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PREFACE

We are developing a new method to comprehend, define, and relate to animals so that they, too, can live full and fulfilling lives by changing how we view animal research and bringing an inclusive perspective to our science and activities. In the current push for evidence-based decision making, welfare and healthcare providers require an evaluation system that is holistic in nature to accurately understand the many factors influencing health and well-being. If you want to get the best grade, it's crucial to get the topic right. In the world of veterinary medicine, qualitative research is infrequently used. Qualitative research will become increasingly important as the veterinary field becomes more complex as we look for answers to fresh, equally challenging research issues. Veterinarians can benefit from evidence-based practise, which uses the best available data to guide treatment and management decisions, by having the ability to comprehend and critically evaluate qualitative research. This will enable veterinarians to find answers to difficult veterinary problems outside of the clinical context in addition to offering the finest treatment possible to fulfil the needs of both clients and patients.

Every area of biomedical health and science is influenced by veterinary research and clinical contributions, including food safety and livestock production, epidemiology, zoonotic diseases, and public health, companion animal medicine and surgery, comparative basic and translational research, animal welfare, wildlife health, the human-animal bond, and ecosystem health. Humans and other animals live in the same environment. Pharmacological and pesticide contamination of food and water, as well as the development of bacterial drug resistance, pose threats to the quality of the environment. Priority one should be given to any potential health hazards for anyone who work closely with animals. Research is also needed to discover interspecies transmission routes, find sources of animal and veterinary medications, and evaluate the function of animals as reservoirs for resistant infections.

Dr. Esra BİLİCİ

CHAPTER 1

THE IMPORTANCE of NEONATAL and PRENATAL LAMB LOSSES in SHEEP BREEDING

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Introduction

Lamb mortality before weaning reduces sheep farming's fertility and profitability. In certain firms, this condition can also develop into a significant welfare issue (Dwyer et al. 2016). Lamb mortality rates can range from 5% to 15% or higher on sheep farms, depending on the production technique (Nowak et al. 2000). According to Hinch and Brien (2014), a significant portion of lamb losses occur in the first seven days following delivery. The number of offspring at birth, the sex of the offspring, and the weak maternity traits in the first givebirth are significant risk factors for the death of the offspring when mortality rates are examined. These risk factors include low birth weight, tough labour, delivery injuries, hunger, and mismothering (Flunn et al. 2020). According to Dwyer and Morgan (2006), a significant factor in infant fatalities in sheep breeding is early delivery, which cannot finish the pregnancy process. However, it is impossible to collect accurate data on this topic since mating and birth records are not frequently preserved, especially in large and semi-intensive companies (Sawalha et al. 2007). between weeks 144 and 150 of pregnancy. The mortality rate in lambs born between the 137th and 139th days of pregnancy can be as high as 90%. This demonstrates that, unlike certain animal species, sheep cannot endure preterm delivery as well. Enhancing sheep's lifelong reproductive performance can be done in general. Because of this, a proper diet may significantly affect how quickly an animal matures sexually and approaches breeding age in addition to genetic merit (McHugh et al. 2016). Numerous concerns surrounding the development of pregnancy in adult animals and the proper upbringing of subsequent progeny till weaning are covered in this circumstance (NADIS, 2011). In addition to underfeeding, unwanted issues in overfed animals include fat gain and a subsequent inability to procreate. Some farms mandate singleton pregnancy of animals to solve the issue and remove the competition that develops during feed consumption and the unfavourable pregnancy outcomes as a result. Due to these issues, infertility, poor-quality embryos, or high embryo loss can be seen (Refshauge et al. 2016). The mating season's length varies by race, and ultimately, differences in sexual maturity age are further problems. If not, there will be a problem with the growth of the placenta and the fetus's requirement for nourishment. Low birth weight lambs are thus born early. Additionally, the need for colostrum declines right away after delivery, and lamb mortality rises.

It was emphasized that the health of the lamb should be regularly watched, correct nutrition should be supplied, and essential aid should be offered against delivery complications when necessary because a study revealed that the death rate of lambs is rather high (62%). High pre-weaning lamb mortality rates continue to constrain industrial-scale sheep production, raising significant economic and welfare issues. Over the past forty years, there have been substantial improvements in genetics, nutrition, and herd management, yet the frequency of lamb mortality has remained between 15 and 20%. There is growing evidence that melatonin may have a beneficial impact in some research, in addition to genetic studies, to address the issue (Kenyon et al. 2014). Melatonin supplementation improves uterine blood flow, fetal oxygenation, and perhaps birth weight and body temperature throughout the neonatal period in pregnant sheep. Melatonin is easily able to pass through both the sheep's placenta and the blood-brain barrier. With superior behavioral outcomes in newborn lambs with low blood oxygen levels, fetal protection is offered throughout chronic and acute hypoxia episodes throughout pregnancy (Wallace, 2019). There is strong evidence that maternal melatonin treatment improves outcomes for lambs with impaired uterine development or extended labor, even though this has not yet been studied in livestock production systems. New research on the effects of maternal melatonin supplementation during pregnancy on pre-weaning mortality under intense production settings might thus be advantageous (Edwards et al. 2016). The reasons and significance of neonatal and prenatal lamb fatalities in sheep breeding are discussed in this article, followed by some recommendations for workable solutions.

The Principles of Lamb Death And Occupation Time

The quantity of lambs born per ewe is a crucial productivity indicator in sheep production systems. Lamb deaths during the raising phase, according to Darwisha and Aashmawy (2011), have a direct influence on the financial performance of sustainable sheep production. To put the issue's significance in numbers, 100,000 more lambs would survive each year if the nation's lamb mortality rate could be decreased by 3%. The creation of herd management techniques is aided by a large decrease in lamb mortality, in addition to the factors that are important (Dwyer, 2008a, b). Table 1 presents some facts relating to lamb deaths' times.

Table 1. Time of death in lambs and their percentages

Death time(hour)	Mortality rates (%)
0	43
24.	15
1-3. days	16
4-7. days	6
Seven and later days	20

Source: Teagasc, 2023. Lamb mortality: the main causes and timing. 20 March.

74% of lamb deaths occur in the first three days after delivery, compared to 80% that occur in the first seven days following birth. When the greatest death rate is included, the percentages are as follows: 43% before or during delivery, 15% from birth to 24 hours, and 16% from 24 to 72 hours (Hinch & Brien, 2014). Table 2 lists several leading causes of lamb mortality. With 32% of all lamb deaths attributable to infection, it follows difficult delivery (20%) as the second most frequent cause of mortality among the variables examined. The total death rate exceeds 52% when these numbers are added to diseases from bacteria and viruses. With effective herd management practices or losses due to disease, it is possible to prevent difficult birth and infection.

Table 2. Reasons of Lamb mortality

Mortality time	Mortality percentage (%)
Infection	32
Dystocia	20
Failure to diagnose	19
The others	14
Congenital	4
Failure to complete the autopsy	6
Mistakes made at birth	5

Source: Teagasc, 2023. Lamb mortality: the main causes and timing. 20 March.

In certain vast or intense farms, the sheep mortality rate might exceed 15%. Given how high this rate is, it could appear challenging for some organizations to reduce it below 3% without sacrificing sustainability and profitability (Kenyon et al. 2019). Khan et al. (2006) assert that the first day following birth is crucial since it is when most deaths occur. Effective sheep breeding depends on identifying possible risk factors and creating mitigation strategies. There are four major categories in which lamb deaths fall. These include, in order, birth complications, lambs that sheep don't take to, infectious disorders, and congenital genetic abnormalities. The causes of lamb mortality are impacted by several risk factors. One of these parameters that is significantly influenced by prenatal sheep nutrition is lamb birth weight. Large or premature puppies run the risk of causing birth trauma and severe oxygen deprivation during delivery. Lambs who are unable to stand, suckle, or make any noise exhibit negative behavioral changes because of oxygen shortage and brain damage (Flin et al. 2020). Pathogen pressure and an elevated risk of infectious diseases are brought on by hygiene management, vaccination practices, and housing in the workplace. Lambs experience lower temperatures just after delivery than their mother's body does. Lambs exposed to cold environments need to use their limited body surface area to control their body temperature. By using their brown fat energy stores and boosting muscular activity through shivering, newborn lambs attempt to maintain body temperature (Nowak et al. 2006). The body weight of the lambs impacted by the prenatal nutrition of the sheep determines the amount of the fat stored as well. It is recognized how crucial the food management of pregnant ewes is because maternal deficiency may impact neonatal lamb fatalities (de Souza et al. 2023). Because of the placenta in the womb, antibody transfer from sheep to lamb is not feasible. Colostrum consumption during the first few hours of life aids in immune system development. The efficiency of passive immunity is significantly influenced by the quantity and quality of colostrum consumed. Early after birth, lambs who have a smaller udder or who exhibit pacifier-seeking behaviour have trouble standing. If sheep aren't permitted to be nursed, they cannot develop enough passive immunity while under stress or fear. It is important to assist lambs, when necessary, since, according to research, 22% of ewes have low colostrum quality (50 g Immunoglobulin G/l) and ewes giving birth to twins frequently have low colostrum quality (Sharif et al. 2005).

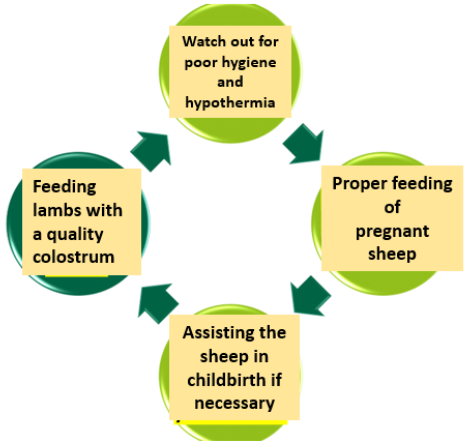

Figure 1. Practices to be done for a successful birth.

To guarantee the ideal birth weight of the lambs, the sheep should get a balanced diet (Figure 1). Compared to lambs with bigger body weights, lambs with exceptionally low birth weights have more difficulty managing their body temperature (Radunz et al. 2011; Liu et al. 2021). The lamb's growth and body condition scores must be measured via ultrasonic scanning for the ratio to be used effectively.

Other Death Causes From Sheep For Lambs

The main reason why sheep lambs die is prolapsed. Vaginal, rectal, and uterine prolapses are the three forms of prolapses that can occur in sheep (Noakes et al. 2001).

Prolapse: Prolapse usually happens at the end of pregnancy. Prolapse, a significant factor in lamb fatalities after birth, can have several effects on the cervix. As a result, it may be crucial to routinely check on the sheep, especially in the final trimester, and the doctor may take prompt action if necessary. Occasionally, prolapsed sheep may require veterinary care. Prolapse is more common in sheep bearing many lambs, and it may also occur more frequently in late-pregnancy overfeeding (Fragkou et al. 2010).

Pregnancy toxaemia: a metabolic disorder called pregnancy toxaemia sometimes referred to as Twin Lambs Disease appears in the last four to six

weeks of pregnancy. It is sometimes referred to as twin lamb disease. This syndrome is frequently present in sheep with low or poor body condition scores (Figure 2). In other words, starvation during the latter several months of pregnancy is extremely dangerous, especially for pregnancies with multiples. Low blood sugar levels and inadequate energy requirements in the ration cause it to happen. These sheep keep their distance from the other animals in the refuge. The eyes appear dull, the hunger has diminished, and the amount of feed consumed has dropped when observed from the outside. The propensity to slouch and lie on the backside increases with a reduction in feed intake**.**

Milk Fever (Hypocalcemia): Dairy cows who are not dried or fed during the dry period get milk fever, also known as hypocalcemia, which is a metabolic disorder. Since milking significantly excretes calcium from the animal's body, this deficit can be resolved by applying a dry spell or drying the animals during the last 4 or 6 weeks of pregnancy. On some intensive and semi-intensive farms, it is a typical scenario for dairy cows, but (Shiels et al.2022). The behaviours of pregnant animals who have milk fever vary. The most frequent symptoms include immobility, especially while lying down, followed by a coma and death. Positive results are shown in animals when this is diagnosed early and with accurate monitoring and diagnosis. It's essential to feed pregnant sheep on pasture-based holdings: For flocks of sheep giving birth on pasture, a body condition score (BCS) of three is recommended. Try to time the lambing to coincide with the spring's height of grass growth to achieve this. Make sure the pasture has grass that is 6 cm tall when the sheep begin to lamb. Excessive feed should not be supplied to the management to avoid disturbing the grazing and lambing habits in the shelter. The viability of the lambs and the quality of the colostrum can both be improved by giving the animals additional meals using various feeders. Internal parasite issues like intestinal worms and liver flukes have a detrimental effect.

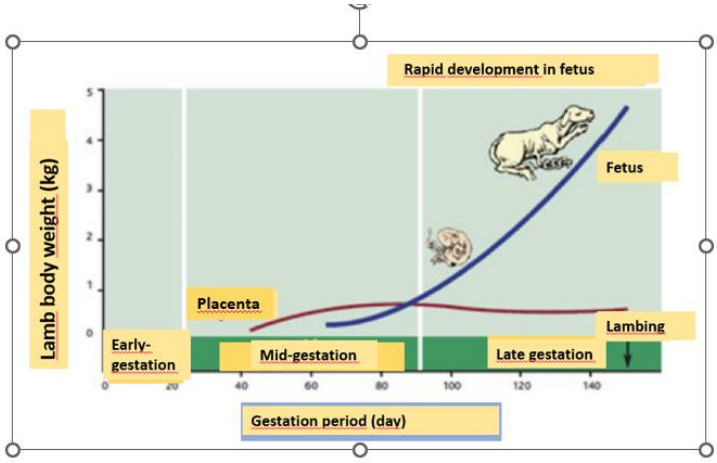

Figure 2. Fetal development rate during pregnancy

Source: https://www.rumenco.co.uk/updates/ewe-nutrition-mid-pregnancy

Relieving Sheep of the Risk of Abortion

Three forms of abortion often account for 86% of abortion incidents in all sheep (Lane et al. 2015; Smith, 2021). respectively, these

Enzootic abortion (EAE) is a disease brought on by the Chlamydia abortus bacteria. Lambs delivered during pregnancy are feeble or stillborn. Because of this, sheep have various birth litter sizes. In other words, different animals have different ratios of lambs born alive to lambs born dead. The illness is only passed from sheep to sheep during the lambing season by sick lambs and pasture/litter-laying animals. Since they are also effective carriers, afflicted sheep and lambs shouldn't be kept with the main herd (Baz and Aydn, 2006). Sheep that have given birth should be kept apart for three to four weeks at the very least. All sheep must have at least a four-week vaccination if the veterinarian believes it necessary.

Toxoplasma gondii is the pathogen responsible for the illness. Early in the pregnancy, the infection causes embryo death; however, if it strikes in the middle, the fetus perishes. Full-term stillbirths or poor lambs are the results of infection at the end of pregnancy (Acoz et al. 2019). Feces from cats are the main method of transmission to sheep, however, it can also happen through feed, water, or pasture. When kittens mature and begin to hunt indoors, they

distribute disease-causing agents. Therefore, it is suggested that pregnant ewes and sheep that have just given birth be housed in different pens. Sheep should be immunized at least four weeks before mating, and cats should be absolutely prohibited from feed and feed storage. a successful immunization campaigns.

Campylobacter: The bacteria Campylobacter is the source of the infection known as campylobacteriosis. Also known as "campylobacteriosis", this disease is one of the most common bacterial diseases in humans and is transmitted through food. It causes stillbirths in animals or weak births of lambs. Sheep to sheep are the primary carriers of the virus, but birds can also spread it. Aborted ewes can be housed alongside lambed ewes but should be kept apart from pregnant ewes. Since there is no vaccine, this intentional propagation of the virus will boost immunity (Yazcolu, 2000).

Causes of newborn lamb mortality

Within the first 28 days of life, newborn lambs die (Figure 3). The age at death in lambs is classified or defined as early (7 days and before) or late (7-28 days) after birth, considering the day of the event (Holmy et al. 2012). According to Steers et al. (2007), neonatal lamb deaths result in considerable financial losses for the sheep industry and reduced animal welfare. Significant losses in some herds might make farmers less inclined to preserve their flock of sheep. Other significant causes that contribute to a rise in lamb mortality include difficult deliveries or birth traumas. The increased life expectancy of lambs and major contributions to mortality reduction will result from feeding and raising practices that consider the growing number of young at birth. While congenital or metabolic problems are not among the major causes of newborn lamb mortality, infections brought on by numerous pathogens are acknowledged to be a significant contributing factor (Binns et al., 2002).

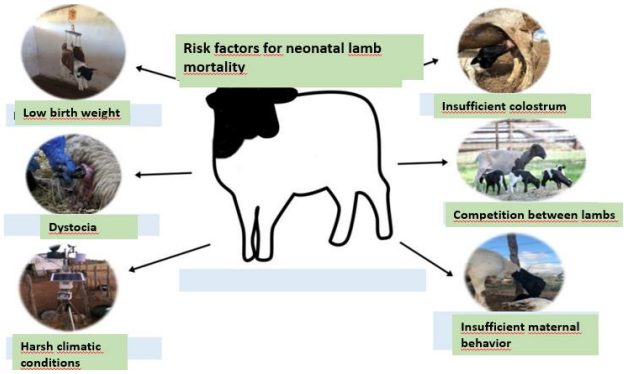

Figure 3. Causes of neonatal lamb mortality

Ruminant Body Temperature Regulation

The level of development at birth is comparable across species in ruminant animals. It's possible that lambs who reach this weight barely 20 days after birth and lambs who reach it 50 days after birth have different rates of brain development. Studies on sheep show that it starts within 20 minutes of birth, not because of a shift in the fetal stage. These animals then exhibit voluntary reactions or the cerebral cortex's participation in perceiving stimuli (Mota-Rojas et al. 2021). Parent-child interaction between 20 and 60 minutes after delivery allows for the detection of subsequent behavioral reactions. When newborn animals greatly prefer their biological dam for up to a month, this phase is known as the sensitive period. As their connection fosters bonding and lowers the amount of heat loss from evaporation by washing and drying the newborn's feathers, trying to get rid of the offspring plays a function, a behavior that plays a part in regulating body temperature (Pollock et al. 2019). The development of a movement (locomotor system) that enables babies to stand up in the initial minutes after birth is another process that aids in preventing heat loss in the body. According to Regueiro et al. (2020), newborn lambs start standing up after an average of 22 minutes, and between 53.9 and 87.9% of them begin nursing an hour after birth. Australian children raised in the bush show comparable results, starting to stand up in an average of 20.2 minutes and sucking in an average of 50.8. The anatomical physiological characteristics of tissues are crucial in mechanisms that prevent heat loss, such as controlling sudden body temperature, skeletal muscle contraction, or heat production through high-energy fat consumption (Muir et al. 2000). This is true even

though neurological development is required to maintain the body temperature of newborns. In the first instance, the ratio of skeletal striated muscle fibres is reflected by involuntary rhythmic contractions. The degree of resistance and ability to sustain muscular contractions make a certain muscle-fibre ratio important. In addition to individual morphology, body fat percentage, food, and degree of cold acclimatization, this variation can change how well chemicals that cause shivering work. Low threshold (4–8 Hz), persistent shivering, and lipid depletion are linked to the recruitment of type I (resulting from aerobic metabolism) or slow oxidative muscle fibers. Type II (rapid glycolytic) fibers, on the other hand, are specialized for the use of carbohydrates and respond to high-intensity bursts (0.1–0.2 Hz) by creating jitters. Because its glycolic fibers may be activated at a lower metabolic rate, type II fibers, often known as anaerobic muscles, play a crucial role in exposure to cold settings. Therefore, during the first few days of life, ruminant newborns with a high percentage of type II muscular fibers may. Because of the features of the hormones that regulate body temperature, type II muscle fibers are particularly important for babies. Research on sheep by Peinado et al. (2004) included the period from 1 to 15 days following birth. On day 30, the proportion of type II fibers decreased by 80% whereas the proportion of type I and type II fibers was comparable. Glucose serves as a significant source of energy for juvenile ruminants. Some animals, like piglets, have less skeletal muscle glucose than others due to the presence of brown adipose tissue deposits.

While the placenta regulates the fetal blood sugar level while the mother is pregnant, newborns or nursing lambs rely on the liver's glucose reserves to regulate their blood sugar levels. This suggests that neonatal ruminants use hepatic glycogen as their main source of thermal energy. Kids, lambs, and calves have low glucose concentrations after birth due to the liver's and muscles' poor capacity to store glycogen, which makes them vulnerable to hypoglycemia because these stores can quickly run out in the first 12 hours following delivery (Habibu et al. 2021). In newborn children, hypoglycemia was discovered in 22.5% of cases. These animals start to use up their muscle reserves during the Cori cycle when glucose concentrations are inadequate. Adipose tissue uses metabolic and cellular processes to control body temperature and energy balance. This tissue is separated into white and brown forms in animals. BAT grows throughout the fetal stage, which helps young ruminants exposed to cold

at birth survive. Although it has been argued that BAT and WAT are comparable, there are significant histological distinctions. Even while BAT is present in all ruminants, each species' distribution varies. 2% of a cow's body weight is made up of BAT, but within the first several days after birth, it gradually changes into WAT (Louveau and Perruchot, 2016). These variations in BAT deposit locations between species imply that levels of thermogenetic activity rely on the amount and distribution of BAT and that a deficiency in BAT makes an organism more susceptible to hypothermia. It enumerates the primary neuroanatomical traits of young ruminants. Because it takes up 2% of body weight, lamb is used in this way, but throughout the first several days of life, it gradually changes into WAT. These differences in BAT deposits between species demonstrate how BAT distribution and volume influence thermogenetic activity, and how a deficiency in BAT increases susceptibility.

Body Temperature Control and Vitality in Newborn Lambs

Newborn lambs are negatively impacted by a rapid shift in the shelter's or the compartment's interior temperature. Newborn pups exert a lot of effort to control their body temperature, especially when heat loss is paired with wind speed and humidity. The lamb's main heat source at birth is its perirenal and pericardial brown adipose tissue (CCD). More than 50% of the entire body temperature is regulated by this resource, with the remaining percentage coming from muscular contraction brought on by shivering and movement (Plush et al. 2016). According to Nedergaard and Cannon (2010), lambs with a 'normal' (4.25 kg) birth weight are more prone to fast heat loss postpartum than lambs with a lower birth weight (2.9 kg). Additionally, a link between birth weight, the capacity to regulate body temperature, and lamb survival from weaning to weaning was found. In addition, low rectal temperature after birth is associated with being unable to stand and reach for the udder. To prevent wet lambs from convectively losing heat to the ground after birth, it's crucial to stand up right away. Lambs' body temperatures are further raised by frequent caring.

The Causes of Perinatal Lamb Death

The perinatal phase is defined as the few weeks before lambing and the first four weeks following delivery (Dwyer, 2003). Late abortions and lamb fatalities within the first month of life are examples of perinatal lamb deaths. If

there is no maternity chamber, particularly in large or pasture-based sheep breeding, 10 to 30% of the lambs die before weaning, whereas most producers may endure an average of 10% to 20% lamb losses each year (Dwyer et al. 2005). The first week following birth is the most hazardous time in a lamb's life. According to several studies involving several breeds of sheep that produce wool, one-third of lamb mortality happened during the first 24 hours (Dwyer et al. 2005). The fact that one-third of lambs are stillborn suggests that about two-thirds of all perinatal lamb, deaths occur within the first 24 hours of the lamb's existence. Eighth-day deaths account for 18% of all perinatal deaths.

To put it another way, more than eight out of every 10 lamb fatalities take place before the end of the first week following birth. Perinatal deaths may be one of the main reasons sheep farms suffer financial losses. The amount lost due to the sheep's pregnancy-related care, feeding, veterinary, and pharmaceutical costs represent the economic burden of this situation. Most perinatal lamb fatality reasons are the consequence of poor herd management decisions or practices. However, it is feasible to reduce the enterprise's early lamb losses by implementing a few straightforward improvements to the herd management system (Dutra and Banchero, 2011).

Stillborn Lambs or Abortion

According to Gitto et al. (2009), infections including toxoplasma, vibriosis, chlamydiosis, or leptospirosis are frequently to blame for miscarriage and stillbirth in lambs. Lambs are feeble when they are born, therefore diseases may cause them to pass away soon after birth. Inadequate biosecurity or poor hygiene are frequent causes of infections in commercial settings (Hatcher et al. 2009). To prevent the illness from spreading to other animals, the membranes and abandoned fetuses from an abortion that occurred among the animals in the holding should be removed. Since many abortions that result in sheep illnesses can be transmitted to humans, gloves should be used while handling abortive sheep fetuses or other contaminated materials. If necessary, thoroughly clean your hands as well. They must as quickly as possible transmit samples of the fetal membranes and the fetus itself to a lab for testing. Pets, birds, rats, and other animals should be kept away from feed and feed storage locations throughout this operation. The water tank and gutters need to be cleaned often. Sheep should be immunized against Chlamydiosis and Vibriosis, however,

there is currently no vaccine authorized for sheep against toxoplasmosis. Pregnancy toxaemia, commonly known as ketosis, is another factor in most stillbirths. Ketosis is brought on by a lack of calories consumed during the latter trimester of pregnancy (Ikeda et al. 2000). Although it is an issue, weak animals can also exhibit it. This is especially true with over-conditioned sheep. Such sheep quit eating their feed, and they walk about more.

Determinants of Perinatal Lamb Losses

Birth weight: The primary factor in perinatal death in lambs appears to be birth weight. According to research by Brien et al. (2014), lambs with birth weights between 3.40 and 4.9 kg had a better chance of surviving their first eight days. Lambs of this body weight stand up to regulate their body warmth and swiftly milk their dams' udder. They also have a decreased death risk from respiratory illnesses. Low birth weight lambs frequently pass away from malnutrition or cold. According to McHugh et al. (2016), lambs with birth weights of 5.5 pounds and above are more prone to experience difficult deliveries and fatal injuries. Fetal growth is most pronounced in the final trimester of pregnancy in sheep. Sheep's dietary requirements nearly increased at this time. By feeding their sheep less during the latter three months of pregnancy, some breeders attempt to keep the birth weight low. However, rather than averting issues at delivery, this strategy of underfeeding results in sheep and fetus losses owing to ketosis. Sheep have a harder time giving birth if they don't get enough nourishment throughout the first three months of pregnancy. In other words, sheep generate lower quantities of worse-quality colostrum. Instead of restricting nutrition, it would be more realistic to choose lambs of average size, ewes giving birth, and mothers who feed lambs with high growth rates until weaning age. Lambs with low birth weights might have a better chance of surviving if they are kept in a warm, dry environment. It must be assured that pups wake up and nurse from their moms as soon as they are born. Otherwise, hypothermia may develop if the mother cannot be located if it is cold.

Sex: Compared to female lambs, male lambs are more likely to pass away between the ages of eight days and delivery. The risk of respiratory mortality increases in male lambs after the first week of birth. Male lambs can

be examined more attentively during their first few weeks of life to increase their chance of survival, even if there isn't much you can do to change the sex of the lamb you buy.

Fertility: All sheep breeders want their animals to produce more than one lamb every year.

If more newborns are delivered, more lambs will be sold during weaning. However, compared to the offspring of a single ewe, the viability of additional lambs is proportionately reduced, especially in the first eight days of life. Only 57% of lambs born to triplets or more survive the first week of life, according to one research. Lambs with many births take longer to stand up and suckle than lambs with a single birth. Newborn pups should also be kept in a warm environment since they often have smaller or lower body weights. They have lower body energy stores, which is a significant additional factor (Morton et al. 2017). Increasing the number of pups at birth also means competition for limited colostrum. Due to the competition that will occur, twin-born lambs can take less colostrum than single-born lambs. This calls for making sure the lambs get enough colostrum to drink. Until they can regulate their own body temperatures, lambs born without multiples must be maintained in a warm environment and fed colostrum which is at least 10% of their live weight. With the aid of ultrasonography in the holding, it is possible to identify which sheep are bearing big offspring, allowing for their separation from the rest of the herd and supplementary feeding. This application will provide appropriate nutrition for the developing fetus, increasing the newborn lambs' survival prospects.

