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REDUCING THE IMPACT OF LIVESTOCK PRODUCTIONS THROUGH INNOVATIVE FEEDING MANAGEMENT

Master´s thesis

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Declaration

I hereby declare that I have authored this master's thesis carrying the name "Reducing the Impact of Livestock Production through Innovative Feeding Management" independently under the guidance of my supervisor. Furthermore, I confirm that I have used only professional literature and other information sources that have been indicated in the thesis and listed in the bibliography at the end of the thesis. As the author of the master's thesis, I further state that I have not infringed the copyrights of third parties in connection with its creation. The data has not been published anywhere as there is a commercial agreement between the University of Pisa and a private company.

In Prague on 19-04-2024 _____

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Reducing the Impact of Livestock Productions through Innovative Feeding Management

Summary:

This study aimed to conduct experiments in a laboratory setting to determine the moderating effects of various combinations of tannin and essential oils (EOs). It has been discovered that the characteristics of tannins and EOs can reduce ammonia and methane levels in the rumen. This is because of their distinct rumen route mechanisms of action. In total, 32 treatments were used in the investigation. Eight additions were used alone (10 mg of EO or 20 mg of tannins/g diet) to determine their basal efficiency, and twenty-four combinations of 20 mg of tannins/g + 10-15 mg of EO were used. The C, Q, and C/Q groups of mixes with EO blends were identified using the tannins of quebracho (Q) and chestnut (C). Citrus peel, carvacrol, thymol, eugenol, a-pinene, bornyl acetate, oregano, limonene, and clove essential oil were used in the formulation of the supplements. For a total of six runs, the supplements were given to a control diet that was likewise left unaltered to serve as a baseline for comparisons with supplemented treatments. The findings demonstrated that the tannin extracts, which suppressed ammonia by as much as 31% and methane output by as much as 15%, had the most moderating effects. The supplements based on tannins produced the largest decreases. However, as shown by decreases in total VFA and in vitro organic matter digestibility, this happened concurrently with the substrate's feeding value. There were only minor impacts on the pH of the rumen, the number of protozoa, and the relative amounts of each volatile fatty acid (VFA). The most effective combinations of C and Q groups were discovered to be six in total. However, more research is required to comprehend the mechanisms of action and the compound-to-compound synergistic effects.

Highlights

- Combinations of tannins and chemicals found in essential oils were screened in vitro.
- It was discovered that certain combinations could reduce ammonia generation and methane yield by up to 31% and 15%, respectively.
- The chemicals found in essential oils strengthened the tannins' moderating effects.

Keywords: Thyme, rumen, oregano, and supplement

Content

1 Introduction

For centuries, the animal agriculture sector has played a critical role in the global agricultural economy by producing essential commodities like beef, milk, and wool and by making a substantial contribution to economic growth and livelihoods (Clutton-Brock, 2017). But in recent years, worries about the industry's environmental impact have grown because of its exponential rise in response to population increase and shifting dietary habits. Conventional cattle feeding methods contribute to greenhouse gas emissions, deforestation, water pollution, and habitat deterioration, among other environmental problems. Addressing the environmental effects of livestock agriculture is becoming more and more crucial as the demand for products generated from animals rises. This is essential to maintain the industry's sustainability and protect the planet's health.

Livestock domestication has shaped human societies and provided essential resources for thousands of years. In the modern era, the livestock sector's economic significance cannot be understated, contributing substantially to GDP and providing employment opportunities for millions (FAO, 2019). Moreover, in developing countries, livestock serves as an invaluable asset and safety net for smallholder farmers, playing a pivotal role in poverty reduction and rural development (Herrero et al., 2016). Nonetheless, there are several environmental issues that this rapidly growing sector must deal with, including greenhouse gas emissions, deforestation for feed production, and water contamination.

As the world's population continues to grow, addressing the environmental impact of livestock production becomes increasingly urgent (Gerber et al., 2013; Steinfeld et al., 2006). Innovative feeding management practices offer a promising solution to enhance resource efficiency and reduce the ecological footprint of the livestock industry (Capper, 2013). By adopting precision feeding, exploring alternative feed sources, and employing feed additives, the industry can progress toward more sustainable and environmentally responsible practices (Kebreab et al., 2016; Makkar et al., 2014). Implementing such measures is crucial to ensure the long-term viability of the livestock industry and to mitigate its impact on the environment.

Overall, there is a need to focus on sustainable and innovative feeding management practices in the livestock industry to address environmental challenges while meeting the growing demand for animal-derived products. By adopting more responsible practices, the industry can ensure its long-term viability and contribute to protecting our planet's health and well-being. Livestock production is essential for meeting the worldwide demand for meat and dairy products; however, it poses significant environmental challenges. The inefficiency of feed conversion in livestock, particularly in ruminants, results in the production of methane during enteric fermentation, a potent greenhouse gas (Hristov et al., 2013). Additionally, the mismanagement of manure also contributes to water pollution, as excess nutrients from livestock waste run into water bodies, leading to eutrophication and algal blooms (Powers et al., 2016). These environmental challenges highlight the urgent need for more sustainable and innovative feeding management practices to reduce the livestock industry's ecological footprint and promote environmental stewardship.

Innovative feeding management practices hold immense promise as a pathway toward mitigating the environmental footprint of livestock production. These practices involve the strategic integration of advanced technologies, alternative feed sources, precision feeding techniques, and nutrient management strategies to optimize resource efficiency, reduce waste, and minimize environmental degradation (Pereira et al., 2018). These environmental challenges highlight the urgent need for more sustainable and innovative feeding management practices to reduce the livestock industry's ecological footprint and promote environmental stewardship. Innovative feeding management practices hold immense promise as a pathway toward mitigating the environmental footprint of livestock production. These practices involve the strategic integration of advanced technologies, alternative feed sources, precision feeding techniques, and nutrient management strategies to optimize resource efficiency, reduce waste, and minimize environmental degradation.

Precision feeding, for example, tailors diets to individual animal requirements based on factors such as age, weight, and growth stage, ensuring optimal nutrient intake while reducing excess nutrient excretion (Pereira et al., 2018). Incorporating alternative feed sources, such as **insect meal, extraction oils, algae, tannins, and, agricultural by-products**, not only diversifies feed options but also reduces the environmental burden of conventional feed production (van Huis et al., 2013). Moreover, the judicious use of feed additives, like **enzymes and probiotics**, can enhance nutrient utilization and decrease methane emissions, thereby addressing one of the main contributors to greenhouse gas emissions in livestock production (Hristov et al., 2015). By adopting these innovative feeding management practices, the livestock industry can significantly contribute to sustainable agriculture and global environmental preservation. A transition to more resource-efficient and eco-friendly feeding systems can not only decrease the industry's ecological footprint but also improve animal health and productivity.

Spices and herbs are examples of plants that produce essential oils, which are intricate blends of secondary metabolites. According to Cobellis et al. (2016a), they have antibacterial and antimicrobial qualities that make them potentially helpful in a range of applications. Essential oils have been shown to have a variety of impacts on rumen fermentation. For example, they can block methanogenesis (Patra and Saxena, 2010), slow down the breakdown of starch-rich substrates (Hart et al., 2008), and diminish intra-ruminal nitrogen turnover and nitrogen excretion (Patra and Yu, 2014). However, because of their broad and non-specific antibacterial actions, their effects on the generation of volatile fatty acids (VFAs) and the breakdown of fiber are sometimes uneven and may even be detrimental. Despite this, there are feed additives on the market that are based on active compounds found in **essential oils and promise to enhance the fermentation and digestion of rumen**. Through either **in vitro or in vivo experiments, or both,** several studies have examined the potential of these products to improve rumen fermentation efficiency (Benchaar and Greathead, 2011; Cobellis et al., 2016a), with varying results even at the same dosage of the products (Kung et al., 2008; Castro-Montoya et al., 2015).

Variations in the outcomes of studies could be attributed to several factors, including differences in the levels of essential oil (EO) active ingredients. It is worth noting that these active components are known to have low chemical stability and high volatility, which could affect their efficacy (Cobellis et al., 2016a). Nevertheless, despite the various effects of the individual compounds, it is regrettable that commercial blends of EO active components (BEO) utilized in studies do not always have their active chemicals correctly identified and quantified (Benchaar and Greathead, 2011). Furthermore, when it comes to EOs, in vitro investigations typically supersede in vivo investigations; nevertheless, in vitro, dose-response studies for commercially available BEOs are not extensively accessible (Cobellis et al., 2016a). Differentiating between the antibacterial and systemic (e.g., metabolic) effects of EO may be made easier with a direct comparison of in vitro and in vivo trials. It is significant to remember that in vitro studies only quantify how EO affects rumen microbial communities. A direct comparison could also aid in understanding how the findings of in vitro experiments can be used to predict EO effects in animals.

1.1 In vivo Experimentation:

1.1.1 **Ethical and Cost-Effective Validation** In vivo, validation of the inhibitory effect is essential for confirming findings from in vitro studies. By strategically designing experiments to minimize the number of animals required while ensuring statistical robustness, researchers can ethically validate inhibitory effects while also being mindful of cost considerations.

1.1.2 **Comprehensive Understanding through Animal Physiology:** In vivo validation allows for a comprehensive understanding of the inhibitory effect within the context of animal physiology. By observing the effect on living organisms, researchers can account for complex biological interactions and environmental factors, enhancing the reliability and applicability of the findings to real-world scenarios.

1.2 In vitro Experimentation:

1.2.1 **High-Throughput Screening and Efficiency:** In vitro methods offer high-throughput screening capabilities, enabling researchers to analyze several samples efficiently within a short time frame. This efficiency enhances the pace of research and facilitates the identification of potential inhibitory effects, laying the groundwork for further investigation.

1.2.2 **Non-Invasive and Cost-Effective Preliminary Assessment:** An efficient and non-invasive way to perform initial evaluations of inhibitory effects is using in vitro techniques. By minimizing the need for animal experimentation at this stage, researchers can reduce costs and ethical concerns while still gathering valuable data to inform subsequent in vivo studies.

2 Scientific hypothesis and aims of the thesis

The research aims to assess how various dietary additives impact rumen fermentation parameters, focusing on gas production, methane (CH_4) emission, and carbon dioxide (CO_2) production, with the overarching goal of understanding their potential to modulate rumen microbial activity, vital for ruminant nutrition and greenhouse gas emissions. Using the Hohenheim Gas Test (HGT) apparatus, the study scrutinized 32 treatments, including a control group and additive-containing ones, with each treatment undergoing six independent replicates to ensure robust statistical analysis. This experimental setting was carefully planned to screen all treatments simultaneously in each run, following recognized guidelines, and included a control diet, rumen blank, and feed standards (hay and concentrate). An alphanumeric coding scheme was used to identify the 32 treatment groups after the additives, which included tannin, and sources of essential oil and essential oil compound (EO/EOC), were included in the control diet at different amounts. Laboratory analyses comprehensively evaluated feed ingredients' compositional properties and rumen fermentation parameters, including gas volume, pH, ammonia concentration, protozoa count, and volatile fatty acid concentration, with gas chromatography utilized for CH_4 and CO_2 concentrations. The daily methane emissions of dairy cattle and the emissions of beef cattle related to body weight were successfully decreased by the application of essential oils. Nevertheless, no matter how the invitro data were replicated, these benefits were not shown in vivo. Future studies should focus on this possibility as it could explain the observed discrepancy between the essential oils' in vivo and in vitro modes of action. The hypothesis suggests that these dietary additives will alter rumen fermentation kinetics, affecting gas production, methane emission, and other fermentation byproducts. Statistical analysis will discern significant differences between treatment groups and the control, offering insights into the additives' efficacy in modulating rumen microbial activity, potentially advancing ruminant nutrition strategies, and mitigating greenhouse gas emissions from livestock production.

