the International Organization for Standardization (ISO) and the American Society for Testing and Materials (ASTM).

Additive Manufacturing Methods;

- Powder bed fusion
- Material extrusion
- Vat photopolymerization
- Material jetting
- Sheet lamination
- Direct energy deposition
- Binder jetting(ISO/ASTM 52900:2021, n.d.)

1.3.1 Powder bed fusion

In this additive manufacturing method, powder particles are partially or completely melted and fused in selected areas in the powder bed laid on the table by a thermal power source using a laser or electron beam. The process of laying a powder layer by a roller is repeated in each layer of production.(Lee et al., 2017)

The powder bed melting process includes selective laser melting (SLM), selective laser sintering (SLS) and electron beam melting (EBM) techniques.

The basic bonding mechanisms of these processes are sintering and melting. In the sintering process, while superficial coalescence occurs as a result of the partial melting of powder particles with thermal energy, a complete melting of the powdered material occurs by using higher energy in the melting process. (Lee et al., 2017; C. Y. Yap et al., 2016)

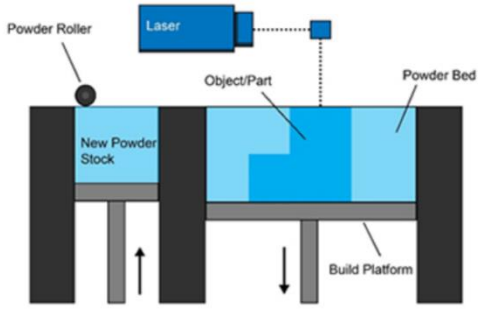

Figure 1: PowderBedFusion (AdditiveManufacturingResearchGroup | LoughboroughUniversity, n.d.)

1.3.2 Selectivelaser melting

This additive manufacturing method, which provides layer-by-layer production by completely melting the powder layers with high laser energy, is quite popular compared to other additive manufacturing methods. The SLM method has many advantages over other production methods. Compared to the traditional casting method, it can produce very detailed complex parts in detail, the produced parts are almost void-free. It uses raw materials efficiently, and the powders left over from melting can be reused. It can produce materials with superior dimensional accuracy by machining a variety of materials and alloys. Thanks to the high mechanical properties and corrosion and temperature resistance of Co-Cr alloys, parts produced by SLM; It is widely used in many fields such as aviation, orthopaedics and dental sector.(Babacan &Seremet, 2022; Wu et al., 2014)

SLM is an additive manufacturing technology that has been widely used in recent years, especially in dental restorations such as personalized crowns, bridges, abutments and screw-retained restorations. SLM can fabricate metal

substructure by melting metal powder into nearly porous layers. This technique uses a focused high-power laser beam and results in products with almost 100% density. Laser may also be the key to hardening metals and ceramics. Additionally, objects with complex geometries can be obtained with high dimensional accuracy. Compared to casting and milling processes, SLM reduces the possibility of operator error, minimizes defects and wastes almost no material as the remaining powder can be used more. (Han et al., 2018)

1.3.3 Material extrusion

In this method, also known as meltdeposition modelling, the filament-shaped material is heated from the nozzle under a constant pressure and remove daccording to a certain pattern. When placed on the substrate, it completely solidifies and the production of objects is carried out layer by layer. (Lee et al., 2017)

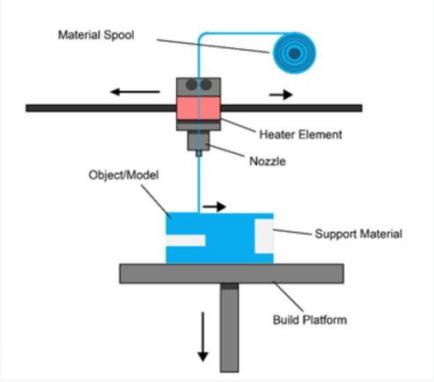

Figure 2: Material Extrusion (Additive Manufacturing Research Group | Loughborough University, n.d.)

1.3.4 Vat photopolymerization

In the pool photo poly merization technique, also known as stereo lithography, a tank is filled with ultraviolet light-sensitive resin. With the light focused on the surface, the areas suitable for the designenter a chemical reaction and solidify in eachlayer, producing the material. (Gibson et al., 2021; Lee et al., 2017)

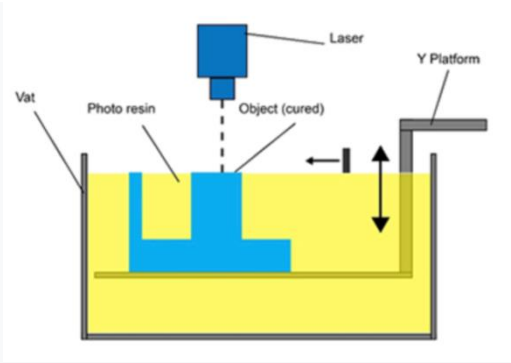

Figure 3: VAT photopolymerization (Additive Manufacturing Research Group | Loughborough University, n.d.)

1.3.5 Material Jetting

In this method, also called material spraying rink spraying, different photopoly merdroplets in liquid form are deposite din to the layers and produce dbyhardening with ultra violet lamps. This 3D printeral lows the production of parts with different materials on thesameobjectbysprayingmaterialswithdifferentmechanicalpropertiesfromdiffe rentends on the samepart. (Y. L. Yap et al., 2017)

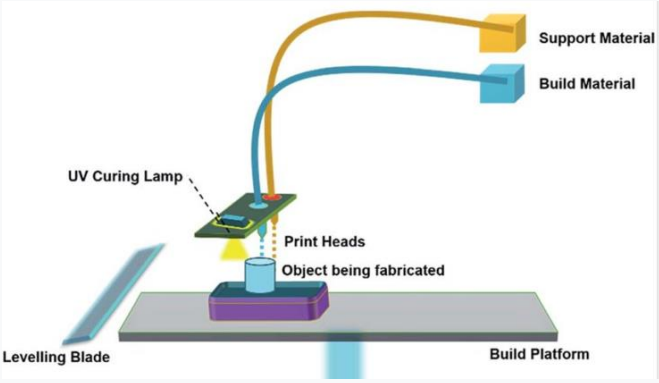

Figure 4: Materialjetting (Additive Manufacturing Research Group | Loughborough University, n.d.)

1.3.6 SheetLamination

It is a production technique in which paper, plasticor metal sheetsare produced in layers, glued, and th edesired shape of the 3D model is cutby a laser source, rather than produced from a material source. Itallows the rapid production of large structures, but the objects produce dare not durable enough

to be a building material, and the cut material pieces cannot be reused and are wasted. (Jadhav & Jadhav, 2022)

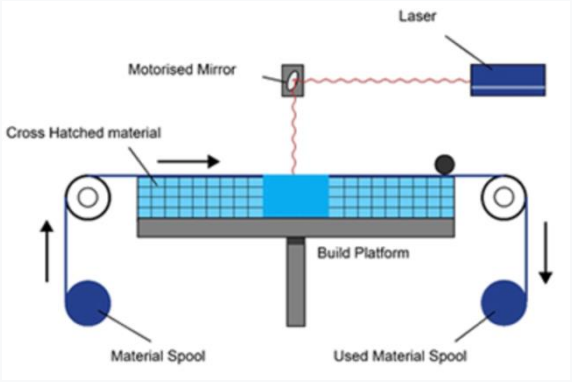

Figure 5: Sheet lamination (Additive Manufacturing Research Group | Loughborough University, n.d.)

1.3.7 Direct energy deposition

This manufacturing technique uses a powerfull aser where energy is focused on a small are atomelt metal powders. Production is carried out by dropping the molten material at theend area of the machine to the surface where it solidifies with multi-axis movement capability. (Lee et al., 2017)

Although it produces less resolution than the powder bedassembly process, it can be used to produce larger structures. Using as much material as needed prevents waste and increases efficiency. It is a fast production technique. (Li et al., 2017)

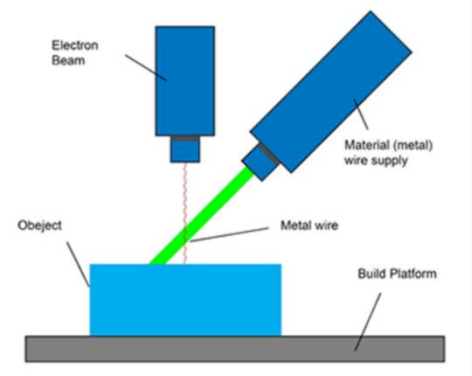

Figure 6: Directed Energy Deposition (Additive Manufacturing Research Group | Loughborough University, n.d.)

1.3.8 Binderjetting

In this method, also called additive manufacturing with adhesives, production is carried out by selectively bonding the material consisting of powder particles, layer by layer, with a liquid-based binder. The function of the liquid drops is to bind the layers in the powderbed. Ineach layer, the powder material is laid on the production table with the help of a roller. Printheaddepositsconnectorsintoselectedareastoproducethedesignbased on CAD data. (Gibson et al., 2021)

The advantages of the binder spraying method include the ability to design without support, produce large materials, fast production and relatively low cost.(Lee et al., 2017)

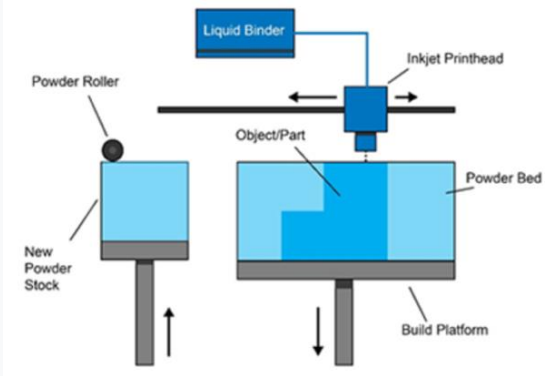

Figure 7: Binder jetting (Additive Manufacturing Research Group | Loughborough University, n.d.)

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CHAPTER 2

INHIBITION OF RUMINAL METHANE EMISSIONS ON LIVESTOCK

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DOI: https://dx.doi.org/10.5281/zenodo.10458482

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INTRODUCTION

Begin by introducing the significance of the issue. Briefly explain the environmental impact of methane emissions from livestock and the role of the rumen in its production. Highlight the importance of finding sustainable solutions to mitigate methane emissions while maintaining animal productivity.

RUMINAL METHANOGENESIS

As a result of anaerobic fermentation, methane is produced in the rumen and intestinal system. Fermentation is particularly crucial in the digestion of lignocellulosic structures. Cellulolytic microbes and protozoa primarily play a role in breaking down lignocellulosic structures, resulting in the production of volatile fatty acids (VFAs), hydrogen (H), and carbon dioxide (CO_2) through chemical digestion. Meanwhile, microbial enzyme activities and enzymes in saliva, collectively, hydrolyze simple sugars, organic matter, and amino acids in the diet, producing volatile fatty acids, hydrogen, and carbon dioxide. The formed carbon dioxide and hydrogen react in two ways.

$$CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O$$

Alternatively, hydrogen can be utilized in the synthesis of VFAs or the synthesis of organic matter by microorganisms, providing significant benefits in ruminant nutrition, particularly in microbial protein synthesis.

The reaction producing 2 H is as follows:

$$\text{Pyruvate} + 2\ H \rightarrow \text{Acetate (C2)} + CO_2 + 2\ H$$

The reaction consuming 2 H is as follows:

$$\text{Pyruvate} + 4\ H \rightarrow \text{Propionate (C3)} + H_2O$$

or

$$2\ \text{Acetate} + 4\ H \rightarrow \text{Butyrate (C4)} + 2\ H_2O$$

When ruminal fermentation models shift from converting acetate to propionate, hydrogen production, and consequently methane production, will decrease (O'Mara, 2004).

Methane in the rumen is generated by methanogenic bacteria and protozoa. The role of protozoa in methane production is complex. Protozoa, by harharbouring than ogenic bacteria, contribute to 25% to 37% of the produced methane gas. Defaunation results in the reformation of rumen bacterial populations, altering VFA production towards propionate from acetate, and reducing methane emissions (Finlay et al., 1994). However, this practice adversely affects cellulose digestion, disrupting rumen metabolism unnecessarily (Demeyer et al., 1982). In an in vitro study conducted by Ranilla et al. (2007), no statistically significant difference was found in methane production between mixed fauna and defaunated environments. They suggested that this lack of difference might be attributed to ration variations.

METHANOGENIC ARCHAEA OF RUMEN

In the studies conducted so far, Methanobrevibacter ruminantium, Methanomicrobium mobile, and Methanosarcina species have been isolated from the rumen of sheep and cattle, and they are considered the most important methanogens in the rumen microbial ecosystem. There are some limitations in performing culture-based analyses of methanogenic microorganisms. One of the most significant constraints is their requirement for an anaerobic environment. Culturing certain methanogens in the laboratory is quite challenging, and conventional methods involve substantial effort and time (Bryant 1959).

There exists a symbiotic relationship between methanogenic bacteria and ciliated protozoa. The hydrogen and CO2 produced by protozoa are vital for methanogenic bacteria as these bacteria require a strictly anaerobic environment to survive (Ohene-Adjei et al., 2007).

Methanobrevibacter ruminantium:

Methanobrevibacter ruminantium is one of the most abundant methanogens and can only thrive in the rumen. Since they cannot meet their nutritional needs from the environment, their development is hindered when released into the surroundings. Therefore, they engage in a symbiotic relationship with protozoa (Joblin et al., 1990).

Methanosarcina barkeri:

Methanosarcina barkeri is an oxygen-sensitive anaerobic methanogen. Unlike other methanogens that only use carbon dioxide, M. barkeri can ferment various carbon sources such as H2, ethanol, methanol, and acetic acid. Due to its ability to utilize a wide range of energy sources, it can adapt better to the environment compared to other methanogens. M. barkeri has been found to contain an amino acid called pyrrolysine, identified as the 22nd amino acid. The presence of pyrrolysine, especially in methanogens, attracts significant interest as it plays a crucial role in methanogenesis.

Methane provides approximately 9 kcal/m3 of energy (35 BTU/L = 0.252 kcal). Assuming that 500 L of methane is produced daily in a dairy cow, 20% of this methane is produced by M. barkeri, resulting in the generation of 900 kcal of energy. This amount of energy is sufficient to melt about 11 kg of ice or run a 750-watt motor for 20 minutes. Considering these factors, M. barkeri appears to be a significant alternative energy source (Öztürk, 2008).

Methanomicrobium mobile:

Identified as the most important methanogen in the rumen ecosystem in 1968, M. mobile is challenging to culture due to its requirement for a heat-resistant growth factor known as the mobile factor, and its slower growth compared to other methanogens. M. mobile cultures begin to degrade and lose viability 2-4 days after incubation (Taner and Wolfe, 1988). In a study, it was determined that total methanogens in the rumen constitute about 3.6% of total rumen microorganisms, and approximately 54% of this is M. mobile (Yanagita et al., 2000).

Here, factors affecting methane emissions will be presented in two main categories: factors related to nutrition (roughage, feed quantity, type of carbohydrates, the concentration of concentrate feed in the ration, and the fat content of the ration), and other factors will be examined.

The physical form of roughage significantly influences methane production. Grinding and pelleting processes reduce methane production. Although the exact mechanism is not well understood, this effect may not be apparent when these feeds are provided in limited quantities. However, with high intake levels, methane production can be reduced by half. Methane

production is also closely related to ruminal particle size and rumen fluid density.

Low-quality roughage increases methane production, as seen in Table 1 (Okine et al., 1989; Boadi et al., 2004).

High-quality roughage, with high digestibility, increases daily methane production because it enhances feed intake. Alternatively, as the digestibility of roughage increases, the rate of energy loss through methane emission decreases (Johnson and Johnson, 1995). Using highly digestible roughage can lead to increased productivity because feed consumption, and consequently, the intake of energy and nutrients, will rise. For livestock grazed on pasture, increasing the digestibility of pastures is crucial to reduce methane emissions (Hegarty, 1999). However, if there is no reduction in the number of animals despite increased productivity, methane emissions from livestock farming may increase rather than decrease (Hegarty, 2002).

Feed Quantity

The impact of an increase in daily dry matter intake on methane emissions occurs in two ways. Firstly, as the energy intake increases, the ration's energy content rises, resulting in a decrease in methane emissions. Secondly, due to improved efficiency, the ratio of energy obtained to energy lost through methane decreases, leading to a relative reduction in methane emissions.

As the daily ration intake consistently increases, the rate of energy loss as methane decreases by an average of 1.6%. As the digestibility of the ration increases, methane production decreases. Despite an increase in the volume of methane produced with increasing feed consumption, the ratio of energy lost as methane to the energy taken in through the feed decreases (Johnson et al., 1993; Johnson and Johnson, 1995). However, laboratory studies attempting to statistically explain this relationship between feed intake and methane production have not reached a consensus. Methane production tends to increase when the ration consists mainly of suitable carbohydrates; however, when feeding with a high amount of highly digestible ration, methane production decreases (Johnson et al., 1993).

Carbohydrate Type

The impact of carbohydrate fermentation on methane production likely occurs due to its effects on ruminal pH and microbial populations. The fermentation of cell wall fibers results in a high acetic acid/propionic acid ratio and increased methane production. Soluble carbohydrates generate less methane than carbohydrates found in the cell wall (Moe and Tyrrell, 1979).

As the proportion of easily soluble carbohydrates increases, the digestibility of energy in the ration also increases. Compared to animals fed at maintenance energy levels, animals fed at twice the maintenance energy level exhibit lower methane emission rates, and those fed at three times the maintenance energy level show even lower rates (Boadi et al., 2004).

Concentrate Feed Ratio

The concentrate feed ratio in the ration is in a negative correlation with methane emissions (Holter and Young, 1992;). As the proportion of high-starch content feeds in the ration increases, the ratio of energy lost as methane decreases. However, feeding in this manner has various negative impacts both on animal health and in terms of cost. From a health perspective, issues such as acidosis may arise, and the production cost per unit yield increases due to the high cost of producing concentrated feed. Additionally, considering that grains in concentrated feed are organic materials that can also be used for human consumption, ruminants competing with humans for food resources could be a concern (Carroet and Ranilla, 2004).

Ration Fat Content

The addition of fat to ruminant rations affects methane production through various mechanisms. During the processing of unsaturated fatty acids in the rumen, the production of propionic acid changes, and the development of protozoa is hindered. The addition of long-chain polyunsaturated fatty acids to the ration provides alternative pathways for carbon dioxide and hydrogen reduction, thereby reducing methane production. A portion of the total metabolic hydrogen produced in the rumen is used for the biohydrogenation of unsaturated fatty acids (1%), a significant portion for carbon dioxide reduction and methane formation (48%), for VFA synthesis (33%), and for microbial cell synthesis (12%) (Jordan et al., 2004).

It is emphasized that the most significant factor affecting methane emissions is the ration (Johnson and Johnson, 1995). Some researchers have indicated that differences among breeds and even individuals could be genetically based. Under the heading of other factors influencing methane emissions, genetics, age, and productivity are considered.

Methane Inhibition

Due to the rapidly increasing methane emissions worldwide, it has contributed to a 1.7 °F increase in global temperatures (EPA 2008). When examining the methane emission inventory of Turkey's Statistical Institute, methane emissions from solid waste can be considered a primary contributor to the globally increasing methane emissions over the past century. However, considering the environmental and economic damages caused by methane emissions, it is widely accepted that emissions from all sources need to be mitigated (Johnson and Johnson 1995, Hegarty 1999, Öztürk 2008a). Inhibiting methane from ruminants is primarily deemed necessary from an economic standpoint and, secondarily, holds ecological significance.

There are various methods for methane inhibition in ruminants. In this seminar, older methods will be discussed first. Almost every method has its disadvantages, and older methods have lost their validity. Following the discussion of older methods, briefly, newer methods with fewer disadvantages, which are planned to be implemented today and in the future, will be explained.

Defaunation

The methane inhibition effect of defaunation stems from the relationship between methanogens and protozoa . After defaunation, the protozoal population is reduced to zero, leading to a decrease in the number of methanogens and subsequently a reduction in methane emissions. However, the most significant side effect is the negative impact on cellulose digestion.

Hegarty (1999) achieved defaunation by intervening in the ration in his study. In the research, chemical substances such as copper sulfate, calcium peroxide, dioctyl sodium sulfosuccinate, and detergents, as well as natural compounds like vitamin A, non-protein amino acids, and steroid hormones, were used for defaunation purposes. A 61% reduction in methane emissions was observed as a result of defaunation.

The effects of defaunation have decreased cellulose digestion, reduced the number of methanogens associated with the decrease in protozoa, and increased hydrogen transfer and the oxygen level in the rumen (Hegarty 1999, 2007).

Fats

Fats exhibit a direct inhibitory effect on methane emissions by showing a toxic effect on the metabolism of methanogens. Additionally, the inclusion of fats in the ration increases the overall energy content, thereby reducing methane emissions. Another effect occurs indirectly by suppressing protozoa development. However, adding fats to the ration comes with several disadvantages. It decreases the milk fat ratio, increases costs, and most importantly, adversely affects cellulose digestion.

