Czech University of Life Sciences Prague

Faculty of Agrobiology, Food and Natural Resources

The Department of Microbiology, Nutrition and Dietetics

Feeding biochar to dairy cows: Effects on feed intake and feeding behaviour

Bachelor's Thesis

Catherine Anne Portway
Agriculture and Food

Ing. Miroslav Joch, Ph. D.

Declaration

I hereby declare that I have authored this bachelor's thesis carrying the name "Feeding biochar to dairy cows: Effects on feed intake and feeding behaviour" 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 bachelor's thesis, I further state that I have not infringed the copyrights of third parties in connection with its creation.

In Prague on 21. 4. 2023 ____________________________

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Feeding of biochar to dairy cows: Effects on feed intake and feeding behaviour

Summary:

In the past decade, several studies have taken place on the use of biochar as a regular feed supplement in livestock. This thesis has focused on the effects of biochar on the feed intake, rumination time and milk production in dairy cows. The effects were tested on 34 cows, which were divided into two groups, a group with a control diet (CTL) and a group with biochar supplementation (BIO) for 30 days after that the diets were swapped between the groups. The total length of the experiments was 60 days. We hypothesized that biochar in the diet of dairy cows would not decrease dry matter intake (DMI) eating intensity, and rumination time. This was confirmed, however the DMI had a tendency to decrease, which might have been due to the high adsorption properties of biochar leading to certain nutrients being bound and becoming unavailable for the cow for digestion. Furthermore, in the cows supplemented with biochar a decrease in milk production has been observed. So far, this thesis is one of few which focused on the effects of biochar on milk production in dairy cows and further research needs to be done to ensure no negative effects on the productivity of dairy cows and further understand the processes occurring in the body.

Keywords: biochar, feed additive, dairy cow, feed, fermentation

Content

1 Introduction

Biochar can be defined as a carbon-rich substance produced by pyrolysis; a thermochemical decomposition of biomass with a temperature about ≤700 °C taking place in anaerobic conditions. Biochar has been proven to improve physiochemical and biological properties of soil, herewith lowering the necessity of the use of fertilizer. With time, studies have taken place on the topic of feeding animals with biochar and then using the manure containing the biochar as an effective soil conditioner. Biochar feed supplements have shown to have several health benefits for the animal and further improving the effectivity of manure and biochar as a soil conditioner.

As a matter of fact, it has been shown that biochar can detoxify mycotoxins in feed, control pathogenicity, regulate heavy metals, organic pollutants and residues from pesticides and herbicides and generally improving the cattle´s immune system, leading to lower veterinary costs. Moreover, many studies have also recorded increase in feed intake, weight gain and generally feed efficiency. Potentially, biochar could work as an alternative to substances such as antibiotics.

Additionally, several studies have taken place on the use of biochar as an animal feed additive with the goal of reducing the production of green-house gas emissions with some success. Biochar has shown potential to reduce the production of green-house gas emissions in the rumen, but further research is necessary to understand the mechanisms behind it.

Overall, biochar has shown the potential to work as a feed supplement in animal nutrition. However, it has been under research a relatively short period of time and it is a relatively new concept, thus part of the results are inconclusive and some mechanisms of action are not yet understood. The aim of this thesis is to contribute to the knowledge concerning the effects of biochar on rumen fermentation and performance of cattle.

2 Objective of the work

The bachelor thesis aims to determine the effects of dietary inclusion of biochar on feed intake and feeding behaviour of dairy cows. We hypothesize that biochar in the diet of dairy cows does not decrease dry matter intake (DMI), eating intensity, and rumination time.

3 What is biochar?

We can define biochar as a carbon-rich substance produced from organic feedstock as a yield of pyrolysis; a thermochemical decomposition of biomass with a temperature about ≤700 °C taking place in anaerobic conditions (Jamaludin et al. 2019) or in other words, biomass is heated up in a closed container with little or no air. During pyrolysis complex chemical compounds are turned into simpler ones. At the end of the process, we obtain gaseous products (e. g. water vapour, carbon dioxide (CO_2), carbon monoxide (CO), hydrogen (H_2), methane (CH_4), ethane (C_2H_6)) and what is often called "char", solid carbon residue. The name "biochar" implies that the product has been attained from biomass. There is a wide variety of materials, biochar can be produced from, influencing its properties. Some examples can be hay, corn stover, bagasse, switchgrass, woodchips, rice hulls, sewage sludge or animal manure (Kalus et al. 2019). Biochar is primarily made up of carbon (60-90 %) and in addition it contains hydrogen, oxygen, and mineral ash from the parent biomass (Basu 2018; Santos et al. 2019).