3 Literature research

3.1 Precision feeding

Precision feeding is a technique used in livestock production to ensure that animals receive the exact amount of nutrients they need based on their age, weight, growth stage, and production goals. This helps to optimize nutrient utilization, reduce nutrient waste and greenhouse gas emissions, and improve animal performance. Precision feeding is particularly useful for ruminant animals, where enteric fermentation contributes significantly to methane production. Advanced technologies like automated feeding systems and sensors can be used to monitor individual animal intake and behavior in real-time, allowing for timely adjustments to the feed ratio. By aligning animal nutrition with environmental stewardship, precision feeding represents a significant advancement in sustainable livestock production.

3.2 Benefits of precision feeding

- Improved Feed Efficiency: Precision feeding allows for a more targeted and balanced nutrient intake, resulting in improved feed conversion efficiency and reduced feed wastage (NRC, 2001).
- Reduced Nutrient Excretion: Precision feeding reduces the amount of extra nutrients, such as phosphorus and nitrogen, that animals excrete into the environment by giving them exactly what they need (Hristov et al., 2013).
- Lower Greenhouse Gas Emissions: According to Pereira et al. (2018), precision feeding has the potential to decrease ruminant enteric methane production, a major contributor to greenhouse gas emissions in the livestock industry.
- Energy Savings: Optimizing feed rations and nutrient intake can lead to energy savings in livestock production, as animals utilize feed more efficiently (Waghorn, 2008).
- Water Conservation: Precision feeding reduces the environmental burden of water usage in livestock farming by decreasing the excretion of excess nutrients that can lead to water pollution (van Krimpen et al., 2015).
- Improved Animal Health and Welfare: Precision feeding can improve animals' welfare and health by precisely addressing their nutritional needs, which leads to healthier and more productive livestock (Bach et al., 2018).
- Decreased Environmental Impact of Feed Production: Precision feeding may reduce the demand for feed ingredients, leading to less land conversion and lower greenhouse gas emissions associated with feed production (Makkar et al., 2014).
- Mitigation of Antibiotic Use: By optimizing feed rations and improving animal health, precision feeding can potentially reduce the need for antibiotics in livestock production (Owens et al., 2015).
- Reduced Feed Costs: Precision feeding enables cost-effective use of feed resources, potentially leading to savings for farmers (Brask et al., 2015).
- Enhanced Sustainability: The overall benefit of precision feeding lies in its potential to contribute to a more sustainable and eco-friendly livestock industry by minimizing resource waste and environmental impacts (De Campeneere et al., 2017).

3.3 Case studies in precision feeding and management

3.3.1 Case Study: Dairy Cattle Precision Feeding in Denmark

De Campeneere et al. (2017) investigated the effects of various feeding practices in cattle farming practices on the environment in Denmark. Precision feeding was implemented to optimize nutrient utilization, reducing nitrogen excretion and greenhouse gas emissions. The study demonstrated that precision feeding improved feed efficiency and significantly lowered the environmental burden of dairy production, making it a successful strategy for sustainable livestock farming.

3.3.2 Case Study: Precision Feeding for Broiler Chickens in Brazil

A study conducted by Vieira et al. (2016) evaluated the effects of precision feeding on broiler chicken performance and nutrient utilization. Precision feeding allowed for accurate nutrient adjustments based on individual animal needs, leading to improved feed conversion efficiency and reduced nutrient waste. The study concluded that precision feeding positively impacted broiler production, making it a promising approach for **resource-efficient poultry farming**.

3.3.3 Case Study: Precision Feeding for Growing Pigs in Canada

Research conducted by Levesque et al. (2015) explored the application of precision feeding in growing pigs. The study utilized real-time monitoring and individual feed adjustments based on the pig's growth rate and body weight. The results indicated that precision feeding improved feed efficiency, reduced feed costs, and decreased nutrient waste. The study demonstrated the potential benefits of precision feeding in optimizing pig production.

3.3.4 **Case Study: Precision Feeding for Beef Cattle in the United States**

Swanson et al.'s (2018) study sought to determine how precision feeding affected the methane emissions and feed efficiency of beef cattle. The researchers were able to minimize methane emissions and maximize nutritional intake without sacrificing animal performance by using individual animal feeding systems. The study's conclusions have a great deal of promise to minimize greenhouse gas emissions from the production of beef since they show that precision feeding can greatly lower cattle enteric methane emissions.

3.3.5 **Case Study: Precision Feeding for Milking Cows in the Netherlands**

The impact of precision feeding on the performance and nutritional utilization of dairy cows in the Netherlands was evaluated by Dijkstra et al. (2017). The feeds were modified by the precision feeding system based on the nutritional needs and production stage of the cow. The outcomes showed increased feed effectiveness, milking yield, and milk structure, underscoring the potential of precision feeding to maximize dairy cow productivity and minimize nutrient waste.

3.4 Alternative feed sources

Livestock production can be made sustainable by exploring alternative feed sources that reduce environmental impact while maintaining animal health and productivity. Insect meal is one such substitute that has drawn inte rest because of its substantial amino acid composition and well-suited nutritional characteristics for animal diets (van Huis et al., 2013). According to Rumpold and Schlüter (2013), insects such as mealworms and black soldier flies and their larvae can be raised on organic waste products and turned into feed ingredients that are high in protein. Another promising alternative is algae, which can be cultivated using minimal resources like water and sunlight, while also providing essential nutrients like omega-3 fatty acids (Makkar et al., 2014). Agricultural by-products such as wheat bran, rice bran, and oilseed cakes can also be used as cost-effective and sustainable feed options, utilizing materials that would otherwise be discarded as waste (Makkar & Ankers, 2014). Additionally, fermentation by-products from the bioethanol and brewery industries can be processed into valuable feed ingredients, contributing to a circular economy approach in livestock production (Spiehs et al., 2002).

The use of these alternative feed sources not only reduces the environmental burden associated with traditional feed production but also diversifies the nutrient composition of livestock diets, potentially enhancing animal health and product quality (Makkar et al., 2016). However, challenges remain in terms of large-scale production, processing, and acceptance by farmers and consumers. Collaborative efforts between researchers, policymakers, and the livestock industry are essential to overcome these barriers and promote the sustainable integration of alternative feed sources in livestock production systems.

3.4.1 Insect Meal: Insects such as mealworms and black soldier flies and their larvae offer high protein content, essential amino acids, and beneficial fats, making them a nutritious alternative feed source for livestock (van Huis et al., 2013).

3.4.2 Algae: Omega-3 fatty acids, important vitamins, and minerals are abundant in algae, providing valuable nutrients for livestock diets while reducing the reliance on fish-based feed ingredients (Makkar et al., 2014).

3.4.3 Agricultural By-products: Utilizing wheat bran, rice bran, and oilseed cakes as feed ingredients not only reduces waste but also provides additional fiber and micronutrients for livestock (Makkar & Ankers, 2014).

3.4.4 Seaweed: According to Jin et al. (2011), seaweeds are a good source of bioactive chemicals, vitamins, and minerals that can enhance animal health and lessen the negative environmental effects of raising cattle.

3.5 Challenges in Livestock Management

- Limited Knowledge and Awareness: Lack of information and awareness about alternative feed practices among farmers and stakeholders can hinder their adoption (Pandey et al., 2019).
- High Production Costs: The initial investment and production costs of alternative feed sources may be higher compared to conventional feed ingredients, limiting their widespread use (Nassu et al., 2017).
- Limited Processing and Storage Facilities: The lack of suitable infrastructure for processing and storing alternative feed sources can hinder their utilization (Farhadnejad et al., 2016).
- Seasonal Availability: Some alternative feed sources may have seasonal availability, making it challenging to maintain consistent feed quality throughout the year (Ibrahim et al., 2015).
- Lack of Quality Standards: The absence of established quality standards for alternative feed ingredients can hinder their widespread use in livestock diets (Girard et al., 2016).
- Regulatory and Policy Barriers: Inconsistent or restrictive regulations regarding the use of alternative feed sources can impede their adoption (Owen & Anderson, 2018).
- Consumer Perception: Consumer attitudes toward animal products derived from animals fed with alternative feeds can influence market demand and acceptance (Pieniak et al., 2017).
- Nutritional Variability: The composition of alternative feed sources may vary, leading to challenges in formulating balanced diets (Stauffer et al., 2017).
- Competing Uses: Some alternative feed sources may have competing uses, such as in the biofuel or human food industries, affecting their availability and cost (Adebayo & Senerwa, 2020).
- Technological Limitations: The use of certain alternative feed sources may require specialized processing technologies that are not readily available in some regions (Stauffer et al., 2019).

3.6 Importance and implications of balanced diets and environmental sustainability

Balanced diets ensure that animals receive the right combination of nutrients, minimizing excess intake and reducing the excretion of nitrogen and phosphorus in manure, which can contribute to water pollution (Baldwin et al., 2004). They can lead to better feed efficiency, reducing enteric methane production, a significant source of greenhouse gas emissions in ruminant livestock (Pereira et al., 2018), (Calder., 2013) states also that Balanced diets provide animals with essential nutrients, supporting their immune systems and overall health, which can reduce the need for antibiotics and other medications. Furthermore, it optimizes the use of feed resources, reducing the demand for land, water, and energy in livestock production (Makkar & Ankers, 2014).

The livestock industry has a significant impact on the environment and biodiversity. However, by promoting balanced diets (Phalan et al., 2011), it can demonstrate its commitment to sustainable practices and social responsibility. Balanced diets can help reduce the reliance on fishmeal as a feed ingredient (Kaushik et al., 2004), contributing to the conservation of marine resources. They can also reduce the use of land for feed production, preserving natural habitats and biodiversity (Zhang et al., 2018). Additionally, balanced diets play a crucial role in reducing greenhouse gas emissions (von Keyserlingk et al., 2013) from livestock production and enhancing the resilience of livestock production systems to climate change impacts (Thornton et al., 2007).

Effective nutrient management strategies, such as precision feeding, dietary manipulation, and feed additives, can help minimize GHG emissions while ensuring animal health and productivity. Precision feeding matches the nutrient requirements of individual animals, thus showing promise in decreasing methane emissions (Hristov et al., 2013). Dietary manipulation, such as using high-quality forages and balanced diets, can improve feed efficiency and reduce methane output (Pereira et al., 2018). The use of feed additives, such as specific tannins or lipids, can inhibit methane-producing microbes in the rumen (Kumar et al., 2014).

Balanced nutrient management in livestock diets ensures animals receive essential vitamins, minerals, and amino acids necessary for optimal growth and overall health(Calder, 2013). Proper nutrition enhances immune function and disease resistance, reducing the incidence of infectious diseases and improving animal welfare. Nutrient management also plays a crucial role in supporting reproductive performance in livestock. Providing adequate energy and nutrients during critical reproductive stages positively influences fertility, conception rates, and litter size in animals. Balanced diets with appropriate nutrient levels ensure efficient growth and weight gain in young and growing animals(Lucy, 2001).

Adequate protein and energy intake are crucial for livestock to achieve target growth rates and reach market weights promptly (Bach, 2017). Nutrient management strategies, such as precision feeding and dietary manipulation, can enhance feed efficiency, which results in better feed conversion rates (Hristov et al., 2013). Precise matching of nutrient requirements to individual animals' needs reduces feed wastage and nutrient excretion, which ultimately leads to better overall feed conversion rates. Providing animals with a well-balanced diet that includes essential nutrients contributes to their mental and physical well-being, reducing the occurrence of behavioral disorders and stress and ultimately leading to better welfare and productivity (Marchant-Forde, 2016).

Proper nutrient management also leads to better health and disease resistance in livestock, resulting in lower mortality rates. Balancing diets with essential nutrients makes animals less susceptible to diseases and health disorders, improving overall survival rates (Wagenaar et al., 2010). Nutrient management practices that optimize the diet composition can also improve the quality of animal products, such as milk and meat, by influencing the fatty acid composition

and nutritional value of these products, providing consumers with healthier and more nutritious food options (Griinari et al., 2001).