Usage of Organic Acids

The use of dicarboxylic organic acids (malate and fumarate) serves as precursors to propionate, facilitating the process of synthesizing succinate using hydrogen from fumarate through propionate. As the hydrogen in the environment decreases, methane emissions also decrease (Mohammed et al., 2004).

However, there are negative effects associated with the use of dicarboxylic organic acids. Firstly, they are expensive chemical substances. Secondly, they are not suitable for animals fed on pastures because they need to be administered daily. Thirdly, they show their effects mainly in situations where feeding is concentrated rather than relying on roughage-based feeding models; in vitro studies conducted with roughage-based feeding models indicate an effect of only 4.8%, which is considered insufficient (Cano and Ranilla, 2004).

Prebiotics

Prebiotics are undigested substances that increase the amount of beneficial microorganisms in the digestive system. Galacto-oligosaccharides (GOS), which are undigestible in non-ruminants, have shown positive effects as prebiotics in ruminants. Mwenya et al. (2004) reported a 10% reduction in methane emissions in sheep fed on roughage-based diets with the addition of 30% concentrate.

The effects of prebiotics are not yet fully understood, and further extensive research is needed to determine whether they are effective in methane inhibition.

Probiotics

Probiotics are microbial-origin feed additives. Their effects aim to increase efficiency by influencing rumen fermentation. The most commonly used probiotics are yeast and Aspergillus oryzae. Some products are sold as containing live yeast cells, while others, sold as live yeast cultures, also contain nutrients that mothers can use for reproduction (Lila et al., 2004).

Although not yet fully understood, probiotics demonstrate their effects in several ways. Firstly, they increase the synthesis of butyrate or propionate. Secondly, they reduce protozoa numbers. Thirdly, they stimulate acetogenesis. Fourthly, they increase animal productivity (Bruno et al., 2005).

Immunization

Immunization is among the biotechnological methods planned for the future and is done in two ways. In the first type, animals are immunized against protozoa (Shu et al., 1999). In the second type, animals are immunized against methanogens (Wright et al., 2004).

It is too early to make a definitive comment on immunization. Researchers suggest that this method could achieve a 70% methane inhibition, but this ratio still seems distant.

Animal Selection

As mentioned in genetic factors, methane emissions vary widely between breeds or individuals. To be able to select, it is necessary to fully identify the source of this variation (Boadi and Wittenberg, 2002; Robertson and Waghorn, 2002). This is a developing subject, and more research is needed.

Hexose Breakdown

Methanogens play significant roles in hydrogen reduction. Beever (1993) reported that hydrogen production and methaneogenesis are influenced by the number of bacteria living in the rumen and the amount of carbohydrates present in the rumen content. Hexose breakdown will adversely affect methane genesis, increasing the availability of carbohydrates for microbial synthesis rather than

fermentation. If the amount of soluble carbohydrates in the diet is increased or the ratio between soluble carbohydrates and cell wall components is reduced, as indicated by in vitro studies, the amount of carbohydrates used for fermentation will decrease, and the amount used for bacterial synthesis will increase, achieving methane inhibition (Moss et al., 2001). More research is needed to make progress on this topic.

Plants and Plant Extracts

Limited studies on this topic suggest that plants and their extracts generally increase feed intake, thereby influencing methane emissions positively. Some plant extracts reduce protozoa numbers, contributing to methane inhibition.

In a study, Bodas et al. (2007) examined 450 different plant samples for methane inhibition properties and reported that Rheum nobile had the highest methane inhibition property among these plant samples that could be used as feed additives in ruminants. Hegarty et al. (2004) reported that tannins from Calliandra reduced methane emissions in vitro. Table 5 shows the effects of some plants on methane inhibition and rumen parameters (Bodas et al., 2007).

Conclusion

In conclusion, it is urgently necessary to create a methane emission inventory in our country, expressing real values and percentages based on animal species and sectors. In countries where pasture-based feeding is common, methane emissions from ruminants can be a serious issue. Unfortunately, since the amounts of methane emissions from our animals are not yet known, it is not possible to assess how ecologically significant these emissions are.

From the perspective of animal feeding and economically, it is indisputable that methane emissions result in economic losses. Therefore, efforts to reduce methane emissions should be initiated immediately and these efforts should be widely implemented.

However, before intervening in nature, an important question that needs to be considered and answered is: Should rumen fermentation and methane emissions inherent in ruminants' nature be completely prevented, or should they be reduced to a certain level? In conducting studies on this matter, it is crucial

to adopt a balanced approach, considering both environmental effects and impacts on livestock productivity.

This book chapter has been summarized from a doctoral seminar with the same title.

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CHAPTER 3

SALMONELLA VACCINES IN POULTRY

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DOI: https://dx.doi.org/10.5281/zenodo.10458571

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Introduction

Salmonella is an important zoonotic pathogen that causes systemic and gastrointestinal diseases in humans and animals. Fowl typhoid and Pullorum disease cause significant economic losses in many parts of the world where intensive breeding occurs. In addition, paratyphoid *Salmonella* serotypes cause gastrointestinal infections in poultry and humans. Poultry products contaminated with these pathogens are among the causes of human *Salmonella* infections. Reasons such as failure to improve hygiene conditions or their cost, deficiencies in management, and increasing antibiotic resistance once again reveal the importance of vaccination in the poultry industry. Vaccination is one of the important strategies used today to reduce *Salmonella* in poultry. Reducing the contamination of poultry products in this way will also reduce Salmonellosis in humans. Although many studies have been carried out on the subject, there is still a need for better defined and more reliable *Salmonella* vaccines.

Salmonella Infections and Immune Response in Poultry

It is known that there are two species of the *Salmonella* genus (*Salmonella* bongori and *Salmonella* enterica) and over 2600 different serotypes of *Salmonella* enterica. Serotypes belonging to S. enterica subsp. enterica constitutes approximately 99% of *Salmonella* infections that cause foodborne infections (systemic infections and enteritis) in humans and warm-blooded animals. *Salmonella* serotypes cause two types of infection in poultry: systemic and gastrointestinal infection. Host-specific serotypes such as S. gallinarum and S. pullorum cause systemic infections with high mortality in poultry. Other paratyphoid serotypes, especially S. Typhimurium and S. enteritidis, have a broad host spectrum and often cause enteritis.

Fowl typhoid affects many poultry species, especially chicken, turkey, and quail. The source of contamination is usually carrier or infected poultry. There is vertical transmission through infected eggs of these animals and horizontal transmission through feces, contaminated feed, drinkers, bedding, and equipment. Both diseases have the ability to spread rapidly and have a high mortality rate.

Although many serotypes can cause paratyphoid Salmonellosis, the most common serotypes are S. Enteritidis, S. Typhimurium, S. Virchow, S. Hadar,

S. Infantis, S. Heidelberg, S. Newport, S. Agona, S. Stanley, S. Derby, S. Thompson. The distribution of serotypes varies from country to country, and different serotypes can be isolated. Non-host-specific paratyphoid serotypes are shed in feces without showing any disease symptoms. Feed is one of the most important sources of contamination. Apart from this, rodents, reptiles, personnel, and poultry equipment also play an active role in contamination. These serotypes, which colonize the poultry intestines and are shed in feces, enter the human food chain through carcass contamination (also through eggs consumed raw) and pose a serious danger to public health.

Since *Salmonella* is a facultative intracellular bacterium, both cellular and humoral immune responses play a role in protecting against the infection. The immune response against the pathogen is generally determined by the innate immune response caused by the interaction between intestinal epithelial cells, M cells, and bacterial components. This interaction causes the production of a potent proinflammatory chemokine (especially IL-1, IL-6, and IL-8) against S. Typhimurium in mice or chicks. In addition, it has been determined that there is serum and mucosal IgA production in chicks exposed to live or inactive *Salmonella*, which forms an antibody response against bacterial LPS and protein determinants. Since fowl typhoid and Pullorum disease have been eradicated in commercial poultry farms in developed countries, little is known about the immune response to infection.

Salmonella Vaccines Used in Poultry and Vaccination Studies

The control strategy of *Salmonella* infections in poultry is primarily carried out on the prevention of infection. Accordingly, the only option other than vaccination for broiler chickens, whose lifespan is limited to five-six weeks, is to prevent disease from entering the chicken coop. In some cases, after vaccination of breeders, maternal antibodies and competitive exclusion products are used to control *Salmonella* in broiler flocks. Both live and killed vaccines provide significant protection against *Salmonella* infections. However, none of the vaccine types can provide complete protection, especially when exposed to high doses of *Salmonella* challenge. Stress sources such as feed, water restriction or heat also reduce the vaccine's effectiveness. Another limiting effect of vaccines is misleading positivity in *Salmonella* infections monitored by serological methods. It is important to diagnose *Salmonella*

infections in flocks where vaccines are used by bacteriological examinations to eliminate the problem.

Live-Attenuated *Salmonella* Vaccines

Live-attenuated *Salmonella* vaccine strains are obtained by classical methods or by creating deletions or mutations in genes required for virulence or metabolism. In order for vaccine strains to stimulate the immune response for a long time, they must remain in the tissues long enough, but be avirulent and be cleared entirely from the host after a certain period of time. Using these strains as vaccines enables stimulation of both cellular and humoral immune responses and oral vaccination of poultry of all ages.

The most important problem of these types of vaccines is their potential to survive in the environment for longer than expected, which may threaten poultry and human health. On the other hand, the permanent nature of live-attenuated vaccines also enables the vaccination of unvaccinated poultry through horizontal transfer in cases where individual vaccinations are not performed. Another disadvantage of live vaccines is that vaccine strains can become virulent and interfere with *Salmonella* testing procedures or require additional testing to distinguish vaccine strains.

So far, there are many studies that have yielded data that will cover different gene regions in different *Salmonella* serotypes and may ultimately have the potential to become a vaccine candidate. Vaccine studies continue to progress with developing technology. Cooper et al. (1994) orally immunized one-day-old chicks with the S. Enteritidis aroA mutant, which was constituted by disrupting the synthesis of an important component of the aromatic biosynthetic pathway, and then exposed them to the virulent strain. At the end of the experiment, it was observed that strain retention in the cecum and internal organs of vaccinated chicks decreased. Hassan and Curtis (1994) constituted the S. Typhimurium cya/crp double mutant by deleting genes encoding adenylate cyclase and cyclic adenosine monophosphate receptor proteins (cAMP). Cya and crp genes regulate the expression of genes involved in expressing cell surface outer membrane protein (OMP), flagella and fimbria, and the transport and utilization of carbohydrates and amino acids. After the chicks were orally inoculated with this mutant strain, it was determined that the virulent strain to which they were exposed was significantly reduced in the

cecum. However, it was not determined how much the virulent strain decreased in the spleen, and it was determined that immunization with this strain did not protect S. Enteritidis. In another study, a heat-sensitive S. Enteritidis vaccine, which was constitued by the genetic attenuation method and could grow at 26-28°C but could not grow at 37°C, was used. In this respect, it has been stated that it induces an immune response by acting similarly to the first step of natural infection. Cerquetti and Gherardi (2000) reported that mice vaccinated with this vaccine strain received a very good immune response against the virulent strain. One-day-old chicks were also immunized intraperitoneally and orally with the same strain, and it was revealed that the virulent strain exposed in both internal organs and cecum was significantly reduced compared to the control group. In another study, mutant strains were constituted by deleting the phoP (part of the phoP/phoQ regulatory system involved in virulence) and fliC genes (involved in *Salmonella* adhesion to the cell) of S. Enteritidis, and chicks were vaccinated with these strains on the first and 21st days. At the end of the study, it was determined that the level of exposed virulent strain was lower in the liver and cecum compared to the control group. In addition, the vaccine strain was compared with the unattenuated strain, and it was determined that the absence of fliC in the vaccine strain was an important advantage for distinguishing vaccinated animals from infected animals. Methner et al. (2011) stated that this mutant strain could be a new vaccine candidate for protection against poultry Salmonellosis. Nandre et al. (2012) constituted the S. Enteritidis Ion/cpxR mutant strain by deleting two virulence genes using the allelic exchange method. By deleting the ion gene, *Salmonella* replication, and systemic spread in the host are harmed, and by deleting the cpxR gene, adhesion and invasion to the host cell are prevented. One-day-old chicks were orally inoculated with this mutant strain and exposed to the virulent strain at four weeks of age. It was determined that the isolation of S. Enteritidis was reduced in the cecum spleen and liver in the vaccinated group compared to the control group. In another study, researchers orally inoculated chicks with a mutant strain constitued by deleting the entire S. Enteritidis pathogenicity island-1 and 2 (or key genes) and exposed them to the virulent S. Enteritidis strain. They found that both pathogenicity islands provide protection against S. Enteritidis, but SPA-1 is more effective in protection, while SPA-2 specifically stimulates the antibody response.

Findings of cross-protection with live vaccine strains against other epidemiologically important *Salmonella* serotypes are variable. It has been demonstrated that an avirulent S. Typhimurium vaccine reduces colonization, organ invasion and egg contamination of S. Enteritidis, whereas the S. Enteritidis aroA mutant does not provide effective cross-protection against S. Typhimurium. On the other hand, it has been demonstrated that even antigenically unrelated *Salmonella* strains provide varying degrees of protection against challenge with S. Enteritidis.

It is thought that this situation may result from a combination of competitive exclusion mechanisms and immunological.

S. Gallinarum 9R vaccine is widely used in Europe to S. Gallinarum and S. Enteritidis. S. Gallinarum 9R strain was produced by passages in a low nutritional quality medium and is known to provide protection against systemic infection in adult chickens. The 9R strain is rough and does not directly stimulate the antibody response against LPS. This is very valuable in that it does not confuse serological tests based on the presence of serotype-specific IgG used in the detection of Salmonellosis. When the fecal swab samples of chicks vaccinated subcutaneously with the S. Gallinarum 9R strain in the sixth and 16th weeks were evaluated, the S. Enteritidis positivity rate of the control group was found to be 11.5%, and that of the vaccinated group was 2.5%. Researchers have reported that this vaccine is effective against S. Enteritidis under field conditions. Penha Filho et al. (2010) found that the shedding of S. Enteritidis and S. Gallinarum decreased in immunization with the S. Gallinarum cobS/cbiA mutant strain, which they obtained by deleting the (cobS/cbiA) genes involved in the cyanocobalamin-B12 biosynthetic pathway. Researchers reported that this strain may be a new live vaccine candidate for the poultry industry.

Although live-attenuated vaccines can significantly stimulate the immune response, concerns remain regarding the safety of live vaccine strains, as some vaccine strains may not be genetically stable and may persist longer than expected in vaccinated chickens.

Inactivated (Killed) *Salmonella* Vaccines

Inactivated vaccines containing the entire bacteria are inactivated using methods such as acetone, formalin and heat. While these vaccines constitute a

strong antibody response, they fail to elicit a cell-mediated immune response. Most inactivated *Salmonella* vaccines are administered intramuscularly or subcutaneously in at least two doses. They partially protect against intestinal colonization, fecal shedding, systemic spread, and egg contamination. Inactivated vaccines constitute a robust immune response by using strong adjuvants. One of the most significant advantages of these vaccines is that they do not pose a danger to human health since they do not remain alive in nature. However, they can be eliminated by the host quickly, and unlike attenuated vaccines, they express a small number of antigens.

S. Enteritidis PT4 vaccine strain produced under iron-limited conditions is a licensed and inactivated vaccine used in the UK, Europe, and other countries. Chickens vaccinated subcutaneously two or three times with this vaccine strain were then exposed intravenously to the virulent S. Enteritidis strain. It was determined that there was a significant decrease in the number of chickens positive for S. Enteritidis in the vaccinated group. Another study examined the protection effectiveness of S. Enteritidis PT4 and S. Typhimurium DT104 vaccine strains, produced under iron-limited conditions, against S. typhimurium. It was determined that the shedding of the bacteria decreased significantly in the vaccinated groups, but there was no decrease in internal organ colonization. The fact that this vaccine has been found to provide protection against S. enteritidis has been stated as valuable for poultry *Salmonella* control. Layers and breeder broilers were vaccinated subcutaneously at five and nine weeks of age with a vaccine containing S. Enteritidis PT 13, 13A, and 28 strains. They were exposed orally to the virulent S. Enteritidis strain at ten weeks of age. It was determined that there was a decrease in the virulent strains in the liver, spleen, and cecum of vaccinated layers, but there was no decrease in broilers.

Compared to inactivated vaccines and live vaccines, vaccination with live *Salmonella* vaccines provides a more robust and longer-lasting immune response. This may be due to the side effects of the preparation process of inactivated vaccines on the protective antigen or to the more permanent presentation of the relevant antigens to the host immune system with live vaccines.

Subunit Vaccines

These types of vaccines can be obtained from synthetic peptides, purified or unprocessed pathogen extracts, or pure DNA or RNA using recombinant DNA technology. These vaccines, which consist of either a single antigen or multiple defined antigens (mostly proteins), are administered by subcutaneous or intramuscular routes unless they are prepared for oral use to stimulate mucosal immunity and minimize the degradation of protein antigens. Like inactivated vaccines, subunit vaccines do not cause regulatory and biological complications that can occur with living organisms. The power of these vaccines to stimulate the immune response is strengthened when given with adjuvants. New generation adjuvants are designed to have minimal side effects, increase the continuity of the immune response, and simultaneously stimulate the cellular, humoral and mucosal immune response.

The ability of S. Enteritidis to cause infection depends on its ability to adhesion, colonize and invade intestinal epithelial cells. Adhesion, which is the first step of pathogenesis, is carried out by proteins known as adhesins. Blocking adhesion is the most effective way to prevent bacterial infection. It has been demonstrated that when S. Enteritidis outer membrane proteins (Omp) are used as a vaccine with adjuvant, fecal shedding is prevented and a high antibody response is formed in chickens exposed to the virulent S. Enteritidis strain. In another study, it was determined that chicks immunized with type 1 fimbrial antigen caused a decrease in the S. enteritidis load in the reproductive organs and eggshell, but did not cause a significant change in the bacterial load in the liver, spleen and cecum. Hanatani et al. (2009), vaccinated chicks twice with a peptide containing *Salmonella* fliC protein (an important surface protein that shapes the flagellar filament) and exposed them orally to the S. Enteritidis strain in their seventh week. At the end of the study, they determined that a specific antibody response occurred with vaccination and that there was a significant decrease in the bacterial level in the caecal content, but there was no change in the bacterial load in the internal organs. T3SS (Type III Secretion System) expressed by *Salmonella* Pathogenicity Island-1 and 2 is an important virulence factor involved in host cell invasion and systemic spread. In studies conducted on T3SS proteins as vaccines, chicks were immunized with these proteins and then exposed to the S. Enteritidis strain. It was determined that vaccination with T3SS-1 produced a specific IgG response, but did not cause

any decrease in the level of virulent strains in the cecum and internal organs. It has been determined that in vaccination with T3SS-2, a high IgG response is formed and this antibody response is transferred to the offspring. Desin et al. (2011) and Wisner et al. (2011) stated that T3SS proteins may provide better protection when combined with other candidate proteins.

Vaccines protect against *Salmonella* serotypes, which cause economic losses along with gastrointestinal and systemic infections in poultry and cause foodborne infections in humans through the consumption of poultry products. However, the effectiveness of vaccines used for poultry is limited depending on the serotype. Developing technology has the potential to develop vaccines that will provide more effective protection against *Salmonella* infections instead of the classical vaccines that are commonly used.

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CHAPTER 4

ENSILING OF ALFALFA (*Medicago sativa L.*)

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DOI: https://dx.doi.org/10.5281/zenodo.10458606

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Introduction

Feeds containing more than 18% crude cellulose in dry matter are called roughage. Roughages are the feeds that form the basis of ruminant rations. Roughages prevent metabolic diseases and digestive disorders in ruminants and help create the desired healthy structure in the rumen microflora. Roughage; It is important in supporting rumen development, stimulating saliva secretion, ensuring rumen contractions and the passage of digestive tract contents through the rumen. At the same time, roughage helps increase milk fat. Although they contain more than 30% fiber content, their protein and energy contents are low. Additionally, nutrient contents can vary greatly (Ergün et al., 2013).

Producers' needs for quality and economical feed lead to the search for various feed storage and evaluation methods. For this reason, many different methods have been developed for storing feedstuffs since ancient times. Drying, the oldest known feed storage method, cannot be used everywhere due to regional and climatic conditions (Ergün et al., 2013). Additionally, nutrient losses occur during drying. Especially during harvest, leaf fall, respiration and washing losses, and microbial activities significantly reduce the nutrient level of roughage (Budak and Budak, 2014).

Despite all these disadvantages, although the drying method is widely used today, alternative methods are also frequently preferred. One of these alternatives is silage. Disadvantages occurring during drying can be eliminated by making silage.

Silage

Silage is a feed obtained by keeping green fodder containing 30-40% DM in an anaerobic environment after harvesting. The process performed is called siloing, and the place used is called silo. The purpose of making silage is to ensure that green fodder is preserved with minimal loss of nutrients by harvesting it in the summer months when it is abundant, and thus to be able to feed it with a product with high nutritional value in the winter months when green fodder is not available fresh (Şahin and Zaman, 2010). Physical losses due to leaf disintegration and defoliation in dry grasses can be reduced by making silage, because moist leaves are less fragile than dry grasses and reduce losses. The risk of rain, which is frequently encountered during hay production

(Collins, 1985), has eliminated the time required for withering in the field thanks to silage production.