Dystocia: Sheep typically give birth in 30.40 minutes. The viability of the lambs will, however, decline if this period is prolonged and the lamb has not yet separated from its mother's body. It may be said that a difficult birth has occurred in other words. Delivery problems are a major factor in the death of newborn lambs. According to studies on the subject, lambs who gave birth in a difficult way died at a rate that was four times higher than lambs who did it easily. Around 10% of lambs that are dystocia-affected at birth die. Another drawback is that difficult-to-give-birth ewes are more likely than average to reject their own lambs (Oldham et al. 2011). Unlike sheep who give birth regularly, these sheep take longer to stand up and have difficulty nursing their young. Because their moms can't lick them dry as quickly or for as long,

newborn lambs take longer to dry. These lambs' metabolic rates are a little bit lower. Additionally, because they are fed less milk, they take longer to stand up. Their moms keep them from nursing for a while, though. The maintenance of body temperature becomes progressively challenging for newborn lambs because of the combination of the negatives. Even though it is necessary to assist the sheep when it has problems giving birth, if it is done incorrectly, the lamb may suffer such severe injuries that it may either not live or perish. Numerous lubricants, latex gloves, and ropes used to remove a lamb are needed for the operation. As far as is practicable, the sheep's birth canal is lubricated. It might be necessary to gently touch the fetus to see if it is in the best position for delivery. If the lamb's head or one leg is bent backwards, it is imperative to softly push the animal back until you can delicately fix it. The lamb's head and forelegs are carefully secured on the prepared rope equipment for removal, which is then gently pulled as the sheep is pushed backwards. It is assumed that the lamb does not move when the sheep is giving birth and experiencing contractions. By using this technique, the birth canal might move backwards along the body of the lamb. Avoid breaking the lamb's legs at all costs. Lambs' bones are still mostly cartilage-filled and exceedingly brittle. A trained veterinarian should perform each of these operations.

Damage: In sheep breeding, the first-born females typically give birth to a single lamb. Compared to lambs from second or later lactation ewes, lambs from first lactation ewes have a lower chance of surviving. Single-bearing ewes require more time to stand still and feed. According to Dwyer and Bumger (2011), lambs born to ewes who gave birth to the first lamb had a higher risk of developing respiratory tract infections. On the other side, if cleanliness or biosecurity procedures are poor, ewes older than six years of age may also have increased lamb losses. To adjust the age of sheep, not much can be done. Some breeders, however, wait until their sheep are 18 months or older before breeding them. Ewes that will give birth to the first lamb can be housed in a separate bin prior to lambing, however, most breeders believe this practice to be unproductive. As a result, it is possible to keep a closer eye on the animals that will give birth to the first lamb and their young. By giving extra nutrition to the sheep that will give birth to the first lamb based on the body condition score in

the final three months of pregnancy, better growth of both the mother and the fetus is assured.

Nutrition: Among other herd management techniques, a robust feeding program in the holding is one of the greatest methods to lower prenatal and postnatal pup mortality there. A poor feeding plan will lead to issues and exacerbate existing metabolic and health issues (Grimson, 2009). Lack of feed during the latter three months of pregnancy in sheep causes low birth weight in lambs, who also have a higher chance of dying.

Maternal Behavior: Sheep keepers with extensive experience are aware that different animals may exhibit different maternal traits. The lambs' chances of surviving are significantly impacted by their primary behavior. With age, this issue gets worse. She'll likely be a good mother for the rest of her life if she takes good care of a newborn sheep when it's her first (Foster et al. 2007). First-born ewes will get better at taking care of their lambs as they get older since mothering is to some part learned behavior. Even if they can grow from their first to second birth, those who do not exhibit acceptable maternal traits should not be employed in breeding. The first few hours following lambing are the most revealing of maternal behavior. Sheep attempt to lick and dry their lambs while producing low-frequency noises. He will also learn to recognize his progeny throughout this time based on these behaviors. Sheep and lambs shouldn't be disturbed as they form a relationship for the previously mentioned cause. If the farm's flock replacement rate is high, the mother-offspring bond may not be as strong or as strong as predicted in dense flocks for whatever reason. When settlement frequency is extremely high, the farm may also experience certain problems, such as not being able to find the mother and adopting another lamb as its own lamb (Refshauge et al. 2016). The temperament of the animal is another characteristic that affects its primary behaviors. In other words, relaxed sheep lick, dry, and bleat their lambs more frequently than tense ones. Because of this, sheep with a calm disposition tend to have more resilient offspring than sheep who are agitated or hostile. Due to health issues, sheep that have trouble giving birth spend less time bonding with their young and are more prone to reject lambs. In fact, ewes that give birth are nearly five times more likely to lose a lamb than ewes that deliver in 30 minutes or less. It's important to approach the sheep quietly and handle them with care.

First, the animal should be carefully observed throughout the delivery without being stressed, and assistance should be given within the first 30 minutes if necessary.

Congenital defects: Congenital defects are hereditary conditions that exist prior to birth. Congenital defects are flaws that develop in the ewe's uterus as the lamb is growing. The defect may be lethal at birth or present challenges to normal function severe enough to result in the lamb's death soon after delivery. Jaw deformities that make it difficult for lambs to suckle or sip milk may be the source of these first issues (Matheson et al. 2012). Other frequent birth defects including spider lamb syndrome and atresia ani, as well as heart and lung disorders where the anus is not formed or is misplaced, can also happen. Congenital problems are inherited; however, some plant poisons may also contribute to abnormalities in the developing fetus. For this reason, it is not advisable for animals, and pregnant animals in particular, to graze in dangerous places where the specific composition of the pasture's flora is unknown. Congenital gastrointestinal tract abnormalities are typically to blame for deaths that occur after the first 24 hours postpartum.

The Influence of Shelters On Lambs' Survivability

Lamb fatalities can reduce the annual income from sheep and goat production by about $100 million in nations with wet climates where sheep husbandry is widespread. Studies on the subject have shown that a 10% increase in the percentage of lambs during the weaning period may result in a 10% rise in the usual gross margin per hectare (King et al. 2012). The survivability of the lamb in the first 48 hours after birth is critical for weaning percentages since 70% of lamb mortality occurs between birth and enumeration/identification. Recent research, however, indicates that crossbred twin lambs have a higher chance of surviving than singleton lambs with the same birth weight Merino genotype (Hocking Edwards et al. 2019). False pregnancy, malnutrition, or challenging birth are to blame for more than 80% of lamb fatalities. The cold index, which considers temperature and precipitation, can be used to gauge the amount of exposure to wind speed within the shelter. A considerable risk for lamb loss has been demonstrated for measurements above 1000kJ/m^2/h.

Utilization Options for Straw Bales and Corrugated Iron

Because sheep rarely use them, straw bales, corrugated iron, and man-made shelters only provide modest advantages. There is little evidence that ewes use these structures for lambing or for their newborn lambs (less than 48 hours when cold exposure is important), while older lambs were shown to utilize shelter in all regions in investigations. In fact, one breeder said that because shelters prevent sheep from seeing their young, mismothering may have become more common (Master et al. 2023). To guarantee that the lambs are always within a safe distance of a shelter wherever the sheep graze, there must be enough shelters spread out over the field.

Telemeters and Their Impact on Vitality

Telomeres, which are non-coding repetitive DNA sequences found at the ends of chromosomes, are essential for maintaining the stability and integrity of DNA (Shay and Wright, 2019). Cellular senescence and apoptosis take place when telomeres drop below a certain threshold. Telomeres shorten during cell reproduction and in response to oxidative stress. As a foundational biomarker of biological ageing, whole-organism health, and ageing, average telomere length (TL) is used. Leukocyte telomeres that are particularly short in humans have been linked to several age-related illnesses, including diabetes, cancer, and cardiovascular disease (7-9), as well as a higher chance of mortality (Monaghan and Ozanne, 2018). Telomere length (TL) is regarded as a significant biomarker that affects the health and ageing of the entire body. Short telomeres have been linked to an increased risk of mortality after death in both humans and other animals, although the exact causes are still unclear. An important unanswered question is whether TL-mortality associations are caused by genes or early life environments that increase a person's mean lifetime TL and lifespan, or whether environmental stressors are responsible for TL-mortality associations because of their more immediate effects on intra-individual TL loss and increased mortality risk. As long-lived creatures like humans, longitudinal TL and life history data across the complete lifetime of many people are needed to answer this topic. Long-term research on wild Soay sheep examined multivariate quantitative genetic models, and it was demonstrated that there was a positive correlation between TL and survival using the information and samples accumulated over almost two decades

(Froya et al., 2021). It appears that TL is not a significant predictor of biological ageing or exposure to environmental stress, but that interindividual variations in mean TL are related to longer lifespans. There was no proof that telomere attrition was linked to a higher risk of death. In conclusion, it demonstrates that telomere length (TL) can change under any condition and suggests that genetics may have a considerable impact on the well-known association between short telomeres and mortality.

Administration of Melatonin to Newborn Lambs

Melatonin has been demonstrated in certain research to benefit the care of newborn lambs. Limited fetal development and severe pulmonary hypertension in newborn animals are consequences of persistent oxygen shortage in tissues during difficult pregnancies. Melatonin (1 mg/kg per day) was given to newborn lambs in high-altitude experiments, and it was found to have some positive effects on vascular function (Astorga et al. 2018). Melatonin may help at-risk lambs recover, according to a study, but in intensive production systems, pregnancy and delivery are the times when oxidative damage to lambs is most likely to happen. The early identification and care of newborns who are hypoxic depend on routine monitoring, which is impossible in this circumstance. For the aforementioned rationale, it would be more advantageous to provide therapeutic intervention to the fetus in the final trimester of pregnancy in order to provide protection during times of chronic hypoxia as well as acute hypoxia after delivery (Boutin et al. 2005).

Intergenerational, Genetic, And Epigenetic Influences

Environmental and genetic variables have an impact on the lamb's survivability. Programs for sheep breeding cannot simply alter environmental conditions, notwithstanding their importance (Nowak et al. 2000; Nowak and Poindron, 2006). In conclusion, the mother must be able to deliver live lambs without any difficulty and must produce an appropriate amount of colostrum, milk, and maternity for the lambs to survive. The lamb, on the other hand, must swiftly adjust to leaving the uterine environment and rely on its capacity to control temperature, stand up, and continuously get nourishment from its mother after delivery. Finally, the creation of the mother-child relationship is just one example of how ewe and lamb factors actively contribute to creating the foundation of viability. Lamb viability variation may be divided into only

two phenotypic categories: lambs survive or do not. However, it is helpful to conceive of a threshold level as an underlying and continually changing threshold character when it comes to determining the viability of lambs (MLA, 2019). According to research, melatonin may aid in the recovery of lambs that are in danger, but in intensive production systems, pregnancy and delivery are the times when lambs are most prone to sustain oxidative damage. Routine monitoring is necessary for the early detection and management of neonates who are hypoxic, but it is impossible in this situation.

Lamb Procedure For Those Who Can't Consume Enough Colostrum

Hunger and cold shock are two major factors in postpartum offspring death in newborn lambs. If these pups receive enough colostrum during the first 6–12 hours after birth, it is feasible to keep them healthy and alive until the conclusion of the raising period (Gökçe et al. 2014). Lambs need around 95 g of colostrum for every 500 g of body weight in the first 18 hours when they are born in an open barn or field (5-10 °C in windy and wet weather). On the other hand, they require around 80 g of colostrum for every 500 g of body weight when they are born in a contained environment (windless and dry) at 5-10 °C.

Certain Tips to Reduce Lamb Death

1. Before Lambing, Test The Feed

The sheep must be in good physical condition and able to generate high-quality colostrum before giving birth. Because the protein and calorie content of a feed-based diet can vary significantly, it should be assessed to determine if silage, straw, or dry hay may be used to adjust diets and improve sheep condition scores (Miller et al. 2010). Any breeding sheep with a body condition rating under 2 should not be used. Sheep with 2.5–3.5 points will produce and be profitable to the greatest extent. Very weak sheep (2.5) will produce little, have a high mortality rate, and have poor offspring. Additionally, to fall outside of the recommended range, highly fat/fat (>3.5) sheep might also indicate.

2. Implementing A Program to Preserve Your Health

With the help of a qualified veterinarian, a production strategy should be created before and after birth, as well as an efficient immunization schedule against bacteria, viruses, and other illnesses. Both this program and the internal-

external parasite control that must be carried out throughout this time should be closely monitored.

3. Sheep Should Undergo Blood Testing, And Screening Outcomes Should Be Carefully Examined

The root of the high infertility rate for infectious disorders or deficiencies should be investigated to reduce the impact of lambing. This procedure becomes considerably more significant, particularly when breeding male and female animals are bought or imported.

4. Sheep Body Condition Score (BCS)

During the lambing season, a condition score of 3-3.5 is appropriate for providing lambs with high-quality colostrum and nutrition. In contrast to farms on the plain, sheep raised in hillside or mountainous locations have an average lambing duration of 2.5 to 3 weeks. If the BCS score is below average, flukes, excessive lameness, or a nutrition issue may be to blame. Taking care of problems as soon as they arise is critical to prevent lambs from being too weak when they approach the third trimester.

5. Give Enough Room for Feeding

As lambing time draws near, providing each sheep with enough roughage and a dense feed consumption area can help to better handle the issue. If intensive feeding is used, the minimum feed route length for sheep farms on the plains should be 45 cm, and for processing on hills or slope areas, it should be 30 cm. A feed route of 10–12 cm should be provided for each sheep if free fodder is offered. If there is space, it may be possible to split the herd or to use additional feeders for these animals. This will make it easier for younger or less robust animals that couldn't compete in the feeder to gain entrance.

6. Make Sure to Utilize A Clean Birth Kit

The company's lambing kit must be examined, and all equipment needs to be cleaned. The medications and solutions in the kit's expiration dates should be carefully monitored. It is imperative to purchase and replace any missing or necessary supplies as quickly as feasible.

7. Quickly consume colostrum

Lambs should receive 20% of their birth weight on the first day of colostrum and 10% of that amount in the first six hours. Using an Ikea bag and baggage scale is a fantastic way to weigh lambs.

For every 3 kg of lamb, give 600 ml (or around 0.56 ml).

For every 4 kg of lamb, provide 800 ml (or around 0.784 ml).

Provide 1,000 ml (or around 0.980 ml) for 5 kg of lamb.

8. Colostrum Quality Needs to Be Regulated

Which lambs may require more colostrum will be determined by the Brix reflectometer's evaluation of the colostrum's quality. According to Alves et al. (2015), the protein level should have a Brix score of over 22%.

Table 3. Some quality characteristics of colostrum

Brix reflectometer value%	IgG value (g/l)	Colostrum quality
<1	0-28	Bad
15-20%	28-50	Medium
20-30%	50-60	Good
>30%	>80	Very good

Colostrum has three crucial characteristics that are, respectively, its quality, quantity, and consumption rate.

• **QUALITY -** The health of the sheep, its BCS, and the pre-lamb diet of the sheep will all affect the colostrum's quality. Colostrum quality may be evaluated using a refractometer.

Lambs should get 200 ml/kg of colostrum after the first two hours, and 50 ml/kg after that.

• **QUANTITY** – Lambs should receive 50 ml/kg of colostrum in the first 2 hours and 200 ml/kg in the first 24 hours. The colostrum share should be increased by 15% to 20% for lambs reared outdoors.

• **FAST** –As soon as possible after delivery, and during the first six hours of life, lambs should be nursed.

Only the best quality colostrum should be taken from sheep and frozen if necessary. During use, attention should be paid to dissolving, otherwise, the proteins may be damaged at high temperatures. Therefore, colostrum should not be heated in the microwave or thawed using boiling water.

9. A Separate Birth Chamber

Sheep and lambs should be carried in separate compartments for washing and disinfection to promote bonding, ease monitoring, and help with colostrum production.

10. Sheep Mastitis Control

Controlling postpartum mastitis in sheep is necessary. Milk flow must be assessed to determine whether the animal has mastitis or whether the nipple opening is closed. Sheep should form strong bonds with their young, and animals that have difficult deliveries should always be under a veterinarian's care.

11. Umbilical Cord Cleaning/Disinfection in Lambs

Against a possible risk of infection after birth, lamb navels should be dipped in iodine as soon as possible and then their eyes should be checked. A condition called entropion or ingrown eyelids can interfere with feeding lambs. Some lambs could have trouble finding their mother's udder, or if the sheep's udder is sagging, the lamb needs assistance finding the udder. Colostrum should be given to the calf as soon as possible via a stomach tube if there is any question as to whether the lamb is receiving enough of it.

Identifying Weak Lambs Is Necessary

When a mother is caring for triplets or is having a challenging lambing, colostrum ingestion might be problematic. In this scenario, it could be challenging for puppies with the lowest birth weight to get colostrum. Start using a bottle or stomach tube as soon as feasible to address the issue.

Furthermore, Colostrum

Colostrum should be given to all lambs in the greatest quantity and quality. Colostrum should be given in the first two hours after delivery at a rate

of 50 milliliters per kilogram of body weight, and then 200 milliliters per kilogram the next day. Agenbag et al. (2021) recommend that all lambs receive adequate colostrum within the first two hours after delivery. If there is any dispute regarding nursing, the sheep's colostrum production should be monitored, and the lamb should be assisted in sucking. Within six hours (after the brown fat that keeps the lamb warm has been depleted), if the lambs have not received enough colostrum or have not swallowed enough of it, they should be supplemented with high-quality, Brix-tested sheep colostrum. If this is not an option, artificial or bovine colostrum replacements may be employed. The donor cow must be clear of BVD and Johne's for cattle replacement. As lambing season advances, animal caretakers or herders should be watchful of mortality and enrollment rates. The fatality rate from pregnancy to sale shouldn't be more than 10%, according to Barry et al. (2008). The most common time for losses is the first 48 hours following delivery. After this period, mortality rates need to be lower than 2%. A check should be made for the presence of viruses or bacteria, such as Clostridium, in any loss greater than this.

Suggestions

One of the main sources of financial loss in the industry might be the mortality of lambs during prenatal or neonatal development. Although several factors might lead to perinatal lamb mortality, potential losses can be significantly decreased with the right herd management arrangements. Due to this, it is best to avoid letting pets like cats and dogs walk free in the herd and to keep them away from food and water. Lambs who are more likely to experience prenatal death should be properly watched. By making a logical feeding decision based on whether the sheep are single or multiple pregnancies, care should be taken to ensure that they are at the appropriate body condition score. Finally, sheep who are calm and have good maternity traits should be chosen as breeders. The strategies or actions suggested above will decrease lamb losses and boost financial gains.

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CHAPTER 2

THE INVESTIGATION of THE EFFECT of SILAGE ADDITIVES on SILAGE MICROBIOLOGY

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Introduction

Silage has become one of the basic components of ruminant rations in many countries. Silage making, like many other fermented products, is a green feed storage method that has been used in animal production for many years. It has been reported that the largest share in the fermentation process applied worldwide is silage making (Jatkauskas and Vrotniakiene, 2016).

Certain types of lactic acid bacteria are found on plant surfaces, which have been shown to occur naturally in plants. Silage making basically depends on these epiphytic lactic acid bacteria. Although the role of lactic acid bacteria on plants is not fully known, it is thought that they protect plants from unwanted microorganisms (by producing antagonistic compounds such as organic acids, bacteriocins and antifungal agents) or limit their proliferation, since they are found in greater numbers in damaged plant parts than in non-damaged plant parts (Onol and Duru, 2021).

Silage is a cheap feed source, but it is also a difficult product to keep and store. It should not be allowed to be deteriorated by bacteria and microorganisms. The main purpose in silage production is to obtain a quality silage. For this, all conditions must be made suitable for silage. Storage conditions should be provided as they should be and contact of silage with external environments should be prevented. It can cause the growth of various microorganisms and spoilage of silage if any problems occur. These are undesirable in silage production and consumption (Atalay and Kaynar, 2023). If animals consume spoiled silage, it can lead to problems such as changes in rumen fermentation caused by harmful yeasts, decreased performance, and the production of undesirable end products by molds and other microorganisms (such as mycotoxins). Silage material that has undergone clostridial fermentation and has a high concentration of butyric acid remains stable when exposed to air. However, clostridial silages are undesirable as they cause high amounts of dry matter and nutrient loss. Additionally, silage material with a high concentration of acetic acid remains stable when exposed to air because acetic acid is highly toxic to yeast. However, high acetic acid concentration is also an undesirable situation as it is an indicator of heterolactic fermentation (Ozcan, 2022).

The use of additives in silage fermentation is a common practice, and there are many biological and chemical products used for this purpose. These

products are generally used to improve fermentation efficiency, aerobic stability, and minimize hygienic risks (Altıncekic and Filya, 2018).

1.Silage Additives

Numerous additives can be utilized in silage production to enhance silage quality. These include carbohydrate sources (such as molasses, sugar beet, turnips, potatoes, and various grain by-products), additives that increase dry matter content (molasses, straw, dried sugar beet pulp, dried forage, cereal bran, etc.), and additives that act as preservatives and ensure silage sterilization (sodium diacetate, sodium benzoate, sodium metabisulfite, sodium nitrate, formaldehyde, hydrochloric acid, sulfuric acid, calcium formate, formic acid, acetic acid, propionic acid, lactic acid, etc.) (Kiraz and Kutlu, 2016a). For the improvement of silage fermentation, biological additives such as bacterial cultures (commercial lactic acid bacteria (LAB) inoculants) and enzymes (amylase, cellulase, hemicellulose, xylanase, etc.) are used in silages (Kiraz and Kutlu, 2016b). Various methods have been employed to classify silage additives. Yitbarek et al. (2014) categorized silage additives into three main classes: biological additives, organic acids and salt additives, and feed components and by-product additives.

1.1. Biological Additives

1.1.1. Fermented Natural LAB Liquid (FDLAB)

In recent years, there has been increasing attention given to Fermented Natural LAB Liquid (FDLAB) as a new silage additive, serving as an alternative to commercial LAB inoculants. FDLAB liquid offers several significant advantages as a silage additive, including its biological origin, ease of preparation and use, cost-effectiveness, safety, non-toxicity, non-corrosiveness to machinery used in silage making, minimal environmental impact, and being a natural product (Jin-ling et al., 2013). The use of fermented LAB liquid as a silage additive has been reported to protect proteins in silage from excessive degradation, reduce silage pH by increasing LAB density, thereby inhibiting Clostridial activity (Nishino and Uchida, 1999). Considering that the number and type of LAB carried by different plants may vary, it has been suggested that fermented LAB liquids prepared from the same plant as the silage material may be more effective in improving silage quality (Ohshima et al., 1997).

1.1.2. Commercial Inoculants

Among silage additives, the most popular fermentation stimulants are bacterial inoculants that enhance lactic acid fermentation, support acidification, and improve aerobic stability. During fermentation, inoculants can also produce ferulic acid esterase enzymes, which facilitate the breakdown of cell wall components (Muruz et al., 2019). The advantages of lactic acid bacteria (LAB) inoculation in silage making have been substantiated by numerous studies. Certain criteria must be met for these bacteria to be used as silage additives. These include the dynamic multiplication of bacteria, their ability to compete with other organisms, and preferably, dominance. To maximize lactic acid production from hexose sugars, bacteria should be homofermentative, and they must be acid-tolerant (e.g., *Lactobacillus acidophilus, L. casei, L. plantarum, L. rhamnosus, Pediococcus acidilactici, and P. pentosaceus*). They should possess the capacity to ferment glucose, fructose, sucrose, fructans, and preferably pentose sugars. These bacteria should not have any impact on organic acids. They should have a wide growth temperature range (up to 50°C) and be capable of thriving in low-moisture silages. Therefore, the success of the inoculant used as a silage additive depends on factors such as the type and characteristics of the harvested crop, climate conditions, epiphytic microflora, silage technique, and the properties of the inoculant (Cai et al., 2014).

Lactic acid bacteria are a crucial component of silage bacterial inoculants. These bacteria expedite silage fermentation and enhance its quality by converting WSC into lactic acid. Lactic acid bacteria are categorized into three main groups based on the amount and type of lactic acid they produce: obligate homofermentative, facultative heterofermentative, and obligate heterofermentative lactic acid bacteria (Bernardi et al., 2019). Obligate homofermentative lactic acid bacteria convert over 90% of carbohydrates into lactic acid and do not produce other organic acids, carbon dioxide, or ethanol. Facultative heterofermentative lactic acid bacteria convert approximately 50% of carbohydrates into lactic acid and can produce other organic acids, carbon dioxide, or ethanol. Obligate heterofermentative lactic acid bacteria convert around 30% of carbohydrates into lactic acid and produce other organic acids, carbon dioxide, or ethanol. Among these groups, obligate homofermentative lactic acid bacteria are the most commonly used. They include species such as *Lactobacillus plantarum*, *acidophilus*, *brevis*, *bulgaricus*, *cremoris*, *curvatus*,

xylosus, salivarus, Enterecoccus faecium, Pediococcus acidilactici, cerevisiae, and *pentosaceus*. Obligate heterofermentative lactic acid bacteria are less commonly used but may have advantages in certain situations due to their production of antifungal organic acids. These species include *Lactobacillus buchneri, Propionibacterium arabinosum, jensenii,* and *shermanii* (Oliveira et al., 2017; Keleş, 2017).

For commercial LAB inoculants to be effective in silage making, the dosage used must exceed the population of epiphytic bacteria in the silage material (Pahlow and Honig, 1986). Otherwise, it becomes challenging for inoculated LAB to establish dominance over the natural microbial population. If the dosage of LAB inoculants is inadequate, natural bacteria may inhibit lactic acid production or produce unwanted fermentation products, reducing silage quality and nutrient value (Jones et al., 2004).

1.1.3. Enzymes

Enzymes play a significant role in animal nutrition, aiding in the digestion of nutrients and enhancing animal performance while reducing environmental pollution by lowering fecal quantity and its quality. The use of enzymes in silage making can improve silage quality and fermentation. Enzymes break down starch and cell wall polysaccharides in silage into simple sugars. These sugars promote the growth of lactic acid bacteria in silage and lower pH. Enzymes can also form enzyme-substrate complexes, which protect against exogenous enzymes that inhibit feed breakdown in the rumen. The efficacy of enzymes depends on the type of feed, lignin content, and plant maturity. Enzymes are most effective in low-lignin and young plants. As plants mature and lignification increases, cell wall hydrolysis decreases. Consequently, the impact of enzyme application on silage quality and animal performance can vary (Yitbarek and Tamir, 2014).

Enzymes are proteinaceous compounds that serve as catalysts for specific biochemical reactions in biological systems and are produced by living cells (Güçlü and Kara, 2009). Enzyme preparations used in ruminant rations are primarily obtained from *Bacillus subtilis, Lactobacillus acidophilus, Lactobacillus plantarum, Streptococcus faecium bacteria, Trichoderma longibrachiatum, Aspergillus oryzae, Trichoderma reesei fungi*, and *Saccharomyces cerevisiae* yeast (McAllister et al., 2001). Enzymes play an

effective role in increasing the activities of microorganisms required for optimal lactic acid fermentation by providing additional energy sources (Güney and Demirel, 2007). The first advantage of cell wall-degrading enzymes used as silage additives is to hydrolyze structural carbohydrates, thereby releasing substrate in plants with low amount of water-soluble carbohydrates (WSC); and the second advantage is to increase the digestibility of plant dry matter and organic matter by animals (Filya, 2003).

1.2. Organic Acids and Salt Additives

Organic acids have an essential role as feed additives in ruminant nutrition. They delay spoilage by increasing feed acidity, reduce mycotoxin formation and effects, improve microbial balance in the digestive system, and enhance nutrient utilization (Güçlü and Kara, 2010). Additionally, they contribute to environmental and animal health by reducing protein loss associated with high ammonia production and methane emissions (Sahoo and Jena, 2014). Organic acids

prevent ruminal acidosis, increase animal performance, stimulate the release of growth hormones, support the balance between pathogenic and beneficial microorganisms (probiotics) in the digestive system, and improve nutrient digestion and absorption. The use of organic acids as feed additives is economically and ecologically beneficial (Ozkaya, 2017). Organic acids enhance silage quality and aerobic stability by preventing aerobic deterioration in silages. They lower silage pH rapidly in the silo environment, inhibiting the growth of microorganisms (yeasts, molds, *Enterobacteria*, and *Clostridia*) responsible for aerobic deterioration. Thus, being effective at low doses as a silage additive by preventing warming, protein breakdown and NH_3-N formation in silages, reducing nutrient loss in silages, increasing the digestibility of silages and animal performance can be counted among the advantages of organic acids (Filya and Sucu, 2005).

The most commonly used organic acids in ruminant rations include formic, acetic, propionic, butyric, lactic, sorbic, fumaric, tartaric, citric, benzoic, and malic acids. Organic acids can be categorized into two groups based on their mechanisms of action. The first group, consisting of lactic, fumaric, and citric acids, takes its effect by indirectly reducing the population of acid-sensitive bacteria by lowering ruminal pH. The second group, which

includes formic, acetic, propionic, and sorbic acids, directly affects bacteria by entering the gram (-) bacterial cells and reducing intracellular pH in the gastrointestinal system (Papatsiros et al., 2013).

Salt (NaCl) is a significant feed additive used to preserve food. Salt can inhibit the growth of harmful bacteria and contribute to the desired fermentation. Sodium and chloride are essential for maintaining osmotic pressure in plants and microorganisms. Increasing the concentrations of these compounds can affect the abilities of organisms without salt tolerance to maintain cellular functions. Adding salt to silage material can reduce water activity and inhibit the growth of butyric acid bacteria during fermentation (Ergin, 2019). A study reported that the addition of salt initially inhibited the growth of *Clostridium butyricum* (Borreani et al., 2018).