Proper nutrition positively influences reproductive efficiency in livestock. Adequate nutrient intake during pregnancy and lactation supports healthy fetal development and milk production, resulting in improved reproductive performance and higher weaning rates (Britt, 2011). Nutrient management strategies that improve feed efficiency and reduce nutrient wastage also contribute to a lower environmental footprint of livestock production. Optimal nutrient utilization leads to reduced emissions of greenhouse gases and nutrient runoff, enhancing the sustainability of the livestock industry (Thornton et al., 2007).

Finally, proper nutrient management practices can lead to economic benefits for livestock producers. Improved feed efficiency, enhanced growth rates, and reduced veterinary costs contribute to better profitability in the livestock industry (Orr, 2014). By adopting nutrient management strategies, livestock producers can improve animal welfare, reduce environmental impact, and increase profitability, ultimately benefiting both the animals and the industry as a whole.

3.7 Feed Additives in Animal Health

Because animal feed additives can supply vital nutrients, improve feed flavor, maximize feed consumption, and boost growth performance, they are extensively utilized for a variety of animals, including poultry, worldwide. Maintaining the health of animals with excellent growth performance requires the use of appropriate additives. In these situations, it's important to make sure the animals eat a nutritious, well-balanced diet, and feed additives can help with that. It has been demonstrated that adding chemicals to animal feed can help to maintain healthy animals and environmentally friendly farming methods.

With the rise of industry standards and consumer awareness, there has been an increased demand for healthier animal-based food products. The industry is under pressure to develop more natural, non-residual feed additive substitutes as a result. The well-being of consumers and animals comes first, so it's critical to identify worthwhile substitutes for additives in animal feed. The focus is on finding solutions that promote the health and well-being of animals while also ensuring that the food produced is safe and healthy for human consumption.

Additives for animal feed come in a variety of forms, including probiotics, prebiotics, enzymes, and herbs. Researchers have determined that these feed additives have distinct benefits, substantiated by both scientific and empirical evidence. Botanicals, or herbs and their extracts, have a broad spectrum of actions that include promoting endogenous secretions, stimulating feed intake, and possessing antimicrobial, coccidiostat, or anthelmintic properties. Utilizing these chemicals is a viable strategy for improving the nutrition and health of animals.

To maintain sustainable livestock production in the wake of the prohibition on the use of drugs as growth stimulants, the animal husbandry industry is resorting to herbal feed

additives. Among the feed additives being utilized to enhance the antimicrobial, anti-inflammatory, antioxidant, digestion, and immune-stimulant qualities of animal feed are herbal extracts, prebiotics, probiotics, and ascorbic acid. The negative side effects of antibiotics are becoming more well-known, and these inexpensive additions are becoming more and more popular. To protect both livestock and individuals who consume their products, more research must be done on the medicinal qualities of herbs.

3.8 Feed additives for environmental Impact mitigation

Supplements to the diet are an essential component of contemporary animal husbandry since they enhance the quality, performance, and health of the animals. These additives are classified into different categories, including nutritional, zootechnical, antioxidants, mold inhibitors, acidity regulators, methanogenesis inhibitors, antiparasitic, and flavoring agents.

Feed additives reduce greenhouse gas (GHG) emissions, which is a significant contribution to animal production. Numerous compounds have been investigated by researchers as possible ways to lower enteric methane generation, a major source of emissions of greenhouse gases in ruminant animals. According to a study by (Chen et al. in 2019), methanogenesis inhibitors have been demonstrated to be a promising approach for lowering emission levels of methane.

Moreover, feed additives contribute to food safety and quality, with some antioxidants improving meat quality and shelf life (Surai et al., 2015). Essential oils should be used with caution in animal diets, though. While essential oils may help to strengthen the intestinal lining, lower the need for antibiotics, increase feed utilization for optimal performance, and even lower methane emissions, proper dosages must be established to avoid harm to animals (Hoskin et al., 2003; Vasta et al., 2020).

Therefore, responsible use, guided by scientific evidence, is crucial for animal welfare, food safety, and environmental sustainability (Patra et al., 2017). It is critical to seek professional advice before implementing any dietary modifications for your pets. With careful preparation and knowledgeable use, the prudent use of feed additives, including essential oils, offers hope for a better, more sustainable future (Danning et al., 2020; Liu et al., 2020).

Thus, the application of feed additives, including essential oils, is essential in modern animal production for improving animal performance, health, and quality while reducing greenhouse gas emissions. Nonetheless, responsible use, guided by scientific evidence, is crucial for animal welfare, food safety, and environmental sustainability.

1. Tannins: It has been demonstrated that the presence of condensed tannins in some plants and feed sources lowers the release of methane by preventing the growth of methanogenic archaea in the rumen (Patra, 2013).

2. Essential Oils: By suppressing methanogens, aromatic compounds obtained from a variety of plant-based sources have shown promise in lowering methane emissions in ruminant animals (Machmüller et al., 2003).

3. Ionophores: By changing the balance of microbes, ionophore feed additives like lasalocid and monensin are being widely utilized to decrease the production of methane in the rumen (Van Zijderveld et al., 2010).

4. Seaweeds: The algae and associated nutrients, such as bromoform as well as phlorotannins, have shown potential in reducing methane emissions by inhibiting rumen methanogens (Machado et al., 2014).

5. 4-Nonylphenol: This compound, derived from essential oils and aromatic plants, has demonstrated significant methane reduction potential in ruminants (Rira et al., 2018).

6. Nitrate: By acting as a substitute hydrogen sink and lowering the amount of hydrogen available for methanogens, the dietary addition of nitrates has demonstrated the potential to reduce the generation of methane from grazing animals (Van Zijderveld et al., 2010).

7. Propionate Precursors: It has been demonstrated that adding specific feed additives, like 3-nitrooxypropanol (NOP), inhibits the activity of methyl coenzyme-M reductase, which lowers the amount of biogas produced in the rumen (Hristov et al., 2015).

8. Enzyme Additives: Including exogenous digestive enzymes in the nutritional intake of animals can enhance fiber digestion and nutrient utilization, leading to improved feed efficiency and reduced methane emissions (Patra & Yu, 2013).

9. Saponins: By altering the structure of methanogenic archaea, saponins, which are present in a variety of plant sources, have demonstrated the capacity to lower the production of methane in ruminant species (Patra, 2010).

10. Yucca Extracts: Yucca plant extracts have been investigated for their methane-reducing properties in the rumen by altering rumen fermentation patterns (Beauchemin et al., 2008).

3.9 Mechanisms for Effective Feed Management

Feed additives function through various mechanisms to improve livestock performance and reduce greenhouse gas emissions. Here are some common mechanisms and their effectiveness,

- **Methanogenesis Inhibition:** Additives like tannins, essential oils, and saponins interfere with rumen methanogenesis by inhibiting methanogenic archaea, leading to reduced methane emissions (Patra, 2013).
- **Hydrogen Sink:** By acting as a substitute hydrogen sink and lowering the amount of hydrogen available for methanogens, the dietary addition of nitrates has demonstrated the potential to reduce the generation of methane from grazing animals (Van Zijderveld et al., 2010).
- **Fiber Digestion Enhancement:** Exogenous enzymes improve fiber digestion, increasing nutrient availability for the animal and reducing methane emissions through improved feed efficiency (Patra & Yu, 2013).
- **Disruption of Microbial Structure:** Certain supplements, such as yucca extracts, change the processes of fermentation and reduce the production of

methane in the gut by upsetting the microbial structure (Beauchemin et al., 2008).

- **Nutrient Utilization Improvement:** Feed additives can enhance nutrient utilization, reducing nutrient excretion and mitigating environmental impacts. Examples include enzymes and tannins, which improve nutrient digestibility and utilization (Patra & Saxena, 2009).
- **pH Regulation:** Some feed additives, such as sodium bicarbonate or potassium carbonate, act as rumen pH regulators, preventing excessively low pH levels. Overall livestock well-being and production are enhanced and the possibility of acidosis is decreased when the rumen pH is kept at its ideal level (Aschenbach et al., 2011).
- **Mycotoxin Detoxification:** Some additives, such as activated charcoal or bentonite clay, have been shown to bind and neutralize mycotoxins present in the feed, decreasing their detrimental effects on the productivity and well-being of animals (Bryden et al., 2012).
- **Immune System Modulation:** Feed additives such as probiotics and prebiotics can modulate the animal's immune system, enhancing disease resistance and promoting better overall health (He et al., 2019).
- **Heat Stress Mitigation:** Antioxidants and electrolytes are two additions that assist lessen the harmful effects of heat stress on cattle. They assist in maintaining proper hydration, electrolyte balance, and cellular function during periods of high environmental temperatures (Collier et al., 2017).
- **Growth Promoters:** Some feed additives, such as growth-promoting antibiotics or synthetic hormone-like compounds, can improve livestock growth rates and feed efficiency. However, the use of such additives has become more controversial due to concerns about antibiotic resistance and consumer preferences for antibiotic-free products (Agunos et al., 2017).

The kind of feed addition, the amount used, and the particular animal species all affect how effective the additive is. Careful thought must be given to ensuring the safety and effectiveness of these additions in livestock diets while also supporting environmentally responsible and sustainable livestock operations.

3.10 Research gaps in innovative feed management

Innovative feeding management has the potential to revolutionize sustainable livestock production. Farmers who embrace these technologies and practices can enhance productivity, reduce environmental impacts, and contribute to a more sustainable and resilient agricultural sector. However, fully realizing these benefits requires continuous research, education, and adoption of innovative solutions.

Research in livestock production management is constantly evolving to tackle new challenges and opportunities. It is important to explore the effectiveness of precision feeding technologies in different livestock species and production systems, as well as their economic

viability and long-term impact on animal health and performance (Houdijk et al., 2018). Further research is required to examine the nutritional benefits, protection, and environmental effects of feeding cattle novel alternative feed ingredients including insect meal and single-cell proteins (Gasco et al., 2020).

Further studies are required to explore innovative approaches to enhance animal welfare, including the impact of environmental enrichment and precision management on behavior and stress reduction in livestock (Ellingsen et al., 2021). There are research gaps in sustainable manure management methods, including enhanced nutrient recovery technologies, decreased emissions of greenhouse gases strategies, and assessments of the practice's effects on plant health and water quality (Chardon et al., 2017).

Studies are needed to identify and validate effective antibiotic alternatives, such as probiotics, prebiotics, and plant extracts, to maintain animal health and performance without compromising antimicrobial resistance concerns (Sugiharto, 2016). To create livestock production systems that can endure the effects of changes in the environment, such as severe weather and shifting feed availability, more research is needed in the domain of climate change resilience (Herrero et al., 2016).

It is becoming increasingly important to explore the potential of digital technologies in livestock management. Studies have indicated that the application of technology like artificial intelligence and big data analytics can significantly improve decision-making and maximize resource use in this domain (Schulze, 2021). To maximize the possibilities of digital technology and enhance livestock management techniques, it is imperative to keep investigating these areas. Further investigation is required to evaluate the benefits of integrated livestock-crop systems in terms of resource use efficiency, nutrient cycling, and resilience to climate variability (Hatfield et al., 2020). One Health approaches that integrate human, animal, and environmental health also need to be explored to address zoonotic disease risks and promote sustainable livestock production (Graham et al., 2020).

Finally, there is a need to examine the socioeconomic impacts of innovative livestock production management practices on smallholder farmers and vulnerable communities, including gender dynamics and income distribution. By addressing these research gaps, we can work towards a more sustainable and equitable future for livestock production (Devendra, 2012).