Alfalfa (*Medicago sativa L.)* Silage

Clover, called the "queen of forage", is one of the most widely grown legume forage plants in the world. Alfalfa is offered for consumption by animals, primarily ruminants, but also non-ruminant animals (mainly horses). Alfalfa has high nutritional quality as animal feed (Karabulut and Filya., 2007). Clover, which has many species in our country, is a delicious perennial forage plant that is rich in protein, vitamins and minerals (β carotene, vitamin B), and has an economic life of 7 years. The first form of alfalfa is recommended as silage and it is recommended to mow when the flowering is 1/10. 1800-2000 kg of dry alfalfa can be obtained from one decare and can be harvested every 6-8 weeks. It is a plant that can adapt to all climate and soil conditions, withstands hot climate conditions, but is seriously affected by the cold in winter and the first days of spring. It is reported that it yields 250-300 kg of grass per decare in dry conditions. Thanks to the Rhizobiums in their roots, they increase the nitrogen rate of the soil due to their ability to bind the free nitrogen of the air to the soil. Clover; It is given to animals in different ways: fresh, dry and silage. Although it is widely used as dry grass, the leaves, which are rich in nutrients, may fall off. The most commonly grown alfalfa species are; purple-flowered clover, yellow-flowered clover, hybrid clover and hop clover (Ergün et al., 2013). It is very difficult to successfully ensile legume green fodder without additives (Aksu et al., 2017). It is reported that this situation, which is especially valid for alfalfa, is due to three main reasons (McDonalds et al., 1991). These; Legumes have a high buffer capacity, low amounts of water-soluble carbohydrates and low dry matter content. Since the amount of water-soluble carbohydrates in alfalfa is low, lactic acid bacteria (LAB) cannot multiply sufficiently during ensiling, and thus lactic acid production is insufficient. Since lactic acid is insufficient in silage, the desired pH for silage cannot be reached and undesirable reactions occur in the environment. One of these reactions is proteolysis. Proteolysis occurs by breaking down the actual proteins in the feed into ammonia. This situation causes the amount of actual protein in the silo to decrease and the amount of ammonia to increase, reducing the efficiency of use of such silages in animals. Nitrogen in the form of

ammonia is difficult to store in the body. Most of the nitrogen in the silage, which undergoes proteolysis, is excreted from the body through urine before it can be converted into microbial protein by rumen microorganisms. In this case, some of the proteins are wasted. In addition, the amount of nitrogen excreted in urine also creates environmental pollution (Winters et al., 2000). In addition, when sufficient Lactic Acid (LA) production cannot occur in Alfalfa silages due to the lack of water-soluble carbohydrates, Acetic Acid, ethanol and Carbon Dioxide production or Butyric Acid formation as a result of Clostiridial fermentation is observed instead. Therefore, various additives need to be added to ensure sufficient LA production in alfalfa silages (Kızılşimşek et al. 2020). he use of lactic acid bacteria and other additives as silage additives is effective in determining the silage quality of different alternative plants. The density of lactic acid bacteria in the silage microflora prevents the proliferation of undesirable microorganisms. The formation of harmful bacterial communities, molds and yeasts in silage feed is an undesirable situation. In addition to the negative effects of such formations on silage fermentation, it can result in loss of productivity, diseases and even animal deaths in animals. For this reason, lactic acid bacteria and inoculants are frequently used to prevent undesirable microbial growth (Filya, 2001b). It has been observed that *Lactobacillus plantarum* and *Streptoccus faecium* bacteria added to alfalfa silage significantly improve feed consumption, milk yield and milk composition in cows (Kurtoglu and Coşkun, 2003).

To facilitate the production of alfalfa silage, additives such as crushed grain, molasses and whey, which enrich the environment with easily soluble carbohydrates, are added. Additionally, it has been reported that 1% diluted acid and the same amount of salt can be added to the material to easily lower the pH below 4.5 (Acar and Bostan, 2016). Adding grain crushed grains to silages is not preferred because their water-soluble carbohydrate content is low and their starch content is high, and most of the starch added to the silage during ensiling remains in the silage medium without any change. Because Lactic Acid Bacteria do not have amylolytic activity, they cannot use starch as an energy source. Therefore, using it as a silage additive does not increase silage quality much (Jaurane and Pichard, 2001). However, since corn has a high water-soluble carbohydrate (WCC) content, significant improvements in silage

quality have been detected in silages made with alfalfa mixtures (Budaklı et al., 2017).

To date, many silage additives have been developed and used successfully to control silage fermentation. In recent years, silage additives are being investigated as 'new generation silage additives' that, beyond controlling silage fermentation, will regulate the digestive system, especially the rumen fermentation of animals after silage consumption, and improve feed utilization (Önenç et al. 2019).

Yakışır and Aksu (2019) carried out their study by adding 5 and 10% of molasses-containing dried sugar beet pulp, which they thought would contribute to fermentation due to its high carbohydrate content, to the fresh alfalfa material harvested at the beginning of the flowering period in order to obtain quality alfalfa silage. It has been reported that ensiling with the addition of beet pulp increases the quality of alfalfa silage.

Researchers have conducted a study aiming to determine the effects of adding thyme and cumin essential oil to alfalfa as antimicrobial additives on fermentation quality and feed value. At the end of the study, it was reported that adding 650 mg/kg of thyme and cumin essential oils to alfalfa had a positive effect on silage fermentation, and that thyme essential oil came to the fore as a preventive against mold development and cumin as a preventive against yeast development. In addition, it has been reported that the addition of thyme and cumin increased the content of organic matter dissolved in the enzyme, and thus there was an increase in the metabolic energy content of silages (Önenç et al. 2019).

Arslan Duru (2019) investigated the effects of adding lavender (Lavandula angustifolia) flowers and stem parts to alfalfa silage at different levels (0.5, 1.0, 1.5 and 2.0%) on silage quality. It was reported that the LAB number of alfalfa silages with 2% addition of lavender, which is preferred due to its high quality essential oil content, was significantly higher than other groups. In order to prevent the development of undesirable bacteria in fermentation, it is reported that sodium diacetate is added to alfalfa, especially in periods when rainfall is abundant and drying is not possible, and ensiling improves fermentation properties and aerobic stability (Hışman et al. 2019).

Okuyucu et al. (2021) investigated the effect of adding lactic acid bacteria and enzyme mixtures to alfalfa silages with different maturity times on

fermentation and feed value. Alfalfa plant, which is the silage material, was harvested in three different periods: at the beginning, middle and end of flowering. Lactic acid + enzyme was applied to the alfalfa plant in the amount of 1 x 105, 5 x 105 and 1 x 106 cfu/g. The silages were opened after 45 days and chemical and microbiological analyzes were applied. It was determined that pH and ammonia nitrogen contents decreased and lactic acid bacteria numbers increased in silages with enzyme 10 inoculants. It was determined that the addition of high amounts of enzyme inoculants decreased NDF and ADF values and increased organic matter digestion and metabolic energy values *in vitro*. They stated that the application of LAB+E inoculants to alfalfa silages improved the feed values and fermentation properties, and that new doses could be applied to the silages.

Azman (2017) reported that the addition of acorns with high tannin content to alfalfa silage partially increased the silage DM level, but had no effect on fermentation.

Daş et al., (2022) added licorice (Glycyrrhiza glabra) plant as an easily soluble carbohydrate source to the alfalfa plant to examine its effect on silage quality. In the study where they determined the raw nutrient compositions and fermentation products of silages, they concluded that licorice can be added to the silage material in order to increase the level of easily soluble carbohydrates in the environment in order to obtain quality silage from alfalfa, which is difficult to ensile, and that licorice can be used to increase the quality of alfalfa silage.

Daş et al., (2023) examined the effect of adding potato chip waste, a by-product of the potato processing industry, to alfalfa plant as an easily soluble carbohydrate source, on silage quality. In the research, trial groups of silages prepared by adding potato chip waste to alfalfa at the rates of 0% (control), 0.5, 1and 2% by wet weight were created. An increase in the total lactic acid bacteria (LAB) levels of the silages was observed with the addition of 1% chips to the silages. When all parameters were examined, they determined that silages prepared by adding 1% chip waste had positive effects on silage fermentation.

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CHAPTER 5

INNOVATIVE APPROACHES IN OBESITY TREATMENT: INTERACTION BETWEEN BARIATRIC SURGERY AND MICROBIOTA

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DOI: https://dx.doi.org/10.5281/zenodo.10458630

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INTRODUCTION

The increasing occurrence of obesity and the related comorbid conditions, including conditions like type 2 diabetes mellitus, heart disease, and various types of cancer, has positioned it as a noteworthy public health concern. Addressing the increasing global burden of obesity and related conditions requires the exploration of efficacious approaches for both intervention and prevention. Strategies for managing obesity encompass lifestyle modifications (such as dietary adjustments and enhanced physical activity), the utilization of medications, and, in certain instances, resorting to surgical interventions (Bray et al., 2016; Ludwig and Ebbeling, 2010). Bariatric surgery has been proven to enhance weight loss efficacy compared to non-surgical methods, and importantly, this success has been demonstrated to be sustainable in the long term (Wolfe et al., 2016). Hence, for those with a body mass index (BMI) equal to or exceeding 40, or with a BMI of 35 or higher accompanied by comorbidities, bariatric surgery is deemed as an efficacious treatment. Nevertheless, research suggests that bariatric surgery has the potential not just to enhance but also to reverse numerous associated conditions, including diabetes mellitus, steatohepatitis, obstructive sleep apnea, and hypertension (Adams et al., 2012; Caiazzo et al., 2014; Ashrafian et al., 2015).

The microbiota, referred to as the intestinal flora, comprises approximately one hundred trillion microorganisms (Barlow et al., 2015). The intestinal flora of obese individuals induces various changes, including an increase in nutrient absorption in intestinal villi, elevated carbohydrate absorption due to increased enzyme activity, reduced lipid metabolism in the liver and muscles, alterations in bile acid metabolism and secretion, and a decrease in hormones released from the intestines. In contrast, bariatric surgery holds the capacity to alter these obesogenic characteristics. Consequently, bariatric surgery not only leads to weight and adipose tissue loss but also has the capacity to prevent metabolic diseases (Barlow et al., 2015; Bays et al., 2016). Studies suggest that, alongside elements like the existence of diseases, stress, age, lifestyle, probiotic or medication use, recent research in both animal and human subjects has demonstrated that bariatric surgery affects the microbiota (Zhang et al., 2009; Furet et al., 2010; Ryan et al., 2014).

Obesity

The World Health Organization (WHO) identifies obesity as a major health concern, ranking it among the top ten most perilous conditions. It is defined by the abnormal and overabundant storage of fat within the body, exerting a substantial impact on human health. In adult males, 15-20% of body weight is composed of fat tissue, while in females, it ranges from 25-30%. When this percentage exceeds 25% in males and 30% in females, it is classified as obesity (WHO, 2018).

Since 2013, the importance of obesity has remained a critical concern affecting not only national but also global health. It remains a risk factor for a range of chronic diseases, including cardiovascular diseases, diabetes, chronic kidney disease, non-alcoholic fatty liver disease (NAFLD) not associated with alcohol, and metabolic syndrome (Mechanick et al., 2020).

Body Mass Index (BMI) is a commonly used simple height-weight index to classify overweight and obesity. This index is calculated by dividing an individual's weight in kilograms by the square of their height in meters (kg/m^2) (WHO, 2023). The classification of BMI in adults is shown in Table 1.

Table 1: The Classification of BMI in Adults

BMI	**Nutritional status**
Below 18.5	Underweight
18.5–24.9	Normal weight
25.0–29.9	Pre-obesity
30.0–34.9	Obesity class I
35.0–39.9	Obesity class II
Above 40	Obesity class III

Body Mass Index (BMI) may not be a completely reliable indicator in all cases. For instance, individuals with well-developed muscle tissue, such as athletes, may have a high BMI despite low levels of body fat. Conversely, individuals who appear thin and have normal BMI values may have an accumulation of fat around abdominal organs and the waist. It is crucial not to overlook this situation, and to determine obesity, reliance on not only BMI values but also waist circumference measurements is necessary. While BMI measurements do not allow separate risk predictions for women and men, it is possible to establish different risk levels for women and men through waist circumference measurements (Rehberi U.T.T., ve Bakanlığı T. S., 2014).

Table 2: Evaluation of Waist Circumference Measurement in Adults (TÜBER, 2022)

Waist Circumference (cm)	**Health Risk Associated with Body Weight**
Male: <94 Female: <80	Health risk associated with body weight is low
Male: ≥94 - <102 Female: ≥80 - <88	Health risk associated with body weight is high
Male: ≥102 Female: ≥88	Health risk associated with body weight is very high

Obesity Etiology

Obesity has a multifactorial and complex etiology, associated with metabolic disorders that affect various systems. Environmental, biochemical, genetic, sociocultural, and psychological factors play a role in the initiation of obesity by interacting with each other (Klancic and Reimer, 2020; Everard and Cani, 2013).

Various dietary elements, including the consumption of energy exceeding the amount expended, consistent consumption of fast food, high intake of refined sugar and fat, inability to be breastfed, and the type and quantity of complementary foods, can create a conducive environment for obesity (Everard and Cani, 2013; Obezite TEMD and LM HCG, 2018).

Prevalence of Obesity

While obesity was previously more common in developed, economically advanced countries, it has evolved into a worldwide public health concern at pandemic levels, observed in countries of all economic levels (Popkin and Ng, 2022).

According to the WHO, in 2016, nearly 40% of individuals worldwide who are 18 years old or older are overweight, and 14% fall into the obese category. From 1975 to 2016, the global occurrence of obesity has nearly tripled. While obesity was initially considered a problem in high-income countries, it has been on the rise in nations with moderate to low income levels. Among the WHO regions, the top three regions with the highest adult obesity prevalence are the Americas, the Eastern Mediterranean, and Europe. There is an observed increase in obesity prevalence with the rise in income in countries. While around 7% of people in nations with low-income levels experience obesity, a quarter of individuals in high-income countries are classified as

obese. From 2000 to 2016, obesity has shown a consistent increase in all WHO regions and income groups, with global prevalence rising from 9% in 2000 to 13% in 2016 (WHO, 2018).

Intestinal Microbiota

Humans are living organisms that can be called a 'superorganism' or 'holobiont,' consisting of ten percent human cells and ninety percent microbial cells. The term microbiota was defined by Joshua Lederberg in 2001 as a "community of microorganisms, including commensal, symbiotic, and pathogenic species, that inhabit our body environment." The intestinal microbiota forms a complex ecosystem closely linked to the physiological environment of the host, evolving in parallel with the host. The human and microbial genomes have co-evolved, and this hologenomic association establishes a shared relationship between human metabolism and microbiota metabolism (Haque and Haque, 2017).

The intestinal microbiota, largely engaged in a symbiotic relationship with human hosts, comprises around 100 trillion microorganisms, containing more genes than those found throughout the entirety of the human genome, making it one of the largest components of our body (Arumugam et al., 2011; Rosenbaum et al., 2015). The gastrointestinal system, with an approximate surface area of 200 m^2 and a rich nutrient environment for microorganisms, provides a suitable habitat for colonization (Haque and Haque, 2017). The microbial cell content in the gastrointestinal system significantly increases spanning from the stomach through the colon. Bacterial content is $<10^3$/ml in the stomach, 10^4/ml in the jejunum, 10^7/ml in the distal ileum, and around 10^{12}/ml in the colon (Torres-Fuentes et al., 2017; Lozupone et al., 2012). Dominant phyla in the intestine include Bacteroidetes, making up approximately 20-25%; Firmicutes constitute around 60-65%; Proteobacteria account for roughly 5-10%; and Actinobacteria contribute approximately 3%, constituting approximately 97% of the intestinal microbial population (Arumugam et al., 2011).

Gut Microbiota and Obesity

The exact explanation of how the gut microbiota affects the pathology of obesity and whether alterations in the microbiota are the root cause or a

consequence of obesity remains inconclusively determined (Zhang et al., 2009). For instance, alterations in the gut microbiota towards Firmicutes, as observed in the case of an uncontrolled high-fat/high-carbohydrate diet, may contribute to increased weight gain and energy intake from the diet. Conversely, a low-fat and low-sugar diet may enhance Bacteroidetes dominance by increasing the regulation of fasting-induced adipose factor expression, subsequently promoting an elevation in the expenditure of energy and a reduction in fat storage (Villanueva-Millán et al., 2015).

In a year-long weight loss program, Ley et al. monitored the fecal gut microbiota of 12 obese participants, randomly assigning them to a low-calorie diet with either fat or carbohydrate restriction. Before the dietary intervention, obese participants exhibited reduced Bacteroidetes levels and elevated Firmicutes abundance compared to lean control participants. After weight loss, the ratio of Bacteroidetes showed an increase, while Firmicutes exhibited a decrease. This finding is associated with the percentage of weight lost rather than changes in the calorie content of the diet. Before the dietary intervention, Bacteroidetes made up around 3% of the microbial community in the gut, increasing to around 15% after weight loss (Amabebe et al., 2020; Ley et al., 2006).

Moreover, in metabolically unhealthy obese individuals, the abundance of Fusobacterium genera has significantly increased. Fusobacterial abundance is associated with atherogenic dyslipidemia and has shown a negative correlation with HDL-C levels before bariatric surgery (Jun et al., 2020).

Table 2. The changes in gut microbiota under conditions of obesity (Kobyliak et al., 2015)

Phylum	Class	Order (Genera)	The trends of changes
Bacteroidetes	Bacteroidetes	Bacteroidales (Bacteroides)	↓
		Bacteroidales (Prevotella)	↑
Firmicutes	Bacilli	Bacillales (Bacillus)	↑
		Lactobacillales	↓
	Clostridia	Clostridiales (Clostridium)	↑
Actinobacteria	Actinobacteria	Actinomycetales	↑
	Actinobacteria	Bifidobacteriales (Bifidobacterium)	↓
Euryarchaeota (domain Archaea)	Methanobacteria		↑

Bariatric Surgery

Excessive weight and obesity treatments include dietary therapy, exercise/behavioral interventions, medications for weight loss, and surgical

interventions for obesity (Sedighi et al., 2017). Despite various traditional non-surgical weight-loss interventions such as exercise, diet, medications, and psychotherapy for obesity, the outcomes are often modest, and patients frequently regain the lost weight. The only method with a long-term impact on weight loss is classified as different types of surgery known as "bariatric surgery." Surgical methods can be malabsorptive, restrictive, or both malabsorptive and restrictive (McTigue et al., 2003; Sjöström et al., 2005).

Restrictive methods include Intragastric Balloon, Gastric Pacing, Vertical Banded Gastroplasty (VBG), Adjustable Gastric Banding (AGB), and Vertical Sleeve Gastrectomy (VSG). Malabsorptive methods are Biliopancreatic Diversion (BPD) and Jejunoileal Bypass (JIB), while combined methods consist of Mini Gastric Bypass (MGB), Roux-en-Y Gastric Bypass (RYGB), and Biliopancreatic Diversion-Duodenal Switch (BPD-DS) (TEMB, 2018). RYGB, VSG, and AGB rank among the commonly utilized varieties of bariatric surgical procedures (Pories, 2008).

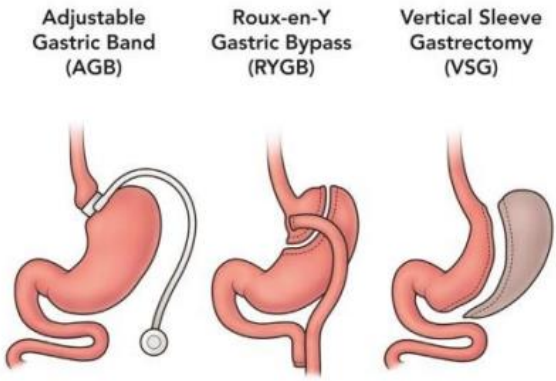

Figure 1. Primary Bariatric Surgical Methods (Pories, 2008)

In the Adjustable Gastric Band method, a silicone band is placed 2-3 cm below the gastroesophageal junction to create a gastric pouch with a volume ranging from 15 to 30 ml. The bottom section of the pouch is stitched to the upper portion of the stomach, positioned beneath it. The continuation of the band is placed under the skin, and when deemed necessary, adjustments can be made to tighten or loosen the band with a sterile saline injection, creating an early feeling of fullness and restricting food intake (Güçlü, 2018).

Roux-en-Y Gastric Bypass (RYGB) is grounded in the principle of connecting a bypassed 30 ml gastric pouch to a 100-150 cm Roux limb. By effectively circumventing a substantial part of the stomach and intestine, this method demonstrates a high rate of success. However, complications such as micro and macronutrient deficiencies may occur over an extended period following this procedure (Deveci, 2013).

Vertical Sleeve Gastrectomy (VSG) involves starting from the gastric antrum and resecting approximately 80-85% of the stomach with a stapler line that ends at the proximal angle of His. The stomach volume is reduced to an average of 100 ml. "It is crucial not to neglect the gastric fundus and to avoid leaving the gastric antrum wide. This method is also known as sleeve gastrectomy due to the tube-like shape given to the new appearance of the stomach (Kılıç, 2016).