1.3. Feed Components and By-Product Additives

Easily fermentable feed ingredients such as sugar or molasses added to tropical forages with low dry matter and sugar content can improve silage fermentation. Since silage quality depends on the fermentation of sugars in the ensiled feed by lactic acid bacteria, sources rich in water-soluble carbohydrates (WSC) have a significant effect on improving silage quality (Muck, 2010). In this context, fermentation stimulants are added to silage to promote lactic acid fermentation. Carbohydrate sources are silage additives added at a rate of <2.5% to increase the

water-soluble carbohydrate (WSC) content in sugar-poor legumes and some tropical plants. In feeds ensiled by adding water-soluble carbohydrates (WSC), lactic acid production is expected to increase after fermentation, while dry matter losses, pH and NH_3-N are expected to decrease (Kaiser et al., 2004). In general, processed by-products such as grains, corn flour, sorghum flour, rice bran, tapioca flour, citrus fruits can be used as silage additives to provide a partially fermentable substrate. These products absorb excess moisture and direct the fermentation process positively (Yitbarek and Tamir, 2014).

2. Silage Microbiology

pH is of crucial importance when determining a quality silage. For this reason, bacteria that multiply in aerobic areas and cause feed spoilage cannot grow in silage feed in an anaerobic environment.

However, there are two types of bacteria that can multiply in an anaerobic environment, one is butyric acid bacteria and the other is lactic acid bacteria. Among these bacteria, only lactic acid bacteria seem to be suitable for proliferation. Lactic acid bacteria cannot multiply when the pH of the silage material is higher than 4.5, and butyric acid bacteria cannot multiply below 4.5 pH. For this reason, the growth of butyric acid bacteria is prevented by ensuring that the pH of the silage material is between 3.8-4.2 (Ergün et al. 2016). Silage bacteria are of great importance in ensuring a successful fermentation process. These bacteria are divided into two groups: lactic acid bacteria (LAB), which are desired in silage and contribute to the quality of silage, and undesirable bacteria in silage, which negatively affect the quality of silage. (Oladosu et al., 2016).

2.1. Bacteria Desired in Silage (Lactic Acid Bacteria)

In silage fermentation, anaerobic microorganisms multiply rapidly, using sugar and starches as energy sources, and they mainly produce lactic acid. They produce small amounts of acetic acid and very small amounts of formic acid, propionic acid and butyric acid. There is almost no butyric acid in well-prepared silage (Sahin, 2019). The term lactic acid bacteria includes the genera *Lactobacillus*, *Pediococcus*, *Lactococcus*, *Enterococcus*, *Streptococcus* and *Leuconostoc* (Pahlow et al., 2003). These bacteria produce primarily lactic acid, which is the fermentation product of sugars, as well as acetic acid, ethanol and CO_2. Lactic acid bacteria grow best at mesophilic temperatures between 20°C and 40°C (optimum 30°C) (Drihehuis and Oude Elferink, 2000). Lactic acid bacteria are divided into two groups, homofermentative lactic acid bacteria and heterofermentative lactic acid bacteria, according to the fermentation products they produce. In other words, lactic acid bacteria that convert sugars into lactic acid are called

homofermentative lactic acid bacteria (Muruz and Yörük, 2000). Heterofermentative lactic acid bacteria ferment water-soluble carbohydrates (WSC) and produce acetic acid, ethanol, CO_2 and lactic acid (McDonald et al., 1991).

- **Homofermentative Lactic Acid Bacteria:** Lactobacillus (*Lb. plantarum, Lb. acidophilus, Lb. casei, Lb. comyiformis, Lb. curvatu, Lb. gasseri, Lb. helveticus, Lb. homohiochii, Lb. maltaromicus, Lb. delbrueckli ssp. lactis, Lb. delbrueckli ssp. bulgaricus*), Pediococcus (*P. acidilactis, P.pentosaceus, P. inopinatus*), Streptococcus (*St. faecalis. St. durans. St. faecium. St. lactis. St. bovis*), Enterococcus (*E. faecalis. E. faecium*)
- **Heterofermentative Lactic Acid Bacteria:** Lactobacillus (*Lb. brevis, Lb. buchneri, Lb. fermentum, Lb. viridescens, Lb. confuses, Lb. collinoides, Lb. hilgardii),* Leuconostoc (*Lue. mesenteroides, Leu. lactis*).

2.2. Undesired Bacteria in Silage

Listeria spp. Salmonella spp. and *Clostridia spp*. together with yeast and mold fungi are undesirable microorganisms in silage material (Basmacıoğlu and Ergül, 2002).

Listeria spp multiplies rapidly, especially in the ventilated upper and side parts of silages, where an anaerobic environment is not provided and therefore the pH is not at the desired level (pH>5.5), and poses a dangerous situation for the animals fed with these silages (Basmacıoğlu and Ergül, 2002). Animals that are infected with *Salmonella spp* bacteria may become ill and, in advanced cases, die. Applying the appropriate fermentation process to silages prevents the development of salmonella species. Cattle, birds, flies, rodents and other farm animals are sources (porters) of salmonella (Jones, 2011). Clostridia type microorganisms are the most important competitors of lactic acid bacteria because they use the water-soluble carbohydrates (WSC) used by lactic acid bacteria during silage fermentation. However, *Clostridia* are among the undesirable microorganisms in terms of silage fermentation because they reduce the nutritional value of silage by creating NH_3-N in the silo as a result of the catabolism of amino acids, cause energy loss, and increase the pH value of silage due to the NH_3-N they create (Basmacıoğlu and Ergül, 2002). Yeasts, which are from the group of aerobic microorganisms, break down the WSC in the silage plant into carbon dioxide and organic acids, especially alcohol. Yeast fermentation occurring in the silo can also be described as alcohol fermentation. It is possible to divide yeasts, which have a significant share in aerobic spoilage,

into two groups. While the microorganisms in the first group (*Candida*, *Endomycopsis*, *Hansenula* and

Pichia) use acids, the other group (*Torulopsis*) use carbohydrates (Woolford, 1990). Candida and Hansenula species of yeast increase the silage pH value by breaking down lactic acid. Thus, suitable conditions are provided for the development of mold fungi after the silo is opened. It is reported that the critical yeast value in terms of aerobic spoilage in silages is 10^5 cfu/gr DM (Jonsson, 1991). Another group of microorganisms that are undesirable to be in the silo are mold fungi. For the development of mold fungi in the silo; factors such as the moisture content being over 13%, the temperature being over 12.8 ^oC, the presence of usable nutrients in the environment, the pH value being over 5, and the presence of oxygen are important. When the presence of mold fungus in the silage reaches the value of 10^6 -10^7 cfu/gr, the fermentation products in the silage are consumed by the mold fungi, resulting in an increase in temperature in the silage. When the temperature inside the silo exceeds 65 ^oC, overheating of the silage material, an increase in the number of yeast and mold, and protein degradation occur (Bolsen et al., 1996; Lin et al., 1992). Mold fungi that become active in the silo consume the lactic acid formed by carbohydrates and proteins in the plant, causing deterioration in the physical and chemical structure of the silage and reducing the quality and digestion values of the silage (Ruppel et al., 1995).

3.Aerobic Stability Microbiology Of Silage

Aerobic stability of silage is defined as the length of time during which the temperature inside the silage remains at a low level and remains intact as a result of contact with air (Çayıroğlu et al., 2016). It is desirable for the silage to remain aerobically stable. After opening, silage begins to deteriorate rapidly when exposed to high amounts of yeast (10^5-10^6), whereas it can generally remain stable for a long time if exposed to low amounts of yeast (10^2-10^4). Considering that the cell number of yeast doubles within 2 hours under suitable conditions, it is estimated that silage that comes into contact with air after opening will begin to deteriorate quickly (Ozcan, 2022). The main yeast species that play a role here are *Candida*, *Hansenula*, *Pichia*, *Issatchhenkia* and *Saccharomyces* (Woolford, 1990). When lactic acid is destroyed by yeasts, the rising pH causes the growth of opportunistic bacteria (*Bacilli*) and molds

(*Aspergillus*, *Fusarium* and *Penicillium*), significantly reducing the quality of silage (McDonald et al., 1991).

Aerobic stability is affected by the type of inoculant added to the silage. Most of the bacterial inoculants contain homofermentative lactic acid bacteria. Such microorganisms ferment sugars predominantly into lactic acid, rapidly lowering the pH and thus improving fermentation (Keleş and Yazgan, 2011). *L. buchneri* is the most widely used lactic acid bacteria as a heterofermentative silage additive. As a matter of fact, Keleş and Yazgan (2011) reported that the addition of *L. plantarum*, homofermentative lactic acid bacteria consisting of *E. Faecium* and heterofermentative lactic acid bacteria consisting of *L. buchneri* to baled corn silages did not affect the fermentation properties of baled silages, but the addition of *L. buchneri* improved the aerobic stability.

The fermentation process affects the aerobic stability of silages. When anaerobic conditions occur in the silo, some changes begin to occur in the ensiled material. First of all, the water of the material becomes free. The enzymes released as a result of this break down the polysaccharides in the plant and produce the sugars necessary for lactic acid bacteria. Keeping the silo feed intact in the silo is achieved by the lactic acid produced by the lactic acid bacteria (Mohd-Setapar et al., 2012). Lactic acid bacteria also prevent the growth of acetic acid and butyric acid bacteria, which are undesirable to multiply in the silo by forming antibiotic-effective substances and prevent the feed from spoiling and rotting (Basmacıoğlu and Ergül, 2002).

4.Conducted Studies

Baytur Atılgan (2023), in their study, left silages of vetch and barley mixtures prepared with the addition of molasses, fermented natural lactic acid bacteria liquid (FDLAB), homofermentative lactic acid bacteria, heterofermentative lactic acid bacteria and fructose syrup for fermentation for 6, 12, 24 and 48 days. It was determined that the homofermentative LAB inoculant additive decreased the pH value, and the highest lactic acid value was observed in the molasses added group. It was observed that the lowest pH value was detected at the end of the 12-day fermentation period, the highest lactic acid value was detected at the end of the 6 and 12-day fermentation period, the lowest yeast value was detected at the end of the 48-day fermentation period, and the highest mold values were detected at the end of the 24-day fermentation

period. A five-day-long aerobic stability test was performed on the silages opened at the end of the fermentation periods, and then the amount of yeast and mold was checked. It was determined that the lowest yeast values were in the heterofermentative LAB inoculant, and the lowest mold value was in the homofermentative LAB inoculant contribution. It has been reported that molasses and heterofermentative LAB inoculant improve aerobic stability and suppress yeast and mold growth.

Erten et al., (2022) aimed in their study to evaluate the re-ensiling of second product corn silage at different times by adding inoculant and organic acid in terms of silage quality and aerobic stability. A seven-day-long aerobic stability test was applied to all silages opened during the ensiling period (60th day). Research results reported that corn silages can be re-ensiled without adding additives, but the use of organic acid in re-ensiling improves aerobic stability.

Eren (2022) added 2% urea, 10^6 cfu/g *Lactobacillus plantarum* (LP) + *Lactobacillus buchneri (LB)* and 10^6 cfu/g *Lactobacillus buchneri (LB)* to high moisture grain corn and they were ensiled for 60 days. The addition of urea decreased the LAB, yeast, mold, lactic acid (LA), propionic acid (PA) content of the silages. Addition of LAB to silages decreased pH, yeast and mold counts, and increased LAB count and LA, PA content. The addition of lactic acid bacteria also improved aerobic stability by increasing the amount of carbon dioxide (CO_2). It was concluded that LAB inoculant is the most suitable additive for nutrient composition, silage microbiology, fermentation, nutrient digestion and aerobic stability. The most effective LAB inoculant was the LP+LB combination. This was followed by LB and 2% urea.

In their study, Filik et al., (2022) determine the potential for use of Chia (*Salvia hispanica L.*) plant in silage production. The effects of 2.5% molasses + 1% salt additive on silage quality were determined. Enterobacter strains were not found in all silage samples examined. (2.5% molasses addition) and (11% salt addition) were excluded from the experiment due to high mold formation. It is thought that yeast-mold development cannot be suppressed with only molasses addition. It is thought that the mere addition of salt suppresses the growth of lactic acid bacteria, and that yeast-mold formation occurs as the silage softens. It was determined that silage containing 2.5% molasses + 1% salt was of better quality.

In a study conducted by Coskuntuna and Gül (2020), the effect of LAB (LAB mixture containing *Lactobacillus plantarum* and *Enterococcus faecium*) and enzyme (cellulase, amylase, hemicellulose and pentosanes enzymes mixture) inoculants added to vetch silage on silage fermentation was examined. Lactic acid values of silages in all groups were found to be similar to each other. At the end of the study, it was stated that the addition of LAB and LAB + Enzyme to vetch silage had a positive effect on the chemical properties of the silages.

In the study conducted by Doğan (2019) to determine the effect of the use of enzymes and commercial inoculants on the fermentation and aerobic stability properties of triticale silage, it was determined that the use of commercial inoculants and enzymes increased the fermentation properties of silages and decreased aerobic stability. In this context, it is understood that the use of heterofermentative lactic acid bacteria in silages with enzymes or additives is more effective.

Ergin, (2019) prepared alfalfa silage by adding salt and lactic acid bacteria to the alfalfa plant separately and together, and the silages were opened on the 7th, 14th, 30th and 60th days of fermentation. It was determined that the total quality score and Flieg score in the lactic acid bacteria added group were statistically significantly higher than the other groups. It was determined that acetic acid and lactic acid increased, and propionic acid and butyric acid decreased with the addition of salt and lactic acid bacteria. It was determined that lactic acid bacteria increased, and the number of yeast and mold decreased in all groups. It was observed that the best result in terms of aerobic stability was in the lactic acid added group. As a result, it was observed that the silage quality, fermentation profile and microbial properties were positively affected by the addition of salt and lactic acid bacteria together or separately to the silage.

Okuyucu et al. (2018), in the study where the fermentation properties and aerobic stability of the use of different levels of lactic acid bacteria + enzyme (LAB+E) in alfalfa silages were examined, added inoculants to the alfalfa yields at the levels of $1x10^5$, $5x10^5$ and $1x10^6$ cfu/g. Chemical and microbiological analyses were performed on the silages on the 2nd, 4th, 8th and 45th days after ensiling. A five-day-long aerobic stability test was applied to all silages opened at the end of the ensiling period. As a result, LAB+E inoculants

decreased the pH and ammonia nitrogen contents of the silages, while increasing their lactic acid content and lactobacilli count. High level ($1x10^6$ cfu/g) LAB+E addition increased the carbon dioxide production of silages and decreased the aerobic stability of alfalfa silages.

Altıncekic and Filya (2018), in their study, aimed to determine the effects of a preservative (FAT) based on homofermentative lactic acid bacteria (LAB) inoculant and formic acid on the aerobic stability and feed value of small bale corn (Zea mays L.) silage (low dry matter). The additives were added to corn respectively and in ratios as follows: LAB 10^6 cfu/g; FAT 3, 4 and 5 g/kg; LAB+FAT 10^6 cfu/g+3 g/kg, 10^6 cfu/g+4 g/kg, 10^6 cfu/g+5 g/kg. Chemical and microbiological analyses were performed on the silages opened 60 days after ensiling, and an aerobic stability test was applied to the silages for 5 days. As a result, homofermentative LAB inoculant, FAT and LAB+FAT combination did not affect the aerobic stability of low DM corn silages.

In another study by Chen et al. (2016), molasses containing oat-common vetch, lactic acid bacteria inoculant (*Lactobacillus plantarum* 10^6 cfu/g), propionic acid (0.3%), molasses + propionic acid (0.3%), lactic acid bacteria + propionic acid (0.3%) additives were ensiled for

45 days. It was determined that all silages had low pH value and high lactic acid level. It was determined that the lactic acid bacteria, molasses + propionic acid and lactic acid bacteria + propionic acid groups contained lower amounts of acetic acid than the control group, and the lowest value was in the group to which lactic acid bacteria was added.

Hashemzadeh-Cigari et al. (2014), in their study, evaluated the effect of the application of molasses and homofermentative and heterofermentative lactic acid bacteria in alfalfa silage on the fermentation quality by using double inoculants. In this study, they reported that the silages applied to molasses deteriorated due to the increase in temperature and the abnormal change in the number of microorganisms. In this context, it was concluded that the use of double inoculants did not further improve fermentation quality and aerobic stability.

5.Conclusion and Recommendations For Further Studies

Some silage additives can be used to increase the quality and efficiency of silo feeds. These additives accelerate the fermentation of silage, prevent the

growth of unwanted microorganisms and preserve the nutritional value of silage. The most common silage additives are lactic acid bacteria, enzymes, yeasts, propionic acid and formic acid. Lactic acid bacteria ensure the aerobic stability of silage by lowering the pH of the silage. Enzymes increase the digestibility of silage by breaking down fibrous components such as cellulose and hemicellulose. Yeast supports fermentation by consuming the oxygen in the silage. Propionic acid and formic acid inhibit the growth of harmful microorganisms such as mold and fungus. In this way, silage additives help with considerations in the preparation of silage feeds. Considering the general principles of fermentation in silages, aerobic spoilage is an inevitable result in all silages, especially during the feeding period. Aerobic spoilage is when microorganisms consume oxygen in the silage, causing the silage to heat up, lose nutrients and decrease in quality. Therefore, necessary precautions should be taken to minimize the contact of silage with air, especially during the feeding period. Silage should not be exposed to air until fermentation is completed.

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CHAPTER 3

AN OVERVIEW of TUBERCULOUS DISEASE FROM A PUBLIC HEALTH PERSPECTIVE

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Introduction

Infectious zoonotic diseases can be transmitted as a result of close contact between animals and humans (Mbugi et al., 2012).

One of the most important of these diseases is tuberculosis disease. Tuberculosis is an important zoonotic disease that affects the health of animals and humans (Amanfu, 2006). In addition, it can cause very heavy economic losses (LoBue et al., 2010).

Mycobacterium bovis is a bacterium that causes bovine tuberculosis. Bovine tuberculosis usually causes chronic diseases and mostly respiratory system diseases.(Özbey et al., 2008).

Mycobacterium tuberculosis is a bacterium that causes human tuberculosis. This disease, which can be chronic or acute in humans, can cause death (Thoen et al., 2014). Human tuberculosis disease, which progresses as a mycobacterium tuberculosis complex in humans, also includes bovine tuberculosis, which is caused by the mycobacterium bovis bacterium (Thoen et al., 2006).

Since tuberculosis disease, which occurs in humans and animals, has a wide host mass, the World Health Organization has reported that this disease is a notifiable disease, taking into account public health (Dibaba et al., 2019).

The first eradication studies on bovine tuberculosis in the world were in Finland in 1889 (Sabar, 1956). Since it causes economic losses and is a zoonotic disease, eradication studies have progressed rapidly in other countries (Diker, 1989). Studies on the eradication of the disease in Turkey started in the 1980s and in the following years, the “Turkish Tuberculosis Struggle Project” was carried out (Atala 2001).

According to researches, more than fifty million cattle in the world have Mycobacterium bovis. However, it causes economic losses of more than three billion dollars annually (Claridge et al. 2012). Although it is known that the potential of TB disease in humans is high in places such as South Asia and Africa, the disease is also seen in other countries due to reasons such as poverty, rapid urbanization, increase in population density, and air pollution (Basnyat et al., 2018).

Many studies have shown that the disease can be transmitted both from animal to human and from person to animal. Ingestion of tuberculosis beef is the most dangerous mode of transmission for humans (Regassa et al., 2008).

Tuberculosis Human Tuberculia and Its Importance of Zoonotics

Tuberculosis, one of the most effective bacterial diseases affecting humanity, can be found in a quarter of people and deaths may occur. According to the report of the World Health Organization, it was stated that the disease was detected in ten million people in 2019 and close to one million people died. It should not be ignored that most of the cases resulting in death have immune system failure (WHO, 2020a; WHO, 2020b).

It is thought that the effects of the 2019 coronavirus pandemic may increase the incidence rate of tuberculosis disease by 20% in the coming years. The fact that both diseases affect the respiratory system is an indicator that supports this (Cilloni et al., 2020; Hogan et al., 2020).

Although tuberculosis remains a global problem, cases are highly concentrated in very specific regions of the world, affecting more densely populated areas. It is not surprising that it is more common in some countries due to poverty, social stigma, poor public awareness, inability to prevent contagion, insufficient resources for treatment and lack of developed health systems due to this density of people (Bapat et al., 2017).

About 90% of human tuberculosis cases are facilitated by HIV-induced immunosuppression in the most populous countries in South Asia, East Asia, Southeast Asia, and Africa, further facilitating the progression of M. tuberculosis (WHO, 2020b).

Cases of tuberculosis in humans often present with common symptoms such as fatigue, weight loss, fever, and night sweats. Chest pain, bloody cough and coughing rash are other symptoms of pulmonary tuberculosis. The location of the infection determines other symptoms of tuberculosis disease in different parts of the body. (https://www.c.d.c.gov/tb/publications/factsheets/general/tb.pdf)

Unfortunately, Turkish data on mycobacterium tuberculosis complex in humans are insufficient. In a study conducted in the province of Elazig, 34 patients out of 60 were found to be positive for this complex disease (Ağaçayak et al., 2007).

Cattle Tuberculosıs and Its Importance of Zoonotics

Cattle are effected by the deadly chronic illness tuberculosis. The disease is asymptomatic in its early stages. But as the disease progresses, there is a progressive weakening, a fever that fluctuates slightly, weakness, and appetite loss. A lung infection may cause tachypnea, a wet cough, or shortness of breath. In the final stage, an animal that is very malnourished may experience acute respiratory distress. Animals with the disease display a wide range of lesions in their upper respiratory system, lungs, and small intestine. While some cows with severe miliary tuberculosis lesions are clinically normal, most may become progressively debilitated with other clinical manifestations. While some animals do not suffer from loss of appetite, some may show signs of anorexia. Although their eyes may appear bright and vibrant, animals who are affected by the temperature may become lethargic and sluggish. These universal signs frequently worsen after calving. Bronchopneumonia-induced chronic cough is a sign of lung involvement. One or two moist, gently repressed coughs. Exercise or pharyngeal squeezing might easily cause a cough. In chilly temperatures and in the morning, the same symptoms frequently occur (Jemal, 2016).

Rarely, direct vaccination against Mycobacterium bovis can result in human infection (Grange, 2001). The danger of human-to-cow transmission by inhalation is minimal because Mycobacterium bovis is either enzootic or sporadic in the majority of developing nations. The pillars in the fight against a disease spread by cows that affects humans continue to be universal pasteurization of milk and measures to eradicate tuberculosis-infected cattle. These precautions are not followed in areas where mycobacterium bovis illness is more prevalent in humans (Sa'idu, 2015).

Animal keepers, veterinarians, farm and abattoir workers, veterinary technicians and technicians, hunters, health workers are the occupational groups at risk from which the disease will primarily be transmitted (Vayr et al., 2018).

Major Treatment Methods in Human and Cattle

Para-aminosalicylic antituberculosis drugs such as isoniazid and streptomycin recommended by the World Health Organization are widely used in the treatment of human tuberculosis. The most important critical point in our

country is that the tuberculosis vaccine is mandatory for newborn babies when they are two months old (Ameni et al., 2010).

Animals are sent to conditional slaughter or are shackled because treating tuberculosis in animals is not a popular or financially viable solution in nations committed to eradicating the disease (Pal, 2011).

Eradication Studies

The tuberculin herd test and auxiliary diagnostic tests performed on cattle are successful in antemortem diagnosis and immunization and in reducing the disease (Torgerson and Torgerson, 2010).

Bovine tuberculosis has been extensively eradicated in many countries through testing and slaughter policy. It also assisted in making the right decisions regarding post-slaughter meats.

(Constable et al., 2018). Performing tuberculin test twice a year in herds can also be beneficial for eradication. In addition to the tuberculin test, which should be done for all cattle older than three months, all hygienic rules should be complied with (Anonymous, 2008).

Where farm animals are housed, wild animals should be prevented from entering the farm. In particular, poultry carrying mycobacterium avium should not be allowed to contaminate animal feed and water (Godfray et al., 2018).

In order to prevent tuberculosis disease in humans, it is necessary to eradicate bovine tuberculosis disease first. In addition, careful consumption of meat, consumption of pasteurized milk and their supply from disease-free enterprises can prevent the formation of the disease (Anonymous 2008). It is also necessary to vaccinate children with Bacillus Calmette Guerin (BCG) to prevent the transmission of tuberculosis infection (Anonymous, 2018).

Conclusion

Planned studies should be conducted for tuberculosis disease, which concerns both humans and animals. It is necessary to carry out the studies throughout the country, to prevent illegal slaughter, to review the animal registration systems and to make the studies done as a state policy. Activities such as raising awareness of the public by organizing social responsibility projects and ensuring environmental cleanliness should not be interrupted. It is possible to prevent zoonotic diseases such as tuberculosis, which affect people's health, by accelerating the studies of the Single Health Concept. However, it

should not be forgotten that good results can be obtained as a result of a patient, determined and correct strategy.

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CHAPTER 4

PROPERTIES of FEEDS USED in TROUT NUTRITION

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Introduction

Aquaculture is a rapidly developing and indispensable food industry on a global basis (Buhan et al., 2010). Aquaculture; is an important production sector in terms of reducing the fishing pressure on natural fish stocks, contributing to healthy nutrition, employment and rural development. From this point of view, the aquaculture sector has become a significant potential in human nutrition in recent years, due to its cheap and high quality animal protein (Gören and Yeşilayar, 2013). For this reason, animal food production is the sector with the fastest growth in terms of sectors worldwide (Tüfek and Yalçın, 2007).

Fish farming in fresh water is at the forefront of fish farming in Turkey. Trout is important among the fish species grown in fresh water in our country as well as in the World (Yiğit and Aral, 1999). Trout species, a member of fish that have the ability to convert their food to meat more quickly compared to land animals; Compared to freshwater and marine fish, it is one of the most delicious fish species (Uysal et al., 2002). The main purpose of trout farming in cages is fattening. With this aim, fish weighing 20-30 grams are placed in net cages, and after 3.5, 4 months of maintenance and feeding, they can be turned into portioned fish weighing 200-300 grams and offered for sale (Ağırağaç and Büyükhatipoğlu, 1998).

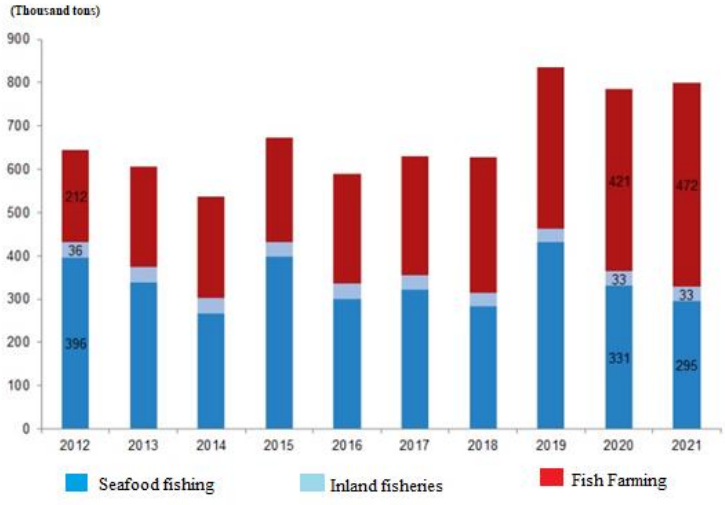

Figure 1. Fisheries Production in Turkey, 2012-2021
(TUIK, 2022)

The basis of fish farming; It is to provide the same or if possible the same natural living conditions of the fish and to feed them balanced with feeds suitable for the composition of the foods they are fed in the natural environment. The nutritional status of the fish has an effect on the characteristics of the meat such as chemical composition, flavor, texture and color. It is known that the consumption of the desired fatty acids by the fish with the feed used has a significant effect on the meat quality (Lovell, 1989).

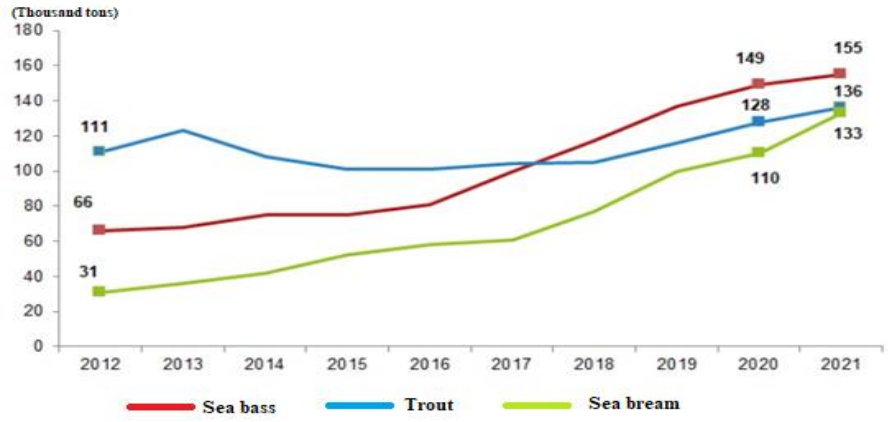

Figure 2. Fishes with the Most Cultivation, 2012-2021
(TUIK, 2022)

In 2021, 335 thousand 644 tons of aquaculture production was realized in the seas and 136 thousand 042 tons in inland waters. The most important fish species grown were trout with 135 thousand 732 tons in inland waters, sea bass with 155 thousand 151 tons and sea bream with 133 thousand 476 tons in seas (TUIK, 2022).