4 Methodology

4.1 Experimental Design & Diet:

A ruminant blank, two dietary standards, and the Hohenheim Gas Test (HGT) apparatus were used in an extensive experiment involving thirty-two cows. Menke and Steingass (1988) provided a detailed description of this configuration, with each receiving a specific diet regimen carefully composed of the following components per kilogram: 370 grams of corn silage, 300 grams of grass silage, 130 grams of grass hay, 190 grams of concentrate (comprising wheat, corn gluten, and soybean meal), and 10 grams of a wheat bran premix containing additives. The cows are divided into four groups, with Group K serving as the control group receiving the standard diet/The control diet, while Groups PC, A, and B are provided with diets supplemented with various additives. Group A receives 8 grams per day of chestnut extract and 6 grams per day of an A EOC blend (including Thyme and Oregano essential oils and limonene), whereas Group B is given 8 grams per day of quebracho extract and 6 grams per day of a B EOC blend (comprising Thymol, Carvacrol, and Eugenol).

Following a fortnight of nourishment, the groups are methodically matched according to important metrics such as milk production (35±5 kg), days in milk (62±27), parity (parity 1, parity 2, and parity 3), dry material consumption, total body weight, and methane emission levels. Carefully balancing each group guarantees comparability and facilitates reliable assessment of the impact of the food supplements on methane emissions, productivity, and cattle wellness. The data used for grouping the cows after the two weeks serves to maintain consistency and integrity in the experimental design, facilitating meaningful comparisons and insights into the impact of the additives on dairy cow management practices.

Table 4.1 Amount (g/kg) of essential oil (EO) and EO compounds (EOC) in the nine formulated blends in relation to total diet dry matter.

EOC blends	Carvacrol	Oregano EO	Thymol	Thyme EO	Eugenol	Clove EO	Citrus peel EO	α-pinene	Bornyl acetate
1	5	–	5	–	–	–	–	–	–
2	5	–	5	–	5	–	–	–	–
3	5	–	5	–	–	5	–	–	–
4	5	–	5	–	–	–	5	–	–
5	5	–	5	–	–	–	–	5	–
6	5	–	5	–	–	–	–	–	5
7	–	5	–	5	–	–	–	–	–
8	–	5	–	5	5	–	–	–	–
9	–	5	–	5	–	5	–	–	–
10	–	5	–	5	–	–	5	–	–
11	–	5	–	5	–	–	–	5	–
12	–	5	–	5	–	–	–	–	5

EO: essential oils; EOC: essential oil compounds.

4.2 Materials and Methods:

The experiment was carried out under the direction of Agroscope in a winterized free-stall barn in Posieux, Switzerland. Throughout the trial, environmental factors including humidity, heat, and illumination were kept an eye on to guarantee uniformity. To clarify the influence on dairy cow performance and metabolic parameters, statistical tests were carried out to evaluate the impacts of food interventions on various metrics. The milking cow's

welfare and well-being were maintained throughout the experiment by adhering to ethical norms, highlighting the significance of animal welfare in research techniques. Overall, the thorough experimental protocol offered profound insights into how nutritional interventions affect the physiology and productivity of milking cows, with implications for improving feeding practices in the dairy industry.

The experimental design spanned a total of 12 weeks and comprised distinct phases aimed at assessing the impact of dietary treatments on dairy cow performance and metabolic parameters. During the initial two-week period, dairy cows underwent adaptation to a basal diet, allowing their digestive systems to acclimatize gradually. Following this adaptation phase, a five-week transition period (weeks 3-7) was allocated for cows to adjust to specific dietary treatments, ensuring a smooth transition and minimizing any potential stressors associated with abrupt dietary changes. Subsequently, the experimental phase encompassed weeks 8 to 12, during which the designated treatments were implemented, and data were collected for analysis.

To check important metrics like consumption of feed, production of milk, and methane emissions, regular monitoring protocols were put in place. Daily measures of milk output offered information on any variations or reactions to the nutritional treatments, and feed intake was documented every day to evaluate consumption patterns and guarantee adherence to the diet. Green feed technology, created by C-Lock Technology Inc., Rapid City, SD, was used to measure methane emissions in real-time during the experiment, following the technique described by Denninger et al. (2019).

To examine changes in nutrient content over time, weekly assessments required assessing the gross composition of milk and feed. This analysis provided important insights into the overall nutritional value of the diet and how it affected the composition and production of milk. Furthermore, some weeks in the experimental schedule required particular sampling and analysis protocols. Before nourishment, intestinal fluid samples were taken, and samples were acidified for a later examination of volatile fatty acids (VFAs). On-site analyses of pH and ammonia (NH3) levels were performed on the samples. This comprehensive analysis provided insights into rumen fermentation dynamics and microbial activity, crucial for understanding nutrient utilization and metabolism in dairy cows. Fecal samples were pooled over 3 days to create representative samples for analysis of gross composition, allowing for assessment of nutrient excretion and digestion efficiency.

Additionally, using the methods outlined by Conte et al. (2016), specimens of milk were gathered and subjected to Gas Chromatography with Flame Ionization Detection (GC-FID) analysis to determine the content of fatty acids. Characterizing the fatty acid profiles in milk was made possible by this technique, which also provided insights into the impact of food on the composition of the fat in milk and possible health repercussions.

4.3 Animals and preparation of rumen fluid:

Three nursing Original Brown Swiss cows were used in this experiment, and their rumen fluid was taken before they were fed in the morning. As previously reported by Terranova et al. (2018, 2020), each donor cow's rumen fluid was utilized for a single run. Because it preserved the natural variance in rumen fluid parameters between individual donor animals, this approach was recommended for generating analytical replicates. It avoided using rumen fluid from the same cow or a combination of rumen fluids and allowed for the inclusion of all treatments in each run and the creation of six real replicates. The diet fed to the donor cows included ryegrass hay (130 g/kg DM), dairy concentrate (50 g/kg DM), grass silage (300 g/kg DM), and corn silage (370 g/kg DM) from UFA-243, UFA AG, Switzerland. Water was freely available to the cows. Following collection, the rumen fluid was immediately filtered through four layers of cheesecloth and kept anaerobically in sealed pre-warmed bottles. Given that the laboratory and the barn were only a few meters apart, this was done. The rumen fluid was introduced to the buffer medium at a 1:2 (v/v) ratio during the incubations. To produce anaerobic conditions, the buffer medium was mixed in accordance with Menke and Steingass' (1988) approach and continually maintained under carbon dioxide (CO2).

4.4 Animals and preparation of rumen fluid:

The experiment was carried out at the AgroVet-Strickhof research center in Lindau, Switzerland, using Menke and Steingass' (1988) modified approach (Soliga and Hess, 2007). An equivalent quantity of basic feed (200 mg DM) was first prepared for each run. The basic feed (control) was then supplemented with feed additives. Since it was believed that the additives' feed value was zero, this action was conducted. After sealing, the HGT glass injections were stored at room temperature. The warm buffered rumen fluid was then added through each syringe's inlet in a set volume of 30 mL. The syringes were immediately put into a 39 °C warmed incubator and left there for a full day. To minimize the impact of the syringe position concerning the rotation axis, the treatments were arranged differently in the HGT incubator for every run.

4.5 Research question and hypothesis formulated

4.5.1 Ethical Approval

The process used to collect samples and dispense rumen fluid adhered to the ARRIVE criteria (Kilkenny et al. 2010). The process (authorization number ZH113/18) was certified by the competent Swiss authority, the Cantonal Veterinary Office Zurich, guaranteeing that all relevant safety and ethical requirements were fulfilled.

4.5.2 Laboratory Analysis

1. Moisture analysis

Moisture content determination is a common process used in industries such as food processing, agriculture, and construction. The process involves measuring the amount of water present in a sample of material. In this case, the method used is oven drying. Taking a 5g sample of grounded material with a significant dry matter percentage is the first stage in the procedure. Ensuring that the sample is representative of the material being evaluated is crucial. The sample is then placed in a dried and clean porcelain crucible. It is important to ensure that the crucible is dry and clean because any moisture or impurities in the crucible can affect the accuracy of the test.

Once the sample is in the crucible, it is spread evenly to ensure that it dries uniformly. The crucible with the sample is then placed in a preheated oven (105 ± 1°C, Forced air ventilation oven, type M 120 - VF) for 4 hours until a stable weight is achieved. The stable weight is achieved when the weight of the sample no longer changes after successive weighings at regular intervals. This indicates that all the water in the sample has been removed. Once the sample is removed from the oven, it is cooled in a desiccator. A desiccator is a container that contains a desiccant, which is a substance that absorbs moisture. The purpose of cooling the sample in the desiccator is to prevent it from absorbing moisture from the air as it cools. After cooling in the desiccator, the sample is re-weighed. The moisture content, given as a percentage, is determined by taking the variation between the initial and end weights.

In cases where the sample is not needed for ash analysis, aluminum-weighing dishes can be used instead of porcelain crucibles. This is because aluminum dishes are less likely to break or crack during the drying process, and they can be reused after being cleaned. Overall, the oven drying method for moisture content determination is a reliable and accurate process for measuring the amount of water present in a sample of material.

$$Moisture(\%) = \frac{(W0 - Wf)}{W0} * 100$$

Where,
W0 = initial weight of the sample (about 5 grams)
Wf = weight of the sample after drying (105°C)

When dealing with forages that have high moisture content, it is important to reduce the moisture content to less than 90%. This can be done by drying the forages. To begin the process, a weighted sample of around 50 grams is taken and left ungrounded. This sample is then baked at 70°C for 24 hours or until it reaches the desired level of dryness. It's important to ensure that the sample has reached the desired level of dryness, as this will affect the accuracy of the results.

After the sample has been baked, it is allowed to cool down to room temperature. Once cooled, the sample is weighed again to determine the final weight. This helps to calculate the dry matter content of the sample. The next step in the process is to pulverize the sample until it

can fit through a 1 mm sieve. Once the sample has been pulverized, it undergoes the same processing steps as described previously.

It's important to note that the accuracy of the results is dependent on following the correct procedures for drying and weighing the sample. Any deviation from the procedure could lead to inaccurate results.

$$Moisture(\%) = \{[\frac{(Wpd - Wf)}{Wpd}] * Wg_{+}[\frac{(W0 - Wg)}{W0}] * 100\}$$

Where:
W_{pd} = about 5 grams of the sample after preliminary drying
W_f = weight of the sample after drying (105°C)
W_g = weight of the sample after preliminary drying
W_0 = initial weight of the sample (about 50 grams)

2. Crude Ash

Ashing is a process in which organic materials are incinerated at high temperatures to yield inorganic residues known as ash. The ash can then be analyzed for its chemical composition. To obtain ash, a clean and dried porcelain crucible, weighing approximately 5 grams, is placed in a cool muffle furnace. The dried samples are then added to the crucible and ashed for three hours at a temperature of 550°C. During the process, the organic material is burned off, leaving behind the inorganic ash. Once the ash is largely white, the ashing process is finished. The crucible is then allowed to cool in a desiccator before being weighed. This weight represents the weight of the inorganic ash produced by the sample. Ashing is a crucial step in many analytical techniques, including elemental analysis and determination of mineral content in food and plant samples.

References:

Regulation (EC) No 152/2009 lays out the official methods of sampling and analysis to be used for the control of animal feed.

3. Crude protein

The Kjeldahl method is used to determine the nitrogen content (N). Next, we calculate crude protein by multiplying N by 6.25. The procedure entails titration, steam distillation, and digestion.

Digestion

To analyze a sample, we need to follow a specific process. Firstly, take 0.2 grams of the substance with an accuracy of 0.001 g and place it into a Kjeldatherm digestion tube manufactured by C. Gerhardt GmbH & Co. KG. This tube is designed to withstand the harsh conditions of the digestion process. Next, digest the organic material by adding 3 mL of 96% sulphuric acid and 1.5 mL of 30% hydrogen peroxide m/m to the tube containing the substance.

The sulphuric acid catalyzes to break down the organic material, while the hydrogen peroxide helps to oxidize any remaining organic material.