Intestinal Microbiota After Bariatric Surgery

Interventions such as gastric surgeries and prebiotic usage can influence the intestinal microbiota and host metabolism. Hence, they offer intriguing strategies for addressing obesity and metabolic disorders in treatment (Lupoli et al., 2017).

The intestinal microbiota is considered a key endocrine organ playing a crucial role in body homeostasis. At present, the improved composition of the intestinal microbiota resulting from bariatric surgery stands as one of the mechanisms elucidating the positive clinical outcomes attributed to this surgical procedure (Dao et al., 2016). Alterations in the gut microbiota post-bariatric surgery are associated with the composition of bacteria and functions, correlating with beneficial results such as weight loss or metabolic improvements facilitated by bariatric surgery (Debédat et al., 2019).

Bariatric surgery contributes partially to the correction of dysbiosis observed during obesity. However, changes in the intestinal microbiota after the operation can vary significantly from individual to individual (Debédat et al., 2019).

After RYGB Intestinal Microbiota

The RYGB procedure includes the separation of the stomach by forming a small gastric pouch and then forming a new digestive organ called Roux by anastomosing this portion with the middle jejunum. This allows consumed food

to bypass a significant portion of the stomach, duodenum, and the beginning of the jejunum (Olbers et al., 2003).

Roux-en-Y gastric bypass results in rapid weight loss, a reduction in fat deposition, and improvement in glucose metabolism. These effects cannot be solely attributed to reduced calorie intake or absorption; however, it is undeniable that the mechanisms explaining these metabolic outcomes are largely unknown (Liou et al., 2013).

In a research study, 13 individuals with morbid obesity who underwent RYGB were meticulously phenotyped. Their intestinal microbiomes were assessed before the RYGB procedure, as well as three months and twelve months after the operation. Through metagenomic sequencing of fecal microbial DNA, the composition of the intestinal microbiota was analyzed. Concurrent with weight loss and metabolic enhancements, there was a notable increase in intestinal microbial diversity within the initial 3 months after RYGB, persisting at an elevated level one year later. As a result of the RYGB process, changes were observed in the relative abundance of 31 species in the first three months ($p<0.05$). Sixteen of these species retained their altered abundances throughout the following nine months. Additionally, 53 microbial functional modules exhibited an increase in the proportional distribution from baseline to three months ($p<0.05$) (Palleja et al., 2016).

An additional study demonstrated that the Firmicutes/Bacteroides ratio, which is high in obese individuals, decreased with medical nutrition therapy and RYGB (Damms-Machado et al., 2015). Zhang et al. (2009) evaluated patients who underwent RYGB 8-15 months postoperatively and found lower levels of Firmicutes bacteria compared to non-surgical individuals with normal and obese body weights.

After VSG Intestinal Microbiota

Sleeve gastrectomy involves the vertical division of the stomach, reducing its size by 75%. The lower section of the stomach, encompassing the pyloric valve, is preserved without altering stomach function and digestion. This procedure is irreversible and can serve as the starting point for RYGB or duodenal switch (Lupoli et al., 2017).

Damms-Machado and colleagues (2015) observed an increase in the Bacteroidetes phylum, a decrease in the Firmicutes phylum, and an elevation in

the Bacteroidetes/Firmicutes ratio following LSG. In individuals undergoing LSG, there was an inverse relationship identified concerning body weight and the number of Bacteroidetes, and there was a positive correlation noted with the number of Firmicutes. Conversely, in individuals on an energy-restricted diet, the opposite trend was noted.

In a study published in 2020, 28 patients with a BMI of 44.2 (6.6) kg/m2 were examined. These patients achieved an excessive weight loss of 53.2 (19.0) percent and showed improvement in metabolic diseases six months after LSG. Additionally, a significant change in the fecal microbial community was observed in conjunction with this improvement (Ikeda et al., 2020).

The abundance of A. muciniphila (Verrucomicrobiaceae family) significantly increased after LSG, and findings in rodent models suggest that A. muciniphila prevents body fat accumulation and metabolic abnormalities (Everard et al., 2013).

Bariatric Surgery: Impacts On Health Through the Microbiota

The microbiota plays crucial roles in regulating disease and health conditions in our bodies, generating necessary signals to promote immune system functions, and contributing to the absorption of undigested carbohydrates. A significant portion of the human microbiota resides in our digestive system (Yılmaz and Altındiş, 2017; Karatay, 2019).

In a recent study, conducted within a year after surgery, only two genera of bacteria producing short-chain fatty acids (SCFAs) showed a notable connection with lipid levels. Among these bacterial types, the Eubacterium eligens group was linked to elevated HDL-C levels, while the Eubacterium xylanophilum group was associated with lower triglyceride and cholesterol levels. A prior study revealed a negative correlation between the Eubacterium eligens group and visceral abdominal fat area (Nie et al., 2020), and the Eubacterium xylanophilum group demonstrated a negative relationship with body weight (Wei et al., 2021).

Eubacterium xylanophilum is a bacterium in the intestines that effectively produces SCFAs. Following RYGB surgery, an increase in butyrate levels has been observed in both humans and urinary models. Additionally, butyrate has been proposed to have a positive impact on diet-induced obesity and atherosclerosis (Liou et al., 2013).

Pre- And Probiotic Use After Bariatric Surgery

The term 'probiotic' and existing literature were subject to review by an Expert Panel assembled by the International Scientific Association for Probiotics and Prebiotics (ISAPP). The resulting publication reiterated the definition with minor changes: 'Beneficial microorganisms that, when given in sufficient quantities, provide health advantages to the host.' This scientifically accepted definition gained widespread global recognition (Hill et al., 2014).

The current scientific definition of prebiotic was developed by a panel of microbiology, nutrition, and clinical research experts assembled by ISAPP in 2016. This consensus definition is as follows: 'A substance that host microorganisms selectively use, resulting in a positive impact on health.' (Gibson et al., 2017).

The optimal composition of the microbiome is a structure that serves as an indicator of human well-being. When embarking on the process of building human well-being, the first step an individual needs to take is a lifestyle change. A proper diet and physical activity form the foundation of this structure. This metaphorical foundation, representing diet and exercise, emphasizes the fundamental role of a healthy lifestyle in maintaining human health and well-being. The columns between the 'roof' and 'foundation' in this metaphor are pre-, pro-, and postbiotics. Currently, much research is focused on determining the ideal ratio and form of each column so that all structural elements work in harmony. It's crucial to acknowledge that elements beyond the scope of this analogy, such as the mode of childbirth, medication usage, or the number of siblings, can also impact the composition of the microbiome (Żółkiewicz et al., 2020).

In a study employing randomization, double-blinding, and a placebo control design involving 100 morbidly obese patients who underwent LSG, the influence of probiotics on hepatic, inflammatory, and clinical results among individuals diagnosed with NAFLD was examined. The findings from this study indicated that the administration of probiotics did not lead to improvements in hepatic, inflammatory, and clinical outcomes at the six-month and twelve-month marks following LSG (Sherf-Dagan et al., 2018). Another study suggested that the use of probiotics and prebiotics in morbidly obese patients undergoing LSG may play a potential role in improving some clinical

outcomes, such as weight loss, blood pressure, and clinical laboratory values (Kazzi et al., 2021).

In a clinical trial involving 46 morbidly obese patients who underwent one-anastomosis gastric bypass (OAGB), a 4-month probiotic or placebo supplementation was compared. The study demonstrated that 4-month probiotic supplementation prevented the elevation of lipopolysaccharide-binding protein (LBP) levels in patients undergoing OAGB, and it resulted in enhancements in serum TNF-α, 25-OH vitamin D3 concentrations, and weight loss. Nonetheless, these effects were not sustained 9 months after discontinuation of the treatment (Mokhtari et al., 2019).

Another study designed to explore the impacts of Lactobacillus and Bifidobacterium supplementation on nutrition and metabolic parameters after RYGB administered probiotic supplementation or a placebo to patients starting 7 days after surgery for three months. After RYGB, serum 25-OH D vitamin increased in both groups; however, this increase was significant only in the probiotic group. B12 vitamin levels were higher in the probiotic group compared to the placebo group, and the decrease in triglyceride levels was significant only in the probiotic group. Additionally, significant reductions in anthropometric parameters and improvements in glycemic profiles were observed in both groups (Ramos et al., 2021).

In a meta-analysis aimed at evaluating the effects of probiotics on post-bariatric surgery outcomes, including body weight, waist circumference (WC), percentage of excess weight loss (%EWL), C-reactive protein (CRP), and BMI, a statistically notable reduction in waist circumference with probiotic use was found 12 months after surgery. Nevertheless, no substantial effects were noted on weight, BMI, %EWL, and CRP. To validate the efficacy and safety of probiotics and to advocate the regular clinical application of probiotics in obese adults undergoing bariatric surgery, higher-quality clinical trials are needed (Zhang et al., 2021).

Despite the widespread use of probiotics after bariatric surgery, uncertainty persists regarding the effectiveness of the probiotics used (Benaiges et al., 2015).

Conclusion

Bariatric surgery plays a crucial role in addressing obesity and related pathological conditions. Obesity, especially the resolution of metabolic disorders like diabetes mellitus, is intricately connected to the variety and quantity of microorganisms in the intestinal microbiome. However, understanding the specific relationship and correlations between changes in the microbiome and the implementation of bariatric surgery requires a thorough examination of the level of interest and specific criteria for each patient. Despite the challenging process of deciphering the exact correlation between microbiome changes and bariatric surgery, these alterations, along with pro-prebiotic applications, may present new treatment options beyond bariatric surgery.

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CHAPTER 6

ALFALFA (*Medicago sativa* L) SILAGE STUDIES CONDUCTED IN TURKEY IN THE LAST 5 YEARS

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DOI: https://dx.doi.org/10.5281/zenodo.10458647

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Introduction

The high cost of feed inputs is one of the most significant problems in our country's livestock farming. The primary sources of feed for ruminant animal nutrition are concentrate and roughage feedstuffs. The high cost of concentrate feed forces producers to focus on roughage-based production. Roughage feeds, whether fresh, dried, or in silage form, are characterized as rich in cellulose, yet poor in energy and protein, serving as feeds that regulate the digestive system (Akyıldız, 1983). Silage is a preserved feed made by fermenting high-moisture green forages and by-products of the food industry in structures called silos, where they are sealed from air contact. It is fed to animals during times when there is no green forage available, without any loss of nutritional value (Kılıç, 1986). Important forage crops in Turkey include corn, alfalfa, alfalfa, vetch, sainfoin, and sugar beet for animal feed. Alfalfa (Medicago sativa L.) is the most widely cultivated forage crop globally. Alfalfa has a higher nutritional value compared to other forage crops and can produce yields for 4 to 8 years depending on environmental conditions. Due to its high protein content, alfalfa is classified as a high-quality silage feed (Coşkun et al., 1997; Ergün et al., 1999). Producing quality silage from such feeds is challenging. Therefore, the use of additives is necessary to ensure fermentation during the ensiling of forage crops rich in protein and low in carbohydrates (Çerçi et al., 1997; Kılıç, 1986).

Alfalfa (*Medicago Sativa L*) Sılage Studıes Conducted In Turkey In The Last 5 Years

Aydın et al. (2023), In this study, it was aimed to determine the effects of the addition of almond hull, which has no economic value and causes environmental pollution, to alfalfa silage as an easily soluble carbohydrate source on silage quality, fermentation characteristics and in vitro organic matter digestion (IVOMD). The groups were designed to contain 0% (control), 1%, 2%, 4% and 6% almond hull, respectively. When the IVOMD and metabolizable energy values of the silages were examined, increases were observed in all additive groups compared to the control group. When the pH, NH_3-N/TN and carbon dioxide (CO^2) values of the silages were examined, the highest values were determined in the control group, while the lowest pH, NH_3-N/TN and CO^2 values were determined in the silage group with 6% almond hull

added. Although an increase was determined in all trial groups in terms of lactic acid and acetic acid values of silages compared to the control group, the highest values were determined in the group with 6% almond hull addition, and a decrease was determined due to the increase in almond hull in terms of butyric acid values. Yeast and mold values of the silages decreased due to the increase in almond hull compared to the control group. As a re- sult, it was determined that the addition of 6% almond hull as an easily soluble carbohydrate source had a positive effect on alfalfa silage quality and fermentation characteristics.

Aydın (2023), In this study, it was aimed to determine the effects of addition of cake waste, which is a food industry waste, to alfalfa silage as a readily soluble carbohydrate source on silage quality, fermentation properties, in vitro organic matter digestion and in vitro CH_4 values. In the study, while silage group without additives constituted the control group, silages with 1%, 2%, 4% and 6% cake waste additions constituted the test groups. Statistically significant differences were found in crude protein (CP), crude ash (CA), dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), in vitro organic matter digestion (IVOMD), metabolizable energy (ME) and in vitro CH4 values of silages. While mold values of the silages were controlled and found in the control group with 1% cake waste addition, it was not found in the other test groups. As a result, it was determined that the addition of 6% cake waste as a readily soluble carbohydrate source had a positive effect on silage quality and fermentation properties.

Aydın (2023), This study examined the addition of wafer waste as a readily soluble carbohydrate source to alfalfa silage for its impact on silage quality, fermentation characteristics, in vitro organic matter digestion, and in vitro CH_4 values. Fresh alfalfa was ensiled with 0% wafer waste (Control), 1% wafer waste, 2% wafer waste, 4% wafer waste, and 6% wafer waste for 60 days. The differences between the groups in dry matter (DM), crude protein (CP), crude ash (CA), neutral detergent fiber (NDF), acid detergent fiber (ADF), metabolizable energy (ME), in vitro organic matter digestion (IVOMD), and in vitro CH_4 values of silages were found to be statistically significant. In comparison to the control group, increases in IVOMD and ME values were seen in all additive-containing groups. Depending on the quantity of wafer waste, the pH, ammonia nitrogen, and carbon dioxide values of the silages declined. In the study, the highest amount of lactic acid occurred in the group with 6%

wafer waste added. Propionic acid was detected only in the control group. Butyric acid values tended to decrease depending on the addition of wafer waste and were not found in the groups to which 4% and 6% wafer waste addition. Yeast and mold values of the silages decreased in parallel with the increase in the wafer waste rate. As a result, it was determined that adding wafer waste as an easily soluble carbohydrate source positively affected alfalfa silage quality and fermentation characteristics.

Aydın and Denek (2023), Within the scope of this study, it was aimed to determine the effect of the groups with the highest LAB numbers determined as a result of storage for one and three months on alfalfa silage quality by freezing fermented lactic acid bacteria (LAB) liquids prepared with different levels of sucrose addition (5-10%) and incubation (2 and 5 days) for different periods of time in deep freezer and by drying via lyophilization process according to the results obtained from the previous study.In the study, groups consisted of control, 2D5%STsL (lyophilized with TRIS (Ts) after 2 days incubation with 5% sucrose addition), 2D10%SDsL (lyophilized with DMSO (Ds) after 2 days incubation with 10% sucrose addition), 5D10%SDsL (DMSO (Ds) additive lyophilized after 5 days of incubation with 10% sucrose addition), and 5D5%STsD (TRIS (Ts) additive deep freezer after 5 days of incubation with 5% sucrose addition).In the study, LAB count, CO2, lactic acid (LA) content, acetic acid (AA) content, pH, NH3-N/TN, and butyric acid (BA) values were statistically significant between the groups at the end of the one-month storage period. Crude protein (CP), pH, LA, and BA values were found to be statistically significant between the groups at the end of the three-month storage period in the study.

Aydın and Denek (2019) aimed to determine the effects of sucrose addition at different levels (1%, 3%, 5%, 10%) and different incubation periods (48, 72, and 96 hours at 30°C) on the total lactic acid bacteria count and the fermentation quality of silages prepared from alfalfa plants after storage at room temperature (25°C) for various durations (fresh, 15, 30, 45, and 60 days). In randomly selected colonies of lactic acid bacteria formed in the fermented natural lactic acid liquids, Lb. Plantarum was observed as the dominant bacterial species with an increase in storage time at room temperature. Overall, for each incubation period (48, 72, and 96 hours), except for fresh liquids, the total lactic acid bacteria count was found to be the highest in fermented lactic

acid liquids prepared with 1% sucrose addition for all storage durations (15, 30, 45, and 60 days). In the study, silages prepared by adding 0.1% (w/v) fresh alfalfa (Medicago sativa) to fermented lactic acid liquids obtained after adding 1% sucrose at the 48-hour incubation stage and stored for 15 (PFJ-15) and 30 (PFJ-30) days showed a decrease in pH, ammonia nitrogen, and acetic acid values, while lactic acid values increased. As a result, it was observed that the addition of 1% sucrose at the 48-hour incubation stage in lactic acid liquids stored at room temperature (25°C) for various durations (fresh and 15 and 30 days) increased the lactic acid bacteria count and improved the quality of alfalfa silages.

Aydın (2023), This study was conducted to evaluate the effect of adding fermented natural lactic acid bacteria (PFJ) obtained from different sources and some lactic acid bacteria inoculants to alfalfa (*Medicago sativa L.*) silage on fermentation, in vitro organic matter digestibility (IVOMD) and in vitro gas production. In the study, (i) pure alfalfa plant constituted the control group, whereas (ii) 2% molasses added group (iii) PFJ (3% molasses) prepared from alfalfa + 2% molasses to alfalfa plant, (iv) PFJ (3% molasses) prepared from meadow grass + 2% molasses alfalfa. plant, (v) PFJ (3% Molasses) prepared from maize + 2% molasses to alfalfa plant, (vi) homofermentative added group + 2% molasses to alfalfa plant, (vii) heterofermentative group + 2% molasses to alfalfa plant, added groups formed the trial groups. Homofermentative and heterofermentative LAB inoculants were added to alfalfa plant at the level of 108 cfu/kg. When the DM, CA, ADF, NDF, IVOMD, ME and CH4 values of the prepared silages were examined, the differences between the groups were found to be statistically significant. When the fermentation characteristics (pH, NH3-N, CO2, LA, AA, BA, mold) of the silages prepared by adding PFJ and some LAB inoculants to alfalfa plant, the differences between groups were found to be statistically significant. When examined in terms of all parameters, it was determined that the addition of PFJ (3% molasses) prepared from meadow grass + 2% molasses to alfalfa plant had positive effects on silage quality, fermentation characteristics and in vitro organic matter digestion.

Doğan Daş et al. (2022), conducted a study to investigate the effect of adding licorice (Glycyrrhiza glabra), a readily soluble carbohydrate source, to alfalfa (*Medicago sativa L.*) with high buffering capacity and, consequently, challenging ensiling, on silage quality. The silage groups prepared for the study

included control alfalfa (without additives) and alfalfa with the addition of licorice root at rates of 2%, 4%, 8%, 16%, and 32% based on fresh weight, creating a total of six different groups. When examining the pH values of the silages prepared with the addition of licorice root, the lowest pH value was obtained in the silage prepared with 32% fresh licorice root addition. Regarding ammonia nitrogen values of the silages, it was observed that ammonia nitrogen values decreased with an increase in the level of fresh licorice root. The highest Fleig score, 73.79, was obtained from the silages prepared with the addition of 32% fresh licorice root. While no statistical differences were found among the groups in terms of structure and color characteristics when examining the physical properties of the silages (color, odor, structure), differences were found to be statistically significant in terms of odor and DLG (digestible dry matter intake) scores among the groups. The average DLG score was highest in the silages prepared with the addition of 32% fresh licorice root. In conclusion, the study suggests that licorice root can be added as an alternative carbohydrate source to silage material to increase the level of easily soluble carbohydrates in order to obtain high-quality silage from alfalfa, which is challenging to ensile. The findings indicate that licorice root can be used to enhance the quality of alfalfa silage by improving its carbohydrate content.

Doğan Daş et al. (2023), This study was carried out to examine the effect of adding potato chips waste, which is a by-product of the potato processing industry, on silage quality, as an easily soluble carbohydrate source, to the alfalfa (*Medicago sativa L.*) plant, which has a high buffering capacity and is therefore difficult to ensilage. In the study, alfalfa (Medicago sativa) harvested at the 10% flowering point was used as the plant material. Four different groups were formed by adding the silage groups prepared in the study, alfalfa plant control (without additives), and potato chips waste at the rates of 0.5%, 1% and 2% on a wet weight basis to the alfalfa plants. When the effects of the silages prepared by adding different levels of chip waste to alfalfa plant on the nutrient value were examined, the differences between the groups in terms of dry matter (DM), crude ash (CA), acid detergent fiber (ADF) and neutral detergent fiber (NDF) content of the silages obtained from the study were statistically significant. While it was not significant in terms of CP content, the differences between the silage groups were found to be statistically significant. In the study, when the pH, ammonia nitrogen (NH3-N), carbon dioxide (CO2) production

and total yeast mold values of the fermentation properties of the silages were examined, decreases were observed due to the addition of chips compared to the control group. When the total lactic acid bacteria (LAB) values of the silages were examined, an increase was observed after the addition of 1% chips. As a result, when all the parameters were examined, it was determined that the silages prepared by adding 1% chips waste had positive effects on silage fermentation.