1.Features of Trout

Rainbow trout (Oncorhynchus mykiss) is one of the most cultivated fish species in our country, which has suitable climatic conditions and ecological characteristics. It is preferred in aquaculture and is an economically important species due to its easy adaptation to environmental conditions, short incubation times, rapid growth under good feeding conditions, living in a wide temperature range, and being resistant to diseases (Akbulut and Keten, 2001). Members of the Salmonidae family, most of which are anadromous, are among the first

species to be grown intensively. Being the first fish species to be cultivated intensively in Turkey, it is the most widely grown species today (Korkmaz et al., 2008). Body shape; is elongated and slightly flattened, with a fat fin (adipose) on the back. The dorsal fin has 10-12 soft rays and the anal fin has 8-12 soft rays. The scales are cycloid and small. Lateral line fully, slightly anteriorly covered with 100 to 150 scales. The body margins are silvery, white or pale yellow-green to grey. The abdomen is silvery white or yellow. There are also many small spots with a fuzzy pink, bluish or wide light pink band on the sides of the body. At the time of spawning in rootstocks, the color is very dark and the lateral line becomes very red (Emre, 2004).

Trouts are affected by various environmental factors (temperature, salinity, pH, oxygen and ammonia) both in the natural environment and in the culture environment, especially in reproduction and growth activities. Although these have extreme and normal limits, these environmental factors can be effective on their own or have an additive effect together. These environmental conditions should be well known before aquaculture (Molony, 2001). Changes in environmental conditions cause stress in the fish, affecting the resistance of the fish and making the fish susceptible to infections. Poor water quality, overstocking, intervention and drugs used in disease treatment are the most important stress factors. Stress usually results in death, but there are also levels of resistance such as behavior change, poor appetite, decreased resistance, growth, inability to withstand additional stresses (Edmonson, 1991).

Although trout are native fish of the cold and temperate regions of the northern hemisphere, a few species have also been widely migrated to warm environments. For example, Rainbow trout (Oncorhynchus mykiss) is found in many countries. This situation reveals that rainbow trout can adapt to wide ecological conditions (Molony et al., 2004). The optimum living temperature for rainbow trout is between 9 and 20 °C. Ideal water temperature should be between 9 and 13 °C in the larval and juvenile stages, and between 12 and 20 °C in the adult period for culturing. At water temperatures below 4 °C and above 26 °C, growth and nutrition appear to be adversely affected. Because cold water fish such as trout have a specific type of metabolism; While its metabolism works actively at low temperatures, at high temperatures above 26 °C, less food is consumed and its metabolism slows down (Avkhimovich, 2013).

Trout belong to the group of carnivorous fish. In natural waters, they constantly feed on animal creatures such as flies, worms, zooplankton, daphnia and other fish. For this reason, they want live feeds at the beginning of artificial feeding. For this, it is beneficial to accustom rainbow trout to feeding with artemia and similar live foods at the beginning. If there is difficulty in obtaining live feed, eating habit is done with liver and spleen paste. Since the amount of liver protein is not sufficient for the desired growth in trout fry, liver can be given by mixing it with fish meal.

Picture 1. Rainbow Trout (Oncorhynchus mykiss)

1.1. Commercial Importance of Trout

Rainbow trout (Oncorhynchus mykiss) in the Salmonidae family is a species of high commercial importance and is extensively consumed in Europe (Çaklı et al., 2006). It is a fish species that is extensively cultivated in many countries due to its rapid development and high nutritional content (Gall and Crandell, 1992; Mashaie, 2001; Gökoğlu et al., 2004; Fallah et al., 2011). It is an inevitable fact that aquaculture, which is an important source in meeting the protein needs of humanity, is provided by aquaculture. In order to achieve this, it is necessary to obtain the highest product from the unit area with the lowest cost and in the lowest unit time. In reaching this goal, great distances have been covered with the introduction of biotechnological methods (Melamed et al., 2002). However, it is also necessary to apply the right feeding program in order to achieve the desired yield. Various feed additives are also used in animal husbandry to increase performance, protect health and positively affect the quantity and quality of animal products.

1.2. Nutritional Characteristics of Trout

Trout fry need 40-50% protein. In order to be successful in fish culture, the protein, vitamin, energy, oil and mineral needs of the grown species should be known very well. If any of these is insufficient, the growth rate slows down or even stops. If the nutrients in question are more than the need, they are wasted and together with financial losses, results leading to the death of the fish may occur due to their toxic effect (Bostan, 2008).

The protein requirement of trout is between 30-55% depending on age, water feature and purpose of breeding. If it is desired to bring the fish in portions in a short time, high protein feeds are used. Accordingly, the fish grow rapidly, but increase the cost. On the contrary, if the breeder is not in a hurry to produce, he uses low protein feeds, and the cost is reduced even though the cultivation is slow. However, the amount of protein should never fall below 30%. Since the growth rate is high in juvenile fish, their protein needs are also high, but the protein ratio should not exceed 55%. It was stated that the crude protein requirement in rainbow trout should be between 31-55%, crude protein requirement should be 49-50% in fry stage, 48% in fingerling stage, 45% in adults and 50% in rootstocks (Çetinkaya, 1995).

One of the basic food elements in the diet of trout is fat. Fats, which have three functions as basic building material, energy and vitamin source, are stored in the muscles of the trout. According to researches, red muscles contain 15%, white muscles contain 2% fat. It is thought that the cultured trout contain more fat than the natural trout, since the natural feed is leaner and the cultured trout are more inactive. In rainbow trout fed a lean diet, symptoms such as hardening of the body, degeneration of the backbone and tail, color change, increased mortality and slowed growth are observed (Aras et al., 1995). The ratio of fat in trout ratios should be around 8-10% optimum, should not fall below 6% and should not exceed 25% in case of suitable type of fat (liquid and unsaturated). In case of excess fat, it becomes difficult to keep the feed, it tires the liver and can lead to death after a certain period of time. In trout, the fat content is at most 15% in fry starter feeds, 12% in grower feeds, and around 9% in others. The moisture content in the feed should be 10% and below. It has been stated that if the humidity level is high, the original form of the feed will deteriorate, it will be attacked by insects and fungi, and the storage period may be shortened by being exposed to burning in hot weather (Çetinkaya 1995).

It is still not clear whether carbonhydrates are important for trout (Aras et al., 1995). It is claimed to be present in some enzymes, liver, body fluid as glucose and glycogen. However, it is recommended to be used at the highest rate as an easily supplied energy source. One of the conditions for carbohydrates to be beneficial to trout is that they are cooked. Cooked starch gives 3-4 times more energy than its raw form. Although it is a cheap source, its excess causes hyperglycemia and leads to death. Certain elements in the waters where trout are grown are required for the mineral needs of the trout. They need Ca (calcium) and P (phosphorus) elements to form their skeletons. Minerals found in natural habitats where trout grow slowly in natural waters are sufficient. In aquaculture, rapid growth must be added to the feed at certain levels of various minerals. The required amounts of some basic nutrients in trout feed; calcium (Ca) 12.0 g/kg, phosphorus (P) 8.6 g/kg, magnesium (Mg) 0.7 g/kg, potassium (K) 5.0 g/kg, sodium (Na) 3.94 g/kg, iodine (I) 0.1 It is stated as -1.0 mg/kg (Aras et al., 1995).

The purpose of adding cellulose to fish feeds; regulating the energy level of the ration, increasing the digestibility by keeping the feed in the digestive organs for a longer time by adding bulk to the feed. Trout feeds contain about 3-5% cellulose. Excess cellulose reduces feed consumption, increases the amount of feces released into the water and causes water pollution (Çetinkaya, 1995).

Rainbow trout meat contains 1.62% crude fat, 16.9% crude protein, 1.42% crude ash, 78.06% moisture and 1.43% carbonates (Uysal et al. 2002).

2. The Importance of Fish Feeds In Nutrition

With the increase in aquaculture, the fish feed industry is also developing. The main expense affecting the cost in a fish hatchery is feed and 50-60% of the total expenses are feed expenses. Therefore, the enterprise should use quality and hygienic feed in order to obtain the desired yield (Kaymak, 2000).

There are many alternatives in aquaculture on the basis of species and aquaculture area. This variety is beneficial in having a wide variety of nutrients used in aquaculture. For this reason, depending on the development of breeding, the feed sector has also shown a rapid development. Especially compound feed production has made great progress. The thought that the rapid development in

aquaculture will continue suggests that feed production will develop at the same rate in the coming years (Korkut 2009).

Due to the wide variety of raw materials used in feed production, the fact that the raw materials to be used come from different regions and are obtained from different processing methods according to their varieties, quality and content differences can be seen among the raw materials. After taking samples from the raw materials taken to the factories and businesses with appropriate methods, their physical and chemical analyzes should be done (Kop and Korkut 2002).

The increase in the number of microorganisms in feed and feed raw materials mostly occurs during storage due to factors such as temperature, humidity, and ambient pH. Under normal storage conditions, the number of fungi in 1 g of feed should not exceed 10^3 and the number of bacteria should not exceed 10^4. However, when microorganisms have suitable growth environments, they can increase their numbers 10 times in a very short time (Ergül 2005).

Most of the species cultivated both in inland waters and in the marine environment in our country are carnivorous fish species. Animal protein-derived feed raw materials constitute 60-80% of the compound feed composition of carnivorous fish. The unit production costs of these fish, which are at the top of the food pyramid, are higher than the herbivorous and omnivorous species due to the widespread use of animal sources, especially fish meal and fat in their feed (Demir, 2011).

In aquaculture conditions, the trout reaches its portion weight (180-220 g) approximately 8-12 months after the food sac is withdrawn and they start to take feed from outside. Previously, 1.5-2.0 kg of compound feed was required for the production of one kilogram of fish, but in recent years, this value has been reduced to 0.9-1.4 kg in feed conversion ratio (FCR). This has been achieved by increasing the knowledge about living biology and water quality, monitoring and controlling the conditions, choosing raw materials for more suitable feed, feed formulation, feed making technique, optimization of environment and care feeding conditions, using technological possibilities and tools (Demir, 2011).

2.1. Trout Fattenıng Bait Ingredıents Of Some Companıes In The Market

Tradem ark	Dımension	Weıght (kg)	Nutrıtıonal Values				Vıtamıns			Macro Elements		
			CP (%)	CF (%)	HC (%)	H (%)	VİT A (UI/kg)	VİTD_3 (UI/kg)	VİT E (mg/kg)	P (%)	Ca (%)	Na (%)
A	10 MM	1000	44,9	18,9	1,4	6,8	3000	750	150	0,75	-	-
	5 MM	1000	41,8	22,3	1,0	7,3	3000	750	150	0,75	-	-
	4 MM	1000	41,8	22,3	2,0	6,2	3000	750	150	1,55	-	-
B	3 MM	25	46	19	1,5	9,5	-	-	-	1,6	2,4	0,3
	4 MM	25	45	20	1,8	9,5	-	-	-	1,5	2,3	0,3
	6 MM	25	45	20	1,8	9,5	-	-	-	1,5	2,3	0,3
	8 MM	1000	45	20	1,8	9,3	-	-	-	1,5	2,2	0,3
	10 MM	1000	45	20	1,8	9,3	-	-	-	1,5	2,2	0,3
C	3 MM	25	46	18	1,5	11	12.000	2.500	250	1,3	2,1	0,4
D	3 MM	25	45,3	21,1	2,2	7,4	-	-	-	1,16	1,68	0,15
E	4 MM	750	44	21	1,7	7,6	-	-	-	1,11	1,90	0,30

CP: Crude protein, %DM **CF:** Crude Fat, %DM CC**:** Crude Cellulose, %DM **CA:** Crude Ash, %DM (DM: Dry Matter)

Although the most suitable water temperature for the breeding of rainbow trout is 15-20 ^{0}C, the water temperature suitable for feeding is 14-16 ^{0}C. It is desirable to have 40% protein in the larval feed of rainbow trout, 30% in the fry feed, and 30% in the feed of portion fish. These rates can be increased up to 50% in larval feed and up to 46% in portion fish feeding. The feeding method is chosen according to the water and farm conditions. The fat content in trout feeds is initially recommended as 4-5%. In the feed mixture (ration), when the protein content is increased and the fat ratio is increased to 8%, the benefit from the feed and the meat quality of the fish are improved. In trout pellet feeds, 8-12% fat and 42-50% protein are accepted as the upper limit (Özdemir, 1994).

3. Bait Forms

3.1. Wet Feeds

All kinds of plant and animal feeds have been used as wet feed. In our country, some trout breeders still use various abattoir residues as fresh feed. This type of feed includes spleen, liver, rumen and intestines, chicken slaughterhouse scraps, chicken brood scraps, eggs, frozen and canned fish, oilseed meal, skim cheese, brewer's yeast and fresh green water plants. Liver comes first in these wet foods. Puppies fed only with spleen cannot develop normally. Because the spleen does not contain some vitamins at all. For this reason, it would be more beneficial to give the spleen and liver together as feed. Liver, spleen, freshwater fish and internal organs of other animals can also be given to fish as a mixture. It is absolutely necessary to add 2% salt to this mixture. In some of the wet feeds, the water rate is at least 60%. The point that is ignored in feeding with spleen and liver in our country is that salt is not added to these feeds. In the case of using wet feed, the pools become more polluted and the cleaning and labor costs increase (Yanık 1997).

3.2. Semi-Wet Feeds

In the production of this type of feed, which is widely used in our country, pasteurization must be carried out. In this type of feed, where the dry feed rate is increased, the water level is reduced to 30-35% (in small enterprises, boiled instead of pasteurized). The biggest problem in this type of feed is the adjustment of fish size and feed size. Various methods have been developed for this. These semi-wet feeds, which are made into dough, can be given by sticking

or spreading on the surfaces of the mangers made in various ways. Or the diameter of the feed can be adjusted as desired by passing it through a meat grinder. The raw materials used in the production of trout feed are generally; fish meal, fish oil, poultry by-product meal, soy and by-products, wheat and by-products, corn gluten, vitamins and minerals. The most proportionally used raw materials in fish feed production are fish meal and fish oil, and their high prices also increase the cost of fish feed and therefore the price of fish feed (Yeşilayer ve ark., 2013).

3.3. Fish and Meat Meal Mix Feeds

As aquaculture developed, the use of different feeds came to the fore. Meat and flour mixture feeds began to be made in the 1940s. In the production of these feeds, animal origin feed materials and vegetable origin feeds are mixed. 4% salt is added to this type of feed. Starch and alginate are used to increase the adhesive property of these feeds. Such feed mixtures are prepared daily and given to the fish. Feeding trials have shown that poultry (chicken) processing products can be used as a 40% substitute for fish meal, without causing any reduction in growth in rainbow trout, but using higher rates leads to reduced growth (Sugiura et al., 1998). It was stated that protein digestibility of poultry processing products in rainbow trout was at the same level (94-99%) as herring meal (Hardy, 2000). Balanced compound feeds are now used in the nutrition of cultured animals. Mixed feed is a homogeneous mixture obtained by the fabrication method of three or more feed raw materials. Aside from the fact that it is prepared with a correct formula in terms of feeding technique and nutritional physiology, the actual feed value of a compound feed depends on whether all the feed ingredients in the formula are present in the same proportion even in a few grams of pellets (Akyıldız, 1992). It has been reported that the addition of 25% chicken abattoir flour to trout feed gives positive results, and the addition of 40% chicken abattoir flour and 10% poultry flour to trout feed gives successful results instead of fish meal. It is reported that 80% of the trout feed protein can be obtained from chicken abattoir residues flour. The researchers stated that it should be taken into account that the essential amino acids of chicken abattoir meal are less than fish meal (Dear and Erturk., 2004). Tacon and Jakson (1985) reported that a mixture of trout feed meat-bone meal with blood meal in the ratio (4:1) was used with 50% success instead of

fish meal. Yanik and Aras (1999) reported that 25-50% of chicken abattoir residues flour can be substituted for fish meal in trout feeds. At the end of a 16-week study in juvenile trout feeds, 28% animal by-products (25% meat-bone meal, 24.5% skin meal, 20% cuttlefish liver meal, 15% feather meal, 7.5% chicken meal) instead of fish meal slaughterhouse residues flour, 0.25% methionine-lysine) reported that the mixture did not have negative effects on growth (Yanik et al., 2003).

3.4. Vegetable Proteins (Meatmeal)

Oilseeds such as soybean, sunflower, cotton, canola are used in animal feeds as a protein source. The addition of oilseeds to animal feeds is advantageous because of its low price and easy availability compared to fishmeal. Soy products have the best amino acid profile after fish meal, which provides essential amino acid requirement in the nutrition of aquaculture species (Hertrampf and Pascual, 2000). It has been reported that soybean meal can be used at high rates in rainbow trout feeds, since trypsin inhibitors are inactivated in soybean meal as a result of heat treatment applications (Cho et al., 1974; Reinitz, 1980). Sunflower meal can be used as a substitute for soy in rainbow trout (O. mykiss). When the ratio of sunflower seed meal is increased in feed, dry matter digestibility decreases due to high cellulose content. Cottonseed meal contains toxic levels of gossypol. It has been reported that cottonseed meal with removed gossypol can be used instead of 80% of soybean meal, and cottonseed meal containing 40% crude protein can be used instead of 60% of fish meal. It is recommended that it should not be used in large amounts in compound feed of breeding fish, as it causes lubrication when used at high rates (Hertrampf and Pascual, 2000). Corn gluten contains 70-80% protein and is a highly digestible source for fish such as rainbow trout and Coho salmon (Sugiura et al., 1998). It is reported that there is no negative effect on growth and feed conversion rate when corn gluten is substituted by 25% instead of fish meal. The disadvantage of adding corn gluten in commercial rainbow trout feeds is that it gives a yellow color to the fish meat when it is added at a high rate (Skonberg et al., 1998).

3.5. Pellet Feeds

Pellet feeds obtained by pressure are stored without freezing and without the need for cold storage. This type of pellets can be stored at room temperature and low humidity for up to 90 days without deterioration. After this period, decomposition begins. The humidity of the warehouse should not exceed 60%. Oil is added to pellet feeds by spraying or spraying. Normally, the fat level in the rations is around 7% to 8%, and this amount can be increased up to 16-20% by using liquid oils (Akyıldız, 1979).

3.6. Extrusion Feeds

The feed industry sector has left the pelleting system it has reached to new methods today. While extrusion was used as a method to neutralize anti-nutritional factors in animal feeds in the 1960s, today it is used as a production technology to produce animal feed in the desired shape and feature. In extrusion, which is a mixed feed production method, higher levels of humidity, heat and pressure are used compared to normal pelleting. Heat sensitive vitamins, especially ascorbic acid, are added to the feed surface just before or after processing, in excess of the need. Feeds produced by extrusion are more compact due to the almost complete gelatinization of starch. For this reason, it is less crumbly and can remain in the water for a longer time without dispersing. It is also stated that this method provides better flavor to the feeds, performs the breakdown of antimetabolites in oilseeds, increases the digestibility and thus increases the nutritional value of the feeds (Akyıldız, 1992; Burn, 1997). Extrusion allows the use of the binder property of inexpensive starch, and the combination of heat and pressure allows greater digestibility of the product. Extrusion can sterilize products completely. This process allows the use of raw materials in large proportions in the formulation of compound feed. Extrusion does not improve the quality of raw materials, but increases the nutritional value (Botting, 1991).

Picture 2. Fish Feed Forms

4. Fish Feed Types

Fish feeds can be listed as starter feed, fry feed, fingerling feed, fattening feed and breeder feed according to their development period.

- Starter Feed: It is the first feed given when the endogenous food source of the larva is exhausted. The transition to exogenous food is critical. It is the period of excessive death due to difficulty in adapting to exogenous

eating. Starter feeds should be nutritionally complete and easily digestible and of appropriate particle size. In many cases, especially shrimp and some marine fish, initial feeding is based on live foods rather than formulated diets.

- Fry Feed: It usually contains a high level of protein. Relatively the fastest weight gain is achieved during this period. It is therefore extremely important to realize potential growth during this period in all breeding systems. Fry feeds are prepared in flake or krembl form.

- Fingerling Feed: It is the period between metamorphosis and 10-20 g size. Their feed can vary from cream to pellet. It contains less protein and energy than fry food.

- Fattening Feed: In this period, weight gain is quite uniform. It is extremely important to ensure that the protein provided in these feeds is used for growth and not for metabolic activity. At this stage, the living mass in the aquaculture has increased considerably. In other words, the need for feed has also increased to the maximum. Therefore, the most savings in feed costs can be made during this period.

- Breeder Feed: During sexual maturity, somatic growth slows down and gonadal development accelerates. Feed quality affects fry quality. Therefore, the breeder feed must be formulated to meet the needs. However, the breeding nutritional needs of many species are not well known. The common practice is to increase the protein level in this period (Anonymous, 2017).

5. Studies on Trout Feeding

Obey Abdalla Gadaın Alnaiem (2020) added rosehip oil at different rates to fry rainbow trout (Oncorhynchus mykiss L.) feeds in his study. For this purpose, the young trout were fed for 90 days by adding rosehip oil at 4 different concentrations (0ml/kg, 2ml/kg, 4ml/kg and 6ml/kg) to their feed. Experimental groups were used in 2 replications and each tank consisted of 200 juvenile fish and a total of 1600 fish were used. At the end of the experiment, success was achieved in the group to which 6 ml/kg rosehip oil was added, and the difference was statistically significant compared to the other groups ($P<0.05$). It has been determined that the addition of rosehip oil to the fry trout feeds increases the rate of injury from the feed and has a positive effect on the development of the fish.

Acar et al. (2018) added lupine flour at different rates (0%, 30%, 45% and 60%) instead of fish meal to rainbow trout fry feed. At the end of the 60-day experiment, blood and liver tissue samples were taken from 15 fish in each group. As a result, it was concluded that the substitution of lupine flour at the rate of 60% in rainbow trout feeds has a positive effect on the immune system of rainbow trout.

Cihangir and Diler (2016), in their study, added thyme essential oil at different rates to fry and juvenile rainbow trout feeds. For this purpose, thyme oil was added to the experimental feeds at 3 different concentrations (0.125 mg/kg, 1.5 mg/kg and 3.0 mg/kg). Fry and juvenile rainbow trout were fed for 90 days. The difference between the groups was found to be significant in the end-of-trial weight and live weight gain in fry trout. The difference between groups in body weight gain, specific growth rate, feed conversion rate and survival rate was significant in juvenile trout ($p<0.05$). In conclusion; In this study, it was determined that thyme essential oil was added to fish feeds and had a growth-enhancing and supportive effect.

Erdogan et al. (2016), in their study, the fish were adapted in fiberglass tanks, acclimated to the feed with fresh rainbow trout meat and fed with 9 mm pelleted rootstock. Counts of dead and unfertilized eggs were made since milking and it was calculated that hatching occurred in approximately 74% of the eggs. The fry that consumed the food sacs were divided into three groups in the same incubators, each with 600 fry. First group fry were fed with brine shrimp (Artemia sp.) as starter food, microdiet (300, 500 and 750µ) and extruded feed (1mm) from the seventh week. The second group of fry were initially fed only 300µ microdiet and later on larger size extruded feeds. The third group fry was given by crushing fresh lamb liver for one month at the beginning and continued to be fed with microdiet and extruded feed in the following stages, similar to the other groups. fry was fed daily at frequent intervals until satiated. In the final calculations, it was determined that a total of 304 fry died, 77 in the 1st group, 104 in the 2nd group and 123 in the 3rd group. As a result, incubation of first generation red-spotted trout eggs adapted to the culture medium was successfully carried out. However, they think that research should continue in order to obtain more detailed information about fry starter feedings.

Altınterim (2010), in his study, investigated the effects of black cumin oil on the immune system parameters of rainbow trout. 900 rainbow trout were used in the study. In the study, black cumin oil was given to the trout by 3 different methods; In the first method, black cumin oil was added to the feed at the rates of 0.1%, 1.0% and 10% in ml and given to the trout for 21 days. In the second method, fish doses of 0.1%, 1.0% and 10% of black cumin oil were injected into the trout. In the third method, 0.1%, 1.0% and 10% doses of black cumin oil were applied to the lateral line of the trout for 3 days. After these procedures, blood samples were taken from the trout on the 3rd, 7th, 14th, and 21st days. As a result, the highest levels of hematocrit, erythrocyte, leukocyte, NBT levels were determined in the application of black cumin oil to the fish in the third method. There was no significant change in the leukocrit levels of the different groups in which the three methods were applied. In the measurement of total protein levels, the highest values were obtained in the groups to which the feeding method was applied. In the analysis of total Ig levels, the highest values were obtained in the injection method, but it did not follow a parabolic line, however, a parabolic curve was determined in the application. It has been determined that the nutritional properties of black cumin oil in the groups given by feeding method are effective at a level to increase protein levels even when used at minimum rates.

Keskin and Erdem (2005), in their study, were fed 1%, 1.5% of live weight of rainbow trout and fed until satiation. At the end of the experiment, the growth rates of the groups were determined as 34.42%, 47.18% and 71.88%, and the feed conversion rates were determined as 1.51, 1.62 and 2.01 in the groups, respectively. The best feed utilization rate was obtained from the group with 1% feeding, and the best live weight gain and growth were obtained from the feed given until saturation. The reason for this is the amount of feed consumption that increases with the feeding rate. According to the economic analysis, the cost of feed is low in the group given 1% feed, and it is high in the group fed until satiation. Feeding should be done as much as the fish needs instead of feeding at a high rate.

Demir and Aybal (2004) fed rainbow trout with 0%, 1%, 2%, 3%, 4%, 5% and 6% clinoptilolite-containing feeds for 60 days. The lowest mean condition factor was obtained from the group fed with 3% clinoptilolite diet.

As a result, the differences between the mean feed conversion rates of the experimental groups were insignificant.

Uysal et al. (2002) fed Abant trout larvae and cultured rainbow trout larvae with extruder pellet feed under culture conditions for 350 days. In order to determine the biochemical compositions of Abant trout and rainbow trout grown under culture conditions, crude protein, crude oil, moisture, ash and carbohydrate analyzes were carried out in experimental fish. Rainbow trout's crude protein (17%), crude oil (1.62%), moisture (78.06%), ash (1.42%) and carbohydrate (2.52%) and Abant trout crude protein (19%), crude oil (1.44%), moisture (78.02%), ash (1.20%) and carbohydrate (2.64%) ratios were determined. It was concluded that Abant trout could not be fed well and remained weak because the condition factor was less than 1. It was found that the difference between species in terms of Condition factor was significant ($P<0.001$).

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CHAPTER 5

PHYSICAL THERAPY and APPLICATION METHODS in HORSES

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Introduction

Physical therapy is an important part of musculoskeletal, neurological and cardio-pulmonary care from paediatrics to geriatrics and sports medicine. For human and veterinary medicine, it is defined as a supportive application that restores mobility/function and quality of life to patients by repairing damaged tissues, functional recovery of these tissues and activating the healing processes of the heart, respiratory, nervous and musculoskeletal systems together. In case of illness or injury, it helps to restore the body's ability to move with the aim of minimising pain and preventing loss of function (Goldberg & Tomlison, 2018).

Equine physiotherapy is used to improve performance and prevent injury, especially in sport horses, and to aid rehabilitation after injury, surgical procedures and neurological or medical conditions. The desired treatment outcome for equine physiotherapy is the highest sporting performance and the ability of the horse to exhibit its natural behaviour as a result of treatment (McGowan et al, 2007). Musculoskeletal physiotherapy is the key area of equine physiotherapy. Musculoskeletal injuries are the most important cause of loss of use and death of sport horses. Treatments should be tailored to each individual horse, taking into account the whole animal, type of riding, short- and long-term performance goals and overall prognosis for recovery (McGowan et al., 2007). Physical therapy applications include massage, stretching exercises, laser therapy, electrical stimulation, magnetic field use, therapeutic ultrasound, rehabilitation exercises, hydrotherapy, and the use of heat and cold (McGowan, 2007).