To heat the substance and speed the digestion process, use the DIGESTION UNIT TURBOTHERM from C. Gerhardt GmbH & Co. KG (as shown in Fig. 1). This device uses a high-temperature reaction chamber to heat the contents of the tube to a precise temperature. The temperature is maintained until the contents of the tube become clear, indicating that the digestion process is complete. Once the digestion process is complete, the nitrogen in the substance will be present in the form of ammonium ion. This is an important step in the analysis process, as the presence of ammonium ions is often used to determine the nitrogen content of a substance. All things considered, the sample preparation procedure is essential for the analysis of organic materials and can yield important details on the makeup of a material.

Distillation and titration

In the process of Kjeldahl analysis, a sample is digested in a tube which is then placed into the VAPOTEST steam distillation system designed by C. Gerhardt GmbH & Co. KG. The system is equipped to automate the addition of sodium hydroxide (NaOH) to the tube which converts the ammonium ions into ammonia. The ammonia produced is then distilled and mixed with 2% boric acid. After that, a pH electrode that recognizes the titration process endpoint automatically is used to measure the pH of the solution. The following formula can then be used to determine the amount of nitrogen in the sample as a percentage of the sample weight (%N):

$$\%N = [\frac{Ceq * (V - VBL) * M}{W0} * 100\%]$$

Where:
Ceq = Titration solutions Normality (mol/L)
V = Titration solution sample Consumption (L)
VBL = Titrarion solution consumption for blank value (L)
M = nitrogen molar mass of (g/mol)

Kjeldahl analysis is a widely used method for determining the nitrogen content in various types of samples such as food, soil, and water. It is a reliable and accurate method for quantitative analysis of nitrogen. The automation of the process using the VAPOTEST steam distillation system makes it easy and efficient to carry out the analysis with high precision and accuracy.

Fig. 4.1: On the left side: Vapotest steam distillation systems for Kjeldahl analysis; on the right side: DIGESTION UNIT TURBOTHERM (C. Gerhardt GmbH & Co. KG).

References:
COMMISSION REGULATION (EC) No 152/2009 laying down the methods of sampling and analysis for the official control of feedingstuffs.
AAVV 1991. manual for plant analysis, Micro-macro publish, Inc. Appendix 4

4. Ether Extract

The Ankom XT10 extractor, manufactured by Astori Tecnica in Brescia, Italy, is a reliable and efficient method for determining the ether extract content of a sample. To perform the test, 0.5 grams of the sample is carefully weighed into an Ankom XT4 filter bag and dried in a preheated oven at 105 ± 1 °C. Once dried, the filter bag and sample are weighed again to obtain the initial weight.

Next, the extraction process is carried out using high-temperature solvent extraction with petroleum ether, following the instructions provided by the Ankom XT10 manufacturer. After extraction, the sample is dried in a ventilated oven at the same temperature as before and cooled in a desiccator. The final weight is then recorded and the ether extract content is calculated based on the weight loss of the sample during the extraction process.

This method follows the official AOCS Am 5–04 method, which is a rapid and accurate way to determine the oil/fat content of a sample. Overall, the Ankom XT10 extractor provides a reliable and standardized method for determining ether extract content in a variety of samples.

$$EE(\%) = \frac{(\mathrm{Wd} - \mathrm{We})}{\mathrm{Wi}} * 100$$

Where:
Wd = weigh of sample + bag after drying and prior extraction
We = weigh of sample + bag after extraction
Wi = initial weight of the sample

Fig. 4.2: Ankom XT10 Extractor (Astori Tecnica)

References:

User manual **https://www.ankom.com/analytical-methods-support/ankom-xt10-extractor.**
Official AOCS method Am 5–04 Method for the rapid determination of oil/grease using high-temperature Solvent extraction.

5. Neutral detergent fibers

An essential analysis for figuring out the nutritional value of diet and forage is the concentration of acid detergent fiber or aNDF. Ankom Fibre Analyzer A200 from Astori Tecnica in Italy and the Filter Bag Technique by the Van Soest et al. (1991) procedure are used for this analysis (Fig. 3). To perform the analysis, 0.5 grams of dried samples are weighed and placed in an F57 filter bag. The bag is then sealed using a heat sealer and the sample is spread evenly inside by shaking. It's crucial to remember that the sample needs to be ground such that it fits through a 1 mm sieve. Low readings could arise from too-finely ground samples causing particle loss from the filter bags.

Up to 24 samples are placed in the 8-bag suspender trays and then soaked in a neutral detergent solution (pH=7±0.1, ANKOM FND20) inside the Ankom 200 Fiber analyzer. 20 grams of Sodium Sulphite Anhydrous (FSS, ANKOM Technology) and 4 mL of alpha-amylase (heat-stable bacterial alpha-amylase: activity=17,400 Liquefon Units/ml; FAA, ANKOM Technology) are added manually, and then the lid is closed. The agitation is started, and the solution inside is gradually heated. Once the extraction is complete, the bags are soaked for 5 minutes in acetone to remove excess water. After that, the bags are allowed to air dry under the fume hood before being fully dried for two hours at 105 ± 1 °C in an oven that has been preheated. After being placed in a desiccator to cool, the bags are weighed. The following formula is used to compute the fiber residuals, which are primarily made up of cellulose, hemicellulose, and lignin:

$$aNDF(\%) = \frac{(W3-(W1*C1))}{W2} * 100$$

Where:

W1 = Weight (g) of the bag tare

W2 = Weight of sample (g)
W3 = Dried bag weight (g) following extraction.
C1 = blank bag correction (final to initial blank bag weight ratio)

In summary, the Filter Bag Technique using the Ankom Fibre Analyzer is a reliable and efficient method for analyzing the concentration of aNDF in feed and forage samples. The process involves several steps, including sample preparation, soaking in a detergent solution, and drying the bags. The resulting fiber residues can then be used to calculate the nutritional value of the sample.

Fig. 4.3: Ankom Fibre Analyzer A200 (Astori Tecnica)

References:

Ankom NDF Method 15 - Neutral Detergent Fiber in Feeds - Filter Bag Technique (for DELTA)
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

6. Acid detergent fiber

The concentration of Acid Detergent Fiber (ADF) is an important parameter in animal nutrition analysis. It is analyzed using the Filter Bag Technique following the Van Soest et al. (1991) protocol and utilizing the Ankom Fiber Analyzer A200 from Astori Tecnica, Italy (Fig. 3). Weighing 0.5 grams of dried sample in an F57 filter bag is the first step. The sample is then dispersed by shaking the bag after it has been sealed with a heat sealer. To make the sample fit through a 1 mm sieve, it must be ground. Low readings may result from over-grinding the sample too fine, which might cause particles to escape through the filter bags.

Up to 24 samples are placed in 8 bag suspender trays and soaked in acid detergent solution (ANKOM FAD20CB) containing cetyl trimethylammonium bromide and H2SO4, inside the

Ankom 200 Fiber analyzer. The lid is closed and agitation begins. Once the extraction is complete, the bags are soaked for 5 minutes in acetone to remove excess water. The bags are then left to dry under a fume hood and then completely dried in a pre-heated oven at 105 ± 1 °C for 2 hours. The bags are dried, allowed to cool in a desiccator, and then weighed. Lastly, the following formula is used to determine the fiber remnants, which are primarily made up of the fiber and lignin:

$$ADF(\%) = \frac{(W3-(W1*C1))}{W2} * 100$$

Where:
W1 = Weight (g) of the bag tare
W2 = Weight of the sample (g)
W3 = weight of dried bag after extraction (g)
C1 = blank bag correction (ratio of final and initial blank bag weight)

It is important to note that the ADF analysis provides information on the amount of cell wall material that is indigestible by animals. This information is used to evaluate forage quality for ruminants and other herbivores. Proper sample preparation, as well as following the protocol carefully, is crucial for obtaining accurate and reliable results.

References:

Ankom ADF Method 14- Acid Detergent Fiber in Feeds - Filter Bag Technique (for DELTA)

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.

7. Acid detergent Lignin

In the process of determining the Acid Detergent Lignin (ADL) content in a sample, the first step involves using an ADF (Acid Detergent Fiber) determination method. Once this is done, the next step is to treat the dried bags containing mostly cellulose, lignin, and insoluble ashes with 72% H2SO4. To carry out this treatment, the bags are placed in a DaisyII jar and covered with 72% H2SO4. The samples are then rotated continuously with the Ankom DaisyII Incubator for 3 hours. Once the acid treatment is completed, the excess acid is washed away with tap water until the pH paper shows a neutral color.

The bags are submerged in acetone for five minutes to extract the extra water. Subsequently, the bags are left to dry under a fume hood before being placed in an oven that has been preheated to 105 ± 1 °C for two hours. The bags are weighed after being allowed to cool in a desiccator until they are fully dry. It is important to note that ADL results are generally expressed net of ash content. Therefore, the bags are placed in pre-weighed crucibles and ashed at a temperature of 550°C for 3 hours. The calculations for ADL are reported as follows:

$$ADL(\%) = [\frac{(\text{WH2SO4} - \text{ashes} - \text{W1} * \text{C1})}{\text{W2}}] * 100$$

Where:
WH2SO4 = weigh after H2SO4 extraction (g)
Ashes = weight of insoluble ashes (g)
W1 = bag tare weight (g)
W2 = initial sample weight (g)
C1 = blank bag correction (ratio of final and initial blank bag weight)

Fig. 4.4: on the left side: Ankom DaisyII Incubator (Astori tecnica); on the right side: DaisyII jar

References:

Ankom Method 9 – determining Acid Detergent Lignin in DaisyII Incubator

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides about animal nutrition. J. Dairy Sci. 74, 3583–3597.

4.5.3 Measurement of Digestiability

1. Digestiability Trials

Digestibility testing is a critical procedure that involves evaluating the capacity of an animal to digest and absorb nutrients from a particular feed. In such tests, the feed to be tested is given to the animal in known quantities, and the excretion of feces is measured. The experiment is usually conducted using more than one animal, usually four, to account for individual variations in digestive capacity, even if they are of the same species, sex, and age. Repeating the experiment also helps to detect experimental errors.

Male or castrated animals are sometimes preferred over females in mammal research because it is easier to separate urine from feces. The animals must be in good health and have a calm disposition. Smaller animals are typically housed in metabolic cages equipped with sieve systems to make it easier to separate urine from excrement. On the other hand, bigger animals like sheep and cattle are equipped with manure-collecting bags made of latex or another

impermeable substance, as well as special harnesses. Urine and feces can be collected separately in female animals using a urinary catheter.

Making sure the feed is evenly mixed before starting a digestibility trial is crucial. Usually, there are three phases to the study, each lasting seven to ten days. The animals are gradually introduced to the experimental feed during the adaption phase. The animals are fed the experimental diet for a while after they have acclimated to ensure total adjustment and to remove any leftover meal residue from their digestive tracts. During the collection period, food intake and fecal excretion are recorded to assess the feed's digestibility.

An indigestible dye, such as ferrous oxide or carmine, is added to the initial and final meals of the collecting period to measure the amount of food consumed by animals with simple stomachs. Then, by delaying the start and conclusion of fecal collection until the dye appears or disappears from the feces, the amount of fecal excretion owing to a particular meal intake can be determined. However, because the colored meal combines with other meals in the rumen, this strategy is not appropriate for ruminants. Feed residues are permitted to pass through ruminants for an arbitrary 24-48 hours. Following the completion of the food intake measurement, the measuring of fecal output starts one to two days later and lasts for the same amount of time.

It is important to maintain consistency in the timing and amount of meals given during digestibility trials, especially in ruminants. Irregular intake can lead to inaccurate results, as an unusually large final meal of the trial period can cause an increase in fecal excretion after the end of faecal collection. This can result in an underestimation of the amount of feces produced from the measured food intake, leading to an overestimation of digestibility. Consequently, it is ideal to make sure that food is served at the same time every day and that daily consumption does not fluctuate.