Tarıq (2021), conducted a study to investigate the in vitro true digestibility of alfalfa silage made by adding orange and mandarin peels. The alfalfa used as the material was harvested at the beginning of flowering and wilted for 24 hours. Subsequently, orange and mandarin peels were added at proportions of 20%, 25%, and 30% to the alfalfa, and the mixture was ensiled for 60 days. No significant difference was found among the silages with different peel proportions. Consequently, it was indicated that the in vitro true digestibility of alfalfa silage would increase when ensiled with 30% orange peel.

In the study conducted by Şerbetçi (2020), the effects of adding waffle crumbs to alfalfa silage at a level of 40-50 g/kg dry matter were investigated. The research reported that this addition positively influenced the chemical and microbiological properties of the silage.

Doğan (2019), investigated the silage quality and mineral content of alfalfa silage obtained by using apple and lemon additions as carbohydrate sources. In the study, apple additions were made to alfalfa silage at percentages of 0, 5, 10, and 15, while lemon additions were made to alfalfa silage at the same percentages. According to the results, as the lemon addition increased, ADF, NDF, ash content, and pH values decreased. Consequently, it was indicated that the quality of alfalfa silage obtained with the application of 15% apple and lemon additions was higher compared to other applications.

In his master's thesis, Yayla (2019) investigated the effects of waste jam mixture addition on alfalfa silage fermentation, aerobic stability, and in vitro digestibility. It was reported that there was an increase in DM amount, DM consumption, and relative humidity compared to the control group in alfalfa silages where jam and jam particles were added.

Karapınar (2019) investigated the effects of kefir addition at different doses on the fermentation development and aerobic stability of alfalfa silage. The study concluded that the addition of kefir improved the fermentation quality and aerobic stability of alfalfa silage.

Özüretmen (2019), investigated the effects of adding whey powder, used as a water-soluble carbohydrate source, to alfalfa silage at different doses on the quality, in vitro digestibility, and metabolic energy content of the silage. The study concluded that whey powder could be used to ensile alfalfa, improving silage quality and increasing digestibility. Additionally, considering whey powder as an additive in silage was noted to play an important role in reducing environmental pollution.

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CHAPTER 7

MELATONIN AS AN ANTIOXIDANT AND ITS PROTECTIVE EFFECTS ON NEURODEGENERATIVE DISEASES

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DOI: https://dx.doi.org/10.5281/zenodo.10458662

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Introduction

The molecule melatonin, which has a significant evolutionary lineage dating back to unicellular organisms, has the ability to induce the transition of algae and primitive photosynthetic bacteria into multicellular organisms. This transformative process occurred towards the later stages of evolution, approximately 2.5 to 3.0 billion years ago. Additionally, melatonin exhibits scavenging properties against free radicals, a mechanism achieved through its transformation into mitochondria and chloroplasts. Furthermore, melatonin plays a role in regulating sleep patterns and possesses properties that are anti-carcinogenic and immunomodulatory in nature (Tan et al., 2014; Zhao et al., 2019). Melatonin has shown the ability to scavenge oxygen-derived free radicals such as hydroxyl (Roberts et al., 1998; Poeggeler et al., 2002; Matuszak et al., 1997) and peroxyl (Livrea et al.,1997; Pieri et al., 1994), which we think is the result of all these properties and effects of melatonin, and melatonin has shown a protective effect in case of in vitro oxidation of low-density lipoprotein (LDL) by copper or macrophages (Livrea et al.,1997; Bonnefont-Rousselot et al., 2002; Bonnefont-Rousselot et al., 2003; Walters-Laporteet al., 1998; Duell et al., 1998; Abuja et al., 1997), similarly, it has been reported in studies that melatonin-lead nanoparticles models, liposomes or microsomes of melatonin-lead nanoparticles show protection activity against lipid peroxidation (Schaffazick et al., 2005), and can neutralize singlet oxygen, peroxynitrite anion and nitric oxide (Allegra et al., 2003; Reiter et al., 2004; Ucar et al., 2007; Topal et al., 2005).

Neurological diseases, as an important area in which both its antioxidant and molecular properties are used, have made melatonin even more interesting. In this review, it has been tried to explain how melatonin shows antioxidant activity and how it acts by affecting which metabolic pathways in neurological diseases, by making use of many literature information, including many meta-analyses.

1. Melatonin as an Antioxidant and Its Relationship with Oxidative Stress

The amino acid tryptophan is converted into the hormone indoleamine known as melatonin (N-acetyl-5-methoxytryptamine). It's produced in the

pineal gland and elsewhere in the body through synthesis (Janas et al., 2013; Genario et al., 2019; Genario et al., 2020) (Figure 1).

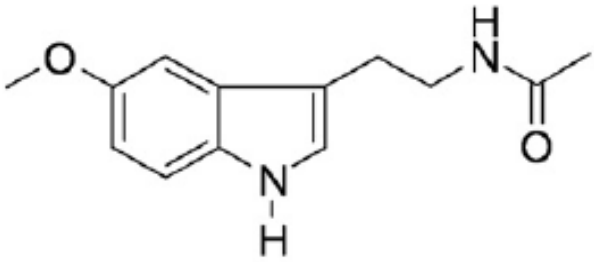

Figure 1. Chemical structure of Melatonin (N-acetyl-5-methoxytryptamine) (Genario et al., 2020).

The nocturnal level of melatonin signaling in day- and night-active animals is thought to differ, in general, most of the knowledge about its function is thought to have been acquired gradually during evolution. It has been documented that this substance possesses the ability to regulate sleep inefficiency, disrupted circadian rhythms, and compensatory immunity. This is attributed to its potent antioxidant and anti-inflammatory properties (Permpoonputtana and Govitrapong, 2013; Tarocco et al., 2019; Brzezinski, 1997), as well as its ability to act as a pluripotent oncostatic agent that specifically targets mitochondria (Um and Kwon, 2010; Reiter et al., 2016). Furthermore, there have been reports suggesting a potential link between aging, age-related neurological diseases, and a decline in the production of melatonin in the mitochondria, particularly within the pineal gland (Hardeland, 2015; Karasek, 2007; Reiter, 1994).

Melatonin has been reported to have positive effects on the regulation of the sleep cycle, and circadian and seasonal rhythms (Mohammadi-Sartang et al., 2018; Yang et al., 2023), but it has been reported that disruption of the proinflammatory and immune modulatory balance may cause circadian rhythm disorder in the elderly as well as the progression of neurological disorders

(Bubenik, 2011). Numerous experimental and clinical investigations have consistently shown that melatonin possesses the ability to regulate cellular stress responses, such as endoplasmic reticulum (ER) stress. Additionally, melatonin has been observed to influence crucial intracellular signaling molecules, including nuclear factor-κB (NF-κB) and NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3), which are involved in inflammatory activation (Chen et al., 2018). Melatonin is an indoleamine with both antioxidant (Tan et al., 2007) and antiestrogenic properties (Alonso-Gonzalez et al., 2008) and many advantages such as low toxicity, high lipid solubility and ease of passage through cell membranes have been reported (Sun et al., 2008; Dun et al., 2021). It has also been reported that melatonin is an excellent antioxidant thanks to its highly efficient reactive oxygen species (ROS) scavenger that can neutralize hydroxyl and peroxyl radicals, activating antioxidant enzymes such as glutathione peroxidase and superoxide dismutase and inhibiting nitric oxide synthase enzymes (Akbulut et al., 2008; Ozdinc et al., 2016) (Figure 2).

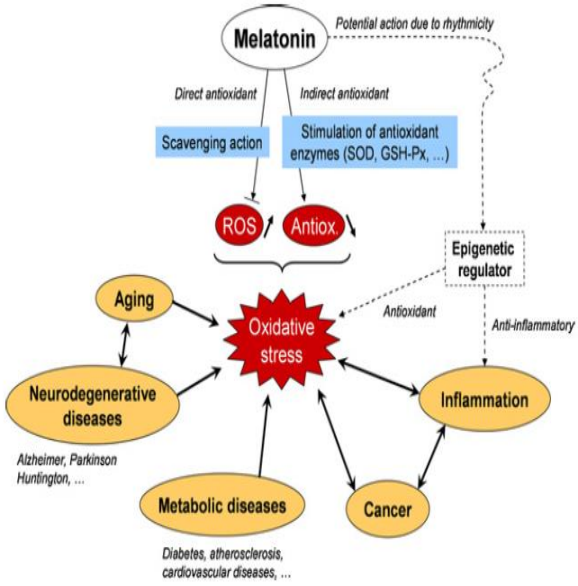

Figure 2. Association of melatonin with oxidative stress and neurodegenerative diseases and other diseases (Bonnefont-Rousselot and Collin, 2010).

The imbalance between the intracellular concentration of ROS and reactive nitrogen species (RNS), which are free radical species, and the intracellular concentration of antioxidants is known as oxidative stress (Thompson et al., 2013). Although the increased concentration of these oxidants in cells damages important biomolecules such as lipids, proteins, and DNA, the immune system protects cells against the destructive effects of these oxidants by scavenging the harmful effects of these free radicals (Akbari et al., 2020) (Figure 2). Oxidative stress has been reported to be associated with various chronic diseases such as neurodegenerative disorders, diabetes, cancer, rheumatoid arthritis, and cardiovascular disease (Modaresi et al., 2015). The strong role of these compounds, which are the cause of oxidative stress, in the process of these chronic diseases has further increased the importance of antioxidant compounds to balance between oxidants and oxidants (Pena-Oyarzun et al., 2018; Loloei et al., 2019). And many dietary recommendations, supplements, and herbal remedies have been recommended to prevent or neutralize the formation of these oxidative agents (Poljsak , 2011; Santos et al., 2020) (Figure 3).

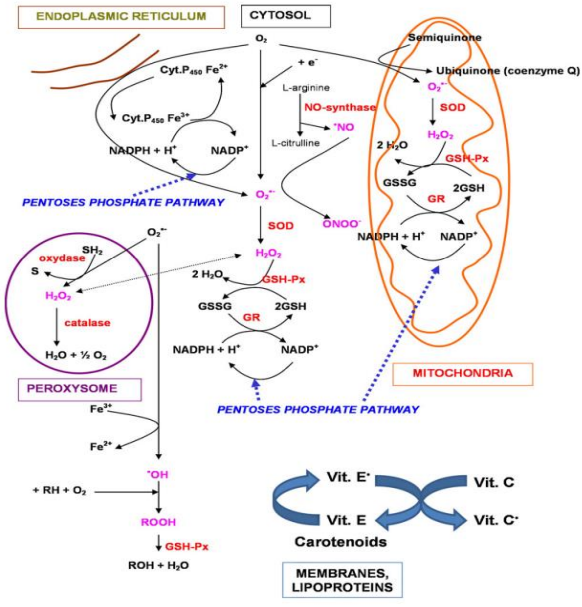

Figure 3. Association of ROS and RNS with antioxidant defenses (Bonnefont-Rousselot and Collin, 2010).

Numerous studies have demonstrated the advantageous effects of melatonin on various metabolic processes, including cell apoptosis (Pingitore et al., 2015), fatty liver disease (Rodriguez et al., 2004), glycemic regulation (Rezvanfar et al., 2017), antioxidant status (Szewczyk-Golec et al., 2017), inflammatory responses, immune system function(Carrillo-Vico et al., 2005; Zarezadeh et al., 2019), membrane fluidity stabilization (Garcia et al., 2014), protection against oxidative stress damage (Poljsak, 2011; Galano and Reiter, 2018), and inhibition of cancer progression (Wang et al., 2012; Jung-Hynes et al., 2010a; Jung-Hynes et al., 2010b). There have been reports indicating that melatonin may play a protective role against neurodegenerative (Joshi et al., 2015) and digestive diseases (Amin et al., 2015). These beneficial effects are thought to be attributed to the antioxidant properties of melatonin, which include the activation of antioxidant enzymes such as glutathione peroxidase, superoxide dismutase, and catalase (Carrillo-Vico et al., 2005, Ortiz-Franco et al., 2017), as well as its potential to capture free radicals (Mohammadi-Sartang et al., 2018).

As previously stated, melatonin exhibits both lipophilic (Roberts et al., 1998) and hydrophilic (Shida et al., 1994) properties, thereby influencing its capacity to traverse physiological barriers, its cellular uptake, and its efficacy in radical scavenging, which are contingent upon its distribution between lipidic and aqueous phases. In a research investigation (Mekhloufi et al., 2007), various model assemblies, including linoleate micelles, phosphatidylcholine liposomes, and LDL, were employed to gain insights into the influence of melatonin on lipid systems. The concentration of melatonin in both the aqueous and lipid phases of each system was assessed using HPLC/UV detection in liposomes and LDL, and fluorescence assays in micelles. The study concluded that melatonin exhibited a higher preference for the aqueous phase of the micelles compared to the lipid phase, with a distribution ratio of 68.4% in the aqueous phase and 30.5% in the lipid phase. In contrast, in phosphatidylcholine liposomes, melatonin was predominantly found in the lipid phase. In the LDL model utilized in the aforementioned study, it was observed that 99.9% of the administered melatonin was detected in the methanol/water extraction phase, which contained phospholipids, unesterified cholesterol, and apolipoprotein B100. Compartmentalization data obtained in such lipid systems may be important in helping to understand melatonin's ability to capture free radicals

according to their site of production and thus protect lipids and/or proteins against the oxidation process. In other words, melatonin's radical trapping efficiency varies according to its partitioning between lipidic and aqueous phases. Melatonin has an advantage over other antioxidants due to its solubility-induced structures that limit its partitioning between intracellular and extracellular compartments (Bonnefont-Rousselot and Collin, 2010).

In the beneficial effect of melatonin in preventing oxidation, another important issue is functional groups. For example, when the acetyl group was replaced with a nonanoyl group, it was observed to significantly increase antioxidant activity (which could be explained by lipophilicity), which was confirmed when it was found that 5-methoxy tryptamine shortened the lag phase time during oxidation of the LDL model (Gozzo et al. 1999). Subsequent examination of this topic has indicated that the presence of the amide function is not an indispensable prerequisite for the antioxidant properties of melatonin. Moreover, the inclusion of an acyl terminal group might serve to inhibit any potential prooxidant tendencies (Tan et al., 1995).

1.1. Effects of Melatonin on Antioxidant Activity (MDA, TAC, GSH, etc.) and Antioxidant Enzymes (SOD, GR, GPx, etc.)

A recent meta-analysis has documented the notable impact of melatonin supplementation on various physiological markers. Specifically, it has been found that melatonin supplementation leads to an elevation in total antioxidant capacity (TAC), glutathione (GSH) levels, superoxide dismutase (SOD) activity, glutathione peroxidase (GPx) activity, and glutathione reductase (GR) activity. Additionally, a significant reduction in serum malondialdehyde (MDA) levels has been observed (Poljsak, 2011). In a previous investigation conducted by Ghorbaninejad et al., it was demonstrated that the administration of melatonin resulted in an elevation of total antioxidant capacity (TAC) and a reduction in protein carbonyl (PCO) and malondialdehyde (MDA) concentrations (Morvaridzadeh et al., 2020). Nevertheless, there exist certain studies that present conflicting findings, displaying inconsistent outcomes concerning the impact of melatonin supplementation on biomarkers of oxidative stress. Additionally, these studies exhibit certain limitations, such as the absence of specific parameters and incomplete information. Certain studies were excluded from prior meta-analyses (Poljsak, 2011; Morvaridzadeh et al.,

2020), which failed to incorporate certain indicators of oxidant/antioxidant status, such as GPx and SOD levels (Morvaridzadeh et al., 2020).

Although significant heterogeneity was observed between studies, some studies (Szewczyk-Golec et al., 2017; Ghorbaninejad et al., 2020; Jamilian et al., 2019; Ostadmohammadi et al., 2020; Ghaderi et al., 2019; Mesri Alamdari et al., 2015; Panah et al., 2019; Raygan et al., 2019; Kari Farzaneh et al., 2019; Zare Javid et al., 2020; Miller et al., 2013) showed that there was no significant decrease in MDA concentrations after oral melatonin supplementation. However, in the study conducted by Zarezadeh et al. to find the possible source of heterogeneity, subgroup analysis was performed, and mean age and intervention duration were reported as high sources of heterogeneity. In the present investigation, the researchers observed a notable reduction in MDA levels following the administration of melatonin supplementation when gender was limited to the male and female subpopulations. The study also found that there was a more pronounced decrease in the observed effect among individuals below the age of 50, as well as those who underwent an 8-week application period (Zarezadeh et al., 2022). The findings of a meta-analysis involving 16 clinical trials examining the effects of melatonin supplementation indicate a statistically significant decrease in levels of malondialdehyde (MDA). Additionally, the analysis reveals a noteworthy increase in total antioxidant capacity (TAC) levels. These results hold true even after conducting subgroup analyses based on various factors such as mean age, gender, dosage, duration of supplementation, and the specific tissue used for measuring MDA and TAC levels, including serum and urine (Zarezadeh et al., 2022). Melatonin takviyesinin TAC düzeyleri üzerindeki etkilerinin incelendiği diğer çalışmalarda ise anlamlı derecede heterojenite olmakla birlikte, oral melatonin takviyesinden sonra TAC düzeyinde önemli artış olduğu rapor edilmiştir [Shida et al., 1994; Ghorbaninejad et al, 2020; , Jamilian et al., 2019; Ostadmohammadi et al., 2020; Ghaderi et al., 2019; Mesri Alamdari et al., 2015; Raygan et al., 2019; Zare Javid et al., 2020; Castano et al., 2018; Elsabagh et al., 2020; Lu et al., 2018). However, in a similar study, Zarezadeh et al. (2019) showed that melatonin had a clinically greater effect on increasing TAC levels in men (40 years of age; treatment dose 15 mg and intervention duration >8 weeks). Another study reported that the supplementation of melatonin resulted in a significant increase in total

antioxidant capacity (TAC) levels and a decrease in malondialdehyde (MDA) levels (Poljsak, 2011).

The concentration of SOD, an antioxidant enzyme, increased significantly after melatonin supplementation (Szewczyk-Golec et al., 2017; Zare Javid et al., 2020; Miller et al., 2013; Elsabagh et al., 2020). Furthermore, it has been documented that melatonin exhibited a noteworthy impact on superoxide dismutase (SOD) levels, which remained significant even after conducting sensitivity analysis. However, the intake of melatonin did not yield a significant effect on glutathione peroxidase (GPx) levels. Nevertheless, subgroup analysis revealed a significant elevation in GPx concentrations among both males and females, particularly at a treatment dose below 10 mg per day. Although this study is also a systematic review and meta-analysis investigating the effects of melatonin on oxidative stress biomarkers (Poljsak, 2011), some analyses were first applied in this study, where major differences in methodology, study population, and statistical analyses revealed significant inconsistencies (Zarezadeh et al., 2022). In 5 separate studies investigating the effects of melatonin supplementation on GPx levels (Szewczyk-Golec et al., 2017; Zare Javid et al., 2020; Miller et al., 2013; Elsabagh et al., 2020), and when the magnitude of the 5 effects obtained from these studies was examined, it was reported that no significant change in GPx levels was observed following oral melatonin supplementation, although significant heterogeneity was found between the studies. Nevertheless, subgroup analysis, gender, and treatment dose, which are thought to be sources of heterogeneity, reported significant increases in GPx levels in studies in which both genders received a treatment dose of ≤10 mg/day. And these results show that these analyses are the cause of heterogeneity. On the other hand, following subgroup analysis, significant decreases in GPx levels were detected in men when the treatment dose was >10 mg/day, indicating that the dose was also important. The average age of the participants and the tissue used had no significant effect on the GPx levels of melatonin, and the use of a single sensitivity analysis did not affect the results of the study (Zarezadeh et al., 2022).

A research study was conducted to examine the impact of melatonin on immunity and the specific activities of antioxidant enzymes in the arthropod crab species Eriocheir sinensis. The results revealed that following the injection of melatonin, there were no significant differences in antioxidant enzyme

activity in the hemolymph among the various test groups. However, the level of malondialdehyde (MDA) was significantly lower in the test groups compared to the control group, except for the group observed 4 hours after injection. Additionally, the superoxide dismutase (SOD) level exhibited significantly higher activity 8 hours after injection compared to the control group. The findings of this study indicate that the outcomes observed in muscle tissue were comparable to those observed in hemolymph tissue. Specifically, it was observed that the activity of antioxidant enzymes in muscle tissue did not exhibit any significant differences between the various time points (1 hour, 2 hours, and 12 hours) following melatonin injection. However, at the 4-hour mark, the group receiving a melatonin injection of 0.01 g/L displayed a significantly lower activity of MDA compared to the control group. Additionally, the groups receiving melatonin injections of 0.001 g/L and 0.0001 g/L exhibited significantly higher activity of SOD compared to the control group. The study also revealed that the catalase (CAT) activity in the group receiving a melatonin injection of 0.0001 g/L was significantly elevated compared to the control group after 4 hours. However, no significant differences were observed between the groups receiving melatonin injections of 0.001 g/L and 0.01 g/L, although their CAT activity was significantly higher than that of the control groups (She et al., 2019).

A study conducted on rodents investigated the effects of melatonin on protein carbonyl formation in the liver, kidney, and brain. The rodents were treated with melatonin to address oxidative stress induced by D-galactose, which was used as a model for natural aging. The study found that melatonin reduced protein carbonyl formation in the aforementioned organs. Additionally, melatonin decreased the production of thiobarbituric acid reactive substances (TBARS) in both serum and brain, and prevented the increase in soluble receptors for advanced glycation end products (sRAGE) caused by D-galactose (Hsieh et al., 2009).