Manual Therapy Applications In Horses

One of the most important areas of physiotherapy applicable to horses is musculoskeletal physiotherapy, which includes the assessment, treatment and rehabilitation of neuromuscular and musculoskeletal disorders (Goff, 2009). Chiropractic, osteopathy, physical therapy, massage therapy and touch therapies are all considered to be manual therapy techniques developed for the treatment of musculoskeletal disorders in humans and transferred for use in horses. Each technique has its own origin and different proposed biomechanical or physiological effects. However, all forms of manual therapy are characterised by the application of varying degrees of manual force and degrees

of soft tissue or joint displacement. The aim of all manual therapies is to favourably influence the repair or healing processes of the neuromusculoskeletal system. Therapeutic effects can be generalised to the whole body by stimulating relaxation. Regional effects may include changes in pain perception or neuromuscular control, or effects may be localised to specific tissues and cellular responses (Haussler, 2009). Manual therapies and massage techniques focus on the role of myofascial tone and connective tissue in supporting muscle, joint, ligament and tendon function. In horses, various manual techniques such as massage, joint mobilisation and manipulation (chiropractic) are applied with the primary therapeutic purpose (e.g. to reduce pain or stiffness) (Haussler, 2018).

Techniques such as joint mobilisation can be applied to the entire joint system, including the columna vertebralis and peripheral joints, together with soft tissue complementary techniques applied to neuromuscular and fascial tissues. Repetitive movement techniques have a beneficial effect on intraarticular, periarticular (joint capsule and ligament) and extraarticular structures, primarily in pain modulation, by affecting passive and active movement limitations. Manual therapy techniques initially produce a treatment-specific local hypoalgesia and sympathetic stimulating effect. It also aids therapeutic exercises and rehabilitation of neuromotor control. The applied forces help to increase flexibility, stimulate proprioception and strengthen the musculature (Stubbs, 2015).

1)Soft Tissue and Joint Mobilisation

Soft tissue mobilisation, also known as massage techniques, focuses on restoring normal movement to the skin, connective tissue, ligaments, tendons and muscles to manage pain, reduce inflammation, improve tissue repair, increase extensibility and improve function. Joint mobilisation is characterised by repetitive joint movements created within the range of passive joint motion to restore joint range of motion, stretch connective tissues and restore proprioception. The aim is to reduce pain, restore the harmony between tissues, and improve joint range and motion (Haussler, 2018). It is also used to assess the quality and quantity of joint range of motion and as a primary means of treating musculoskeletal disorders (Haussler, 2010).

Soft tissue mobilisation is defined as a therapeutic technique in which superficial and deep layers of soft tissue are manipulated manually. Tissues addressed include muscle, tendon, ligament and fascia. It is often grouped under the term "massage" (Coates, 2017).

Joint mobilisation manually applies a force to create passive physiological or accessory movements and active mobilisations of the joints. Each joint must be mobilised in a specific way. Therefore, this technique should be performed by well-trained professionals. Mobilisation techniques involve small rhythmic oscillations and gliding movements across the joint, directed perpendicular or parallel to the normal direction of movement of the joint to improve movement and normalise joint function. As a result, stiffness and pain are reduced (Atalaia et al., 2021).

Tissue mobilisation includes massage, myofascial release and neural tissue mobilisation techniques to break down myofascial adhesions such as scar tissue, move blood and tissue fluids, relax muscle tension and optimise muscle function. In Bromiley's narrative review, massage techniques such as effleurage, petrisage, tapotement, friction, performed in equine rehabilitation settings were described (Atalaia et al., 2021).

Efleurage is used at the beginning and end of each massage session. It can also be used to link one technique to another and is performed using one or both hands. The technique requires long and slow rhythmic strokes and pressure is applied preferably in the directional flow of venous return, while the operator's hands move away from the body (Scott and Swenson, 2009).

Petrisage means "kneading" and is designed to compress and then loosen the underlying tissue, including deep tissue (Bromiley, 1999).

Tapotement refers to three types of rapidly repeated percussive movements with alternating hands. It is a two-handed technique and is achieved by using the edge of the hand with the hands and fingers in a relaxed state. The effects of this application include stimulation of superficial blood flow (Bromiley, 1999).

In addition to the techniques described in Bromiley's narrative review, some of the common therapeutic techniques used today in massage therapy methods applied to horses include compression, direct pressure, friction and myofascial release (Scott & Swenson, 2009).

Compression is applied as rhythmic pressure, such as the heel of the hand, a loose fist or a pumping motion with the fingertips. This pressure repeatedly compresses a muscle against the underlying bone, which has the effect of flattening and separating the muscle fascicles (Scott and Swenson, 2009).

Direct pressure is a form of compression that involves applying pressure with the thumb, fingers or elbow. This pressure is held for 5 seconds or more as felt by the practitioner. The purpose of direct pressure is to alter fluid distribution and blood flow to an area. By creating a temporary ischaemia and slowly releasing the pressure, extracellular fluid and blood are redistributed within the area. After this redistribution of fluids, the practitioner will often feel that a previously tense area becomes more flexible when palpated (Scott & Swenson, 2009).

In friction, the thumb, finger, tense fingers, fist or heel of the hand is used to apply deeper pressure to the muscle tissue. This technique is typically performed in a cross-fibre pattern perpendicular to the long axis of the muscle group (Scott and Swenson, 2009).

Myofascial release treatments affect the three-dimensional subcutaneous fascial network that surrounds the muscles, tendons and ligaments. The aim is to address the limited fascia coverage or transition between muscles in an effort to lengthen and thus allow greater freedom of movement of the affected muscles (Scott and Swenson, 2009).

2)Manipulation

Manipulation is characterised by a high-speed manual push in a specific direction. It involves a sudden, powerful push beyond the patient's control. It is performed at the physiological joint motion limit (elastic barrier) and in the pathophysiological space. It should only be performed after appropriate training (Adair & Phillips, 2017).

The primary biomechanical difference between joint mobilisation and manipulation is the presence of a high-speed thrust or impulse. In addition, manipulation can specifically stimulate receptors in deep intervertebral muscles, whereas mobilisation techniques affect more superficial axial muscles (Haussler, 2009). Manipulation involves the application of controlled stimuli to joint structures within the axial skeleton with the aim of reducing pain and

muscle hypertonicity and increasing range of motion (Haussler, 2009). The application is often used to relieve localised pain and joint stiffness, with less focus on the surrounding soft tissues (Haussler, 2018).

A "cracking" or "popping" sound can be heard during joint mobilisation or manipulation as the applied force exceeds the elastic resistance barrier (Haussler, 2009). Joint mobilisation and manipulation can be combined with sedation or general anaesthesia providing increased relaxation and analgesia for the assessment of mild joint movement restrictions or for the treatment of joint contractures and spinal pain. The risks of manipulation under sedation or general anaesthesia include the inability of patients to provide feedback about pain or to resist excessive manipulation. Because there are no intrinsic protective mechanisms associated with voluntary muscle contraction, this may lead to an increased risk of iatrogenic injury (Haussler, 2009).

3)Passive Stretching Exercises

Range of motion (ROM) refers to the movement that occurs in a joint (flexion, extension, abduction, adduction, internal rotation, external rotation) (Haussler, 2018).

ROM can be restricted by various structures and conditions such as swelling, muscle/tendon, ligament, capsular impingement, pain, meniscus and joint mouse. It is important to identify the limiting structure so that the treatment can be directed correctly (Haussler, 2018).

ROM is created by both osteokinematic and arthrokinematic movements. Osteokinematic motion occurs when bony segments move around a joint axis. Arthrokinematic motion is the movement that occurs between opposing articular surfaces within the joint (Alvarez et al., 2008).

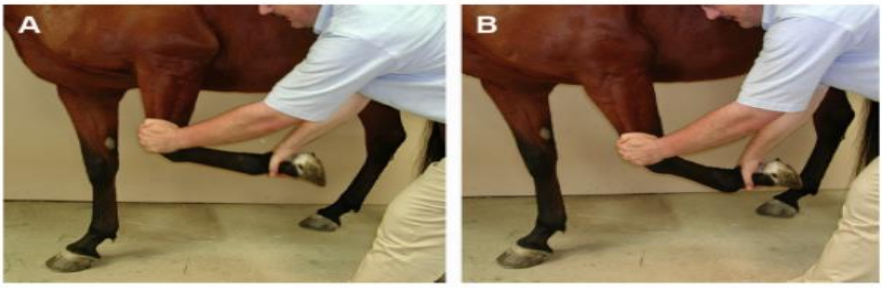
Joint movement can be either passive or active. Passive ROM (PROM) requires a second party (assistant) to move the joint along the desired ROM without patient participation. With PROM, there is no muscle contraction. If the patient assists or resists the movement, it is no longer "passive". Active ROM (AROM), on the other hand, requires the patient to contract their muscles to move the joint through its current range (Haussler, 2018).

Passive Range of Motion (PROM), maintaining or improving joint range of motion, is important in preventing the negative effects of immobilisation. PROM is a manual technique applied to maintain or restore AROM. The

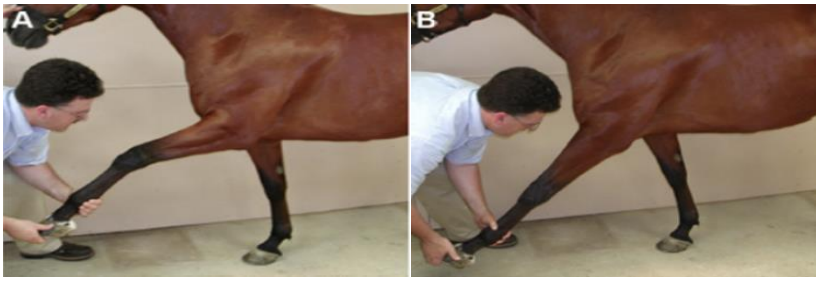
technique used during PROM includes stabilising the proximal bone segment, moving the distal bone segment to its end range, maintaining the proper plane of motion (not allowing abduction/adduction or rotation), and working in a pain-free range of motion (Haussler, 2009).

Manual contact or hand placement is very important when performing PROM. It must be remembered that the distal bone is being moved over a stabilised proximal bone. Therefore, one hand should be set to stabilise the proximal bone segment and the other hand should be set to support the limb while moving the distal bone segment. The support of the limb usually occurs at the joint distal to the treated area (Haussler, 2018).

Objective changes in ROM are measured with a protractor and documented in degrees. Goniometric measurements can be performed before and after treatment to determine the effectiveness of the treatment. A goniometer is a tool used to measure joint angles. In the field of physical therapy, goniometry is used to measure the total amount of movement available in a given joint. Goniometers come in various sizes and shapes and are usually made of plastic or metal (Haussler, 2018).



Pic.1: Pasive (A) and active-helpful (B) thoracic limb retraction stretch
Source: (Haussler, 2018).



Pic.2: Pasive (A) and active-helpful (B) entire thoracic limb protraction flexion. The thoracic limb is fully extended and held at the end of the movement to encourage soft tissue elongation and increase neuromuscular activation
source: (Haussler, 2018).

Electrotherapy (Electrophysical Methods)

Physiotherapy fields that use electric current to treat animals or humans are characterised by different methods and techniques (Buchner and Schildboeck, 2006). Electrical stimulation can be used to manage many disorders, including musculoskeletal, soft tissue, vascular and neuromuscular injuries (Sprague and Goldberg, 2017).

Electrical stimulation (ES) is widely used in physical therapy and is effective for many purposes such as increasing muscle strength, increasing range of motion (ROM), correcting structural abnormalities, improving muscle tone and function, pain control, accelerating wound healing, reducing oedema, reducing muscle spasm and transdermal administration of medication (iontophoresis) (Levine & Bockstahler, 2013).

Electrical stimulation methods have the potential to benefit a horse with joint disease by providing pain relief, controlling muscle atrophy due to muscle disuse and increasing muscle strength (Porter, 2005). The most commonly used forms of electrical stimulation are neuromuscular electrical stimulation (NMES), a term used interchangeably with transcutaneous electrical nerve stimulation (TENS) and functional electrical stimulation (FES) (Sprague and Goldberg, 2017).

Electrotherapy applies varying waveforms and frequencies of electric current to the body depending on the desired effect. TENS causes temporary pain relief by releasing endorphins and activating inhibitory interneurons at the spinal cord level. When the muscle has atrophied as a result of disuse,

immobility or nerve damage such that a patient is unable to contract the muscle, NMES can be applied to generate 80% to 90% of the maximum contractile force. This helps to maintain neuromuscular control and muscle development (Stubbs, 2015).

Therapeutic electric current provides pain relief through stimulation of large myelinated afferent neurons that override nociceptive transmission in the dorsal neck of the spinal cord. In addition, pain relief is also achieved by stimulation of endogenous opiates such as endorphins. Through these two mechanisms, electrical stimulation can provide immediate and long-lasting relief of pain associated with joint disease. Skin surface electrodes can be placed on associated acupuncture points as well as the joint to provide rapid and long-lasting pain control (Porter, 2005).

1)Transcutaneous Electrical Nerve Stimulation (TENS)

TENS uses pulsed alternating current of variable frequency and intensity applied through surface electrodes. The aim of TENS is to relieve pain (Paulekas & Haussler, 2009).

TENS devices produce a low-intensity electrical signal designed to reduce or control pain but do not produce motor stimulation (Bromiley, 1999).

With electrostimulation, pain relief is achieved primarily by segmental inhibition through pain transmission mechanisms. This is based on the activation of larger diameter fibres in peripheral nerves and helps to block nociceptive activity in smaller afferent nerves. Secondary electrical stimulation of peripheral nerves stimulates the central release of endogenous opiate-like substances, which may have a decreasing inhibitory effect on pain. The treatment parameters are based on electrical stimulation in the low frequency range (<250 Hz) using appropriate pulse durations and intensities to activate the desired nerves. Due to their proximity to the skin surface, primarily large diameter sensory nerves are activated. Secondarily, motor nerves are activated. The nociceptor nerves are then affected via pain transmission mechanisms. If the contacts are left too long, it can cause skin irritation. Care should therefore be taken to check the treatment area regularly. Otherwise, the method has minimal complications when used correctly (Schlachter and Lewis, 2016).

TENS is a useful modality when used for pain control. It is an alternative form of electrical stimulation that in the past was applied at high frequencies to

relieve pain, but is now used at very low frequencies (2-10 Hz). The presence of pain alone has been shown to cause changes in muscle activity and subsequently reduce muscle forces throughout the body. A reduction in pain will result in an improvement in function. TENS is an excellent adjunctive pain management treatment. It can be used in combination with pharmaceuticals in acute pain conditions such as after trauma or surgery and in chronic pain conditions associated with degenerative change (Goldberg and Tomlinson, 2018).

It should be noted that pain modulation only continues when the current is switched on and has no residual effects after treatment. The source of pain must first be isolated. Then the area should be moisturised and a bonding medium should be applied. The machine must be tested. The electrodes should be applied (1) to the site of discomfort, (2) to the same dermatoma as the location of the injury being treated, or (3) to trigger or acupuncture points. The horse should be treated once a day for 20 to 30 minutes. The electrodes can be held in place with microporous or surgical tape and the unit can be mounted on a roller or surcingle (a strap made of leather-like synthetic materials that secures around the horse). The horse's skin should be cleaned after the electrodes are removed (Bromiley, 1999).

The reason that short duration pulses can be used to achieve these effects is that the targets have sensory nerves that tend to have relatively low thresholds (i.e. they are fairly easy to stimulate) and will respond to a rapid electrical change. The lower frequency option is most often used in cases of chronic pain. Higher frequency is used in acute pain conditions (Watson & Lawrence, 2016). Indications for the use of TENS in horses include:

- -Diagnosed soft tissue pain
- -Joint capsulitis
- -Influenced spinous processes
- -Lumbosacral discomfort
- -Sacroiliac joint disorder (Bromiley, 1999).
- -Electrical stimulation for pain modulation
- -Acute pain associated with surgery or trauma
- -Chronic musculoskeletal pain
- -Muscle stimulation via alpha motor nerve

- Stimulation of non-working muscles
- -Iontophoresis
- -Edema reduction
- -Wound healing (Schlachter and Lewis, 2016).

Contraindications

- -Direct open wounds
- -Areas of skin irritation
- -Areas with high fat content
- -Sensory loss areas
- -Over the carotid sinus or pharyngeal area
- -Directly over the heart
- -Patients with a history of seizures
- -Areas of thrombosis or thrombophlebitis
- -Infected areas
- -Ocular use (Sprague ve Goldberg, 2017).

Pic.3: TENS device
Source: (Schlachter ve Lewis, 2016).

Pic.4: Application of the TENS device to the cervical area

Source: (Schlachter ve Lewis, 2016).

2) Neuromuscular Electrical Stimulation (NMES)

NMES is the application of electric current generated by a stimulator that travels to electrodes placed on the skin to depolarise the motor nerve and produce a skeletal muscle contraction. It causes nerve depolarisation and subsequent activation of muscle fibres (Levine and Bockstahler, 2013).

NMES works on the principle of muscle contraction through motor nerve stimulation using an intermittent direct current. It is the most appropriate method among electrotherapy techniques as it produces both superficial and deep controlled motor and sensory responses while maintaining a high level of compliance of the horse (Schills, 2009). Electrical muscle stimulation improves venous and lymphatic drainage, prevents muscle atrophy, prevents the formation of unwanted adhesions, reduces scar tissue formation, builds and reconstructs damaged or weak muscles, and promotes nutrition of the affected area (Adair & Philips, 2017).

Using the same electrical stimulus as a TENS unit, NMES units utilise a longer pulse duration (width; 200-600 ms), variable amplitude required to achieve muscle contraction and a frequency of more than 50 Hz. Since the aim of the treatment is to induce muscle contraction, appropriate placement of the electrodes and increasing the pulse amplitude is an effective way to create a treatment plan. The intensity should be such that the animal can tolerate it. The number of repetitions should start low (8-15 contractions per session) and be applied over 3-5 weeks with one to five sessions per week, depending on the

purpose of treatment and the injury. Some horses react against this application. The same skin irritation can occur as with a TENS unit, but is less common as the treatment time is usually shorter (Schlachter and Lewis, 2016).

It is usually applied at higher frequencies (20-50 Hz) for muscle strengthening purposes. NMES provides muscle tetany and contraction that can be used for functional purposes. Thus, an electrical potential is provided to the muscle group to elicit an involuntary contraction. The electric current depolarises the motor nerve and produces a muscle contraction.

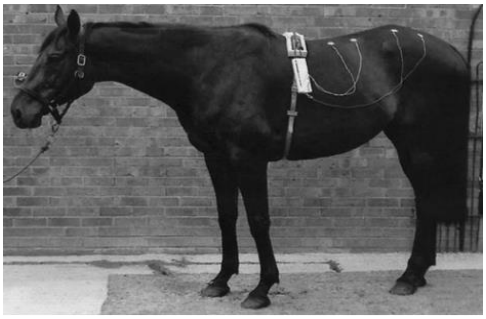
- NMES helps to reduce tissue oedema. Muscle contractions during normal movements provide a pumping action that assists venous return. Venous return is inhibited as a result of weakness, pain, trauma, bed rest or other neuromuscular problems. Muscle pumping can be achieved using low frequency (10 pps) twitch-like contractions in a continuous setting or tetanic contractions (frequency greater than 25-30 pps) with a rapid on-off cycle. Electrical stimulation applied to the affected muscles can improve circulation by increasing muscle pumping action. When electrical stimulation is used to reduce oedema, it is applied to large muscle groups around the oedema to promote mutual contraction and relaxation of agonist and antagonist muscles (Levine and Bockstahler, 2013).

Indications

- -Increase joint mobility
- -Reducing joint contracture
- -Reduce oedema
- -Increase circulation
- -Minimising muscle atrophy
- -Increase muscle strength
- -Delaying voluntary loss of control
- -To develop sensory awareness
- -Reducing plasticity
- -Reduce pain
- -Correcting gait abnormalities (Levine and Bockstahler, 2013).

Contraindications

- High-intensity stimulation directly on the heart
- -In animals with heart pacemakers
- -In animals with seizure disorders
- -On areas of thrombosis or thrombophlebitis
- -On infected areas or neoplasms
- -On the carotid sinus
- -Active movement is contraindicated at any time
- -On the body during pregnancy
- -In areas with sensory impairment
- -In areas with skin irritation or damage
- -near electronic sensing devices such as ECG monitors (possible interference) (Levine and Bockstahler, 2013).



Pic.5: NMES application to Longissimus and Gluteus medius muscles
Source: (Bromiley, 2013).

Pic.6: NMES application for muscle strengthening
Source: (Goldberg ve Tomlinson, 2018).

Thermal Therapy Methods

The application of cold (cryotherapy) or heat (thermotherapy) to the skin has been a fundamental physiotherapy method since ancient times. Usually cold therapy is used to reduce inflammation and pain in acute soft tissue injuries, while heat is used to increase blood flow, tissue metabolism and extensibility (Buchner and Schildboeck, 2006).

Cryotherapy and thermotherapy are useful adjunctive applications for the treatment of musculoskeletal injuries. Although these treatment modalities reduce both pain and muscle spasm, their effects on tissue metabolism, blood flow, inflammation, oedema and connective tissue extensibility are in contrast to each other. Cryotherapy reduces these effects while thermotherapy increases them. Continuous low-level cryotherapy and thermotherapy are newer concepts in therapeutic modalities. Both modalities provide significant pain relief with a low side effect profile (Nadler et al., 2004).

Thermal therapies are an additional form of treatment for musculoskeletal and soft tissue injuries. It is applied on the skin to change the temperature of cutaneous, intra-articular or other soft tissues. Heat application can increase skin and joint temperature, improve circulation, relax muscles and reduce joint stiffness. In addition, deep heating can reduce the sensitivity of nerves and muscle spindles. Cold applications can reduce pain, reduce swelling, constrict blood vessels and block nerve impulses. Heat and cold are important treatment modalities because they are mostly inexpensive and easy to perform by ordinary people with only a few measures (Atalaia et al., 2021).

Pic.6: Use of cold or hot water compression therapy boots to treat cellulitis, lymphangitis, and other inflammatory conditions of the limb
Source: (Kaneps, 2016).

1) Cryotherapy (Cold Application)

Cryotherapy treatment is the therapeutic application of any substance to the body that causes a decrease in tissue temperature by removing heat from the area. The application of cold causes vasoconstriction, reducing tissue blood flow and decreasing tissue metabolism, oxygen utilisation, inflammation and muscle spasm. It also causes effects both locally (at the site of application) and at the spinal cord level through neurological and vascular mechanisms. Topical cold therapy lowers the temperature of the skin and underlying tissues to a depth of 2 to 4 cm, reducing the activation threshold of tissue nociceptors and the transmission rate of pain nerve signals. Current research shows that cryotherapy reduces tissue blood flow and cell metabolism. Continuous cryotherapy has been shown to have a protective effect on injured tissue (Nadler et al., 2004). Excessive haematoma formation at the site of injury is prevented as the cold causes a transient vasoconstriction, thus preventing excessive oedema (Bromiley, 2013).

Cold therapy finds indications for physical problems associated with pain, heat and swelling and is an important conservative treatment for horses with joint disease. This therapy can take many forms, such as ice baths or frozen bandages and ice massage. Ice massage is a simple but effective method of pain relief by applying ice directly to the tissues with massage movements until the local area cools down. In the case of joint disease, it provides pain relief in the periarticular tissues and may reduce or eliminate the need for anti-inflammatory drugs (Porter, 2005).

The application is particularly effective during the first 24 to 48 hours after injury or surgery. Cold can be applied by ice water immersion, ice packs or cold packs and circulating bandages or boots filled with ice water. The most beneficial therapeutic effects of cold occur at tissue temperatures between 15 C and 19 C. The average duration of cold application is 20 to 30 minutes. Treatments are best repeated every 2 to 4 hours during the first 48 to 72 hours of injury or surgery if the aim is to reduce tissue inflammation. Direct contact of ice water with the skin is the most effective method of cold therapy (Kaneps, 2016).

Effects of Cryotherapy

- -Reduces pain
- -Reduces blood flow (vasoconstriction) and bleeding

- -Reduces inflammation and oedema formation
- -Reduces muscle tone (spasticity)
- -Reduces metabolism and histamine release
- -Reduces nerve conduction velocity
- -Increases connective tissue stiffness
- -Temporarily increases muscle viscosity (decreased ability to perform rapid movements).

Contraindications and warnings: Cold therapy should not be used in these cases:

- -In advanced cardiovascular disease
- -In acute febrile illness
- -People sensitive to cold
- -In some acute skin conditions, e.g. eczema, dermatitis.
- -If there are areas of ischaemia
- -Frost date or impaired thermoregulation
- -Radiotherapy or other ionising radiation in the last 6 months
- -Open or infected wounds (without appropriate precautions)
- -Malignant tissue
- -Extensive scar tissue - poor blood flow can lead to cell damage through overcooling
- -In cold therapy, ice should never be applied directly to the skin and should be covered with a damp towel when using ice packs (ice massage is an exception).
- -Be careful when applying cold packs directly onto superficial metal plates (the high conductivity of metal allows the effect of the cold to be prolonged even when the cold pack is removed, thus potentially preventing healing) (Lindley and Watson, 2010).

Pic:7: Application of two fluid administration bags with ice water between them to cool the distal extremity.
Source: (Kaneps, 2016).

Thermotherapy (Heat Application)

Thermotherapy is the therapeutic application of any substance to the body that causes an increase in tissue temperature by the application of heat to the body. Heat therapy, which can be superficial (up to 1-2 cm deep) or deep (approximately 4-5 cm deep), is similar to cryotherapy in that it provides analgesia and reduces muscle tonicity. Differently, thermotherapy increases tissue temperature, blood flow, metabolism and extensibility of connective tissue. Heat therapy is provided by three mechanisms: conduction, convection or conversion. Increased blood flow facilitates tissue healing by supplying protein, nutrients and oxygen to the injury site. Continuous low-level heat therapy directly on the skin has been shown to be safe and therapeutically effective in the treatment of musculoskeletal disorders. It has also been found to be effective in the treatment of acute muscular and low back pain. The applications are widely used in the treatment of various musculoskeletal disorders (Nadler et al., 2004).

The main physiological benefits of heat therapy are increased local circulation, muscle relaxation (and thus reduction of muscle spasms and associated pain) and increased tissue extensibility. Increased local blood flow mobilises tissue metabolites, increases tissue oxygenation and increases the metabolic rate of cells and enzyme systems. For a tissue temperature increase of 10 C, the metabolic rate increases 2 to 3 times. These responses to heat therapy are particularly beneficial for wound healing. Soft tissues can stretch more effectively when they are warm, so it can be said that heat reduces tissue

viscosity and increases tissue elasticity. Low-load, prolonged stretching of tissues heated from 40 C to 45 C results in increased extensibility of tendons, joint capsules and muscles. Heat is best applied after acute inflammation has subsided. It is useful to reduce muscle spasms and pain resulting from musculoskeletal injuries. The application of heat, especially before active stretching, is used to improve joint and tendon mobility. Superficial heat is most commonly applied using hot packs and hydrotherapy. These methods provide heat penetration into the skin to a depth of approximately 1 cm. The most profound physiological effects of heat occur when tissue temperatures rise between 40 °C and 45 °C. Tissue temperatures above 45 °C cause pain and tissue damage.

For deeper tissues such as tendon or muscle, 15 to 30 minutes is required to raise the tissue temperature to the therapeutic range. When using heat sources hotter than 45 C, the source should be wrapped in several layers of damp towels before application. Heat from these sources is usually applied for 20 to 30 minutes. Warm water is probably the most accessible method of heat treatment. The rule of thumb is that the water should have a temperature of 38 C to 41 C, hot enough for your hand to comfortably withstand. Tissue heated with water at such a temperature can only reach the lowest tissue therapeutic range. Therefore, the target temperature should be above this level, but as already mentioned, horses will often be uncomfortable with water that is 45 C and hotter. Heat can be used to relax tense muscles in the back before exercise. Simply using a thick wool blanket or exercise rug can be used to relax muscle spasm and prepare the back for stretching exercises or riding. The use of magnetic blankets has been another treatment method used to treat muscle stiffness and pain by increasing localised blood flow (Kaneps, 2016).

Heat therapy can be used to relieve pain, relax muscle spasm, increase blood flow to an area and increase soft tissue extensibility. Subacute and chronic inflammatory or traumatic conditions such as contusion, sprains, strains and myositis may also benefit from heat therapy (McGowan and Goff, 2016).

Superficial heating agents may include: hot packs, heat wraps, hosing with warm water, whirlpools, paraffin baths, circulating warm water blankets, electric heating pads and infrared lamps. The last two are thought to have a higher risk of burns in animals (Millis and Levine, 2013).