In conclusion, after conducting a thorough evaluation of an animal's ability to digest and absorb nutrients from a specific feed, digestibility testing is considered an essential process. The experiment is typically conducted using several healthy and well-natured animals, and the feed is mixed thoroughly to ensure consistency. To ensure accurate results, it is highly advised that meals be delivered at the same time every day and that the amount consumed be consistent. The study should be conducted across three periods. The process of digestibility testing is crucial for assessing the nutritional value of animal feed, and it helps researchers determine the optimal diet for animals.

The nutritional digestibility coefficients of the hay fed to cows are calculated as follows: To find the digestibility of hay, a trial of digestibility involving three cows was carried out. For ten days during the collection period, feed intake and feces output were monitored. Hay and fecal samples were subjected to laboratory analysis. The following is the standard formula used to determine digestibility coefficients:

$$\frac{\text{nutrient consumed} - \text{nutrient in faeces}}{\text{nutrient consumed}}$$

Oats serve as the test food, and hay serves as the baseline diet. The following general formula can be used to determine the test food's digestibility:

$$\frac{\text{nutrient in test food} - (\text{nutrient in faeces} - \text{nutrient in faeces from basal diet})}{\text{nutrient in test food}}$$

Ruminant diets frequently include fat supplements, albeit in small doses. In order to ascertain the digestibility of a fat supplement, we can presume that the basic constituents of a diet including the fat supplement and the long-chain fatty acids (LCFA) in a control diet have the same digestibility coefficients. We can determine the LCFA intake from the fat supplement by knowing its composition and ingestion. Similar to this, we may calculate the output of fatty acids derived from the fat supplement by subtracting the LCFA output from the basal ingredients from the total LCFA output of the animals fed the supplemental fat, based on the digestibilities of the LCFA in the control diet. This technique has an advantage over other procedures involving total fat or gross energy since the proportion of LCFA provided is often larger relative to the basal diet. It is especially helpful when the LCFA composition of the test fat and the basal elements change.

2. **Stastistical Analsysis**

The linear mixed model used for ANOVA analysis was implemented in R software version 4.2.0 by the R Core Team. The model is represented by the equation:

$$\mathbf{Yijn = \mu + Ti + wkj + Tixwkj + parity\_groupedW + A(T)k + eijwkn}$$

where:
Yijn = Response variable
M =The overall mean
Ti = Fixed effect of treatment (K, PC, A, B)
Wkj = Random effect of the week (8-12)
Tixwkj = Interaction between treatment and week.
Parity_groupedW= The fixed effect of parity grouped (1,2,3)
A(T)k = The random effect of the animal within the treatment.
Eijwkn =The Residual error term.

In addition, the following information is relevant for understanding the model: 4% FCM (Fat-corrected milk, kg) is calculated using the following formula:

0.4 x milk (kg/d) + 15 x milkfat (kg/g) (NRC, 2001)

CH4 yield is expressed as g per kg of DMI (dry matter intake), and CH4 intensity is expressed as g per kg of FCM (fat-corrected milk)

Indicator methods

Sometimes the design of the study or a lack of appropriate equipment makes it impractical to assess food intake or feces output directly. For instance, it might not be able to gauge each animal's intake when they are fed in groups or while grazing. If the food contains an indicator chemical that is known to be entirely indigestible, digestibility can still be determined. Digestibility can be calculated by measuring the amounts of this indicator material in each animal's meal and in small samples of its feces, then calculating the ratio between these concentrations. For example, half of the dry matter would have been digested and absorbed if the indicator's concentration rose from 10 g/kg DM in the food to 20 g/kg DM in the stool. In terms of dry matter digestiability, this can be expressed as an equation like this.

$$\frac{indicator\ in\ faeces\ (g > kg\ DM)\ -\ indicator\ in\ food\ (g > kg\ DM)}{Indicator\ in\ faeces\ (g > kg\ DM)}$$

There are two categories of indications that can be utilized to evaluate food digestibility: internal and external. Natural dietary components like lignin, acid-insoluble fiber, and acid-insoluble ash (which is mostly silica) are examples of internal indicators. Long-chain hydrocarbons (n-alkanes, C25–C35) discovered in the waxy cuticle of leaves have also been employed as internal markers more recently, particularly in research on grazing.

External indicators, on the other hand, are things that are added to food. Because chromic oxide (Cr2O3) is so insoluble and indigestible, it is one of the most widely used external indicators. Furthermore, most foods do not naturally contain chromium (Cr). Titanium oxide (Ti2O3) is a common external indicator in non-ruminant nutrition.

Instead of measuring digestibility, external indicators like chromic oxide can be used to estimate the amount of feces produced. For this purpose, the indicator is typically given in fixed amounts for 10-15 days, usually in a gelatin capsule. Once the excretion of the indicator becomes stable, its concentration in fecal samples is determined. Based on this, the amount of dry matter in the feces produced per day (in kg) can be calculated as:

$$\frac{\text{Indicator dose (g/day)}}{\text{indicator in faeces (g/kg DM)}}$$

It is a difficult undertaking to measure the digestibility of the herbage that grazing animals consume. The challenge of obtaining representative samples of pasture herbage complicates the use of lignin as an internal indicator to assess herbage digestibility. Grazing animals favor the leaves of plants over the stems, and they also choose new plants over old ones. As a result, it is doubtful that a sample of the sward that was manually picked or clipped with a mower accurately represents the food that the animal ate.

An animal having an oesophageal fistula can be used to collect representative samples of herbage consumed. The oesophageal fistula creates a passageway from the lumen to the skin's surface. This passageway can be momentarily opened to gather the herbage the animal

has eaten and store it in a bag suspended beneath the fistula. The internal indication can then be determined by analyzing the grazed herbage samples that were obtained as well as the feces samples.

The n-alkane approach is another helpful method for determining the makeup of grazing animals' diets. This technique depends on the significant and discernible variations in the n-alkane concentration among different plant species. The method makes it possible to evaluate the composition of grazing animals' diets by comparing the pattern of faecal n-alkane output to the n-alkane pattern in various plant species.

Laboratory methods

Determining the digestibility of foods through digestibility trials can be time-consuming and expensive. As a result, many attempts have been made to replicate the reactions that occur in an animal's digestive system in a laboratory setting. Although simulating digestion in non-ruminants completely is difficult, it is possible to determine the digestibility of food protein by assessing its susceptibility to pepsin and hydrochloric acid. Additionally, digestive tract secretions can be collected through cannulae and used to digest foods in vitro.

A two-stage in vitro method can be used in a laboratory to precisely test the digestibility of diets for ruminants. First, a finely ground sample of the food is combined with buffered rumen fluid and left to incubate anaerobically for 48 hours. Adding hydrochloric acid to pH 2 kills the bacteria in the second stage, after which pepsin is used to digest the material for a further 48 hours. During this phase, some microorganisms and the undigested dietary protein are also digested. To determine how much digestible organic matter is in the food, the leftover residue is filtered out, dried, and burned. Corrective equations can be used to connect in vitro and in vivo digestibility, despite the fact that the former is somewhat lower.

Collecting rumen liquid for laboratory procedures can be challenging due to various factors. The most common method of collecting rumen liquid is through the use of a rumen fistula or stomach tube, but both techniques have animal welfare implications. Additionally, the fermentative characteristics and solids content of the rumen liquid can vary depending on the diet of the animal it is obtained from. To achieve more consistent estimates of digestibility, fungal cellulase preparations may be used as an alternative to rumen liquid.

A laboratory technique for assessing bovine food digestibility uses rumen liquid. Here, the volume of gas produced during fermentation in the rumen or test tube is used to indirectly estimate the amount of food digested. The amount of food that ferments determines how much gas is created, with carbon dioxide making up around half of the gas. The remaining material is a combination of carbon dioxide and methane that is created when proteins and carbohydrates ferment to make volatile fatty acids. Compared to other laboratory techniques, this method has the benefit of being able to be applied to a large number of food samples, particularly if the creation of gas is automatically recorded. But since it doesn't account for the breakdown of

biomass produced by bacteria, measures of gas production must be correlated with the amount of nutrients that remain after fermentation.

Apparent total tract digestibility:

In animal nutrition research, apparent total tract digestibility (ATTD) is a frequently used statistic to evaluate how well animals utilize nutrients. It is the proportion of a nutrient in the feed that the animal truly absorbs and digests rather than excretes in its feces. The following is the formula to determine ATTD:

A method for determining the apparent total-tract digestibility in live animals, originally described by (Van Soest, 1994), has been modified for field use. To determine apparent nutrient digestibility, an indigestible ADL (% of DM) was measured in both fecal and TMR samples, and used as an internal marker. The sample ADL was determined using the Ankom Fibre Analyzer A200 method, which was described by (Van Soest, 1994), The following equation was used to calculate the apparent total-tract nutrient digestibilities:

$$Total - tract\ nutrient\ digestibilities := 100 * (1 - \frac{([\text{nutrient}]\text{faeces})}{[nutrient]feed} * \frac{[\text{ADL}]\text{feed}}{[\text{ADL}]\text{faeces}})$$

Where:

[nutrient] feces = The amount of nutrients excreted in the faces

[nutrient]feed = The amount of nutrients provided in the feed

[ADL]feed = The amount of acid detergent fiber in the feed

[ADL] feces = The amount of acid-detergent fiber in the feces

Table 4.2: Nutrient intake and total apparent digestibility in mid-lactation cows fed a blend of essential oils and Tannins

	C-10	K	PC	Q-2	C-10	K	PC	Q-2	SEM	P	P	P
	10	10	10	10	12	12	12	12		t	wk	w*wk
DM_dig	66.560	72.359	79.648	82.076	85.981	77.838	67.215	80.625	3.132	0.20	0.07	0.00
OM_dig	67.741	73.162	80.438	82.671	86.425	78.685	68.528	81.244	3.101	0.23	0.06	0.00
CP_dig	50.887	64.764	70.267	76.418	80.531	71.590	56.952	75.655	4.078	0.06	0.01	0.00
NDFom_dig	70.081	72.299	74.687	78.137	79.393	66.917	50.196	69.123	4.460	0.11	0.00	0.00
NDF_dig	70.366	72.680	74.245	77.539	79.663	67.446	50.749	69.223	4.391	0.10	0.00	0.00

Table 4.3: Ingredient and chemical composition (g/kg of DM) of TMR provided to cows in both experiments Diet gross composition of weeks 8, week 10, and 12.

TMR	G/KG
Corn silage	370
Grass silage	300
Grass hay	130
Concentrate (wheat, corn gluten, soybean meal)	190
Wheat bran premix-containing additives	10

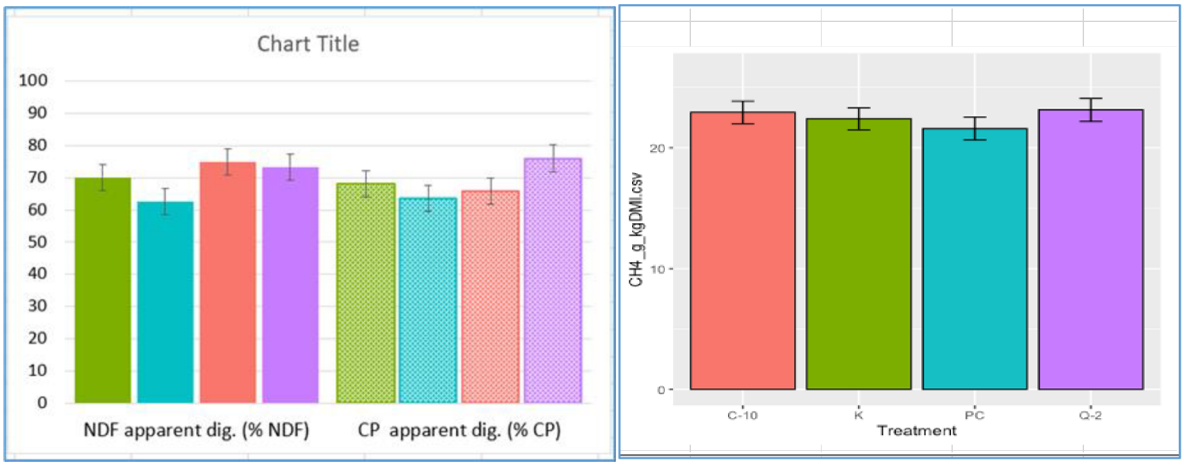

Figure 4.5: Total-Tract apparent digestability

3. Factors Affecting Digestibility:

I. Food composition:

The chemical makeup of food has a direct bearing on its nutritional value, and certain foods like barley display a consistent composition that translates into predictable digestibility. On the other hand, fresh or preserved herbs have a less constant composition, leading to greater variation in their digestibility. The amount and quality of fiber in food play an important role in determining its digestibility.