2. Melatonin and Neurodegenerative Diseases

The role of melatonin (N-acetyl-5-methoxytryptamine), known as an endogenous hormone synthesized and secreted by the pineal gland in the brain, in regulating circadian rhythms has been reported in previous studies (Vasey et al., 2021; Yin et al., 2022). Such a role has been reported by demonstrating that

exogenously administered melatonin can easily cross the blood-brain barrier with low toxicity and may have neuroprotective effects in various central nervous system (CNS) diseases. Melatonin has been reported to exert this effect by using different signaling pathways such as pro-survival PI3K/AKT and SIRT1, TLR4/NF-κB and MAPK which are associated with inflammation, Nrf2 and Endothelin-1 which are reported to be associated with oxidative stress (Andrabi et al., 2015; Watson et al., 2006; Alghamdi , 2018). Additionally, melatonin has been reported as a promising neuroprotective agent that can modulate microglial activation and polarization by interacting directly or indirectly with microglia in the CNS (Azedi et al., 2019; Hardeland et al., 2021) ıt was reported that melatonin inhibits intrinsic apoptotic pathways in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and MS (Wang et al., 2009).

2.1. Alzheimer's Disease (AD)

It has been reported that patients with Alzheimer's disease exhibit evidence of macromolecular damage, specifically to nucleic acids, proteins, and lipids, which is believed to be mediated by reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Pratico et al., 2008; Sultana et al., 2010). The process of aging is associated with a gradual decline in the endogenous antioxidant system, leading to an augmented occurrence of oxidative damage to cellular structures. This phenomenon has been particularly observed in the brain regions of individuals (Floyd et al., 2002; Ansari et al., 2010). All of these pieces of evidence have made it more attractive to investigate oxidative stress (Rosales-Corral et al., 2012; Pevet, 2012; Carpentieri et al., 2012) and indirectly antioxidant systems and substances with antioxidant properties for the prevention or treatment of Alzheimer's disease. In relation to this, Shikha et al. reported that oxidation products increased in the brain tissue of mice with Alzheimer's disease (Ansari and Scheff, 2010). The use of an antioxidant such as melatonin for the protection of neurons from oxidative stress, prevention, or treatment of Alzheimer's disease may therefore be therapeutically promising. It is hypothesized that the neuronal loss observed in Alzheimer's disease may be attributed to the apoptosis of neuronal cells induced by radicals. Consequently, the antioxidant properties of melatonin are believed to have the potential to

safeguard neurons against degeneration, thus preventing cell death (Pena-Oyarzun et a., 2018). The efficacy of melatonin in the treatment of chronic diseases is demonstrated by a study that provides evidence of the beneficial effects of melatonin administration on patients with Alzheimer's disease. The study reveals a significant delay in disease progression and a reduction in brain atrophy, as assessed through magnetic resonance imaging (MRI) analysis (Brusco et al., 1998).

In contrast, the regular administration of melatonin in patients with neurodegenerative conditions, such as Alzheimer's disease, at doses ranging from 1-300 mg or 4.3-1291.5 mol (Jan et al., 2000; Seabra et al., 2000), does not appear to induce any toxic or adverse effects upon repeated use. Consequently, melatonin can be employed with confidence in these individuals.

In addition to its direct scavenging of free radicals, melatonin has been found to possess the ability to induce the production of various endogenous antioxidant enzymes. This property contributes to its potent antioxidant activity and potential therapeutic application in the treatment of Alzheimer's disease (AD) (Reiter et al.,2010; Reiter, 1998; Pandi-Perumal et al., 2013; Linet al., 2013). Recent research has demonstrated that melatonin has the potential to augment the growth and specialization of neural stem cells in the hippocampus of adult mice (Ramirez-Rodriguez et al., 2009; Liu et al., 2013). This finding implies that melatonin could be a promising candidate for the development of new therapeutic approaches for Alzheimer's disease.

In contemporary times, particularly in the past few years, there has been a development of multifunctional melatonin-based hybrids that incorporate an anticholinesterase motif. One instance involves the development of novel tacrine-melatonin hybrids, which have been designed as advanced inhibitors of acetylcholinesterase with additional antioxidant properties (Rodríguez-Franco et al., 2006). In addition, there have been efforts to develop novel melatonin N, Nedibenzyl (N-methyl) amine hybrids that possess multiple functionalities, including antioxidant, cholinergic, and neuroprotective properties (Lopez-Iglesias et al., 2014). Furthermore, carbamate derivatives of indolines and cholinesterase inhibitors have been explored as potential therapeutic agents for Alzheimer's disease, as well as for their antioxidant activity (Yanovsky et al., 2012). Also, another new melatonin derivative of the benzyl pyridinium bromide series, inhibiting cholinesterase, In a study in which they were

designed and synthesized as multifunctional anti-AD (Alzheimer's disease) agents with antioxidant and neuroprotective activities, it was shown that most of these compounds exhibited strong inhibitory activity against human-AChE (acetylcholinesterase) and human-BuChE (butyrylcholinesterase) and good antioxidant capacity in ORAC assay (Xiao-Ting et al., 2015).

A comparative study was conducted to investigate the protective effects of vitamin C and melatonin on neuroblastoma cells induced by okadaic acid in Alzheimer's disease (AD). The findings revealed that when cells were exposed to 50 nM okadaic acid for a duration of 2 hours, there was a significant reduction in cellular glutathione transferase, glutathione reductase, and catalase activity. Additionally, an increase in lipid peroxidation was observed. However, treatment of cells with either melatonin or vitamin C effectively mitigated the detrimental effects caused by okadaic acid. The findings of this study demonstrated that the administration of okadaic acid led to an imbalance in oxidative stress. However, the co-administration of melatonin and vitamin C effectively mitigated the oxidative stress induced by okadaic acid. The findings of this study also indicate the significant significance of oxidative stress in both the experimental model and the progression of neurodegenerative diseases, particularly Alzheimer's disease. Furthermore, it is observed that melatonin exhibits greater efficacy in mitigating oxidative stress compared to vitamin C. Additionally, a lower dosage of melatonin is found to be adequate in producing effects on lipid peroxidation comparable to those achieved with vitamin C (Montilla-Lopez et al., 2002).

2.2. Parkinson's Disease (PD)

Parkinson's disease (PD) ranks as the second most prevalent neurodegenerative disorder, following Alzheimer's disease (Nussbaum et al., 2003). The characteristic feature of Parkinson's disease (PD) is the gradual degeneration of dopaminergic neurons in the substantia nigra, accompanied by the aggregation of a-synuclein and the accumulation of Lewy bodies (Cacabelos, 2017). This disease is characterized by a dysfunction in the protein quality control system, which includes protein aggregates, autophagy, the ubiquitin-proteasome system (UPS), and molecular chaperones (Hartl et al., 2011). Therefore, the clearance of protein aggregates or the enhancement of the

protein quality control system represents a crucial objective in addressing this particular disease (Chowdhury et al., 2008).

Melatonin is widely recognized for its potent antioxidant properties and its potential to mitigate the onset of neurodegenerative disorders, including Parkinson's disease. This is attributed to its ability to easily traverse the blood-brain barrier (Chowdhury et al., 2008). Over the past decade, numerous studies have yielded substantial scientific evidence supporting the protective effects of melatonin against Parkinson's disease (PD). Among the notable evidence, it has been observed that individuals with Parkinson's disease exhibit reduced levels of circulating melatonin in their serum (Breen et al., 2014). Additionally, there is an inverse correlation between melatonin levels and the severity of Parkinson's disease (Breen et al., 2016). In a rat model of Parkinson's disease induced by homocysteine, melatonin has been demonstrated to improve mitochondrial complex I activity and protect dopaminergic neurons from oxidative stress (Paul et al., 2018). This is achieved through various mechanisms, including the amelioration of oxidative stress, scavenging of hydroxyl radicals, restoration of glutathione levels, and increased activity of antioxidant enzymes. Additionally, there have been studies indicating that melatonin has the ability to mitigate oxidative stress and the cycle of mitochondrial fragmentation in an in vitro model of Parkinson's disease induced by MPP+ (Chuang et al., 2016). It has also been found to prevent the upregulation of autophagy mediated by CDK5 and reduce the aggregation of a-synuclein in an in vivo model of Parkinson's disease induced by 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) (Su et al., 2015).

Several studies have also presented evidence of the neuroprotective properties of melatonin in animal models of Parkinson's disease induced by 6-hydroxydopamine (Sharma et al., 2006), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Patki et al., 2011), and rotenone (Saravanan et al., 2007).

The study examined the potential protective effects of melatonin against MPP+-induced dopaminergic cell death in an in vitro PD model. The findings indicated that melatonin effectively reduced the levels of reactive oxygen species (ROS) induced by MPP+ and mitigated apoptotic signaling through the involvement of heat shock protein 70 (HSP70). The present study provides empirical evidence supporting the neuroprotective function of melatonin in an in vitro model of Parkinson's disease (PD). According to reports, melatonin has

the potential to mitigate the detrimental impact of MPP+ on dopaminergic cell death by increasing the expression of the HSF1/HSP70 pathway. It has been suggested that augmenting HSF1 and HSF70, which play a crucial role in Parkinson's disease (PD), through the use of melatonin could serve as a promising therapeutic approach for PD (Jung et al., 2022).

The existing literature suggests that melatonin's protective effects in Parkinson's disease (PD) are attributed to its ability to inhibit various pathways associated with oxidative stress, apoptosis, neuroinflammation, autophagy, and a-synuclein aggregation. However, further studies are required to gain a comprehensive understanding of the precise mechanism by which melatonin exerts its effects in PD models.

2.3. Huntington's disease (HD)

The etiology of this pathological condition was documented as a duplication of the cytosine-adenine-guanine (CAG) protein in exon 1, leading to initial impairment of the striatum and cortex (Macdonald et al., 1993) The precise role of this protein remains incompletely understood to date (Ross and Tabrizi, 2011).

As antioxidants are frequently used in the treatment of neurological diseases, melatonin has again been the focus of studies in this sense. Experimental models in experimental studies of this disease usually used mitochondrial complex II inhibitors (3-nitropropropionic acid) or quinolinic acid (Tunez et al., 2004).

One study demonstrated the impact of melatonin on protein carbonyl, superoxide dismutase (SOD), and succinate dehydrogenase activities in a rat model of Huntington's disease induced by 3-nitropropropionic acid. The study reported that melatonin's antioxidant properties exhibited a delay in the onset of the disease (Tunez et al., 2004). Previous studies have demonstrated that melatonin has the ability to mitigate oxidative damage induced by quinolinic acid in both rat brain homogenate (Cabrera et al., 2000; Southgate and Daya , 1999) and brain tissue culture (Garcı´a et al., 2005). Another study has reported on the potential influence of melatonin dysregulation on the pathogenesis of Huntington's disease (Aziz et al., 2009). Other mechanisms involved in the pathogenesis of HD include intracellular calcium accumulation leading to

mitochondrial blockade, induction of the N-methyl-D-aspartate (NMDA) receptor, and the presence of oxidative stress (Brusco et al., 1998).

However, the administration of quinolinic acid (Southgate and Daya, 1999), which is also involved in the pathogenesis of various degenerative, infectious, and inflammatory human neurological diseases, has been reported to produce striatal lesions similar to those indicative of Huntington's disease (Bruyn and Stoof, 1990) such as stereotyped motor changes (Rossato et al., 2002).

Due to the limited number of experimental studies on the prevention and treatment of HD disease, more studies are needed to observe the antioxidant and neuroprotective effects of melatonin on HD disease.

2.4. Amyotrophic lateral sclerosis (ALS) Disease

Amyotrophic lateral sclerosis (ALS), a debilitating neurodegenerative disorder, impacts the primary and secondary motor neuron pathways, leading to impairment in the anterior horn of the spinal cord and disruption in the myelination of motor neurons. From a pathophysiological perspective, it has been documented that amyotrophic lateral sclerosis (ALS) disease can be attributed to three primary causes. The foremost and most significant mechanism involves a mutation in the superoxide dismutase (SOD) 1 gene, resulting in the accumulation of toxic substances such as tyrosine nitration. Additionally, this mutation leads to a decrease in the antioxidant effect by interfering with zinc binding capacity. Another contributing factor is selective motor axon degeneration, which is associated with mutations in neurofilament genes and the phosphorylation of cytoskeletal proteins. Lastly, increased glutamate levels in the cerebrospinal fluid and impaired function of excitatory amino acid transporters have been identified as the third pathophysiological mechanism. These conditions also give rise to excitotoxicity (Difiglia et al., 2007).

Prior research has indicated that the presence of ROS/reactive nitrogen/oxygen-induced oxidative stress is notably significant when analyzing the pathophysiological mechanisms of the disease. Consequently, therapeutic approaches may prioritize the inhibition of these radicals associated with oxidative stress (Reiter, 1998). As previously stated, melatonin has been suggested as a potential therapeutic option for individuals with amyotrophic

lateral sclerosis (ALS) who are able to tolerate elevated dosages. This recommendation is based on the observation that high doses of melatonin do not appear to elicit any adverse effects. The initial investigation into the utilization of melatonin in this particular patient population was documented in 2002, involving a sample size of three individuals (Jacob et al., 2002). A study documented the preventive effects of administering high-dose oral melatonin on disease progression and survival in SOD1 (G93A)-transgenic mice (Polimeni et al., 2014). Another study observed alterations in SOD1 expression in the lumbar spinal cord of newborn rats following melatonin administration (Rogerio et al., 2005). Additionally, a separate study reported the reduction of oxidative parameters through the use of high-dose melatonin (Weishaupt et al., 2006). In a separate clinical investigation, it was documented that the rectal administration of melatonin at a high dosage over a period of two years was well-tolerated. Notably, the inclusion of melatonin led to a reduction in the levels of oxidative stress markers, which are typically elevated in individuals with amyotrophic lateral sclerosis (ALS). Consequently, the utilization of high-dose melatonin in human subjects may be deemed appropriate for the treatment of ALS (Weishaupt et al., 2006).

Numerous studies have demonstrated the effectiveness of melatonin in experimental models of ALS, including those conducted on humans, animals, and cellular systems., these studies are limited, and more comprehensive clinical studies are needed.

2.5. MS Disease

Multiple sclerosis (MS) is a disease associated with immune mediators, which commonly affects the white matter and, to a lesser extent, the gray matter (Wootla et al., 2012). Similar to numerous other inflammatory diseases, MS predominantly affects women in the age range of 20 to 30 years. The disease exhibits a range of symptoms, which may vary in accordance with seasonal fluctuations (Farez et al., 2015).

Although melatonin is known to accumulate in various parts of the cell, especially in the mitochondria, recent studies have shown that melatonin levels are lower in MS patients (Natarajan et al., 2011). Early in the 1990s, Sandyk and Aweerbuch conducted a series of studies emphasizing the possible role of melatonin in the pathogenesis of MS, first reporting that melatonin levels were

low in 44% of MS patients before steroid administration (Sandyk and Awerbuch, 1992), and that there was a correlation between melatonin levels and age of disease onset and epiphyseal calcification, while there was an inverse correlation between melatonin levels and disease duration (Sandyk and Awerbuch, 1992; Ramos González et al., 2018; Sandyk and Awerbuch, 1994b). A subsequent cohort study of 32 patients found that at low melatonin levels, disease duration was 5 years longer than normal (Sandyk and Awerbuch, 1994 a). In a subsequent study by the same researchers, a higher incidence of pineal calcification and brain atrophy was reported in MS patients (Sandyk and Awerbuch, 1994).

Further research confirmed that serum (Sandyk and Awerbuch, 1994b; Akpinar et al., 2008; Bahamonde et al., 2014; Escribano et al., 20016) and plasma levels of melatonin (Bahamonde et al., 2014) as well as urinary levels of 6-sulfatoxymelatonin (aMT6-s)-a major melatonin metabolite-are significantly lower in MS patients compared to healthy subjects (Farez et al., 2015; Ghareghani et al., 2018; Farhadi et al., 2014; Damasceno et al., 2015; Gholipour et al.,2015). However, in a study by Ghorbani et al., it was reported that no difference was found in serum melatonin levels between MS patients and controls (Melamud et al. 2012). A similar study reported an inverse correlation between melatonin and fatigue severity, number of relapses, Expanded disability status scale (EDSS score), and disease exacerbation contributing to the seasonality of MS (Farhadi et al., 2014; Damasceno et al., 2015).

An additional noteworthy aspect of the association between melatonin and this particular disease is the heightened prevalence of multiple sclerosis (MS) among individuals engaged in nocturnal employment. It has been postulated that individuals engaged in night shift work exhibit an inherent reduction in melatonin levels, which may potentially contribute to the onset of multiple sclerosis (MS). Consequently, it has been documented that diminished melatonin levels could potentially serve as a risk factor for the development of MS (Schernhammer et al., 2004; Hedström et al., 2011). Research investigating the impact of melatonin on the quality of life of individuals with multiple sclerosis (MS) has demonstrated that melatonin administration leads to enhancements in quality of life. One study, comprising a sample of 102 individuals diagnosed with multiple sclerosis (MS) and 20 healthy volunteers,

administered a daily dosage of 5 mg of melatonin. The findings of this study indicated a decrease in levels of malondialdehyde (MDA), an improvement in the quality of life among the patients, and an increase in Multiple Sclerosis Impact Scale (MSIS) scores (Adamczyk-Sowa et al., 2014). Subsequent investigations have indicated that the administration of melatonin exhibits a mitigating effect on multiple sclerosis (MS) through the inhibition of cytokines that promote inflammation. This effect is primarily mediated by the modulation of various receptors, particularly the reduction of IL-17 secretion by TH17 cells via the MTNR1A receptor, as well as the augmentation of IL-10 secretion by Tr1 cells. Consequently, the administration of melatonin contributes to the reduction of inflammatory processes associated with MS (Pevet, 2000). There have been reports indicating that melatonin can enhance the release of IL-27 from dendritic cells, resulting in the release of IL-10 (Mascanfroni et al., 2013). Additionally, melatonin has been shown to elevate the levels of cytokines such as IL-2, IL-12, IFNg, and TNF, and has demonstrated efficacy in the treatment of patients with multiple sclerosis (Alvarez-Sanchez et al., 2017).

Conclusion and Recommendations

As mentioned in this review, melatonin is a powerful antioxidant, free radical scavenger, anti-inflammatory, circadian rhythm, and neuroregulatory hormone. We have also reviewed the existing literature and meta-analyses that explain melatonin's scavenging actions by neutralizing free radicals and converting them into less harmful species through enzyme activity. We have also tried to analyze and show the positive effects on neurological diseases through studies. Based on the available data, it can be suggested that melatonin is a reliable therapeutic model to strengthen the protective and therapeutic antioxidant defense system against neurological diseases.

Although all these effects depend on the biological and chemical properties of melatonin, it was mentioned in the literature analysis that the metabolites of melatonin have the same beneficial properties. While many studies provide evidence that the effects of these metabolites are stronger than melatonin alone, detailed studies on this subject may provide answers to the questions of which metabolites of melatonin may be more effective or more effective against which diseases.

Abbreviations

AD: Alzheimer's Disease

ALS: Amyotrophic lateral sclerosis

CNS: central nervous system

DG: D-galactose

GPx: glutathione peroxidase

GR: glutathione reductase

GSH: glutathione

HD: Huntington's disease

LDL: low-density lipoprotein

MDA: malondialdehyde

Melatonin: N-acetyl-5-methoxytryptamine

NLRP3: pyrin domain-containing protein

PD: Parkinson's Disease

PCO: protein carbonyl

RNS: reactive nitrogen species

ROS: reactive oxygen species

SOD: superoxide dismutase

TAC: antioxidant capacity

TBARS: Thiobarbituric acid reactive substances

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CHAPTER 8

NAVICULAR SYNDROME IN HORSES

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INTRODUCTION

Navicular syndrome arises from closely related structures such as the navicular bone (distal sesamoid bone), musculus flexor digitorum profundus, bursa navicularis, distal sesamoidean ligament or collateral sesamoidean ligaments (Waguespack and Hanson, 2010; Gerdes, 2015). Collectively, these structures are called the podotrochlear apparatus. Navicular disease is a common cause of forelimb lameness (Gerdes, 2015; Fraser, 2016; Salinas et al., 2021). It is a chronic, degenerative and progressive, bilateral disease characterized by an increase in the force exerted by the musculus flexor digitorum profundus tendon (DDFT) on the distal sesamoid bone and therefore on the distal interphalangeal joint. It also includes the collateral sesamoid ligament, distal sesamoid ligament, and podotrochlear bursa (Waguespack and Hanson, 2010; Dyson, 2011a; 2011b; Dyson et al., 2011; Coomer et al., 2013; Salinas et al., 2021). It is characterized by structural changes such as fibrillation in the flexor tendons, adhesion formation, and the presence of metaplastic fibrocartilaginous areas rich in elastic fibers, especially in the proximal part of the bursa navicular (Dyson et al., 2011; Salinas et al., 2021).