Indications

- -Reduces pain
- -Reduces blood flow (vasoconstriction) and bleeding
- -Reduces inflammation and oedema formation
- -Reduces muscle tone (spasticity)
- -Reduces metabolism and histamine release
- -Reduces nerve conduction velocity
- -Increases connective tissue stiffness
- -Temporarily increases muscle viscosity (decreased ability to perform rapid movements)

Contraindications

- -In chronic cardiovascular disease
- -In acute febrile illness
- -People sensitive to cold
- -In some acute skin conditions (eczema, dermatitis)
- -If there are areas of ischaemia
- -Radiotherapy or other ionising radiation in the last 6 months
- -Open or infected wounds (without appropriate precautions)
- -Malignant tissue
- -Extensive scar tissue - poor blood flow can lead to cell damage through overcooling

Laser Therapy

The term "laser" is an abbreviation for Light Amplification by Stimulated Emission of Radiation. A laser device is a device that produces coherent, parallel and monochromatic light through an optical amplification process based on the stimulated emission of electromagnetic radiation. Laser therapy utilises light in the red and infrared parts of the electromagnetic spectrum (Zielinska et al., 2020), which is the only wavelength of light to stimulate reactions within cells. It is in the far red to near infrared spectral range (R750 nm). This treatment is primarily used to reduce pain and improve the quality of repair in soft tissue wounds. Its mode of action has been attributed to the activation of components of the mitochondrial respiratory chain, resulting in a series of signalling events that promote cellular proliferation (Mimi Porter,

2005). In principle, laser light is applied at a low intensity/energy level. The purpose of its use is to have a biostimulatory effect rather than the destructive effect achieved at higher levels, as in surgical and ablative procedures (McGowan and Goff, 2016).

Laser light is absorbed in tissues mainly by chromophores (in mitochondria). This results in increased cellular reaction levels. The energy thus transmitted in a manner similar to ultrasound leads to cellular regeneration, various secondary reactions, modulation of cellular functions and typically stimulation of tissue repair mechanisms. Laser irradiation increases the production of adenosine triphosphate (ATP) within cells and thus mediates various events, including the release of growth factors and cytokine reactions. The ultimate effect of these events is accelerated tissue healing (Mcgowan ve Goff, 2016). Several studies have shown that laser light has anti-inflammatory, analgesic and anti-edema effects. It can also stimulate fibroblast activity and proliferation and increase adenosine triphosphate production (Zielinska et al., 2020).

There are two general groups: high power (hot) and low power (cold) (Bromiley, 2013).

High power lasers cause thermal changes in the tissue. For example, the CO2 (carbon dioxide) laser is used both to cut tissue and to coagulate blood (Bromiley, 2013).

Low-power laser therapy (power output <500 mW) has been used in both human and veterinary medicine for decades. These are therapeutic lasers. Low power laser therapy (LLLT) is a treatment option for tendon and ligament damage (Pluim vd., 2018).

These low-level lasers operate in a range of less than 500 mW and have wavelengths ranging from 540 nm to 1060 nm. Similar to therapeutic ultrasound and shockwave, laser therapy has anti-inflammatory and analgesic effects (Schlachter and Lewis, 2016).

Indications include wound treatment, treatment of soft tissue injuries, osteoarthritis and local pain relief. The biological effects of laser include anti-inflammatory effects such as reduced IL-1 levels, reduced nerve depolarisation, reduced pain sensation through endorphin release and increased ATP production. The dose of energy required for treatment depends on the shape of

the injury, the depth of the tissue and the desired effect (healing or stimulation of tissues for anti-inflammatory and pain relief effects) (Kaneps, 2016).

Laser wavelengths for wound treatment should be near 650 nm, whereas treatment of deeper tissues requires the use of wavelengths between 805 and 980 nm. Lasers with energy outputs of less than 500 mW and up to 15 W are available. Higher energy outputs reduce treatment time, but can cause tissue burns if used incorrectly. The recommended laser dose for soft tissue injuries is 4 to 12 J/cm2 (Kaneps, 2016).

If the working mechanism of laser therapy is summarised;

-Stimulates cell metabolism,

-Provides direct activation of mitochondrial calcium channels,

-Provides regulation of ATP production and synthesis,

-Increases fibroblastic activity,

-Stimulates increased cellular division, fibroblast migration and cellular matrix production,

-More specifically, prostaglandin E2, tumour necrosis factor-a, interleukin-1b, plasminogen activator and cyclooxygenase-1 and 2 have all been shown to be manipulated using laser therapy, resulting in a reduction in inflammation. The anti-inflammatory effect occurs through stimulation of prostaglandins leading to vasodilatation (Schlachter and Lewis, 2016).

Indications

- -Performance maintenance
- -Prevention of injury recurrence
- Synergistic with stem cell and platelet-rich plasma therapy
- -Tendon and ligament injury
- -Chronic and acute musculoskeletal disorders
- -Edema control
- -Chronic joint disease
- -Synovitis
- -Osteoarthritis
- -Back pain/injury
- -Wound treatment and healing
- -Painkillers

- -Neurological injuries
- -Acupuncture/acupressure alternative (Carver, 2015).

Contraindications

- -Not to be used in pregnant animals (mares).
- -Young, growing animals (physiologically unknown effect)
- -Malignite
- -Hematological disorders
- -Febrile patients
- -Ocular use (Schlachter and Lewis, 2016).
- -Laser therapy should not be used in animals with neoplasia.
- -Laser treatment should not be applied on the thyroid region (Carver, 2015).

Pic.8: Laser treatment for superficial digital flexor injury
Source: (Schlachter and Lewis, 2016).
Pic.9: Low level laser therapy application
Source: (Adair and Philips. 2017).
Pic.10: Low level laser application in the torcolumbar region
Source: (İnt. ref. 1)

Therapeutic Ultrasound

Following the tragic sinking of the Titanic in the early twentieth century, research into the use of sound waves to identify objects began. The first recorded use was on a person suffering from sciatica in 1938. Unlike diagnostic ultrasound, therapeutic ultrasound is specifically designed to have a biological

effect on tissues. Ultrasound uses cyclic vibration frequencies of 1 to 3 MHz. The mechanical energy produced creates a wave of acoustic energy that the human ear cannot hear. This energy passes through the tissues and is absorbed by deep tissues through molecular vibration without changing the temperature of the skin surface (Schlachter & Lewis, 2016).

Like laser, ultrasound has multiple uses in medicine. It is a therapeutic range used for scanning, measurement, diagnosis and treatment that can be applied after injury. Unlike massage, muscle stimulation and heat, but similar to magnetic field and laser therapy, the effects of ultrasound occur at the cellular level (Bromiley 2013).

Therapeutic ultrasound uses sound waves to stimulate healing, relieve pain, reduce tissue oedema and reduce fibrous scarring, and is a method that can also allow heat to reach deep tissues (Kaneps, 2016).

Alternating electric current drives the vibration of synthetic crystals within a sound head that produces pressure waves known as ultrasound. The sound waves associated with therapeutic ultrasound penetrate the skin and subcutaneous tissues to a depth of 3-5 cm with minimal to moderate attenuation or absorption. Tissues with high protein or collagen content or tissue interphases (e.g. periosteum or entheses) readily absorb the sound wave. This results in energy transfer to the surrounding tissues and a localised increase in tissue temperature. The primary biophysical effects of therapeutic ultrasound can be classified as thermal or non-thermal effects. Non-thermal effects may be the result of the expansion and compression of small gas bubbles present in tissue fluids and the movement of fluids across the boundaries of cell membranes (Paulekas and Haussler, 2009).

Therapeutic ultrasound is a form of acoustic energy used to treat musculoskeletal injuries, including inflammation and wounds. It provides deep heating without destroying the skin. It is also used to reduce pain and muscle spasm, promote wound healing, help absorb haematoma, reduce oedema and accelerate cicatrisation. It increases blood flow in the treated area. Increases cell membrane permeability to ions and other substances. Inhibits signal transmission in nerves. Reduces muscle spasms. It has been shown in clinical and scientific studies that it increases collagen extensibility and collagen production, improves collagen remodelling, increases heat and blood flow in

deep tissues, increases range of motion, reduces pain and muscle spasm, and accelerates wound healing (Adair & Phillips, 2017).

The therapeutic effects of ultrasound are both thermal and biological. Thermal effects are achieved by heating tissue using continuous sound waves or continuous ultrasound. Thermal effects are caused by the energy carried by the ultrasonic waves being attenuated and absorbed by the tissue as the waves pass through it. Non-thermal (biological) effects result from the mechanical alteration of the local, cellular environment provided by the ultrasound waves. It is observed to stimulate cell behaviour and activity. To avoid heating the treated tissue and to achieve non-thermal effects, pulsed ultrasound is used where pulse velocities intercept sound waves by 50%, 80% or 90% (Porter, 2005). Changes in the cellular environment can lead to changes in cellular function resulting in a shorter inflammatory healing phase, increased vascularity at the treatment site and increased fibroblast proliferation (Medina and Davies, 2017).

In the frequency of sound waves that determine the penetration depth, the absorption of sound increases as the frequency increases. Common wavelengths of ultrasound devices are 1 and 3 MHz. At 3 MHz, most of the energy is absorbed at a depth of 1-2 cm. It makes it useful in the treatment of superficial structures such as tendons and ligaments. At the low frequency of 1 MHz, minimal absorption by the skin and superficial tissues occurs and more energy is used to heat the tissues to a depth of 3-5 cm (Paulekas and Haussler, 2009).

The intensity of sound waves, which is the speed of propagation of the sound wave, is measured in Watts/cm2. The higher the intensity, the faster and larger the temperature increases (Paulekas and Haussler, 2009).

Ultrasound energy is attenuated in air and completely reflected at air-tissue interfaces. Therefore, a coupling medium (e.g. water-soluble gel) is used to eliminate as much air as possible to maximise the transmission of the sound wave from the transducer head to the tissue surface. Patient variables such as the size of the treatment area, the depth of the lesion, the characteristics of the tissue to be treated and the stage of healing will determine the clinical parameters that the therapist will choose (Paulekas and Haussler, 2009).

A caveat of ultrasound application in veterinary medicine is that sound waves are largely absorbed by the hair layer. The attenuation of the sound wave

by even a short horse hair is important to reduce thermal effects on the underlying tissues. For adequate transmission of the sound waves, the hair on the skin in the area should be cut as short as possible (Paulekas and Haussler, 2009).

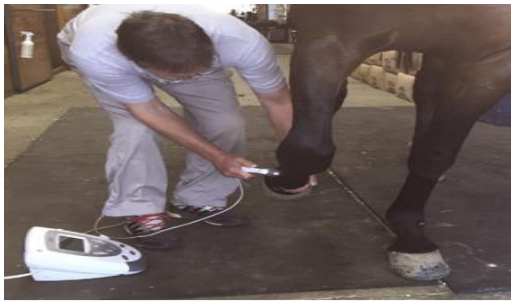
The most commonly used frequencies in therapeutic ultrasound are at 1 and 3 MHz, with 1 MHz used to reach deeper tissues (up to 5 or 6 cm) and 3 MHz used for more superficial lesions (up to about 2 cm deep). The absorption of ultrasound energy is optimal in dense collagen-based tissues (tendon, ligament, fascia, joint capsule and established scar tissue) and is most effective in these tissues. It does not have an anti-inflammatory effect in the inflammatory phase. It stimulates cells responsible for maintaining inflammatory events through the release of numerous growth factors, cytokines and other chemical messengers. In the proliferative (early repair) phase, the effect of ultrasound is to stimulate fibroblasts and myofibroblasts, both of which are important contributors to the formation of newly formed scar tissue. In addition, ultrasound has been shown to enhance the angiogenic (neovascular) response, which provides essential support to cells producing new scar tissue (collagen) (Watson and Lawrence, 2016).

Indications for Use

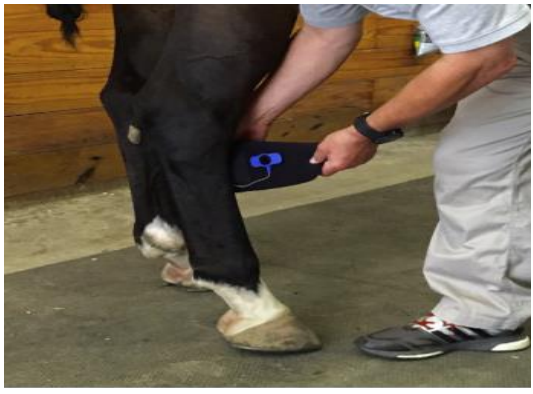
- -Tendon and ligament: Minimises contraction with healing
- -Facial wounds
- -Joint capsule: Improves range of movement
- -Scar tissue: Softens scar tissue
- -Osteophytes/enthesiophytes: Reduces pain but has no effect on the bone itself.
- -Muscle spasms: Slows down gamma fibre conduction
- -Wounds: Increases protein synthesis in fibroblasts (use 2 weeks after injury)
- -Edema: Reduces tissue oedema
- -Nerve injuries: Remyelination and regeneration of damaged axons (Schlachter and Lewis, 2016).

Contraindications for Use

- -Ophthalmic use: Poor vascularisation of the lens causes heat intolerance
- -Pregnant mares: Especially on the breeding areas
- -Cardiac: Power outage potential
- -Growth plates: Potential for developmental abnormalities
- -Fractures: May delay the healing process
- -Sensation blocked like nerve blocked places
- -Vascular insufficiency
- -Thrombophlebitis/coagulation dysfunction: Embolic potential
- -Infection/cellulitis: Potential for spread of infection through vasodilatation
- -Malignancy: Potential to spread
- -Immediately after exercise: tissues are already at high temperature (Schlachter and Lewis, 2016).
- -Avoid diseases of the brain, eyes and reproductive organs, tumours, thrombosis, heart disease, circulatory and intestinal disorders.
- -Too high a dose may cause periosteal pain. Always use a low output dose.
- -If any haemarthrosis or sepsis other than sinusitis is suspected, it should not be treated with therapeutic ultrasound.
- -It causes no problems as long as the dosage is not too high and is well tolerated by most horses.
- -If many animals are treated for too long a period of time (3 months) this is harmful and can lead to demineralisation of the bone.
- -One month is recommended as the maximum duration for a treatment regime - with a break of at least two weeks before restarting treatment (Bromiley, 2013).



Pic.11: Conventional therapeutic ultrasound allows manipulation of the transducer over various anatomical regions and adjustment of treatment output, but requires a trained person to administer the treatment
Source: (Kaneps, 2016).



Pic.12: Low-level therapeutic ultrasound with preset wavelength and power output is used to deliver the treatment over several hours. The transducer (black disc in blue holder) is attached to an elastic sleeve that is secured over the treatment area
Source: (Kaneps, 2016).

Hydrotherapy

While the benefits of exercising in water have long been recognised in humans, evidence has recently been provided that hydrotherapy is beneficial for pain, function, joint mobility, strength and balance (Monk, 2016).

Hydrotherapy uses the physical properties of water such as temperature, pressure, viscosity and water fullness for therapeutic purposes in the treatment of disorders such as pain relief, blood circulation and joint, muscle and nerve diseases (Atalaia et al., 2021). It is a commonly prescribed treatment option to manage musculoskeletal injuries and reduce or limit gait abnormalities.

Exercising in water is known to provide an effective environment to increase joint mobility, support normal motor functions, increase muscle activation and reduce the incidence of musculoskeletal injuries caused by joint pathology (King, 2016).

By utilising the properties of water, an ideal environment is provided to restore the function of animals with poor motor functions. In healthy animals, it is used to provide resistance and muscle strengthening effect (Carver, 2016).

Hydrotherapy involves several different mechanisms of action with particular benefits in the treatment of equine musculoskeletal disorders. Current human and veterinary medicine literature suggests that hydrotherapy has beneficial effects on many musculoskeletal morbidities, such as reduced pain and increased range of motion (King, 2016).

The main aim of hydrotherapy is to make the patient more functional in a faster time period compared to other treatments. Swimming and underwater treadmill walking are considered the most common treatment methods in veterinary medicine. In order to understand the difference between treatments in water and treatments on land, it is necessary to understand the properties of water and the effect of these properties on the patient during hydrotherapy (Egan & Fitzpatrick, 2018).

The practice of hydrotherapy in horses primarily involves the use of underwater treadmills, swimming pools (circular or flat) and standing saltwater spas or whirlpools. underwater treadmill units have features such as changing treadmill speed, water temperature and solute concentration. A period of 3 to 5 days is required for horses to acclimatise and be trained to exercise in the UWTM units (King, 2016).

Aquawalkers are mechanical walkers placed in a circular pool containing a consistent depth of water. The diameter of the Aquawalker determines how many horses can be run at a time. Most systems can run 6-8 horses at the same time. The horses are not in a fully swimming state and are separated from each other by dividers that create a separate "compartment" for each horse. The depth of the water is determined by the system design (King, 2016).

Horse pools should be designed so that carers on either side of the horse's head can walk side by side during each exercise session. Water depth should be greater than 12 feet to ensure full flotation. Linear pools can reduce cardiorespiratory stress as the horse is allowed to recover at the exit while

walking back to the entry point. Conversely, continuous lap swimming in circular pools does not allow cardiorespiratory recovery until the exercise session is complete. Horses are not natural swimmers and usually use their thoracic limbs to maintain balance, while the pelvic limbs are mainly used for propulsion. In addition, upon entry into the water, horses often adopt a posture that results in cervical, thoracolumbar and pelvic extension. According to the authors, swimming horses with thoracolumbar, sacroiliac, hip, stifle and knee injuries should be approached with caution (King, 2016).

Based on the evidence available for human and animal hydrotherapy, the proposed benefits of hydrotherapy or aquatic exercise for animals include the following.

- Reduces the load on painful structures - exercise can be started early with less muscle activation required and less load on the limbs.
- Provides additional support to the limbs, reducing the likelihood of injury to muscles, tendons and ligaments.
- It facilitates the performance of difficult activities and movements thanks to its buoyancy.
- Water provides more resistance than air.
- Prevents atrophy.
- Increases muscle mass and strength.
- Increases cardiovascular fitness and endurance.
- Increased joint range of motion reduces stiffness.
- Increases soft tissue extensibility.
- Reduces muscle spasm and hypertonicity.
- Increases tone in hypotonic body parts.
- Allows gradual progression and return to more normal function
- Helps oedema management through hydrostatic pressure.
- Provides relaxation.
- Reduces pain in joints with degenerative joint disease.
- Increases circulation and helps promote healing (Monk, 2016).

Contraindications of Hydrotherapy for Animals

- Open, infected or oozing wounds
- Unhealed surgical incisions without a waterproof sheath
- Active gastrointestinal disease (vomiting and/or diarrhoea)

- High body temperature, infection
- Systemic problems such as severe heart, liver, kidney disease, hypotension or hypertension
- Respiratory distress
- Uncontrollable epilepsy (Monk, 2016).

Pic.13: Horse Pool
Source: (McGowan ve Goff, 2016).

Pic.14: Pools designed for horses to walk and exercise in water (aquawalker)
Source: (McGowan ve Goff, 2016).

Pic.15: Underwater Treadmill
Source: (McGowan ve Goff, 2016).

Pic.16: Cold Mineral Water Therapy
Source: (Adair ve Philips, 2017)

Extracorporeal Shock Wave Therapy (Eswt)

Extracorporeal shock wave therapy (ESWT) is a non-invasive treatment involving the application of high-energy sound waves or acoustic energy to the affected area (Adair and Philips, 2017). This treatment was developed in the 1970s to break stones in the urinary system in humans (Jann & Fackelman, 2010). Commonly known as shockwave therapy, it uses high-energy sound waves called "pulses" or "shock waves" to stimulate and accelerate the body's own healing process (Goldberg & Tomlinson, 2018). Shockwave therapy was initially used in human medicine to provide a non-invasive treatment for urolithiasis. It was later used as a treatment for orthopaedic and soft tissue injuries, including skin wounds (Goldberg and Tomlinson, 2018).

After the observation of the effects of shock waves on the pelvis during animal lithotripsy studies, applications to increase bone healing began. These studies have shown that after exposure to extracorporeal shock waves, a marked osteogenic response occurs, especially callous formation and bone remodelling (Levine and Millis, 2013).

Today, ESWT has become a preferred treatment method in many musculoskeletal problems (Jann & Fackelman, 2010).

Mechanism of Impact of Shock Waves

Extracorporeal shock waves are acoustic waves of high pressure and velocity generated outside the body. These pressure waves differ from ultrasound waves due to their lower frequency, minimal tissue absorption and lack of thermal effect (Adair and Philips, 2017).

As acoustic sound waves pass through tissue, energy is dissipated when they encounter denser tissues such as bone, cartilage, tendons and ligaments. As energy accumulates in tissues, its mechanical force is transformed into a biological response that promotes healing (Goldberg & Tomlinson, 2018).

The mechanism of action seen in the tissue and its relationship with the clinical effect of treatment is not fully understood. Current theories include cytokine induction, increased osteoblast activity, stimulation of nociceptors that block afferent pain signals, stimulation of neovascularisation, synthesis of nitric oxide and thus bone healing/remodelling. ESWT is generally considered safe. No adverse effects on skin, tendons, ligaments, nerves or bone have been recorded (Adair and Philips, 2017).

ESWT is not recommended for acute injuries. Sufficient time should be allowed for the inflammation to subside and, in the case of tendons and ligaments, for the lesion to adjust. Indications effectively treated with ESWT are insertion desmopathies, navicular syndrome, dorsal spinous process impingement, arthropathies (fetlock, pastern and hock), exostosis, constriction of the annular ligament, tendinopathies with or without calcification, calcification of the nuchal ligament, splint bone fractures and dorsal metacarpal disease. For the treatment of the affected area, 1500-2000 blows given at intervals of 10 to 14 days are used. Horses are rested during treatment and may require a rest period of up to 6 months depending on the condition being treated (Adair and Philips, 2017).

Shock waves cause neovascularisation in both bone and soft tissue (Jann and Fackelman, 2010). Extracorporeal shockwave therapy promotes bone healing and enhances tendon and ligament fibre remodelling in vivo. Shock waves can be focussed on a specific area in the body and have a lower frequency compared to ultrasound waves but have minimal tissue absorption and no thermal effects. ESWT is used in the treatment of a variety of musculoskeletal disorders including suspensory desmitis, tendonitis, stress fractures, periostitis, osteoarthritis, navicular disease, dorsal spinous process impingement, sacroiliac injuries and general back pain (Waguespack et al., 2010).

The primary biological effects of ESWT include decreased levels of inflammatory mediators, increased levels of angiogenic cytokines resulting in vascular proliferation, increased levels of growth factors resulting in tissue healing. It also involves the recruitment of osteoblasts and mesenchymal stem

cells. As the shock wave crosses tissue interfaces, tissue compression and shear loads occur. This results in stimulation of bone and soft tissue healing (Kaneps, 2016).

ESWT treatment of arthritis of the equine distal tarsal joints (bone spavin) resulted in improvement in the degree of lameness in 59 of 74 treated horses. 3 Chronic hanging desmitis was successfully treated in 24 of 30 horses after ESWT treatment. ESWT is indicated for the treatment of insertional desmopathy (such as origin or insertion of the suspensor ligament), dorsal cortical stress fractures, incomplete fractures of the proximal sesamoid bone, arthritis and navicular disease and has also been used for the treatment of tendonitis (Kaneps, 2016).

Contraindications

- Applications to the neck area should be avoided
- If ESWT is used in the cervical region, the focal point should not be over a joint or articulation due to its proximity to the spinal cord
- Foals may develop swelling and petechiae (white areas) when used at high settings
- Excessive energy or pulses may cause tissue necrosis resulting from cavitation of cells
- Shock wave areas in gas-filled organs such as the lungs, trachea, oesophagus and intestines can cause tissue damage and thus perforations
- Focussing on large nerves and blood vessels can cause damage to the vessel walls, leading to rupture, leakage or narrowing of the arteries
- High energy delivering an extremely high number of shocks to the bone can cause micro fractures
- In the presence of neoplasia or infection, it may cause dissemination of neoplastic cells or bacteria in the area (Jann ve Fackelman, 2000)

Pic.17: The horse should be positioned with the fetlock flexed and tendons displaced axially or axially for optimal energy exposure of the proximal suspensory ligament during extracorporeal shock wave therapy
Source: (Kaneps, 2016).

Pic.18: Shock wave therapy applied to the medial distal interphalangeal joint collateral ligament
Source: (Kaneps, 2016).

Magnetic Field Therapy

Magnetic and electromagnetic fields, a bioelectric phenomenon, have existed since the beginning of the universe. These natural energies have existed long before the emergence of the plant and animal world and have had a great influence on the basic life functions of all bodies (Jastrzebska, 2020).

One of the treatment modalities used in physiotherapy is electrotherapy or electrical stimulation, which is characterised by various techniques using electric current. Pulsed electromagnetic fields (PEMF) are often included as part of this group and are widely used in human and veterinary medicine (Biermann et al., 2013).

Modern electromagnetic field therapy began in 1971 when Friedenberg and colleagues described the healing success of direct current delivered to non-union fractures (Schlachter and Lewis, 2016).

Pulsed electromagnetic field (PEMF) stimulation has been used clinically for more than twenty-five years in patients with fracture healing, delayed healing and non-union (Inoue et al., 2002). In the sixteenth century, the Swiss physician Paracelsus used magnets in the treatment of epilepsy, diarrhoea and bleeding. Today, pulsed magnetic fields (PEMF) and static magnetic fields (SMF) are used to treat horses with inflammatory and degenerative musculoskeletal disorders. Several scientific studies have suggested that magnetic fields affect the microcirculation of the body parts to which they are applied (Rindler et al., 2014).

PEMF therapy signals are the most widely used electromagnetic signals for therapeutic applications. This therapy works through the creation of pulsed magnetic fields that have been found to have therapeutic benefit in the healing responses of various tissues. PEMF therapies provide therapeutic microcurrents to enhance the natural regenerative pathways utilised by the body. The signals come in the form of an electromagnetic field. A magnetic field is the region surrounding a magnet or electric current. The electric current transmitted to the magnet through a wire provides an electric current that affects the targeted tissue to create a healing response by creating a pulsed magnetic field (Gaynor et al., 2018).

The use of PEMF is gradually increasing. In current clinical veterinary practice, electromagnetic field techniques have been shown to have therapeutic benefits for bone and wound repair and acute and chronic pain relief (Gaynor et al., 2018). It is a safe, simple and cost-effective method with minimal or no side effects. Therefore, it is becoming a popular treatment choice in veterinary rehabilitation (Goldberg and Tomlinson, 2018).

Pulsed magnetic fields have been used to treat a variety of conditions including inflammation, osteoarthritis, soft tissue injuries, chronic pelvic pain and tendonitis and have been reported to be beneficial for nerve regeneration, healing of bone defects, bone grafts, fracture repair and prevention of osteoporosis (Steyn et al., 2000).

The use of pulsed and static magnetic field therapies in horses has also been investigated and it has been found that pulsed magnetic field therapy in

horses has a significant positive effect on the healing of cancellous bone grafts (Steyn et al., 2000).

Indications for Use

- Bone healing
- Increase blood flow in superficial and deep tissues
- Reducing pain and muscle spasm

Contraindications for Use

- Application on any implant
- Pregnancy
- Open wounds
- Cancer
- Infection
- Acute inflammation and joint effusion (Schlachter and Lewis, 2016).
- Sepsis
- Kidney damage
- Laminite (Bromiley, 2013).

Pic.19: Coil blanket used for PEMF therapy
Source: (İnt. Ref. 2).

Pic.20: Application of PEMF with a wire coil used to transfer the magnetic field in the trapezius muscle
Source: (İnt. Ref. 4).

Pic.21: Use of magnetic field for Navicular Syndrome treatment
Source: (İnt. Ref. 5).

Therapeutic Exercise

Therapeutic exercises are a very important component of any patient's rehabilitation programme, regardless of the problem or diagnosis. Exercises should be tailored to the individual patient, taking into account the clinical diagnosis, the patient's age, physical condition and available resources. Exercises are used to improve a patient's range of motion (ROM), strength, weight bearing and walking pattern, flexibility, balance and proprioception, reduce pain, accelerate recovery. Exercises also improve strength, aerobic capacity (endurance) and performance (Huntingford and Bale, 2016).

Therapeutic exercise is often used during a rehabilitation programme. The amount and intensity depends on the condition being treated, the extent of the injury, the recovery time and the facilities available. Each exercise programme is individual and may need to be adapted several times during the rehabilitation programme. The basic principle is to reduce force and strain on the injured tissue while the normal repair process continues. Ground obstacles (ground poles, cavalettis) can also be incorporated to improve co-ordination and agility. In addition, a change in terrain can be incorporated to target specific areas (slopes to strengthen the hind limbs). The aim of therapeutic exercise is to provide a gradual return to function, improve strength and co-ordination and provide mental stimulation (Adair and Philips, 2017).

The Role of Exercise Physiology

Skeletal muscles perform movements guided by the nervous system. Skeletal muscle performance depends on the type of muscle fibre. In general,

muscles are classified as type I (oxidative or slow twitch) or type II (glycolytic or fast twitch) (Huntingford and Bale, 2016).