Biochar has a wide range of applications such as but not limited to flue gas cleaning, building material, metallurgical applications, environmental remediation, medical use, and what this thesis is focused on, use in agriculture and animal husbandry (Weber & Quicker 2018). Biochar has many favourable characteristics e. g. high carbon content, large surface area enabling adsorption of heavy metals, pollutants, etc., high cation exchange capacity and stable structure. These and other properties have intrigued many, showcased in the increased number of published studies and articles concerning this topic in the last decade.

Properties of biochar can differ, as already mentioned, according to the biomass it has been produced from and production conditions such as the temperature used. Generally, with increasing temperature also the surface area increases leading to the improvement of its adsorption capacity. The higher temperature causes removal of volatile organic compounds leading to an increase of micro-pore volume. But at the same time higher temperature causes lower biochar yields, therefore there needs to be a certain strategy in the production regarding the adsorption capacity and yield. The physiochemical properties of biochar can be also modified by the use of acids, alkali, oxidizing agents and metal ions. Goal of these modifiers can be the increase of surface area, change of functional groups or the increase of its catalytic capacity (Wang & Wang 2019).

In addition, when comparing biochar to activated charcoal an important difference is their final purpose. The debate on biochar originally began with research concerning highly fertile anthropogenic soils rich in pyrogenic organic matter such as the Amazonia dark earth, leading to the idea of its use as a soil conditioner. In comparison, the activated charcoal is mainly used for its absorptive properties, which have been utilized already for several thousand years. For example, the Romans made use of charcoal to purify water. The production of activated charcoal includes the process of activation, which can be the chemical or structural alteration with the aim to increase its surface area, therefore increasing its absorptive properties (Hagemann et al. 2018).

All in all, biochar can be developed through a wide range of conditions and from many different materials influencing its properties. The conditions and biomass used is determined by the utilization of the biochar.

4 Use of biochar in agriculture

An important function of biochar in agriculture is its use in soil remediation. It has been shown that biochar improves physiochemical and biological properties of soil e. g. it increases water retention, increases pH and microbial activity. Herewith lowering the necessity of the use of fertilizer, therefore biochar can play a role in mitigating climate change in the future (Hagemann et al. 2018). The benefits of using biochar as a soil conditioner have been recognized already for some time. An example can be a statement from the President of Highland Agricultural Society of Ohio in 1850 stating: "We have evidence upon almost every farm in the county in which I live, of the effect of charcoal dust in increasing and quickening vegetation. The spots where charcoal pits were burned 20, and some say even 30 years since, still produce better corn, wheat, oats, vegetable, or grass, than adjoining lands" (Trimble 1851).

The idea of adding biochar to soil originates mainly in the studies of sustainably fertile anthropogenic soils rich in pyrogenic organic matter originally found in Amazonia carrying the name "Terra Preta" soils (Hagemann et al. 2018) or also known as Amazonian Dark Earths (ADE) (Lehmann 2009). These soils are not only rich in nutrients such as nitrogen, phosphorus, potassium, and calcium, but they also contain high amounts of stable organic matter such as charcoal and highly aromatic humic substances. It has been shown that the Terra Preta soils contain 70 times more black carbon in comparison to the surrounding soils. The black carbon has a polycyclic aromatic structure, which makes it chemically and microbially stable enabling it to persist in the environment for centuries. It can undergo oxidation, during which it produces carboxylic groups on the edge of the aromatic backbone leading to an increase of nutrient-holding capacity (Glaser et al. 2011).

These soils also differ from others in their unique microbial composition, which can form in the specific conditions the ADE offers. It is possible, that the unique microbial communities change soil nutrients and carbon dynamics encouraging the sustainability of Terra Preta soils (Lehmann 2009).

The research has shown that black carbon is a key element for sustainable and fertile soils, especially in humid tropics. It can be used as an agent to transform poor soils into highly productive agroecosystems (Hagemann et al. 2018). Besides the already mentioned benefits of biochar, when adding biochar together with organic or inorganic fertilizer, the biochar may cause a slower release of nutrients and a lower risk of leaching losses (Lehmann 2009), which can be especially beneficial in humid tropics where high risk of leaching losses is present due to high rainfall when applying inorganic fertilizer (Syuhada et al. 2016).

Biochar was originally described as carbonized biomass or even charcoal utilized for agricultural purposes, mainly for soil amendment. With time studies have taken place on the topic of cascading uses of biochar. An example can be that instead of applying biochar directly to soil, it could be mixed into manure or directly incorporated to the manure through feed additives. Biochar feed supplements have shown to have several health benefits for the animal and further improving the effectivity of manure and biochar as a soil conditioner. The biochar in manure reduces odours and nutrient losses not only in fresh form, but also during its composting and the end-product functions as a slow releasing fertilizer (Hagemann et al. 2018).