However, the hindlimb can also be affected. Navicular syndrome causes intermittent chronic lameness with various etiologies (Dyson et al., 2011; Gerdes, 2015; Lee at al., 2021). Therefore, it is not surprising that its clinical presentation varies significantly. While some horses show acute, sudden onset and severe, unilateral lameness, sometimes it shows insidious onset, mild, slowly progressive bilateral forelimb lameness (Dyson et al., 2011;Waguespack and Hanson, 2011; Gerdes, 2015).

Although adult riding horses are most affected, navicular disease can also be diagnosed in young horses that begin working at an early age. Navicular syndrome is also seen in different horse breeds and horses with different foot structures; For example, it can be seen in "flat feet" Thoroughbred horses with long pastern/low heel structure, as well as in warmblood horses with upright or narrow foot structure (Gerdes, 2015).

1. ETIOLOGY

The hoof contains a small bone called the distal sesamoid (navicular bone), located behind the joint of the short pastern bone and the coffin bone. The primary function of the navicular bone is to provide an insertion angle to

stabilize the musculus flexor digitorum profundus tendon (DDFT) (Stephens and Hoopes, 2023). Significant forces are applied to the navicular bone when the foot is weight-bearing, which can lead to degeneration of overlying structures (Bowker et al., 2001; Wilson et al., 2001; Stephens and Hoopes, 2023). Over time, it creates pain and lameness. Other biomechanical factors that may contribute to this syndrome include large body weight, incorrect shoeing technique, improper hoof trimming and defective hoof structure, as well as overly intense training. It has also been suggested that vascular changes in the nail may cause navicular syndrome. Reduced blood flow to the hoof can lead to pain and bone necrosis. It can be said that the narrow nail structure with long heels prevents adequate blood flow to the digital vessels and increases the risk of degeneration (Stephens and Hoopes, 2023).

The exact cause of pain and lameness in horses with navicular disease is not fully understood (Gerdes, 2015). Biomechanically, the navicular bone is subjected to great stress during landing and hyperextension of the distal interphalangeal joint. Different foot conformations can change the forces applied to the foot (Wilson et al., 2001; Eliashar et al., 2004; Gerdes, 2015). For example, low heels cause increased dorsiflexion and higher pressure on the palmar and distal aspects of the navicular bone. Recently, lesions in the musculus flexor digitorum profundus (DDFT) in the finger region have been noticed clinically. However, available information on the pathophysiology of these injuries is limited. Ruptures, dorsal fibrillation or abrasions, and core lesions can be detected in DDFT. Primary lesions may result from repetitive stress or acute trauma superimposed on pre-existing degenerative changes. More common are secondary lesions such as dorsal fibrillation with navicular syndrome. Proteoglycans have been shown to increase in the distal DDFT and distal sesamoidean ligament in response to stress in older horses. It has also been suggested that low or weak heels make horses more susceptible to DDFT lesions (Gerdes, 2015).

The footing of horses with navicular syndrome varies compared to healthy horses (Gerdes, 2015; Salinas et al. 2021; Stephens and Hoopes 2013). In a study conducted by McGuigan and Wilson (2001), they examined the effect of palmar digital nerve anesthesia on the compression forces applied to the navicular bone in horses with navicular syndrome. In these horses, a paradoxical reaction occurs, such as contraction of the musculus flexor

digitorum profundus, in an attempt to evacuate the heel. This causes increased force on the navicular bone and musculus flexor digitorum profundus. This increased force was reduced when local anesthesia was administered to the horses' palmar digital nerves. The study also showed that there was no difference in the actual amount of force achieved during the stance phase between intact horses and horses with navicular syndrome. The main difference was that peak force was reached much earlier in the lame group, resulting in a higher loading rate in horses with navicular syndrome. Radiological studies on the shape of navicular bones (Dik and Van den Broek, 1995; Salinas et al., 2021) have shown that the shape of the proximal joint edge in the dorsoproximal-palmarodistal oblique view may affect the risk of developing navicular disease. Horses with a concave or wavy-shaped proximal margin, as commonly seen in warm-blooded horses, show more radiological changes associated with navicular syndrome and are at higher risk of developing the disease (Gerdes, 2015).

2. DIAGNOSIS

Navicular syndrome can be difficult to diagnose and often requires a combination of several diagnostic tests (Gerdes, 2015; Salinas et al., 2021).

2.1. Clinical Examination

On clinical examination, horses with lesions proximal to the musculus flexor digitorum profundus tendon in the digit region may be sensitive to palpation in the distal palmar pastern region. Some horses will respond to static flexion of the distal limb (Coomer et al., 2013). Lameness examination should be performed as usual. Walking on hard surfaces is important to accentuate slight lameness. In horses with greater or equal lameness on soft surfaces, significant soft tissue damage is suspected (Stephens and Hoopes, 2023).

2.2. Diyagnostik Anestezi

Lameness caused by navicular syndrome is significantly improved by blockade of the palmar digital nerves. Sometimes it may be necessary to block the palmar digital nerves at the level of the abaxial sesamoid. Due to the proximity of structures that could potentially cause inferior lameness, extreme caution should be exercised when interpreting blockage results. It is important to exclude possible pathology in the pastern and fetlock areas, especially in

cases where the abaxial sesamoid nerve block is positive. Primary pastern or fetlock joint pain can be ruled out by intrasynovial anesthesia of these joints in a separate case. In horses with bilateral lameness, alternation of lameness is observed in the contralateral limb following a positive block. Horses with navicular syndrome may respond fully or partially to anesthesia of the distal interphalangeal joint and/or navicular bursa. Positive blockades can help determine a treatment plan as well as make a diagnosis. For example, horses that respond favorably to diagnostic anesthesia of the navicular bursa are more likely to recover with drug therapy in the same area. A negative response to analgesia of the distal interphalangeal joint or navicular bursa reduces the likelihood of the horse developing navicular disease (Gerdes 2015).

In a study by Dyson (2003) examining 46 horses with tendonitis in the musculus flexor digitorum profundus of the fingers, lameness disappeared in 24% of the horses after palmar digital blockade. A total of 68% of horses with primary tendinitis responded to intra-articular anesthesia of the distal interphalangeal joint, while 92% of horses with navicular bone abnormalities improved significantly. A report by Ross (1998) found that 164 horses responding favorably to diagnostic local anesthesia of the medial and lateral palmar digital nerves were caused by a range of conditions (pedal bone fractures, laminitis, other diseases of the foot), including navicular syndrome, pathology in the distal and proximal interphalangeal joints. He reported having a lameness. This highlights the uncertainty of palmar digital nerve blockade in the localization of lameness in the foot. Bowker et al. (2001) showed that the majority of sensory fibers of the navicular bone and collateral sesamoidean ligaments are located dorsal to the suspensory ligaments of the navicular bone and directly subsynovial of the distal interphalangeal joint. Therefore, it is important to consider the possibility of desensitization of these nerves when applying local anesthesia to the distal interphalangeal joint.

2.3. Diagnostic Imaging

2.3.1. Radiography

Hoof radiography is the standard imaging method of choice for navicular syndrome. To fully evaluate navicular disease radiographically, the horseshoes should be removed and the feet should be thoroughly cleaned to avoid artifacts created by dirt on the soles of the feet (Gerdes, 2015).

Correct positioning during image acquisition is of great importance for image interpretation. A complete lateromedial (LM) position is necessary to evaluate the sagittal projection of the flexor aspect of the navicular bone, to determine the proximal to distal thickness of the flexor cortex, the uniformity of the opacity of the flexor cortex, the boundary between the endosteal surface of the flexor cortex of the navicular bone and the trabecular bone of the spongiosa, and the presence of dorsoproximal periarticular osteophytes. Correct positioning of the navicular bone in dorsoproximal-palmarodistal oblique (DPr-PaDiO) views is important. Thus, the distal border of the bone does not superimpose on the distal interphalangeal joint. Over- or underflexion of the fetlock are the most common causes of overlapping of the distal border of the navicular bone over the distal interphalangeal joint. Two images at slightly different angles may be required to evaluate the dorsal and palmar aspects of the proximal and distal borders of the bone (Dyson, 2011a; 2011b).

Correct position of the extremity and angle of the x-ray beam are crucial to obtaining the diagnostic palmaroproximal-palmarodistal oblique (PaPr-PaDiO) view of the navicular bone. To prevent the fetlock from overlapping the navicular bone, the foot should be positioned caudal to the opposite foot with the fetlock joint extended. The x-ray should be tangential to the flexor aspect of the navicular bone. The exact angle depends on the shape of the foot. In horses with low heels, a shallower angle with the ground is appropriate, while in horses with upright ankles, the angle should be steeper. Improper angulation results in artificially increased opacity of the spongiosa and lack of definition between trabecular and cortical bone (Dyson, 2011b).

2.3.2. Magnetic Resonance Imaging (MRI)

MRI is frequently performed in cases with navicular syndrome. It is the ideal advanced imaging method used in the evaluation of the podotrochlear apparatus. MRI is particularly useful in cases where radiographs are inconclusive, but is also performed to evaluate the full extent of pathology in cases with obvious radiographic findings (Dyson and Murray, 2007a; Gerdes, 2015). MRI reconstructs a three-dimensional image of the extremity, which can then be viewed as two-dimensional slices in any plane (normally sagittal, frontal and transverse). Three-dimensional cross-sectional reconstructions can also be created (Dyson et al., 2003; Dyson and Murray, 2007a; Coomer et al.,

2013). Standing low-field scanners allow performing MRI in the sedated horse and obtaining high-quality MRI images (Dyson et al., 2003; Gerdes, 2015).

2.3.3. Computed Tomography (CT)

CT is used to evaluate localized lameness in the hoof. Like MRI, CT reconstructs a three-dimensional image of the extremity, which can then be viewed as two-dimensional slices in any plane (normally sagittal, frontal and transverse) (Dyson and Murray, 2007a; Coomer et al., 2013). CT and MRI demonstrate abnormalities that cannot be identified on radiography and ultrasonography, making CT and MRI superior diagnostic tools (Dyson and Murray, 2007a; Lee et al., 2021).Vallance et al. (2012) compared low-field MRI and CT (plain images and contrast-enhanced images) techniques for both anatomical visualization and lesion identification of horses with localized lameness in the foot. Similar visualization scores were assigned to most structures of the navicular apparatus for MRI and plain CT. MRI had an advantage in imaging the distal aspects of the DDFT and the distal sesamoidean ligament. Results from comparison of lesion identification between methods showed that CT was poor at recognizing DDFT lesions distal to the proximal aspect of the navicular bone. MRI focusing on the podotrochlear apparatus may fail to identify a proximal soft tissue lesion such as mineralization.

2.3.4. Nuclear Scintigraphy

Naviküler hastalığı olan atların naviküler kemiğinde artmış kemik döngüsü görülür. Bu artan radyonüklid alımıyla gösterilir. Gama sintigrafisi artan kemik döngüsünü tespit etmek için hassas bir yöntem sunmasına rağmen, bu bulgunun mutlaka patolojik değişiklik veya mevcut ağrı ile ilişkili olduğu söylenemez. Bazı atlar, distal interfalangeal eklem ve/veya naviküler bursa analjezisine, tespit edilebilir radyolojik anormallikler olmadan, ancak naviküler kemikte artan radyofarmasötik alımıyla pozitif yanıt verir(Dyson and Murray, 2007b, Gerdes, 2015). Primer kemik nekrozu veya son dönem naviküler hastalığı olan vakalarda kemik taraması görüntüleri normal olabilir.

2.3.5. Ultrasonography

Ultrasound of the foot is not routinely used to evaluate horses with navicular syndrome. There are numerous publications on the anatomy and use

of foot ultrasonography. The method has significant limitations in visualizing and identifying lesions affecting the podotrochlear apparatus (Gerdes, 2015).

3. TREATMENT

Once it is determined that navicular syndrome is the cause of lameness, many treatment options are available. The choice of treatment for each case depends largely on the exact diagnosis and the structures involved. The approach will depend on many factors, including the severity and chronicity of the lesion, as well as the horse's age, intended use and current exercise level. In this case, other orthopedic conditions such as hind leg lameness or back pain should be considered. The most common treatment options are listed below (Waguespack and Hanson, 2011; Gerdes, 2015).

3.3.1. Conservative Treatment/Management Options

- Long-term rest
- Organizing exercises
- Therapeutic nail care (nail cutting and shoeing), regulation of biomechanics
- Systemic NSAIDs
- Corticosteroid injection into navicular bursa
- Intrasynovial injection of IRAP (interleukin I receptor antagonist protein) or other biological agents into the navicular bursa
- Tiludronate (bisphosphonate) – intravenous infusion

3.3.2. Surgical Treatment Options

- Navicular bursoscopy
- Palmar digital neurectomy

Therapeutic foot and hoof care and reorganization of biomechanics are an important part of the treatment of horses with navicular syndrome (Waguespack and Hanson, 2011; Gerdes, 2015). Therefore, a good working relationship and teamwork between the farrier and the veterinarian is important. Both parties must agree on the trimming and shoeing protocol for each case. In general, certain principles should be applied, taking into account the structure of the horse's foot, and changes should be implemented gradually over several

trimming/shoeing periods. Sudden or radical changes may cause increased lameness. The positive effect of hoof care on the degree of lameness will be greater in horses with improper shoeing or poor balance at the time of diagnosis. The general principles to be applied in horses with navicular syndrome are to correct the dorsopalmar and lateromedial foot balance, to shorten the nail tip to alleviate breakage, to protect the heel support and to protect the palmar foot from shaking. The axis of the sole of the foot should be straight. However, this may not be achieved depending on the conformation. Elevating the heels can provide relief to the horse in the short term, especially in horses with tendon injuries. However, the negative effect of heel height on heel mass in the long term should be taken into consideration.

4. CONCLUSION

Navicular syndrome can be a serious disorder, especially because its etiology is unknown and it is often chronic and progressive. Although the prognosis is poor in most cases, correct nail trimming and care, maintaining hoof balance, and appropriate medical treatment will help relieve pain, reduce lameness, and minimize the degenerative process.

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CHAPTER 9

THE IMPORTANCE OF VETERINARY PUBLIC HEALTH IN ONE HEALTH APPROACH

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DOI: https://dx.doi.org/10.5281/zenodo.10458721

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Throughout history, Veterinary Medicine, Human Medicine, and Public Health have been interconnected due to the close relationship between humans and animals needs (Steele, 1991). The integration of health services for the community is seen as crucial for promoting a healthier and happier society. Similar to how health centers and family medicine play a significant role in human medicine within public health, Veterinary Public Health service units serve as the foundation for Veterinary Medicine in public health and protection, focusing on preventive medicine principles (Serpen and Temizyürek, 2010). This collaboration between Public Health and Veterinary Public Health is now recognized as the One Health Concept. The concept was initially proposed by Prof. Dr. Rudolf Ludwig Karl Virchow, who believed that the distinction between humanitarian and veterinary medicine should not exist. This idea was further supported by Sir William Osler, a Canadian humanitarian physician, who highlighted the complementary nature of Veterinary Medicine and Human Medicine. This One Medicine Concept emphasizes the importance of cooperation between different medical fields to benefit of both humans and animals (Cevizci and Erginöz, 2009; Serpen and Temizyürek, 2010).

1. PUBLIC AND VETERINARY PUBLIC HEALTH

The concept of public health goes beyond medicine and encompasses a wide range of professional groups. However, in the 20th century, there was an increased awareness among societies about animal diseases and zoonoses, thanks to certain reforms. This led to the recognition of the field of Veterinary Public Health, highlighting the contributions of veterinarians to public health, particularly in terms of epidemiology and zoonoses. Veterinarians play a crucial role in protecting public health through their work on domestic animals, wildlife, and environmental health. The disciplines of Public Health and Veterinary Public Health have become highly intertwined and parallel. Veterinarians are responsible for various tasks, including ensuring the safety of animal foods for human consumption, controlling zoonoses, combating bioterrorism, maintaining ecological balance, implementing vaccination protocols for infectious diseases, treating chronic diseases that affect human and animal welfare, and conducting medical research for social welfare. (Capua and Alexander, 2008; Cevizci and Erginöz, 2009).

In previous years, the importance of studies and epidemiological data on human health provided by veterinarians in Turkey was poorly understood and ignored. However, in recent times, both in the world and in Turkey, some viral zoonosis outbreaks (Crimean Congo Hemorrhagic Fever, Avian Influenza, Zika virus, Covid-19, etc.) have highlighted the importance of joint work, and in this respect, especially Veterinary Public Health organizations have gained momentum.

In order to express the importance of the concept of One Medicine, it is necessary to examine the stages of zoonotic diseases in the world until today and to emphasize the importance of zoonoses in terms of Public Health. In this context, the total number of zoonotic pathogens has increased from 86 in 1950 to over 200 today, and new ones are constantly being added to these (Horby et al., 2014). The fact that some of these have regained importance is among the biggest reasons for this process, and the term One Medicine to come to the agenda (Cardiff et al., 2008; Zinsstag et al., 2011).

2. IMPORTANCE OF ONE HEALTH CONCEPT

The "One Health Concept" emphasizes the link between animal and human health, highlighting the importance of animal health for global health. As many diseases that affect humans are transmitted through animals, it is crucial to prioritize animal health. This concept is supported by scientific authorities globally. The "One Health Concept" recognizes that healthy animals and animal products are essential for public health. Quality animal products and animal health positively affect human health and overall well-being. To successfully implement the One Health Concept, veterinarians play a vital role in ensuring animal health and welfare and the safety of animal food. They also play a crucial part in protecting Public Health concerning zoonotic diseases, which can be transmitted between animals and humans. The statements of the prominent responsible persons of veterinarians are also given below.

- The increase in the number of zoonoses every year and the recurrence of these diseases on earth, as well as the re-emphasis of some diseases.
- The need for personnel trained in the environmental, human, and animal origin of many diseases and the complexity of relationships.

- The fact that Veterinarians and Humanitarians form common knowledge pools in basic medical sciences such as Physiology, Pathology, Epidemiology, and Veterinarians have a broad scope of knowledge due to the wide range of species in zoonotic diseases.
- Increasing problems regarding food safety/food safety and provision of protein sources.
- The need to save time by ensuring an integrated surveillance system for the early detection of pathogens that can be transmitted from animals to humans.

The concept of "One Medicine", and "One Health" is crucial in addressing zoonotic and infectious diseases that threaten public health. It involves collaboration between veterinarians, humanitarians, and other health professionals to achieve total health for people, animals, and the environment. The One Health Approach emphasizes the need for different disciplines to collaborate locally, nationally, and globally. Zoonotic and vector-borne diseases, which involve human-environment-animal components, can only be effectively controlled through this approach. The concept also highlights the importance of seamless interaction between veterinary and human medicine, with clinicians, researchers, institutions, and governments all working together to benefit domestic and wild animals, human health, and the global environment (Day, 2011). In 2010, the World Health Organisation (WHO), the Food and Agriculture Organisation of the United Nations (FAO), and the World Organisation for Animal Health (OIE) issued a joint declaration to endorse interdisciplinary cooperation and introduce the concept of "One Health".

3. ZOONOTIC DISEASES

Historical records show that animals were domesticated by humans around 6000 BC. People regularly used animals for work, food, transport, clothing, shelter, and companionship. Even though animals have served humanity well, there is a darker side to the human/animal relationship, and that darker side is disease (Bryant, 2009). In this context, today, there are more than 200 diseases that can be transmitted from animals to humans and are known as zoonoses. Some scientists suggest that many new infectious diseases emerged in the Neolithic Age or later due to close contact with domestic animals and their pathogens (Trueba, 2014). In the investigation of zoonotic infections,

cooperation between professional associations is provided by the "Medical-Veterinary-Network (Med-Vet-Net)" within the European Union (EU) (Cevizci and Erginöz, 2009).

Zoonotic diseases and public health problems related to wildlife, the risk of transmission of zoonotic infections from the past to the present has manifested itself with accidents caused by toxins or bites of arthropods/vertebrates. Following the establishment of WHO in 1948, which is the only organization that deals with the health problems of people in the world without discrimination after the Second World War, American Veterinary Virologist Prof. Dr. Martin M. Kaplan was assigned to establish the Veterinary Public Health Department within WHO and the Veterinary Public Health Department was established by Kaplan. Immediately afterward, the first zoonosis meeting in the world was held jointly in Italy in 1950 by WHO and FAO experts. In this meeting, the definition of Veterinary Public Health was put forward, and it was declared to the whole world as "Veterinary Public Health covers all social events that are affected and affected by Veterinary Medicine for the prevention of diseases, the protection of life and the productivity and welfare of humanity (Kaplan and Bogel, 1991; Steele, 1991)." The first zoonotic diseases that the World Health Organisation focused on after its establishment are known as Rabies and Brucella (Kaplan and Bogel, 1991). This was followed by Q fever, Hydatidosis, Leptospirosis, Bovine Tuberculosis, and other diseases. Today, it is possible to talk about many zoonotic infections transmitted by bacterial, viral, parasitic, or vector-borne agents. Especially in recent years, in addition to Crimean-Congo Haemorrhagic Fever, zoonotic diseases transmitted by vectors such as leishmaniasis, which are also accepted as "neglected tropical diseases," are frequently studied diseases. Veterinarians and humanitarians should work in cooperation and coordination in the fight against and control of all these zoonoses. Establish a protection-control strategy as People is vital for Health.