Type I muscle fibres are recognised as endurance muscle fibres and type II as sprint muscle fibres. When muscles are immobilised, muscle strength decreases rapidly. Up to 50 per cent of strength is lost in the first week of non-use (Huntingford and Bale, 2016).

Normal posture and movement involves proprioceptive and mechanoreceptive afferent feedback from joints, tendons, ligaments, fascia and skin that modulate efferent neuromuscular control. For these reasons, musculoskeletal and neurological rehabilitation requires not only the management of pain and dysfunction but also the training of motor skills. This allows the appropriate neuromuscular pathways to be stimulated and strengthened over time, allowing the horse to return to optimal function (Stubbs, 2015).

Sensory integration techniques involving tactile stimulation during exercise are reported to be a very useful clinical tool in horses. These techniques modulate and coordinate motor function by stimulating afferent proprioceptive or mechanoreceptive input. The techniques may be potentially effective in horses due to the equine skin mechanoreceptive system, which works in conjunction with the underlying cutaneous trunci myofascial ligaments. Through this system, horses can sense even a small tactile stimulus and respond by fasciculation of the cutaneous trunci. Tactile stimuli can be applied to the skin over targeted areas, including limbs or specific muscles, to provide mechanoreceptive and proprioceptive feedback, altering motor control (Stubbs, 2015).

Forms of sensory integration that can be used during training include bandages known as body wraps, the Pessoa and Equiband systems. These can be wrapped around the horse's hindquarters, abdomen or chest. The band system can be clinically useful as it increases the horse's body awareness (kinesthesia) and ability to use its core muscles. (Stubbs, 2015).

Another clinically effective sensory stimulation method is the functional proprioceptive taping technique, which is widely used in human rehabilitation, sports medicine and athletic performance. In veterinary literature, it has been reported that rigid mechanical athletic taping of the fetlock joint does not alter the kinematics of the forelimbs during stance but limits flexion of the fetlock

during the swing phase. However, there was a reduction in peak vertical force, which may be a result of the increased proprioceptive effect. These bands are elastic, hypoallergenic and semi-waterproof. Thanks to its porous texture, it allows the horse to sweat. The tape is stretched and placed along the length of the muscle that the practitioner wants to stretch. The inhibitory effect provided by the stretch in the tape increases kinesthetic awareness along the desired muscle fibres (Stubbs, 2015).

Poles, cavals, obstacles, balancing boards, various surfaces (asphalt, grass, gravel, sand and water), swimming pools and treadmills or water treadmills can be used to improve the horse's motor control and train the neuromuscular and cardiovascular system (Stubbs, 2015).

Maze or obstacle exercises are low-level neuromotor training exercises used to stimulate proprioceptive awareness (Paulekas and Haussler, 2009).

Kavaletti poles or ground poles are widely used for training horses. These poles are used to train the gait, improve proprioception and strengthen the flexor muscles of the front and hind limbs. They are also used to improve active HA. The cavalettis are placed in a series and are height adjustable. They are placed low to the floor or on the floor when the patient begins exercise or has significant muscle weakness. The height can be adjusted as the patient progresses through the rehabilitation process (Huntingford and Bale, 2016).

Incline work facilitates abdominal contraction and strengthens the muscles responsible for propulsion; it brings the pelvic limbs further under the body and lifts the back (Paulekas and Haussler, 2009).

The main goal of rehabilitation is to return the horse to its previous level of performance and exercises are used to gradually improve proprioception, neuromuscular control and to load and strengthen the musculoskeletal tissues (Tabor and Williams, 2018).

Exercise protocols are established during rehabilitation after injury or during return to work after a long period of hospitalisation. According to ultrasound and lameness examinations, controlled exercise is gradually increased depending on the horse's fitness level or injury status. For most soft tissue injuries, manual walking should begin immediately after injury to promote optimal fibre alignment and prevent restrictive adhesions. Exercise is started with 5-10 minutes of walking once or twice a day (depending on lesion severity). Ultrasound and lameness assessments should be repeated every 8 to

10 weeks and exercise levels can be increased as parameters improve. All exercises should be adjusted according to the level of soundness. If there is increased lameness, swelling at the site of injury or ultrasound parameters deteriorate, the exercise level should be reduced. Speed work should begin after 10 to 15 minutes of solid hand walking for warm-up and should be performed in short segments of 1 to 1.5 minutes (Kaneps, 2016).

Floor exercises are exercises performed in the hand or while riding. They usually target a specific area such as improvement in proprioception and coordination, strengthening of specific muscles, improvement of joint mobility and improvement of general body condition (Paulekas & Haussler, 2009).

Before designing an appropriate controlled exercise programme, a full and accurate diagnosis is essential because an incorrect diagnosis leads to an inappropriate and potentially harmful post-injury treatment programme. Without a proper diagnosis and treatment, there is a higher risk of re-injury or inability to return to athletic function. Diagnosis involves determining which tissue was injured and the severity of the injury (Davidson, 2016).

Other considerations when planning a controlled exercise programme include the horse's tolerance to prolonged periods of stall rest. Cardiac and muscular function does not undergo significant deconditioning for at least 4 weeks after injury and, therefore, horses with minor injuries can return to full function with no or minimal loss of condition (Davidson, 2016).

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CHAPTER 6

ANTIBIOTICS and RESISTANCE to ANTIBIOTICS

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Introduction

Antibiotics are natural or synthetic molecules that can inhibit the growth of bacteria (bacteriostatic) or kill some bacteria (bactericidal) (Gamboa et al, 2022). They exert a specific effect on certain structures or functions of the microorganism. It not only helps save patients' lives, but it also helps human and veterinary medicine and surgery succeed greatly (Gould et al., 2013). Patients with chronic illnesses like tuberculosis, diabetes, renal disease, and rheumatoid arthritis as well as those undergoing or recovering from major surgery like joint replacements, heart operations, or organ transplants benefit from its ability to prevent and treat infections (Ventola, 2015).

The most often used class of antibiotics are beta-lactams, which include penicillins, cephalosporins, and carbapenems. Antibiotics are widely utilized in medical, veterinary, and agricultural systems for therapeutic, preventive, metaphylactic, and growth-promoting objectives (Kumar and Pal, 2018). Their range of activity varies considerably. Some are "narrow spectrum," which means that they are harmful to just a few pathogens. Others tackle a variety of infections "broad spectrum" (Fang et al., 2018). Antibiotics are used to treat bacterial infections. In accordance with Grenni et al. (2018), these antibiotics function by impairing protein synthesis, blocking the synthesis of nucleic acids, modifying cell membranes, and possessing antagonistic and antimetabolite properties. They also hinder the creation and repair of cell walls.Antibiotic use and the emergence of antibiotic resistance are related, according to epidemiological studies. Antibiotic resistance grows faster as a result of excessive antibiotic use (Read & Woods, 2014; CDC, 2015).

Antibacterial Resistance

Global health is seriously threatened by antimicrobial resistance. By 2050, it is anticipated that 10 million people will perish annually as a result of resistant infections unless immediate action is taken (Lim et al, 2020). Bacterial resistance to antibiotics is an important issue in the field of antimicrobials. One of the main causes of resistance is antibiotic use, and according to reports, antibiotic use is rising daily around the world (Klein et al., 2015). One of the key factors in the emergence of resistance is the use of antibiotics in some viral infections and unidentified disorders (Llor et al., 2014).

The prevalence of bacteria that are resistant to antibiotics makes it more difficult to regulate bacterial contamination and has serious negative impacts on both the human and ecological environment (Lu et al., 2023). For bacteria to develop resistance and the requisite time for mutation, a dynamic environment is required (Sommer et al., 2010). According to Fernández and Hancock (2012), the major mechanisms of bacterial resistance include the inactivation of the antibiotic compound, the lack of target molecules, or the impermeability of the bacterial cell to the antibiotic molecule.

General Mechanisms of Molecular Development and Antibiotic Resistance

Antibiotic resistance is brought on through stable genetic change, such as mutation, transformation, conjugation, and transduction (Holmes et al., 2016). There are two ways that antibiotic resistance might develop: intrinsically and acquiredly. Intrinsic resistance arises from the bacterial chromosomes' pre-existing genes being mutated as a result of environmental adaptation through lower antibiotic affinities for their targets (Ventola, 2015). Genetic mistakes build up in the genes (plasmid or chromosome) of bacterial cells during this evolutionary process and result in intrinsic or innate resistance by being transferred to new generation cells through vertical gene transfer (Founou et al., 2016). According to Hayek et al. (2015), acquired resistance arises as a result of conjugation, transformation, or transduction between different or related bacterial species. It may also occur if the mutation site picks up mobile extrachromosomal DNA elements like transposons, integrons, and plasmids. The development of genes for beta-lactamase, an enzyme that destroys beta-lactam drugs like cephalosporins and penicillins, is an example of acquired resistance (Doyle et al., 2006). When bacteriophages, which are viruses that affect bacteria, are used to transmit DNA encoding resistance genes, transduction takes place (Roca et al., 2015). Bacteria change when they take in free DNA from the environment that contains resistance genes.

Functional proteins, like ribosomal proteins and enzymes, are the targets of antibiotic reactions (Blair et al., 2014). An antibiotic's target site and it interact specifically (Blair et al., 2014). When these target areas are altered by mutation, antibiotics may lose their effectiveness (Blair et al., 2014). Antibiotics are also rendered ineffective due to decreased drug flow due to

variations in porin penetration in the outer cell membrane (MacLean et al., 2010).

Gene Expression and Antibiotic Resistance

Gene regulation helps bacteria withstand antibiotic stress. Gene expression-altering mutations may lead to antibiotic resistance when control is subpar (Palmer et al., 2018). Optimality simply refers to something that is "as good as possible". The ability of bacteria to withstand antibiotics and other stimuli is influenced by the regulation of gene expression (Storz and Hengge, 2010; Poole, 2012). The degree to which an organism's genes are expressed and its system for controlling their activity can change over time in response to environmental changes and disturbances that are common in its ecological niche. A specific stress may not influence the expression of some survival-related genes, and even if it does, the expression levels caused by the stress may not be tailored to maximize growth and survival under the stress (Bolenbach et al. 2009). A gene may not express optimally under stress if mutations that change its level of expression can increase its capacity to withstand stress. Genes' optimal levels of expression can fluctuate considerably when given an antibiotic.

Conclusion

Antibiotics stop vital cellular pathways, either killing germs or preventing their growth. Additionally, it enables the host's immune system to combat invasive bacteria as part of our natural defenses. No matter how a new antibiotic treatment works, resistance to it has been noted soon after it was initially used in a clinical setting. Antibiotics are overused in both humans and animals, which has encouraged the emergence of resistance. Bacterial pathogens adapt to survive and evade medications intended to kill them, just as they have against natural antibiotics produced by rival species in their environment over millions of years. This is the outcome of natural selection. The evolutionary, genetic, and physiological factors that underlie frequent deficits in response to antibiotic stress are not well understood. To find areas where antimicrobials are utilized excessively, comprehensive antimicrobial use datasets should be created. In addition, methods for assessing the risks associated with disease prevention, particularly the emergence and spread of bacteria resistant to antibiotics, must be developed.

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CHAPTER 7

SOUTHEASTERN ANATOLIA REGION AWASSI SHEEP

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Introduction

Livestock is an important sector of the general economy in many countries, especially in developed countries. In Turkey, although a significant proportion of the population in the agricultural sector makes a living from livestock, livestock is not able to make the desired contribution to the national economy. The most important indicator of this is that livestock in Turkey has a share of about 30% in the total value of agricultural production (Akman and Tatar, 2006).

The aim of animal production is to produce and increase animal products economically. It is reported that sheep and goat breeding is the first animal production area of humans (Zeder, 2008). Sheep have many advantages over other farm animals and can be raised in quite different environmental conditions. Sheep, by making use of the stubble fields and transforming the vineyard and garden residues that no animal can benefit from, into products, in areas where crop production is not possible, during the periods of the year when crop production is made, make a significant contribution to human nutrition by making use of only these areas with a very low cost in terms of feeding. Therefore, it is one of the most important farm animals that contribute to the country and world economy with the least expense and expenses (Sönmez, 1974).

Sheep and goats have an important place in the world due to their many different characteristics. The adaptability of these animals is very high and they can be turned into a variety of products for human consumption, such as meat, milk, leather and fleece. In addition to its economic importance, it is also of great importance due to its biological and physiological properties. “Domestication”, one of the turning points for humanity, started in the Neolithic age, approximately 11,000 years ago, in the lands known as the Fertile Crescent (Zeder, 2008). 4 species are mentioned in the domestication of farm animals. These are pigs, cattle, sheep and goats. Although the domestication dates are close to each other, it is estimated that the sheep and goat were the first domesticated species (Zeder, 2008).

Sheep Breeding in The World

In world trade, the importance of sheep meat, sheep milk and fleece is very important. These products cause significant economic mobility between

countries. Due to this mobility of countries, some countries have natural and climatic conditions, and some countries have distance in their marketing. In addition, the culture of the nations, heep care and feeding have been effective in the development of the desired sheep breed type and different yields. As a result, when talking about the right of sheep breeding in the world, it is not enough to explain only the existence of sheep, but the level and type of sheep breeding should also be mentioned (Ensminger et al., 1986). In terms of animal production in different countries of the world, sheep farming is at the forefront in terms of economic value, and in some countries it has a great importance as national income. One of the most typical examples of these countries is Australia. Sheep breeding is not only our nutritional needs, but also sheep leather and fleece, which are used as raw materials in textiles and have an important position in world trade, are also important (Sönmez, 1974).

Sheep Breeding in Turkey

Increasing production in sheep farming depends on increasing the yields obtained per animal head. The yield levels of animals are determined by two main factors. hese are determined as genetic structure and environment. 97% of sheep in Turkey are made up of domestic breeds with low genetic capacity. The existing sheep stock is largely devoid of scientific, modern practices, but rather is bred based on tradition. In particular, the nutrition of sheep is inadequate and unbalanced. The total amount of meat, milk and fleece obtained from sheep raised in Turkey will increase significantly with the improvement of the environment and genetic structure. This situation shows that the place of sheep breeding in the country's economy will continue to be important in the future (Kaymakçı and Sönmez, 1992).

The most important condition for successful sheep breeding is to determine the efficiency of the breeding direction, considering the economic opportunities and geographical conditions of the regions where sheep breeding will be carried out, and to select the appropriate sheep type and breed for this purpose (Akçapınar, 1994). Since the establishment of the Republic, breeding studies on our domestic sheep by pure or crossbreeding are limited. Karacabey merino, Tahirova sheep, Central Anatolian Merino and Ramlıç are the main breeds of developed culture sheep (Akçapınar, 1983; Kaymakçı and Taşkın 2001). The fact that cultured sheep breeds are at the level of 3% in our total

sheep stock indicates that sheep breeding is facing many serious problems among all farm animals. In our domestic sheep breeds, there are no breeder herds either in private farms or in public agricultural enterprises due to the improvement studies in the direction of pure breeding. Awassi breed, which is one of the domestic sheep breeds, is mostly grown in the Southeastern Anatolia Region (Akçapınar, 2000).

Classification of Sheep

In various regions of the world, many sheep breeds with different characteristics and purpose of breeding have emerged due to the effect of nature and climatic conditions with different wild breeds. By classifying these breeds according to their yield characteristics, it will facilitate their examination, recognition and identification, as well as making it easier to understand their subjects (Bilgemre, 1950). Various measurements are taken into account in the classification of sheep breeds. Among them, the most and most widely used ones are those that are classified according to tail structure, fleece and hair cover, and yield aspects (Sönmez. 1974).

Classification according to queue structures:

Short-tailed sheep (Northern European Sheep and Martian Sheep)

- Fat-tailed sheep breeds (Karaman, Dağlıç and Awassi)
- Lean long-tailed sheep breeds (Curly, Karayaka and Merino)
- Oylu fat sheep breeds (Kalmuk, Kazakh and Hisa

Classification according to fleece and hair cover:

- Hair sheep breeds (Cameroon, Senegal)
- Fur sheep breeds (Karagül)
- Coarse and mixed wool sheep breeds (Turkish domestic sheep)
- Fine and uniform wool sheep breeds (Merinos)
- Medium wool sheep breeds (Hampshire, Suffolk and Dorset)
- Long wool sheep breeds (Leicester and Lincoln)
- Hybrid wool sheep breeds (Targhee and Corriedale)

Classification according to yield aspects:

- Fleece-meat or meat-fleece sheep breeds (German meat fleece merino, columbia and targhee)

- Meat sheep breeds (Suffolk, Lincoln, South Down and Leicester)
- Wool sheep breeds (Merinos)
- Domestic Sheep Breeds (Kıvırcık, Dağlıç and Karaman)
- Fur sheep breeds (Karagül)

Awassi Sheep

Awassi Sheep is one of our fat-tailed sheep breeds in our country and is bred in the Southeastern Anatolia Region of Turkey, in the provinces of Şanlıurfa, Hatay and Gaziantep, which are close to the Syrian border (Özcan, 1997). The heads of Awassi sheep are seen as dirty yellow or black in color, their bodies are brown in color and they are our fat-tailed sheep. Queue structures; round, the ends take the form of an upward curved shape and form a cavity, and it is seen only as a piece with a lean tail in the part where the cavity is finished (Sönmez, 1974). The fleece weight ranges from 1.5 to 2.0 kg. Its fleeces are used in making carpets, blankets, rugs and beds.

The live weights of Awassi sheep are between 35-45 kg in sheep and 55-60 kg in rams. Meat fibers of ewes, especially of very old or milked queen ewes, are coarser and longer. Fat accumulation between meat fibers is less. For this reason, their meat is not of very good quality (Sönmez, 1974).

Pic 1. Awassi Sheep (Taşan, 2016).

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CHAPTER 8

Histophilus somni: AS an ETIOLOGIC AGENT of BOVINE RESPIRATORY DISEASE COMPLEX (BRDC)

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Introduction

The bovine respiratory disease complex (BRDC) is one of the leading causes of morbidity and mortality in cattle related to pneumonia. Many of the bacterial agents found in BRDC are associated with other predisposed environmental variables, physiological stresses, or concomitant illnesses. Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, and Mycoplasma bovis have been identified as the most common bacterial agents causing BRDC and inflicting the most catastrophic losses. These bacterial pathogens can cause illnesses on their own or in combination. Although numerous virulence and pathogenicity variables contribute to infection formation, the pneumonic lesions they induce may be identical.

One of the key bacterial pathogens in the multifactorial etiology of BRDC is Histophilus somni, a gram-negative bacterium. It is classified as a random disease of cattle around the world. H. somni is a bacterium that is widely isolated from instances of BRDC and other disease syndromes in domestic cattle (septicemia, thrombotic meningencephalitis, myocarditis, arthritis, abortion, and infertility). It is a commensal bacterium that can be an opportunistic pathogen, worsening viral and other bacterial diseases.

Etiology

H. somni shares 60% genetic homology with Haemophilus influenzae and 44% with Actinobacillus lignieresii (Rosendal and Boyd, 1986). H. agni and H. ovis protein profiles are remarkably similar to bovine H. somni isolates (Ogunnariwo et al., 1990). H. ovis isolates are often indistinguishable from H. somni isolates. H. somni, H. agni, and H. ovis were recently determined to be the same species, and it was proposed to assign them to a new Pasteurellaceae family genus entitled Histophilus somni (Angen et al., 2003). H. somni is the only member of the rearranged genus that is Gram-negative, nonmotile, and pleomorphic rod bacteria. For growth, it requires carbon dioxide (5-10%) and thiamine monophosphate. It lacks a polysaccharide capsule and does not express pili or flagella. It is typically a yellowish pigment. On blood agar, the majority of the isolates are -hemolytic. While the colonies are the size of a pin head after 24 hours of incubation, they reach a diameter of 1-1.5 mm after 48 hours.

The routine identification of H. somni by biochemical testing is problematic due to its low unique biochemical features. H. somni fermentation processes include urease, arginine dihydrolase/ornithine decarboxylase, alkaline phosphatase, nitrate, and glucose. Catalase and Voges-Proskauer reactions are both negative. They frequently have oxidase readings that are positive. Pyridoxine, flavin mononucleotide, riboflavin, and thiamine monophosphate can be added to the medium to increase the likelihood of bacterial growth (Inzana and Corbeil, 1987).

Epidemiology and Pathology

H. somni infects cattle and causes infertility, septicemia, abortion, myocarditis, arthritis, and meningoencephalitis. The chief symptoms of the illness are thrombosis, vasculitis, placentitis, thrombotic meningoencephalitis, and pneumonia (Gogolewski et al., 1988). It can be found asymptomatically as a commensal bacterium in the reproductive and respiratory mucosa and is typically transferred by direct touch and/or aerosol through contaminated respiratory and reproductive secretions, particularly in close-contact livestock facilities (Kwiecien and Little, 1992).

Even though nasal cultures are negative, bronchoalveolar lavage fluids may contain a substantial number of germs. H. somni survives better in the bronchoalveolar region than in the nasal mucosa, so coughing is a probable route of infection transmission in animals with negative nasal cultures (Gogolewski et al., 1989). Urine is a major source of the organism's propagation. Even one-month-old calves can become sick and nasal carriers of the pathogen without displaying any clinical indications. The cow is the calf's primary source of nourishment.

H. somni is occasionally seen in young calves with enzootic pneumonia (Harris and Janzen, 1989). The infection lasted 6-10 weeks (Gogolewski et al., 1989). H. somni may be isolated from bronchoalveolar lavage fluid rather than nasal swabs, indicating that H. somni may colonize the lower respiratory tract rather than the nasal mucosa.

Acute pulmonary lesions can manifest themselves in a variety of ways. Acute fibrinosuppurative bronchopneumonia is one of these. The histological alterations seen in this pneumonia are similar to acute pasteurellosis caused by P. multocida. As a result, based on the changes in the lung, it is impossible to

draw a firm judgment concerning the likely involvement of H. somni. The ensuing bilateral lobular lesions impair the cranial ventral sections of the lungs. The afflicted parenchyma congregates, and gray to red exudate is observed in the narrow airway lumen. Numerous tiny abscesses form in and efface the bronchioles in areas of consolidation (Tegtmeier et al., 1995). The lymph nodes in the mediastinum and tracheobronchium are mildly swollen. A small percentage of patients of acute histophilosis develop big lesions that resemble acute Mannheimiosis, as well as florid bronchopneumonia and pleuritis. Pleuritis caused by H. somni may manifest as a single lesion with varied quantities of exudate in the pleural sacs (Andrews et al., 1985).

Concurrent pneumonia lesions are distinguished by the production of inflated, homogenized nuclei with no discernible cytoplasmic borders, as well as a significant degeneration of leukocytes (Gogolewski et al., 1987). These could be early neutrophil extracellular traps produced in leukocytes after in vivo exposure to H. somni (Hellenbrand et al., 2013). These observed changes may differ from the nuclear flow characteristic of Mannheimiosis.

Vasculitis and thrombosis are common symptoms of experimental pneumonia (Clark, 2005). Histologically, the image is consistent with purulent bronchiolitis and bronchopneumonia. The alveolar gaps are filled with fibrin-rich exudate. Bronchiolar epithelium and muscle are affected by necrosis, resulting in bronchiolitis obliterans in survivors. Tegtmeier et al. (1999) found epithelial ulcers 4-7 hours after infection. Necrotic foci emerging from the distal airways are found throughout the affected (anteroventral) lung regions. Tegtmeier et al. (1995) discovered H. somni in sites of acute inflammation and necrosis. Bacterial antigen is seen in the bronchi/bronchioles and necrotic foci as extracellular aggregates. Bacterial antigen is also detectable intracellularly at the live edges surrounding such foci. The presence of H. somni in necrotic foci may explain some of H. somni 's ability to persist chronically in the lungs.

From the infected materials, P. multocida, M. haemolytica, M. bovis, and/or Trueperella pyogenes can also be isolated (Panciera and Confer, 2010). In calves with chronic pneumonia, M. bovis and H. somni are frequently discovered together (Booker et al., 2008). Pneumonias caused by M. bovis and H. somni have many characteristics, including early feeding media illnesses, concomitant arthritis, tenosynovitis, otitis, a tendency to become chronic

following failure antibiotic therapy, and anteroventral distribution. It is critical to ascertain whether necrotic foci comprise one or more components.

Interstitial pneumonia caused by septicemia/endotoxemia is a reasonably common pulmonary lesion of histophilosis. This pneumonia is distinguished by alveolar edema and bleeding (O'Toole et al., 2009). Vascular alterations caused by left heart failure may potentially play a role in this circumstance.

Protection-Control and Vaccination Strategies

Metaphylactic treatment, vaccination against H. somni, and vaccination against other agents predisposing to Bovine Respiratory Disease (BRD) complex are important for preventing and controlling infection of H. somni in cattle (O'Toole and Sandgeroth, 2016). Although the use of antibiotics in the prevention of bovine pneumonia is critical, it is not without challenges. Because bacteria acquire antibiotic resistance if the suitable antibiotic is not picked or comparable antibiotics are administered repeatedly (Step et al., 2007).

If a disease epidemic is expected, metaphylactic treatment is administered in addition to quick individual treatment of infected animals. The administration of long-acting oxytetracycline for metaphylaxis at the outset of the disease had little effect on disease mortality. This method reduces losses caused by BRD. Tilmicosin, tulathromycin, ceftiofur, enrofloxacin, trimethoprim-sulfadoxine, and florfenicol are used to inhibit the establishment of the BRD illness complex (with or without nonsteroidal anti-inflammatories such as fluxin meglumine) (Yaeger, 2007; Aytekin et al., 2010).

To protect calves in the risk category from respiratory system infection, some precautions should be taken. A sufficient amount of colostrum should be given promptly after birth. In the first 6 hours after birth, calves should be given 4 liters of colostrum. After 8 hours, the calves should be given a second meal of 2-3 liters. Colostrum, whether fresh or refrigerated, contains the greatest combination of antibodies (immunoglobulins), immune cells, other key immunological components, vitamins, and minerals for the calf's immune system.

The calves' resting locations should be away from dampness and wetness and should not be exposed to air currents. Bedding should be clean, dry, and comfortable. Calves should not be allowed to interact with other calves. Natural ventilation should be allowed at the rear and front of the calf hutches.

Ventilation should be designed to reduce the amount of germs in the air, hence lowering the risk of illness (Boyles et al., 2007). Increasing the number of animals in a limited space raises the likelihood of disease spreading in calves. Calves will learn to compete once they are separated from their mothers. This can significantly raise stress and, as a result, the occurrence of BRD. Weaned calves should be kept in small groups since respiratory illnesses account for 46.5% of losses in weaned female calves (Duff and Galyean, 2007).

Farmers should be able to identify respiratory system infections in their livestock at an early stage. Such animals should be separated from healthy calves and removed from community breeding grounds. Coughing and runny nose spread respiratory illnesses through the air, therefore an affected cow can readily spread the disease to healthy calves in the same barn or calves in open areas.

There are several commercial vaccines available on the market for respiratory issues. There have been insufficient studies to demonstrate the effectiveness of vaccinations against bacterial pneumonia in newborn calves. Cattle vaccination strategies against disease pathogens should be created.

The efficacy and economic utility of vaccinations against respiratory system infections in cattle, particularly calves, are still unknown. Despite numerous investigations on the matter, no comprehensive scientific consensus on the vaccine has been obtained. Because of the nature of bovine respiratory illnesses, evaluating vaccine effectiveness and interpreting field data is particularly difficult.

Another technique for combating histophilosis is to provide vaccinations against different BRD complex agents. Vaccination of beef cattle against infectious cofactors (Mannheimia haemolytica, Pasteurella multocida, bovine respiratory syncytial virus, and bovine herpesvirus 1) is advantageous in the event of a probable H. somni infection (Wollums, 2015).

Commercial vaccines are available to prevent and control respiratory illnesses caused by H. somni in cattle. While immunization may lower the frequency of respiratory disease in at-risk animals, it may not give long-term protection. These vaccinations' usefulness in preventing meningoencephalitis, polyarthritis, reproductive, or heart disease in cattle is unknown.

Vaccination has been shown to be the most cost-effective intervention for protecting animals against infectious illnesses and increasing cattle

productivity. Vaccines for H. somni -associated disease are currently ineffective in preventing disease (Guzmán-Brambila et al., 2012). To prevent pneumonia, commercial H. somni vaccines typically contain dead cells or outer membrane proteins. Reverse vaccination, in conjunction with modern bioinformatics and next-generation whole genome sequencing techniques, has paved the way for the discovery of gene reservoirs encoding entire surface-exposed proteins that are more likely to be possible antigenic vaccine candidates (Sette and Rappuoli, 2010). Surface-exposed proteins (e.g., outer membrane proteins) are ideal vaccine candidates because they can elicit an immune response in response to natural infection. When compared to standard vaccination procedures, reverse vaccination has the advantage of identifying many target gene products that can generate the required immunogenicity in a shorter time.