The contents of the cells dissolve when feed is treated with a neutral detergent solution, but the cell walls—which are composed entirely of cell wall material—remain as a residue known as neutral detergent fiber (NDF). Acid-detergent lignin (ADL), which represents lignin, and acid-detergent fiber (ADF), which represents cellulose and lignin, are two more divisions of the cell wall fraction. The cell contents have a true digestibility of 1.0, meaning they have been nearly entirely digested. However, because metabolites are excreted into the digestive tract, their perceived digestibility is reduced by around 10% to 15%. Conversely, the degree of lignification, or ADL, determines the digestibility of cell walls and is far more variable. Nonetheless, the digestibility of cell walls is also influenced by the tissue structure of the plant. For example, because tropical grasses have more vascular groups and therefore more lignin in their leaves than their calm counterparts, they are often less digestible. Additionally, they feature dense cell masses that prevent microbial invasion.

II. Ration Composition:

The edibility of food can be affected by many factors, including how it is made and what it is eaten with. These factors can either positively or negatively impact the food's edibility. Positive impacts occur when combining certain foods enhances their edibility. For instance, mixing protein with low-quality feed like straw can improve the digestibility of the straw, by helping the microorganisms in the rumen break down the straw more efficiently. Negative impacts occur when combining certain foods reduces their edibility. For example, adding a carbohydrate supplement like starch to feed can decrease the digestibility of the feed. This is

due to the rumen's pH dropping to 6 or lower as a result of the quick fermentation of starch to volatile fatty acids. The action of cellulolytic microbes is inhibited by this low pH, which lowers the digestibility of the fiber in the feed. Apart from its pH-related impact, starch may also adversely affect cellulolysis. While certain microbes are capable of fermenting both cellulose and starch, when both are available, they may preferentially ferment starch. This means that diets high in starch can reduce cellulolysis, even when buffering agents like sodium bicarbonate are added to the feed.

III. Food Processing:

Foods are frequently treated to maximize and improve their digestibility prior to feeding. Typically, the most common treatments used are crushing, grinding, chaffing, and chopping. Cereal grains should normally be ground for pigs and crushed for cattle to prevent them from passing through the digestive tract whole. Cattle that are fed cereal grains that have been ground may have a higher rate of fermentation, which puts the animal at risk for rumen acidosis. Contrarily, sheep can efficiently chew entire grains during rumination, which lessens the need for mechanical processing and other factors impacting digestibility. However, this seems to be overlooked by the dynamics of regurgitation, which are influenced by the kind of cereal grain and the composition of the basal diet. Regurgitation appears to be more challenging when cereal grains are offered in conjunction with forages such as silage; oats, on the other hand, tend to regurgitate more effectively than barley. This implies that trapping in interlaced particles and grain shape might be important contributors. For this reason, if cereal grains are given silage, they should be ground into a powder.

Cereal straws are one example of a forage that can be chemically treated to extract the cellulose and lignin constituents. The main substances used are alkalis, namely sodium and ammonium hydroxides, which cause cereal straws' dry matter digestibility to rise dramatically from 0.4 to 0.5–0.7.

IV. Enzyme supplementation of foods:

Among animals that are not ruminants, the stomach related framework is not suitable to manage a few food sources because the creatures need fitting catalyst frameworks. Compound arrangements (ordinarily of contagious beginning) might be added to food sources to increment supplement accessibility. β-glucanase has proven to be the most consistently effective component added material when used in poultry slims down containing grain. A significant portion of the endosperm cell mass in grains is made up of β-glucans, which are usually inedible. If they manage to elude absorption, they manifest as gels in the excreta that result in unintentional "tacky droppings." β-Glucans also prevent the assimilation of other food components. As a result, their enzymatic destruction improves edibility overall.

V. Animal factors:

The food's capacity for digestion has more to do with it than the animal eating it. However, this does not mean that different animals will absorb food in the same way. The main animal factor affecting digestibility is the species. While both ruminants and non-ruminants are capable of digesting low-fiber diets equally as well, ruminants are better at digesting high-fiber foods. Because pigs excrete less metabolic fecal nitrogen than ruminants, their apparent digestibility coefficients for protein are often greater in pigs. Since the differences in digestibility between sheep and cattle are typically negligible and unimportant, digestibility values derived from sheep are frequently transferred to cattle. But extremely digestible foods. Meals that are less easily digested, like inexpensive roughages, digest better in cattle, while meals that are more easily digested, like cereal grains, digest better in sheep. For instance, because whole grains pass through the digestive tract intact in cattle as opposed to sheep, the digestibility of the grain component in whole-crop cereal silages is lower in cattle. However, levels for digestibility determined in sheep may not always translate to cattle.

VI. Level of Feeding:

The pace at which food moves through an animal's digestive tract quickens with increased consumption. As a result, the food's digestibility is decreased because it is exposed to digestive enzymes for a shorter amount of time. The quantity of food needed to keep an animal in balance, or "maintenance," is commonly expressed as multiples of the animal's feeding level, which is equal to one. The food level for animals that are growing and fattening is usually two to three times their maintenance requirement, but the feeding level for nursing animals is three to five times their maintenance requirement.

Hay, silage, and grazed grass are examples of high-fiber diets whose digestibility decreases very little when the feeding amount is increased by one unit (e.g., from maintenance to double maintenance). For these diets, the digestibility decrease is only 0.01-0.02. On the other hand, digestibility drops by 0.02-0.03 for mixed diets and smaller particles for every unit increase in feeding level.

Dry matter digestibility for a normal dairy cow diet might drop from 0.75 at maintenance feeding levels to 0.70 at three times maintenance. Negative associative effects which become more prominent at higher feeding levels are the cause of this decrease in digestibility. The digestibility of ground and pelleted forages and some fibrous by-products decreases the most when feeding levels rise (0.05 per unit change in level). This is because longer forages require more extensive fermentation in the rumen before they can be further processed through the digestive system, whereas meals with smaller particle sizes pass through more quickly.

5 Results

The purpose of the study was to evaluate the effects of essential oil (EO) administration on various ruminal and milk parameters. The study found that the administration of EO had no significant influence on a multitude of ruminal parameters, including pH levels, limonene, carvacrol, and eugenol concentrations, as well as the total amino acid (AA) content, total volatile fatty acids (VFA), and the essential oil compositions of Thyme and Oregano. Similarly, the protozoal population and polysaccharide-degrading activities remained unaffected by EO supplementation.

Interestingly, the study did observe a notable 40% elevation in ruminal ammonia concentration amongst cows subjected to EO treatment, but this elevation did not reflect any significant treatment effect. The EO supplementation also did not have any substantial impact on crucial metrics such as dry matter intake, milk production, 4% fat-corrected milk (FCM) output, or FCM feed efficiency. Although there was a marginal improvement in feed efficiency, its significance in treatment outcomes was inconclusive.

Furthermore, milk constituents, including lactose, protein, and fat, exhibited no notable alterations in response to treatment. While there were slight increases in lactose and milk protein yields with EO supplementation, the overall difference was marginal. Notably, dietary nutrient composition and dry matter intake remained consistent across diets during digestibility assessments.

The apparent total-tract digestibility of nutrients remained largely unchanged, except for a discernible increase in neutral detergent fiber (NDF) digestibility. However, EO supplementation failed to induce any notable effects on various parameters, including feed efficiency, milk urea nitrogen (MUN) concentration, dry matter intake, 4% FCM yield, milk composition, as well as milk fat, protein, and lactose yields.

Moreover, when compared to the control group, milk yield in EO-treated cows frequently exhibited a slightly lower trend, albeit without significant deviation. These findings suggest that EO administration has a limited effect on ruminal and milk parameters, and further research is necessary to explore the potential benefits of EO supplementation in dairy cow diets.

5.1 In vivo experiments

Due to technological difficulties, the methane production data for week eight were unreliable. Consequently, the outcomes of weeks 0 through 12 served as the foundation for the analysis and interpretation of this parameter. The experiment demonstrated that, as the study came to an end, milk output dropped linearly. However, during the trial, there was no change in the amount of fat, protein, or lactose in milk. Measurements were also made of the absolute daily enteric CH4 production (g/d), the CH4 relative to DMI (g/kg DM), and the CH4 relative to milk production (g/kg milk). In comparison to the period without the addition (week 0 vs. weeks 10 to 12), the results indicated that the addition of a blend of essential oils tended to

diminish CH4 generation in g/d and g/kg DM. By the end of the experiment, the reductions in CH4 production were responsible for 14% (g/kg DMI) and 15% (g/d). Nevertheless, when CH4 was expressed about milk production, these disparities vanished. It is important to note that a comparison in these units might be faulty because of the current experimental setup, which evaluated the supplementing impact throughout time and recorded the decline in milk production as lactation progressed.

5.2 In vitro organic matter digestibility and net dissolved hydrogen

When coupled with either of the two substrates, none of the tested concentrations during the experiment showed any effect on the production of CH4. Furthermore, the inclusion of essential oils did not affect the overall or specific VFA amounts. The Concentrate+Corn silage substrate, however, produced more CH4 than the Concentrate+Corn silage+Grass silage substrate due to a substrate impact that was noticed. However, the discrepancies vanished when CH4 was represented with the overall amount of VFA produced. Additionally, there were no interactions between the essential oils and the substrate. The kind of substrate or the interaction between the substrate and essential oils did not affect the overall synthesis of VFAs. The concentrate+grass silage+corn silage substrate was used in all ensuing trials because there were no interactions between the essential oils and the substrate.

The computed IVOMD was lowered by the addition of tannins, either alone or in conjunction with carvacrol. Comparing the C/Q addition and the C/Q group (4.5% vs. 7.2%) and the Q addition and the Q group (3.5% vs. 7.1%), the reduction in IVOMD was comparable between the C addition and the C group (5.7% vs. 5.5% on average). Nevertheless, no discernible variation was seen amongst the various tannin-containing additions (data not shown). In comparison to the control diet, the net H2 concentration dissolved in the incubation liquid decreased by up to 12% in the majority of the C and Q group mixes.

5.3 Fermentation gas production and composition

With most treatments, there was a noticeable reduction in gas production (GP) with the addition of supplements. With single additives or mixtures of additives, such as group C treatments (6.7 to 9.8%), group Q treatments (6.8 to 14.9%), and group C/Q treatments (7.6 to 12.2%), the GP decreased by 5.5 to 8.1%, with no discernible variations between the mixtures. Along with the addition of C, C/Q, and carvacrol, as well as with all mixes other than C-11, GY (green yield) also reduced. In terms of mitigating potential, methane production and CH4 yield exhibited a similar trend to that of GP. With very few exceptions where there was no discernible difference, tannins either as single additions or in combination with an EOC blend decreased CH4 generation by 6.4 to 13.9% and CH4 yield by 7.9 to 15.0%. Only CH4 output was considerably impacted by treatment Q-6; yield and CH4 production were not significantly affected by Q-10, Q-11, or Q-12.