3.1.Q Fever

Q fever is a zoonotic infection caused by Coxiella burnetti, an obligate intracellular microorganism. The reservoirs of Coxiella burnetti are numerous and include domestic and wild mammals, birds, and arthropods. The most important reservoirs are cattle, sheep, and goats (Özbey et al., 2009). Ticks are

the vector of Coxiella burnetti in nature. The transmission of the agent from ticks to both reservoirs and humans can be transstadial, and transovarial transmission, known as transmission to the next generation of ticks, is also possible. The fact that this agent has been found in more than 40 species of ticks so far enables us to understand better the importance of the role of ticks in the transmission and spread of the disease (Porter et al., 2011). However, inhalation of contaminated aerosols, consumption of fresh products of infected livestock, and contact with infected animals are more critical than tick-borne infections in humans (Kanaute et al., 2017; Mobarez et al., 2017). In areas endemic to this disease, control, and elimination may be possible through interventions that simultaneously assess animal housing and human interactions from a single health perspective (Kanaute et al., 2017).

3.2.Crimean-Congo Haemorrhagic Fever

In recent years, viral diseases transmitted by ticks have been among the infectious diseases of increasing importance. Crimean-Congo hemorrhagic fever (CCHF) has the widest distribution area among tick-borne viral diseases. It has been reported from more than 30 countries in Africa, Asia, the Balkans, Ukraine, Crimea, South Russia, and the Middle East (Whitehause, 2004; Yılmaz et al., 2009). CCHF is a life-threatening hemorrhagic disease caused by tick-borne viruses. Transmission to humans is caused by blood-sucking or crushing of infected ticks, as well as contact with blood or tissues of viremic animals and infected humans (Whitehause, 2004; Maltezou, 2010). The CCHF virus belongs to the genus Nairovirus in the Bunyaviridae family. CCHF virus persists in nature in the tick-vertebrate host-tick cycle. CCHF affects many organs and systems, causing ecchymoses and hemorrhages in internal organs; the liver is a disease characterized by dysfunction (Ergönül, 2006; Tonbak et al., 2006).

The disease was serologically detected for the first time in Turkey in 1980 in the Aegean Region, and after this date, there were no reports until the outbreak in the Eastern Black Sea Region in 2002 (Serter, 1980). The first case reports made in Turkey were those detected during this outbreak. The disease was initially observed in the Central Black Sea and Northern/Northeastern Anatolia regions of Turkey, and then its presence was reported in different provinces of Turkey (Midilli et al., 2007; Güneş et al., 2009). Turkish CCHF

isolates from patients or ticks were found to be close to isolates from Kosovo and Russia and different from isolates from Iran, a neighboring country. These results support the hypothesis that the origin of the disease is the transport of vector ticks on the migration routes of birds and the formation of disease foci rather than animal and human movements (Tonbak et al., 2006).

Ticks are known as the primary carrier and harbourer of the disease in nature. The virus remains in ticks for a lifetime and can multiply. It has also been reported that the virus can be transmitted in ticks for generations. CCHF virus has been isolated from 2 species of Argasidae family and 7 species of ixodidae family ticks and small flies (Culicoides spp). However, about 10 tick species are known to be proven vectors (Turell, 2007). It is accepted that tick species of the genus Hyalomma play a very active role in CCHF epidemics (Gargılı, 2013). Hyalomma marginatum is reported to be the primary vector of the Crimean-Congo virus in the Balkans, Crimea, and Caucasus; H. anatolicum in Iran, Pakistan, Turkmenistan, and Tajikistan; H. asiaticum in Central Asia and China; and H. rufipes in Africa (İça and Çetin, 2006). The presence of CCHF in ticks in Turkey was first demonstrated in 2006, and the agent could be isolated from H. marginatum, which is considered to be the primary vector, and also from Rhipicephalus bursa (Tonbak et al., 2006).

In line with the data and information obtained, a protocol was signed between the Ministry of Agriculture and Forestry, the Ministry of Health, the Ministry of Environment, Urbanisation and Climate Change, and universities, including academicians working on this subject, and symposiums were organized where both the follow-up of the emerging cases and the epidemiology of the disease and the measures to be taken at the point of protection and control were discussed. At the same time, it was ensured that the necessary measures were taken in the fight against this disease by raising public awareness. In addition, the International Hemorrhagic Fevers Symposium was organized in Istanbul in 2008 in cooperation with WHO, and the public health dimension of the issue was emphasized in addition to veterinary medicine.

3.3.Leishmaniasis

Leishmaniasis is the general name of diseases caused by more than 20 species in the genus Leishmania, which are obligate intracellular protozoa with zoonotic characteristics and manifested with different clinical symptoms

(Bakırcı et al., 2016). The parasites are transmitted to humans during blood-sucking by infected female sandflies (Phlebotominae). Reported by the World Health Organisation as one of the most essential vector-borne infections, leishmaniasis is found in 98 countries on five continents worldwide (Alvar et al, 2012; Novo et al., 2016). Leishmaniasis presents clinically in three different presentations: visceral, cutaneous, and mucocutaneous (Toz et al., 2013). It is thought that an average of 2,000,000 new cases occur each year, of which 500,000 are visceral leishmaniasis (VL) and 1,500,000 cutaneous leishmaniasis (CL), of which only 600,000 are reported cases (Souto et al., 2013). It is reported that 90% of VL cases, also known as kala-azar, occur in Bangladesh, Brazil, Ethiopia, India, Nepal, and Sudan. In contrast, CL cases are mostly detected in Afghanistan, Algeria, Brazil, Colombia, Iran, Pakistan, Peru, Saudi Arabia, Syria and Tunisia (WHO, 2013). In leishmaniasis infections, dogs play a role as reservoirs as well as hosts and thus lead to the continuity of infection foci in natüre (Aysul et al., 2012). Since L. infantum, which causes canine visceral leishmaniasis, is zoonotic and human visceral leishmaniasis caused by this species affects children or adults who are immunosuppressed due to other reasons such as AIDS, the detection of dogs carrying the disease is closely related to public health as well as veterinary medicine (Atasoy et al., 2010).

3.4. Babesiosis

Babesiosis is a protozoan blood disease caused by Babesia species of the Apicomplexa family, widespread in domestic and wild animals living in tropical, subtropical, and temperate regions, and transmitted by some tick species of the Ixodidae family (Kuttler, 1988). Since some Babesia species (B. divergens, B. microti, B. venatorum, and B. duncani) are also seen in humans, babesiosis is a severe disease that threatens human and animal Health. The first human case of babesiosis was detected in Zagreb, Croatia, in 1957. In a young farmer grazing his cattle in a tick-infested pasture, the infection started with symptoms of fever, anemia, and hemoglobinuria, and the patient died in the second week as a result of renal failure. Investigations revealed that the causative agent of the disease was Babesia bovis, probably B. divergens, which initially infected cattle (Vannier et al. 2012). Subsequently, in 1968, B. divergens was confirmed as the aetiological agent in an asplenic person infected while on holiday in rural Ireland (Fitzpatrick, 1968). While these asplenic cases

were attracting the attention of physicians in Europe, babesiosis was diagnosed in several individuals on Nantucket Island off the coast of Massachusetts, and the causative agent was identified as B. microti infecting mice and other small rodents (Vannier et al. 2012). Spielman et al. (1985) later identified the white-tailed deer (Odocoileus virginianus) as an essential natural host for B.microti, demonstrating that the vector of the agent is Ixodes dammini (syn. I. scapularis). In the following years, Babesia duncani (WA1) was identified as the causative agent in human cases reported from the North Pacific coast (Vannier et al. 2012). In studies, B. microti was identified as the most common causative agent in human babesiosis cases in the United States of America, while B. divergens, which uses Ixodes ricinus as a vector, was identified more frequently in Europe (Morch et al., 2015). As a result, considering that babesiosis disease threatens both animals and humans, it is essential that human physicians and veterinarians act together to establish diagnosis, treatment, and control methods for this disease.

CONCLUSION

The responsibility for worldwide health security lies with all countries, as ideal public health can only be achieved through cooperation between humans and animals. "One Health" aims to strengthen the relationship between humans, animals, and the environment. Understanding the epidemiology of diseases and developing protection and control strategies can improve public health and protect animals and humans. Protecting animal health ultimately safeguards human health and vice versa. To achieve this, the fields of Human Medicine and Veterinary Medicine should share knowledge, skills, and data through interdisciplinary studies and joint projects. This collaboration between human and veterinary public health disciplines is essential for a healthy and safe future. Overall, embracing the concept of One Health will ensure the integration of human and veterinary health for the benefit of the global community and public health.

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CHAPTER 10

THE IMPORTANCE OF VACCINE IN THE FIGHT AGAINST ANIMAL DISEASES: THE EXAMPLE OF ARDAHAN PROVINCE

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DOI: https://dx.doi.org/10.5281/zenodo.10458731

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INTRODUCTION

The importance of preventive veterinary medicine becomes evident when considering the potential losses that may arise due to any disease. These losses encompass genetic material loss, expenses for medication and veterinary care, productivity and production loss, time and labor loss, among others. Preventive veterinary medicine involves a series of measures and practices implemented in animals before the onset of various infectious (viral, bacterial, fungal, and parasitic) or zoonotic diseases. These measures can be summarized as the use of antiparasitic drugs against internal and external parasites and specific vaccine applications targeting viral, bacterial, and fungal agents. Among these general preventive principles, vaccination stands out due to its practical and economic advantages, forming the cornerstone of preventive veterinary medicine practices.

This chapter aims to evaluate the results of vaccinations administered to ruminants in the hands of the public within the Ardahan province under the Ardahan Agriculture and Forestry Directorate.

1- What is a Vaccine?

A vaccine is a biological substance developed to be administered to healthy individuals, both humans and animals, with the aim of providing protection against microorganisms such as viruses, bacteria, etc., which have the potential to cause diseases. These microorganisms are either purified from their disease-causing capabilities or the effects of certain microorganisms' toxins are neutralized (Altuğ et al., 2013). The substances used as vaccines can consist of the inactivated (dead) or attenuated (weakened) forms of the disease agents, their antigenic structures, or the toxins they release outside their cells (Sahms, 2005). In today's global livestock practices, the majority of vaccines used are either inactivated or attenuated vaccines. (Altuğ ve ark., 2013).

2- Types of Vaccine

2.1. Live Vaccine (Attenuated Vaccine)

Live vaccines are vaccines in which the microorganisms inside the vaccine are alive but rendered harmless for the host by repeated passages in tissue-cell cultures and/or embryonated eggs (Altuğ et al., 2013). As live vaccines use weakened strains of the microorganism, vaccine reactions resembling a mild form of natural infection can occur (Aytekin et al., 2011). Additionally, live vaccines, being able to replicate in the body, can stimulate the immune response for an extended period and generate high levels of antibodies. Consequently, live vaccines often do not require repeated administration, unlike inactivated vaccines (Shams, 2005).

There is no need for the use of adjuvants, substances used for protective purposes to enhance the antigenicity of live vaccines. However, if there is a subclinical disease present in the vaccinated animals, it may progress to a clinical form (Shams, 2005; Büyüktanır, 2010).

The advantages of live vaccines include the rapid onset of the immune response, the sufficiency of a single dose, and lower production costs due to the absence of additional processes such as concentration, purification, and the use of adjuvants. However, attention should be paid to storage conditions as live vaccines are sensitive to environmental factors(Aytekin et al., 2011).

2.2. Inactivated Vaccine (Dead Vaccine)

Inactivated vaccines, also known as dead vaccines, are produced by chemically (using substances like formalin, beta-propiolactone) or physically (UV radiation, heat) inactivating the antigenic characteristics of disease agents and adding adjuvants (Altuğ et al., 2013). Since the microorganisms are inactivated, they do not cause disease in vaccinated animals, and the risk of transmission is low. However, because

inactivated vaccines do not lead to replication in the body after vaccination, the immune response may not reach a sufficient level. Therefore, unlike live vaccines, multiple administrations may be required, and sensitivity reactions to adjuvants can occur (Shams, 2005).

2.3. Toxoid Vaccine

Some bacteria cause diseases solely through the toxins they release, and in vaccine production, the microorganisms themselves are not used. In this context, the toxins produced by bacteria are processed with various chemical substances, and after eliminating their disease-causing effects, they are utilized (Altuğ et al., 2013). Toxoid vaccines establish a safe humoral immunity (Shams, 2005).

3- Properties of an Ideal Vaccine

Vaccination is the most effective method, especially in livestock operations, for protecting animals against diseases that could lead to production and performance losses, cause permanent damage, or result in death. Vaccination practices are a more efficient and cost-effective measure compared to treating these diseases after they occur. Vaccines have been used for a long time in the context of preventive veterinary medicine against many viral/bacterial diseases. However, it has not been possible to develop vaccines with desired characteristics against all diseases threatening animal health (Aytekin et al., 2011).

The desirable characteristics that a good vaccine should possess are as follows:

- It should provide 100% protection in animals.
- The immunity it confers should be long-lasting.
- It should affect the variant strains of the pathogen.
- It should not be influenced by the levels of maternal antibodies at a specific threshold.
- The benefits provided by the vaccine should outweigh its cost (having a higher economic benefit).

4- Side Effects that Can be Observed in Vaccination Practices

Sometimes, undesirable situations may occur after vaccine administration. Due to the potential side effects, the benefit-risk relationship should be assessed before the use of vaccines (Altuğ et al., 2013).

The most commonly observed side effects after vaccination are:

- Swelling, redness, local swelling, or abscess at the injection site
- Abortions in pregnant animals
- Fever
- Anaphylactic reactions
- Stress

5- Reasons for Vaccination Failure

Vaccination failure can be examined under 5 main categories. In this context, veterinarians or animal owners can take measures to minimize vaccination failures by considering these factors.

5.1. Host-Related Factors:

Immunocompromised individuals may not mount a sufficient immune response to the vaccine. Presence of maternal antibodies at levels that interfere with the vaccine's effectiveness**.**

5.2. Vaccine-Related Factors:

- Inappropriate storage conditions leading to the loss of vaccine potency.
- Improper handling or administration of the vaccine.
- Use of expired vaccines.

5.3. Pathogen-Related Factors:

- Genetic variations or mutations in the pathogen.
- Presence of multiple strains or serotypes of the pathogen.

5.4. Environmental Factors:

- Stressful environmental conditions affecting the animal's immune response.
- Exposure to a high level of the pathogen in the environment.

5.5. Administration-Related Factors:

- Incorrect timing or spacing of vaccine doses.
- Inadequate coverage of the target population.
- Poor injection technique leading to local reactions.

6- Livestock and Vaccination Practices in Ardahan Province

Ardahan province has an approximate population distribution of 60% in rural areas and 40% in urban areas (TUIK, 2023). Due to the majority of the population residing in rural areas, the economy of the province is predominantly based on livestock farming. According to the Briefing Reports of the Ardahan Agriculture and Forestry Directorate, there were 13,214 registered farms in the Farmer Registration System (ÇKS) in the year 2021. The livestock inventory in Ardahan province over the years is provided in Table 1.

Table 1. Animal Asset of Ardahan Province

Year	Bovine Animal Asset				Small Ruminant Animal Asset			
	Ardahan	%	Türkiye	%	Ardahan	%	Türkiye	%
2018	306.925	100	17.220.903	100	74.238	100	46.117.399	100
2019	348.895	113,7	17.872.331	103,8	99.240	133,7	48.481.479	105,1
2020	330.168	107,6	18.157.971	105,4	94.807	127,7	54.112.626	117,3
2021	331.468	108,0	18.036.117	104,7	118.527	159,7	57.519.204	124,7
2022	337.958	110,1	17.023.791	98,9	150.926	203,3	56.265.750	122,0

Reference: TÜİK, 2023

When Table 1 is examined, it is observed that in the year 2022, the cattle population in Ardahan province increased by 10% compared

to 2018, reaching 337,958 head, while the cattle population in Turkey decreased by 1.1% compared to 2018, falling to 17 million 24 thousand.

When the small livestock inventory is analyzed in Table 1, an increase in sheep and goat population is noticeable both in Ardahan province and nationwide in Turkey. Despite a significant decrease in sheep and goat populations due to livestock policies implemented after 1980, sheep farming has regained importance since 2010, thanks to state support provided for sheep farming and the recent increase in demand for sheep meat. The sheep and goat population has increased by approximately 90% compared to 2010, rising from 29,383 to 56,266 heads.

In Ardahan province, the recent increase in small livestock can be attributed to both subsidies and the demand and price increase for sheep meat. Indeed, the natural structure of the province, along with the presence of grasslands and pastures, provides a suitable environment for small livestock farming. Additionally, the easier care and lower cost of small livestock farming compared to large livestock farming contribute to income and employment for producers in the region, making the increase in small livestock another significant factor (Demirbaş et al., 2009; Aksoy and Yavuz, 2012).

In Turkey, the Ministry of Agriculture and Forestry conducts vaccination programs regionally and/or nationally to achieve increased production and efficiency in livestock, a strategically important sector with socio-economic aspects. These programs aim to control and eradicate various animal diseases, including zoonotic diseases. Based on data obtained from the Ardahan Provincial Directorate of Agriculture and Forestry, Table 2 presents the vaccinations conducted in the province over the years, along with the numbers of outbreaks in Ardahan.

Table 2. Vaccinations and Total Outbreak Numbers in Ardahan Province between 2018-2022

Hastalığın Adı	**2018**	**number of outbreaks**	**2019**	**number of outbreaks**	**2020**	**number of outbreaks**	**2021**	**number of outbreaks**	**2022**	**number of outbreaks**	**2023**	**number of outbreaks**
FMD (Bovine)	665.087	8	635.449	2	607.513	10	614.528	NA	698.675	5	661.357	NA
FMD (ovine)	6.500	0	3.829	0	9.357	0	9.924	NA	2.564	0	NA	NA
Brucella (bovine)	50.165	54	56.179	107	107.209	3	92.888	NA	104.356	0	77.802	NA
Brucella (ovine)	9.586	0	9.954	0	17.777	0	18.017	NA	30.736	0	42.802	NA
Antrax (Bovine)	51.586	7	70.299	12	83.976	11	93.121	NA	64.240	7	48.175	NA
Antrax (ovine)	--	0	2.090	0	2.240	0	2.025	NA	1.265	0	NA	NA
Rabies	2.218	6	1.228	10	937	1	1.963	NA	584	1	NA	NA
Sheep Pox	1.675	0	1.543	0	2.190	0	-	NA		0	NA	NA
PPR	28.181	0	26.274	0	28.153	0	40.301	NA	43.864	0	44.564	NA
LSD	338.173	0	376.111	0	345.283	0	-	NA	281.357	0	NA	NA

Reference: Ardahan Provincial Directorate of Agriculture and Forestry,

NA: Not Available **FMD:** Foot and Mouth Disease LSD: Lumpy Skin Disease, PPR:peste des petits ruminants

Within the scope of the Ministry of Agriculture and Forestry's "Foot and Mouth Disease Control and Eradication Action Plan Based on Gradual Reduction of Regional Risks," intensive vaccination efforts are being carried out with the aim of designating livestock enterprises in Eastern Anatolia as vaccinated Non- Disease Animal Farm status. Indeed, when Table 2 is examined, it is observed that as a result of intensive vaccination campaigns conducted in spring and autumn months in the province, foot and mouth disease cases in the region have significantly decreased.

In Turkey, systematic vaccination efforts are implemented nationwide to reduce the incidence and achieve the eradication of Brucellosis disease, particularly in the eastern regions near the border. In the context of intensive vaccination conducted within the province, Table 2 shows that the number of outbreaks of the disease in the province has dropped to zero. Similarly, as a result of vaccination activities in the fight against Peste des Petits Ruminants (PPR) and pox, no cases of these diseases have been encountered in the province between 2018-2023.

As part of the Ministry of Agriculture and Forestry's "Control and Prevention of Lumpy Skin Disease (LSD) in Cattle" Project, supported by the European Union, it is observed that successful vaccination efforts in the province have resulted in zero outbreaks between 2018 and 2023.

Furthermore, the Ministry of Agriculture and Forestry provides support for "Programmed Vaccination Applications" to facilitate more effective combat against animal diseases, with contributions from licensed veterinarians.

In conclusion, Ardahan province is one of the provinces with a high risk of infectious diseases due to its high meadow and pasture areas, traditional pasture-based breeding and being one of the border provinces. In this context, preventive veterinary medicine and regular vaccination practices become crucial to mitigate the production and productivity losses associated with animal diseases and to make livestock farming profitable. Continuation of the Ministry's free vaccination programs in this regard, along with providing awareness-raising training for producers and enforcing penalties for those who do not comply with vaccination, is essential. Additionally, at the provincial level, conducting technical and economic feasibility studies aimed at reducing the incidence and achieving the eradication of infectious diseases,

implementing protection and control protocols, can help minimize economic losses.

THANK

We would like to thank the Director and staff of Ardahan Provincial Directorate of Agriculture and Forestry for their great contribution to our study.

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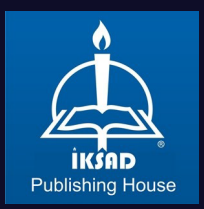

ISBN: 978-625-367-616-2