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CHAPTER 9

EVALUATION of PREGNANCY TOXEMIA in SHEEP in TERMS of PROGNOSIS GENETICS and FLOCK MANAGEMENT

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Introduction

Metabolism is the basis of life in living things. The nutrients in the ration undergo metabolic processes and change and energy is obtained (Brosnan, 2005). Healthy animals have to regulate their individual reactions, acid-base balance and biochemical events in order to maintain their physiological events normally (Wu, 2020).

With the deterioration of the physiological state, abnormal findings occur in animals. Many factors such as malnutrition, malnutrition and environmental conditions play a role in the deterioration of this physiology. As a result of this deterioration, many diseases, especially metabolic diseases, occur (Dwyer et al., 2016).

Metabolic disorder called pregnancy toxemia is the most common disease in small ruminates (Brozos et al., 2011).

Pregnancy toxemia usually occurs close to birth and is a disease characterized by hypoglycemia, hyperketonemia and hypoinsulinemia (Bergman, 1973). The disease occurs as a result of not meeting the energy need of the growing fetus in the uterus of the sheep due to reasons such as insufficient feeding and malnutrition (Pethick and Lindsay, 1982). The disease, which is a common metabolic disorder in sheep during late pregnancy and early lactation, often occurs in the last 4-6 weeks of pregnancy and causes significant financial losses due to the high mortality rate in pregnant sheep. It is more common in ewes that are pregnant with two or more fetuses, in very fat ewes, and also in ewes with a single large fetus. As pregnancy progresses, the energy needs of the fetus increase. At the same time, the rumen capacity of the female sheep is reduced because the developing fetus takes up more space in the uterus and leaves less room for the rumen (Bickhardt et al., 1989; Drackley et al., 1989). The metabolic condition known as pregnancy toxemia, which is exhibited in sheep at the conclusion of pregnancy, is linked to nutrition and is brought on by a change in the metabolism of fatty acids and carbohydrates. The biggest risk factor for the condition brought on by insufficient energy in the food or a decrease in rumen capacity owing to offspring growth in sheep during late pregnancy is nutritional deficit.

The disease affects between 5% and 20% of animals, and untreated animals can die at rates as high as 80%. Furthermore, despite aggressive treatment plans, 40% of sheep still perish (Rook, 2000).

The disease can cause abortion or stillbirth, as well as cause toxemia and death in sheep due to the death of the fetus in the uterus of the sheep.(Kemp et al.,2013)

The susceptibility of ewes to pregnancy toxemia is also related to the fact that the total weight of pregnant ewes with triplet fetuses can be as high as 27% of their body weight during mating (Alonso et al., 1997).

Pregnancy Toxemia Pathogenesis

Similar to other ruminants, sheep mostly meet their glucose needs through gluconeogenesis rather than through the direct absorption of carbohydrates. Dietary carbohydrates are initially digested in the rumeno-reticulum to produce acetic acid, propionic acid, and butyric acid. In the liver, propionate is converted to glucose. Long-chain fatty acids are produced during the metabolism of acetate and are then deposited in bodily tissues as lipids. The majority of butyrate is transformed into hydroxy-butyrate (BOHB) and absorbed. Additionally crucial for gluconeogenesis are dietary protein and certain amino acids produced during protein catabolism (Duehlmeier, 2013).

Because sheep cannot alter the amount of glucose that their fetuses need, when the ration is insufficient to meet those needs, the sheep deplete their body's reserves of glucose. Lipids are mobilized under these circumstances. Acetyl coenzyme A (acetyl CoA), a source of energy, is created when the free fatty acids generated are transported to the liver and oxidized via the tricarboxylic acid cycle. In order for this metabolic pathway to function, propionate's oxaloacetate must be continuously produced. The diet's low carbohydrate content prevents this condition from occurring, which results in incomplete oxidation and the generation of aceto-acetyl CoA. Acetoacetate and BOHB are produced as a result of the hydrogenation of aceto-acetyl CoA, while acetone is produced as a byproduct of decarboxylation (Mitruka and Rawnsley, 1977). These ketones can be digested, but because of their excessive synthesis and hepatic failure brought on by the buildup of triglycerides linked to lipolysis, they accumulate in proportion to the dietary carbohydrate shortfall (Fraser et al., 1997).

BOHB and acetoacetate are strong acids. In addition, prolonged urinary excretion of ketone bodies reduces the plasma alkaline reserve, resulting in loss of sodium and potassium ions. The resulting keto-acidosis causes dyspnea,

exacerbates hypoglycemic central nervous system depression, and becomes irreversible. Dehydration and uremia also occur here. Fetal death and uterine autolysis usually occur. It combines with toxemia and septic metritis in the blood (Sargison, 1995). In addition, prolonged hypoglycemia leads to hyperactivity of the adrenal glands, increased cortisol secretion, antagonism of insulin action, and effective inhibition of glucose utilization (Herdt, 1988).

Results

Hypoglycemia and ketosis are frequent side effects of pregnancy toxemia (Rook, 2000). Dehydration and issues with electrolyte metabolism may result from the disease's elevated levels of ketone bodies (Kolb et al., 2021). According to Lorenz et al. (2011) and Vasava et al. (2016), sick sheep exhibit a variety of symptoms including decreased appetite, depression, shaky walk, rough skin, blindness, muscle tremors, teeth grinding, lying on the ground, convulsions, and ultimately coma and death.

Although clinical necropsy findings of deceased sheep are not specific, carcasses are usually emaciated and there is more than one fetus in the uterus. The liver is swollen, pale, orange, brittle. This is indicative of severe hepatic lipidosis. The adrenal glands may be hypertrophic, pale, or hyperemic. In the brain, lesions consistent with hypoglycemic encephalopathy consisting of hypertrophy, proliferation, and cerebrocortical neuronal necrosis and vacuolation of cerebral or cerebellar white matter have been described (Jeffrey, and Higgins, 1992).

Genetic Factors

Pregnancy toxemia may be influenced by genetic variables, which are more common in sheep than goats (Moallem, 2012). For instance, extensively bred sheep are more likely to experience a negative energy balance during pregnancy, which can lead to problems with lipid metabolism and pregnancy toxemia since the mother and baby are competing for the same nutrients. These findings imply that the development of the multiparous condition may be significantly influenced by genetic variables (Han, 2017). According to Zheng (2019), the FecB gene is a significant contributor to sheep productivity and a prospective candidate gene for genetic regulation of sheep reproductive performance. It has been demonstrated that sheep with many pregnancies are

more sensitive to pregnancy toxemia, as are sheep with the type BB (mutant pure) gene of FecB (Wang et al., 2015).

Prognosis and Death Rates

A lengthy course and significant mortality characterize pregnancy toxemia. According to Cal-Pereyra (2015), current treatments are not particularly effective. Toxemia in late pregnancy cannot be effectively treated. The rest of the flock should be treated while a sheep that is already unconscious should be put to sleep (Duehlmeier, 2013). The mortality rate can reach 80% and the herd's morbidity rate can exceed 20% during severe pregnancy toxemia epidemics (Ismail et al., 2008).

In most cases, treatment is not economical. Because when the prognosis is poor, euthanasia should be considered to avoid further suffering. In a study conducted on 53 sheep with pregnancy toxemia, only 33% survived despite intensive treatment (Sargison, 1995).

In very critical cases, cesarean section may be recommended, but treatment costs should also be considered. Even in this situation, the prognosis may be poor. Because it may encounter dead puppies or autolyzed fetuses in the uterus, in which case the chance of survival of the sheep decreases. Even in cases where cesarean section is performed, the mortality rate is around 40% (Edmondson, 2012).

Effect of Flock Management on Disease Prevention

First of all, the pregnancies of the sheep should be determined and even after it is determined that they are single, twin or triplet pregnancies, group feeding according to the gestational month and the number of fetuses in the uterus can significantly prevent the occurrence of the disease (Browning and Correa, 2008; LeValley, 2010).

In herd management practices, it is also important to prepare the appropriate rations to keep the sheep away from stress, without allowing them to become excessively fat or weakened (Qing, 2021).

Physiological and biochemical indicators of sheep should be monitored during pregnancy. In addition, training can be given to breeders on how to understand the symptoms of pregnancy toxemia at an early stage (Silva et al., 2022).

Synthetic vitamin B12 compounds can enhance the energy balance and metabolism of pregnant sheep when provided medically on a regular basis. These substances include 10% crude glycerol or intravenous butyl-phosphate (6 mL/sheep) (Barimanloo, 2021). Additionally, recombinant bovine somatotropin 160 mg injections are recognized to have the capacity to prevent pregnant toxemia in sheep (Arajo, 2018).

Conclusion

Sheep pregnancy toxemia is a distinct and complicated condition. Some aspects of the disease's pathophysiology are yet unknown. Due to this, it is an extremely lethal condition, and treatment rarely results in the patient recovering their health. Since there is now no proven approach for disease prevention or treatment, molecular studies of disease regulatory pathways are required. Preventive medicine, animal breeding, herd management, and other areas need to be improved in addition to the diagnosis and treatment of pregnant toxemia. To promote healthy reproduction and reduce financial losses in the sheep farming business, more research is required to understand the mechanism of pregnancy toxemia in sheep.

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CHAPTER 10

NON-APOPTOTIC REGULATED CELL DEATH

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Introduction

Karl Vogt's observation of dying frog cells in 1842 marked the beginning of the scientific study of controlled cell death. When John Kerr, Andrew Wyllie, and Alastair Currie invented the term "apoptosis" in 1972, research into controlled cell death continued to grow. Apoptosis was described by Kerr et al. as planned cell death that is distinguished from necrosis by morphological changes. Tang et al., 2019; Kerr et al., 1972. Cell homeostasis, tissue remodeling, and tumor formation are just a few pathological and physiological processes that are mediated by apoptosis and other forms of controlled cell death (Singh et al., 2019). Numerous types of cell death can happen in response to various stimuli, most notably oxidative stress. According to Berghe et al. (2014) and Linkermann et al. (2014), diseases including cancer, dementia, autoimmune disorders, and infectious diseases can all be attributed to a loss of control over the forms of cell death.

Cell Death

The morphological and structural characteristics of tissues and cells are used to classify early cell death techniques. In order to categorize cell death in prenatal tissues exposed to different embryotoxic chemicals as categories I, II, and III, Schweichel and Merker developed a morphological marker in 1973 (Schweichel & Merker, 1973; Tang et al., 2019). Apoptosis, or type I cell death, is characterized by a number of characteristics, including DNA fragmentation (karyorrhexis), chromatin condensation, cell shrinkage (pyknosis), the development of cell surface protrusions, the formation of apoptotic bodies, and cell surface protrusions. Type II cell death is also known as autophagy-induced cell death, which is characterized by the growth of cytosolic components and organelles with enormous autophagic vacuoles. Although autophagy undeniably supports cell survival in the majority of situations, it can occasionally result in autophagy-induced cell death (Kroemer et al., 2010; Liu et al., 2013; Nassour et al., 2019). Type III cell death, also known as necrosis, can be distinguished by the incidence of membrane breakdown and organelle growth, or "oncosis." It is well known that the necrosis is an example of uncontrolled cellular death. Regulated necrosis, such as necroptosis, on the other hand, takes place under controlled conditions (Berghe et al., 2014; Linkermann et al., 2014; Weinlich et al., 2017). It is believed that cell death is

a natural byproduct of cellular life. Cell death is a physiologically unpredictable but regulated process connected to disease that frequently happens in reaction to oxidative stress, which activates signaling pathways in numerous ways and can cause disease (Hasani et al., 2022). Accidental and controlled cell death fall under two different categories (Gao et al., 2022). Accidental cell death is a disease process that is biologically uncontrolled. On the other hand, controlled cell death involves well calibrated and organized signaling cascades, which are recognized as effective molecular processes (Tang et al., 2019). Programmed cell death is the term used to describe the physiological type of controlled cell death, which is fully dependent on physiological processes (Galluzzi et al., 2018). Regulated cell death is essential for preserving homeostasis and preventing the onset of illness. Based on many morphological, biochemical, immune, and genetic traits, it is categorized into apoptotic and non-apoptotic categories. Autophagy, ferroptosis, pyroptosis, and necroptosis are several types of non-apoptotic controlled cell death (Koren & Fuchs, 2021; Gao et al., 2022; Peng et al., 2022). There is a need to accurately differentiate between the other types of cell death that take place in tissue and to show that non-apoptotic cell death signaling pathways are now present in tissues because oxidative stress can cause several types of cell death. Additionally, in light of this knowledge, it is necessary to clarify the mechanisms underlying each cell death subtype in a variety of disorders (Hasani et al., 2022). It may be possible to find novel treatment targets to lessen abnormal cell death and excessive cell proliferation by identifying these deadly pathways and the effects of their interaction (Cui et al., 2021).

Autophagy

Cells undergo a closely controlled process known as autophagy, which literally translates to "self-eating," in which their cytosolic components are broken down and recycled inside lysosomes, which can result in cell death (Galluzzi et al., 2018; Schwartz, 2021a). Autophagy controls lipid metabolism and balance in eukaryotic cells. Autophagic membrane structures are created when a variety of stressors are present and activated in order to engulf and destroy intracellular structures, such as damaged organelles, unfolded proteins, and pathogens (Morishita & Mizushima, 2019; Xia et al., 2021; Gao et al., 2022). Recently, a lot of research has been done on the process of autophagy,

which involves the lysosome in the destruction of large molecules. Through the selective destruction of both positive and negative apoptosis regulators, autophagy can govern cell death, notably apoptosis (Miller et al., 2020). This controlled cell death doesn't involve chromatin condensation or phagocytes like apoptosis does. Numerous autophagic vesicles containing cytoplasmic material for lysosome breakdown amass during autophagic cell death. In contrast to apoptotic cells, it disrupts late cytoskeleton and early organelles (Stringer et al., 2023). Maintaining cell function and homeostasis depends on the biological process known as autophagy, which can destroy harmful proteins or organelles through lysosomal fusion (Xiang et al., 2020). According to recent research, apoptosis or other controlled cell death processes are not necessary for autophagy-induced cell death to take place. Apoptosis and autophagy are highly interwoven during developmentally regulated cell death, according to solid evidence (Stringer et al., 2023). The double-membrane structure known as an autophagosome, which mediates autophagy, encloses and organizes proteins and organelles (Li et al., 2021). To disassemble internal components, these autophagosomes then combine with liposomes. When the autophagic structures are united, acidic hydrolases in the lysosome can destroy them, and the residual components are then released into the cytoplasm for cellular recycling. Genetically engineered cell death has been demonstrated to require some autophagic processes (Kroemer & Levine, 2008; Schwartz, 2021b). Genes associated with autophagy regulate the autophagic process. The regulation of autophagy involves the genes Beclin-1, ULK1, LC3, p62, FoxO, and other autophagy-related genes (Yoshida, 2017; Peng et al., 2022).

Proptosis

A new variety of planned cell necrosis is called pyroptosis. Pyroptosis is recognized as lytic inflammatory cell death, which is mediated by inflammatory caspases (caspase-1/4/5), in contrast to immune-silencing apoptosis. Cellular swelling and membrane rupture characterize the morphology of pyroptosis (Man et al., 2017). The initial perception of pyroptosis is that it is caspase-1-dependent planned cell death (Cui et al., 2021). Caspase-1 and the release of proinflammatory cytokines cause a kind of cell death known as pyroptosis, which results in karyokinesis, cell edema, plasma membrane rupture, and cell death (Hasani et al., 2022). Apoptosis and pyroptosis differ morphologically.

In vitro, DNA fragmentation does not occur during pyroptosis; instead, cells swell and nuclear condensation results in the creation of sizable bubbles in the plasma membrane that will eventually burst (Chen et al., 2016; Rathkey et al., 2018).

Numerous cytokines and danger signaling molecules are generated during pyroptosis, stimulating the immune system and causing an inflammatory reaction. Non-infectious triggers like microbial infection and host factors created during myocardial infarction can trigger pyroptosis. Pyroptosis, a lytic proinflammatory process that results in the release of inflammatory intracellular contents and the generation of activated inflammatory cytokines, happens when the plasma membrane ruptures quickly. Because they trigger the proinflammatory cytokines IL-1 and IL-18, inflammatory caspases are essential in pyroptosis (Cui et al., 2021). The cell death executor protein gasdermin is necessary for pyroptosis. By creating membrane holes and unleashing the inflammatory response, inflammasomes can activate gasdermin-D to guide pyroptosis (Jorgensen & Edward, 2015; Liu et al., 2016). Depending on which caspase is involved in the activation, inflammasomes are classified as conventional or unconventional. Inflammasomes, both common and uncommon, can directly mediate gasdermin-D cleavage. They are pyroptosis executors that control whether pyroptotic cell death takes place by cleaving the pore-forming protein gasdermin-D in response to inflammatory caspases (Shi et al., 2015; Amarante-Mendes et al., 2018). Caspase-1, which has a high affinity for gasdermin-D and proforms of IL-1 and IL-18, is activated by conventional inflammasome activation. Caspase-4/5/11 are activated by non-traditional inflammasomes to cause pyroptosis. There are currently five common inflammatory sensors: Pirin, NLRP1, NLRP3, NLRC4, and NOD-like receptor (NLR) family pyrin domain-containing 1 (NLRP1). (2015) Kaneganti et al. After sterile and infectious attacks, the innate immune system serves as the first line of defense for the host, and inflammasomes play a crucial part in this system. In response to varied stimuli, the sensor molecule starts the building of this macromolecular complex. The development of inflammasome multiprotein complexes to process and activate caspase-1 occurs according to the established mechanism when pathogen-associated or dying cells produce stimuli (Cui et al., 2021).

Ferroptosis

Typical inflammasome activation activates caspase-1, which has a strong affinity for gasdermin-D and proforms of IL-1 and IL-18. Pyroptosis is brought on by non-traditional inflammasomes that activate caspase-4/5/11. Pirin, NLRP1, NLRP3, NLRC4, and NOD-like receptor (NLR) family pyrin domain-containing 1 (NLRP1) are the current five widely used inflammatory sensors. (2015) Kaneganti and others. The host's innate immune system, which is the first line of defense after sterile and infectious attacks, is heavily dependent on inflammasomes. The sensor molecule initiates the formation of this macromolecular complex in response to various inputs. When pathogen-associated or dying cells emit stimuli, the creation of inflammasome multiprotein complexes to process and activate caspase-1 takes place in accordance with the documented mechanism (Cui et al., 2021). Glutathione (GSH) levels drop during ferroptosis, glutathione peroxidase-4 (GPX4) is inhibited, and iron buildup and lipid peroxidation rise. This causes the outer mitochondrial membrane to tear, the mitochondrial crista to shrink or disappear, and the mitochondrial membrane to condense (Bartos & Sikora, 2023). Transferrin specifically induces ferroptosis by mediating iron uptake through the transferrin receptor (TFRC). By destroying intracellular iron-storing proteins and the iron exporting soluble carrier family member 1 (SLC40A1), autophagy brought on by increasing iron accumulation initiates and increases ferroptosis (Geng et al., 2018; Brown et al., 2019; Wang et al., 2020).

Accumulated iron and lipid peroxidation lead to ferroptosis. According to Dixon et al. (2012) and Friedmann et al. (2014), the morphological process is dysmorphic small mitochondria with reduced cristae and no typical necrotic or apoptotic features like decreased cell volume, membrane damage, increased mitochondrial membrane density, release of cytochrome C from mitochondria, caspase activation, and chromatin fragmentation.

GSH depletion and lipid peroxidation caused by iron are the key biological characteristics of ferroptosis. One of the two primary antioxidant systems is inhibited by the classical pathway, which then causes ferroptosis. The disulfide-linked heterodimer System Xc-, one of them, takes up enough cysteine (the extracellular oxidized form of cysteine) to transport intracellular glutamate. GSH synthesis necessitates cysteine. According to Hassannia et al. (2019), the tripeptide antioxidant GSH works as a crucial cofactor of GPX4 to

detoxify lipid hydroperoxides. GPX4, a vital ferroptosis regulator and phospholipid hydroperoxidase, is another antioxidant system. By promoting the GSH-dependent reduction of lipid peroxides, GPX4 can directly lower the formation of phospholipid hydroperoxide. Ferroptosis may be brought on by the direct or indirect targeting of GPX4. Depletion of intracellular GSH and direct inhibition or depletion of GPX4 both indirectly inactivate GPX4 (Hassannia et al., 2019; Yang et al., 2014). Unusual ferroptosis is defined as ferroptosis that is brought on by an elevated intracellular pool of labile iron as a result of an overactive heme oxygenase-1 (HO-1) enzyme. When the GSH or the GSH-dependent antioxidant enzyme GPX4 production is suppressed both in vivo and in vitro, ferroptosis is brought on. Antioxidant enzyme GPX4 is critical in preventing excessive lipid peroxidation in a variety of cell types, including neurons (Galluzzi et al., 2018; Hassannia et al., 2018). For ferroptosis, glutamine and glutamate play key regulating roles. Human tissues and serum typically contain significant quantities of glutamine. The Krebs cycle can be powered by glutaminolysis, whereas some synthesis processes (like lipid synthesis) are inhibited. Ferroptosis brought on by cysteine deficiency necessitates glutaminolysis (Gao et al., 2015; Gao et al., 2019). High levels of extracellular glutamate can impair the Xc- system's performance and cause cell death. This is due to the imbalance in intracellular cysteine caused by excessive glutamate buildup. Intracellular cystine deprivation cannot cause reactive oxygen species (ROS) buildup, lipid peroxidation, or ferroptosis in the presence of glutamine insufficiency or suppression of glutamine synthesis. Ferroptosis is also closely related to lipid metabolism. Polyunsaturated fatty acid (PUFA) peroxidation is susceptible to lipid peroxidation in the development of ferroptosis. Ferroptosis is suppressed by providing PUFAs to cells in order to stop this peroxidation. The amount of lipid peroxidation and the distribution of PUFAs control the severity of ferroptosis. According to Yang and Stockwell (2016) and Yang et al (2016), iron is one of the key ingredients for lipid peroxide buildup and the ferroptosis process. By excessively creating ROS through the Fenton reaction to encourage lipid peroxidation in ferroptosis, iron can damage lipids and DNA. In addition, non-heme iron-containing enzymes such lipoxygenases that increase lipid peroxidation can be activated by iron (Tang et al., 2021). Ferroptosis may therefore be influenced by proteins involved in the entrance, excretion, storage, and circulation of iron. These

proteins are nuclear receptor coactivator 4 (NCOA4), transferrin, transferrin receptor, iron-sensitive element binding protein 2, transferrin, and divalent metal transporter 1 (Cui et al., 2021). Increased concentrations of iron ions and metabolic problems are caused by abnormal or defective expression of these proteins. According to Yang et al. (2016), excessive iron buildup in tissues results in the production of ROS and lipid peroxide and causes ferroptosis. In vitro and in vivo, iron chelators like Deferiprone Deferasirox stop the development of ferroptosis. Cellular labile iron typically increases when ferroptosis is induced. In addition, providing exogenous iron sources makes cells more susceptible to substances that cause ferroptosis (Dixon et al., 2012; Hou et al., 2016; Cui et al., 2021). Ferroptosis can potentially be impacted by autophagy since it alters iron metabolism. Ferroptosis is seen as an autophagic process of cell death, and ferrotinophagy, which affects ferroptosis, is the selective autophagic conversion of ferritin. As an autophagy transporter receptor that binds and targets ferritin for lysosomal degradation, nuclear receptor coactivator-4 (NCOA4) is a key modulator of ferritinophagy, according to biochemical investigations (Cui et al., 2021; Gao et al., 2022).

Necroptosis

A brand-new form of planned necrosis called necroptosis includes the activation of cell signaling pathways and takes place in response to particular stimuli. When it comes to morphology, necroptosis exhibits necrosis-related symptoms such as swelling of organelles and cells, rupture of the plasma membrane and release of intracellular components, and the absence of the pyknosis associated with apoptosis during chromatin condensation (Cui et al., 2021). Necroptosis is a type of planned necrosis that exhibits morphological characteristics of necrosis. It was first discovered in 1996 in bovine pox virus-infected pig kidney cells that expressed the Caspase-1 and Caspase-8 inhibitor cytokine response modifier A (CrmA) (Ray & Pickup, 1996; Pasparakis & Vandenabeele, 2015). It was established that L-M cells were susceptible to tumor necrosis factor-alpha (TNF-alpha)-induced necrotic cell death. Further research revealed that Caspase-8 has a detrimental regulatory effect on this specific type of cell death (Tang et al., 2019). ATP levels fall as a result of necroptosis. Cell death is brought on by the activation of genes in the necroptotic pathway, such as receptor-interacting serine/threonine kinase-1

(RIPK1), RIPK3, and mixed lineage kinase domain-like pseudokinase (MLKL) (Weinlich et al., 2017; Nassour et al., 2019; Singh et al., 2019; Hasani et al., 2022). The activation of death receptors, toll-like receptors (TLR3 and TLR4), nucleic acid sensors, interferons, and adhesion receptors are only a few of the stimuli that can cause necroptosis (Tang et al., 2019; Cui et al., 2021). Numerous factors, including as cytokines, viral infection, toxins, or damage-associated molecular patterns (DAMPs), can trigger necroptosis. The cytokine TNF- is currently the stimulus that is most carefully and thoroughly investigated, and it is also likely the most significant initiator of necroptosis. The activation of RIPK1 kinase activity in TNF-stimulated cells is critical in determining whether a cell will undergo necroptosis or apoptosis. As an executor, phosphorylated MLKL (pMLKL) can penetrate organelles and the plasma membrane, rupturing the membrane and releasing their contents as DAMP (Duan et al., 2020). In situations where apoptosis is insufficient, necroptosis is triggered. TNF- can cause apoptosis or necroptosis in cells, among other responses. Nuclear factor-B (NF-B) and mitogen-activated protein kinase (MAPK) signaling are primarily triggered by necroptosis (Cui et al., 2021). The identification of RIPK1 as a regulator of Fas ligand-induced necroptosis in T cells launched the field of molecular necroptosis research in 2000 (Holler et al., 2000). The word "necroptosis" was first used when the pharmacological RIPK1 inhibitor necrostatin-1 was discovered. The downstream mediator of RIPK1 in death receptor-induced necroptosis, RIPK3, was later discovered. Our knowledge of the molecular process of necroptosis has been greatly enhanced by the later identification of MLKL as a necroptosis effector 2019 (Tang et al.). RIPK1, RIPK3, and MLKL are the three main participants in necroptosis-related signaling (Cui et al., 2021). The necrosome is a complex made up of RIPK1 and RIPK3 that takes role in necroptosis activation. Cell death is dependent on MLKL involvement in plasma membrane rupture, and MLKL is an effector of necroptosis (He et al., 2009; Sun et al., 2012). RIPK3 that has been activated phosphorylates MLKL and draws it to the plasma membrane to form a protein complex. First, by directly encouraging pore formation, these oligomers may contribute to the instability of the plasma membrane. Additionally, they might act as a platform for the inadvertent deregulation of Ca+2 or Na+ ion channels (Cui et al., 2021).

By modulating the production of inflammatory cytokines and inhibiting the release of DAMPs, which are involved in the onset of many diseases, necroptosis can significantly enhance inflammation (Duan et al., 2020). A multifunctional signaling kinase known as RIPK1 is involved in cell stress, immunity, cell survival, and cell death. 2019 (Tang et al.). The RIPK3 protein is distinguished by an N-terminal kinase domain where it phosphorylates both itself and other substrates and a C-terminal region with a receptor-interacting protein homotypic interaction motif (RHIM) where it binds to other proteins to form an oligomer. The pseudokinase MLKL, which is necessary for membrane permeabilization during necroptosis, is phosphorylated and activated by RIPK3 after it has been activated (Morgon & Kim, 2022).

Conclusion

The various impacts should be able to be distinguished with a better understanding of the fundamental workings and functions of non-apoptotic autophagy, pyroptosis, ferroptosis, and necroptosis in controlled and programmed cell death. Both in the presence and absence of apoptosis, non-apoptotic forms of programmed cell death can significantly contribute to physiological cell death. Therefore, understanding how cells kill themselves in a caspase-independent manner goes beyond mere academic curiosity and may enable us to therapeutically manage this in a variety of processes, such as cancer and autoimmune illness. It is necessary to establish whether tissues contain signaling pathways for non-apoptotic cell death. The mechanism of each cell death subtype in various diseases must also be revealed in light of this information. It may be possible to find novel treatment targets to lessen pathological cell loss and excessive cell proliferation by having a thorough grasp of these deadly subroutines and the effects of their interaction. The study of these pathways' roles in numerous physiological and pathological processes brings forth the fundamental significance of this mechanism.

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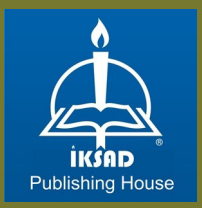

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