In one investigation, the addition of EO/EOC sources significantly affected CH4 production. When CH4 was expressed per unit of digestible organic matter (DOM) or moles of

total volatile fatty acids (VFA) generated, however, the impact pattern shifted. Tannins reduced CH4 per unit of DOM when applied as a single additive, but not per mole of total VFA generated. Of the thirty-six multiple mixtures, nine from group C, four from group Q, and two from group C/Q were among the fifteen mixture additives that were successful in reducing CH4 per unit of DOM. Nine, comprising two from group C, five from group Q, and two from group C/Q, likewise showed a decrease in CH4 expressed per moles of VFA. Regardless of how it was expressed, only six treatments—C-4, C-10, Q-2, Q-7, Q-8, and C/Q-8—lowered CH4. Ten supplements, five single EO sources, and five combinations were ineffective, but 38 of the 48 treatments considerably decreased the absolute amount of CO2 produced after 24 hours, with an average reduction of 9.8% compared to the control. Similar results were observed when the pattern was expressed per unit of feed dry matter. However, none of the EO/EOC sources that were supplemented by themselves produced a noticeable decrease. Although the ratio tended to be larger in the a-pinene and Q-4 treatments compared to the control ($P<0.10$), none of the treatments affected the CH4-to-CO2 ratio.

6 Discussion

Research has indicated that the rumen can experience a decrease in CH4 and NH3 through the action of tannins and essential oil compounds (EOC). The combined effects of these substances, whether favorable or unfavorable, have not yet been fully investigated. The goal of a recent in vitro screening method was to find the optimal tannin and EOC combinations that would have the least negative impacts on CH4 and NH3 mitigation (1526 G. FOGGI ET AL). The pure EO/EOC exhibited no discernible effects on CH4 and NH3 at the concentration tested, in comparison to the control group, suggesting that the tannin content of the supplements was important in decreasing CH4 and NH3. When contrasting treatments that contained pure tannins versus a combination of tannins, the results were typically not significantly different. However, the generation of NH3 was significantly reduced in six supplement combinations that contained chestnut extract. The study also showed that while EO/EOC contributed to the overall VFA reduction, tannins are responsible for the feed value drop seen when mixes are supplemented. The additives with the least amount of feeding value loss attracted the attention of the researchers since the ruminant environmental impact cannot be mitigated by additions that merely lower absolute CH4 production. As a result, the study, where feasible, examined CH4 per mole of VFA generated as well as per unit of digestible OM (DOM).

6.1 Tannins

6.1.1 General effects of tannins

Research has indicated that the impact of consuming tannins is mostly related to their chemical makeup and the amount consumed (Cardoso-Gutierrez et al. 2021). A minimum effective dose of tannins (equal to 20 g/kg or 133 mg/L incubation fluid) was determined to be necessary for the research conducted by Jayanegara et al. (2012) to considerably reduce CH4 generation. In a similar vein, this investigation also used tannins at a dosage of 20 g/kg, which significantly reduced the generation of CH4 and NH3. Other research, however, has only shown an impact on CH4 at levels more than 50 g/kg DM. Benchaar and Hassanat (2013). It is noteworthy that Mueller-Harvey (2006) found that the maximum suggested dosage is likewise 50 g/kg to avoid negative effects on feed value, especially in CT. Numerous studies have demonstrated the dose-dependent link between tannin supplementation and the depression of CH4 and NH3, wherein larger dosages result in a reduction of feed value. However, the fermented diet and the in vitro conditions both affect how tannins affect the generation of CH4 and NH3. Half of the dosage utilized in this study, or double the dose of chestnut wood extract added to the basal diet, produced comparable effects on the synthesis of CH4 and NH3, according to a recent in vitro investigation (Cappucci et al. 2021). Menci et al. (2021) observed in a different investigation that adding 30 g tannins/kg feed decreased absolute CH4 output by 6% but did not affect CH4 production per gram of degraded feed.

The lack of substantial differences in the digestibility of dry matter between treatments may be related to the concurrent decline in fiber degradation in vitro. Some studies' findings suggest that the crude protein (CP) content of the feed may have an impact on the effective

tannin content. Put otherwise, the higher the concentration of CP, the higher the amount of tannins needed to have a moderating effect. It is imperative to consider the potential decrease in feed value that can arise at elevated tannin concentrations.

6.1.2 Effects of combinations of condensed and hydrolyzable tannins

The purpose of the study was to see whether it would be possible to combine various chemicals to increase their ability to mitigate rumen fermentation. This was accomplished by fermenting a 1:1 combination of C and Q, both by itself and in conjunction with EOC combinations. The effects of combining quebracho and chestnut extracts on the properties of rumen fermentation have been examined in earlier research. Menci et al. (2021), for example, found no discernible difference in performance between adding quebracho alone and adding a blend of chestnut quebracho. However, the combination caused NH3 to be more attenuated in vitro. In a similar vein, Aboagye et al. (2018) examined the effects of supplementing calves fed a high diet with chestnut extract alone or in combination with quebracho extract (1:1) at doses of 2.5 and 15 g/kg. Their investigation revealed that NH3 levels in the rumen decreased irrespective of the kind and quantity of ingestion. Nevertheless, there was no impact on CH4, and neither did animal performance nor overall VFA output decline. These outcomes are in line with studies that discuss quebracho or chestnut supplementation alone. However, the addition of 18 g/kg of a chestnut:quebracho (1:2) to dairy cows in a prior study (Duval et al. 2016) yielded a difference of 2.6 g/kg TMI of CH4. Interestingly, the difference became substantial only after 90 days of supplementation, indicating that, unlike NH3 in the rumen, which was significantly lowered at the 45-day initial measurement time point, a longer exposure is needed to accomplish CH4 reduction.

6.2 Essential oils

6.2.1 General effects of essential oils

The effects of different essential oil sources on hyper-NH3-producing bacteria and methanogens have been the subject of numerous investigations, as the literature (Benchaar et al. 2007; Patra and Yu 2012; Joch et al. 2015; Rofiq et al. 2021) describes. It is crucial to remember that the efficiency of the mixture can be significantly impacted by the quantity of essential oil applied. Instead of indicating the quantity of intake based on the diet's DM, the amount of essential oil provided was often reported as the concentration in the buffered rumen fluid in research. Therefore, in this study, the amounts investigated for individual essential oil and essential oil compound sources were 67 mg/L (equivalent to 10 g/kg feed), while EOC mixtures were tested at concentrations of 67-100 mg/L (equivalent to 10-15 g/kg feed) in the incubation fluid (Patra and Yu (2012) have emphasized the importance of dosage, as increasing the amount of essential oil from 250 to 1000 mg/L led to a consequent reduction in CH4 and NH3 formation, but also drastically impaired the feed degradability.

6.2.2 Effects of essential oil mixtures

Note that mixes of EOC are used in the formulation of some commercial additives, including Agolin RuminantVR and CrinaVR Ruminant, to lower CH4 emissions (Tomkins et al. 2015; Belanche et al. 2020). In research by Castro-Montoya and al. (2015), coriander oil, geranyl acetate, and eugenol were examined in cattle (0.2 g/day) and in vitro (30 mg/L incubation fluid) under low concentrations of Agolin RuminantVR. While the in vivo study reported a significant reduction in CH4 production (-15% with 0.2 g/day, the recommended dosage by the manufacturer), the in vitro study demonstrated that the dosage was insufficient to detect any significant effects. This finding was recently confirmed in a meta-analysis by Belanche et al. (2020). Conversely, Crina Ruminants or Agolin Ruminants at 16.7 mg/L (i.e., 1 g/head and day) did not affect CH4 emissions (Pirondini et al. 2015), but the former decreased total VFA by more than 22%.

The low dose levels examined were the reason for the in vitro study's findings indicating that Agolin Ruminant's moderating effect on CH4 was not substantial, according to the study's authors (Castro-Montoya et al. 2015). On the other hand, as noted in other research (Newbold et al. 2004; Tomkins et al. 2015), Crina supplementation in cattle (1-2 g/day) and sheep (0.1 g/day) did not affect rumen fermentation, CH4, or NH3 production. There have been several attempts to augment triple EO mixtures—eucalyptus, cinnamon, rosemary, and oregano—in vitro. When these mixes were evaluated at a concentration of about 800 mg/L, feed degradability was very little impacted, although CH4 and NH3 levels decreased (Cobellis et al. 2016). The quantities of the EO/EOC mixture (67-100 mg/L) in the EO-tannin mixes were less than the amounts that had effectively reduced the production of CH4 and NH3 in earlier investigations. However, because the mixtures in this investigation included a tannin source and the EO and EOC were blended in a different way than in other testing, the results were unique.

6.2.3 Effects of mixtures of tanning agents and essential oils

Under the present experimental setup, it was found that the addition of EOC mixes to tannins improved these compounds' capacity to reduce CH4 (particularly when expressed per total mole of VFA) and NH3 production. It's interesting to note that the addition of tannin-EO mixtures had no discernible effect on the degradability of OM in the feed. Except for three combinations that mixed mixes 10, 11, and 12 with Q extract, it was found that the majority of the tannin-EO mixtures decreased absolute CH4 generation and yield. These concoctions included citrus peel, a-pinene, bornyl acetate, and essential oils of oregano and thyme. Notably, the other characteristics that were considered were consistent with the other combinations that exhibited mitigating properties, making it difficult to explain this discrepancy. In addition, CH4 was expressed as a mole of total VFA produced (CH4/VFA) and as a unit of digestible OM (CH4/dOM) to account for the modest feed value decreases caused by the tannin-EOC mixtures. Comparing the mixes to the control diet, only six (C-4, C-10, Q-2, Q-7, Q-8, and C/Q-8) demonstrated a simultaneous decrease in CH4/dOM and CH4/VFA.

It is noteworthy that certain combinations of essential oil compounds (EOCs) with tannins (both C and Q) produced similar moderating outcomes. Similar outcomes were seen, for example, when C tannins were coupled with EOC mixes 4 and 10, or when Q tannins were paired with EOC blends 2 and 8. Interestingly, EOC blends 2 and 8 shared eugenol content and blends 4 and 10 shared citrus peel content. This shows that regardless of the existence of thymol-carvacrol EOC (blends 2 and 4) or thyme-oregano EO (blends 8 and 10), there may be an ideal synergistic effect between limonene (the primary active element in citrus peels) and C extract, or between eugenol and Q extract. It's also important to note that Q-8 was the only therapy that concurrently decreased CH4 and NH3 levels and had no detrimental effects on overall VFA production.

Studies have demonstrated that even at low concentrations, specific combinations of tannins present in quebracho and chestnut extracts can effectively reduce methane gas in the rumen. It was discovered that these tannin-containing additions were very effective at reducing the production of ammonia in the rumen, with certain C-group treatments exhibiting the greatest potential. The tannins' ability to bind proteins and the extracts' ability to directly inhibit NH3-producing bacteria were probably the main causes of these mixtures' inhibitory effects on NH3 synthesis. It's interesting to note that adding some EO-tannin mixes from the C/Q group unexpectedly increased the generation of iso-butyric acid, indicating more valine deamination than in the control. Overall, our results point to the possibility of a synergistic interaction between tannin extracts and specific essential oil components in lowering methane production and preventing ammonia synthesis in the rumen.

7 Conclusion

When applied in combination treatments, tannin-containing extracts from quebracho and chestnut have been shown to have the best results in reducing the production of CH4 and NH3. Even though EO/EOC's primary function is to assist attenuation, particularly in the context of combining digestible OM and total VFA with CH4 generation, it does have a negligible detrimental impact on feed value. There are just six mixed remedies that have shown promise, and further research is required to comprehend the working principles and complementary benefits of these formulations. It is also crucial to remember that in vivo investigations are necessary to verify the effectiveness of the combinations under investigation in living animals and to evaluate how long the beneficial effect lasts on the lowering of CH4 and NH3.

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