5 Reasons to add biochar into animal feed

Adding biochar into animal feed is not as unnatural as one might think. It has been found that some mammals eat biochar or charcoal in the wilderness. Charcoal residues can be found in forests after wildfires and can stay there for years. An example could be deer and elks eating from charred trees in Yellowstone National Park or domestic dogs eating charcoal briquettes. And a very specific example is also a monkey called Zanzibar red colobus (*Procolobus kirki*), which lives on a Zanzibar Island and regularly eats charcoal to help with the digestion of young Indian almond or mango leaves, which contain toxic phenolic compounds (Biser 1998). Activated charcoal has already become quite a common feed additive in cattle and it is used as a preventative method thanks to its adsorbing toxins, or it can be mixed with wood vinegar as treatment for several diseases in calves. It has also been known as an emergency poisoning treatment in humans and animals for centuries (Schmidt et al. 2019; Nanda et al. 2015). But there have not been many studies conducted for the use of biochar as a regular component of everyday feed. One of the reasons is that the feeding of biochar connects two fields that do not have much in common and that is the veterinary field and biochar research. Despite that, the practice is rapidly spreading in countries such as Switzerland, Austria, and Germany (Kammann et al. 2017).

6 Effects of biochar on the cattle digestive system

Biochar has several benefits when used as a feed additive in animal nutrition. It has been shown that biochar can detoxify mycotoxins in feed, control pathogenicity, regulate plant-derived toxins, heavy metals, organic pollutants and residues from pesticides and herbicides. It has also shown effects on the rumen microbiome and the process of rumen fermentation leading to improved feed digestibility, feed efficiency and weight gain. Potentially, biochar could work as an alternative to substances such as antibiotics (Lao & Mbega 2020).

6.1 Adsorption of toxins

The structure of biochar and activated charcoal is extremely porous with a large internal surface area. This internal surface enables the removal of contaminant through adsorption or as toxin bounder (Hansen 2012). The process of adsorption of toxins by charcoal in the gastrointestinal tract has been characterized by Schirrmann (1984). The process has been described as follows:

1. Adsorption of proteins, amino acids, and amines.
2. Adsorption of digestive tract enzymes, as well as adsorption of bacterial exoenzymes.
3. Binding, via chemotaxis, of mobile germs.

4. The selective colonization of biochar with gram-negative bacteria might lead to decreased endotoxin release as these toxins could be directly adsorbed by the colonized biochar when gram-negative bacteria die off.

Furthermore, biochar possesses the ability to adsorb lipophilic and hydrophilic toxins and directly remove them from the blood plasma, as the adsorption power of the large surface area interacts with the permeability properties of the intestine. This ability is known as "enteral dialysis" (Schirrmann 1984).

6.1.1 Adsorption of mycotoxins

Mycotoxins, secondary metabolites produced by specific fungi, represent a high health hazard in both animal and human consumption. Exposure to mycotoxins is related to several acute and chronic diseases both in animals and humans (Iheshiulor et al. 2011). Mycotoxins also pose an economic threat. All contaminated products need to be destroyed, which can lead to large economic losses. To the most frequently occurring mycotoxin groups in animal feed belong aflatoxins (AFs), fumonisins (FBs), ochratoxin A (OTA), trichothecenes (TCs) and zearalenone (ZEN) (Tolosa et al. 2021).

Nowadays, there are two main strategies, in one, efforts are being made to control mycotoxins during pre-harvesting and the other focuses on remediation of contaminated commodities during post-harvesting. One of the methods used during post-harvest treatment is the addition of adsorption agents (binders) to the livestock feed, which can restrict the passage of mycotoxins into the animal´s blood and organs by creating a complex between the binders and the mycotoxins leading to lesser harmful effects. Commonly used are mineral or organic adsorbents such as smectite, montmorillonite, sodium aluminosilicates and one example can be also activated charcoal. The ability of the adsorbents to bind mycotoxins is influenced by the structure of the binders and mycotoxins, or in other words, how compatible are they in the terms of charge distribution, pore size, polarity, shape, etc (Zhu et al. 2016).

Activated biochar has shown potential in absorbing different types of mycotoxins in animal feed. An example can be a study conducted by Galvano et al. (1996a) where they researched the adsorption behaviour of biochar and its capability to reduce the passage of toxins into the digestive tract. They added 2% activated biochar to a pelleted AF-spiked feed for dairy cows and it lowered the concentration of extractable AF in animal feed by up to 74%, in milk by 45%. However, the study has also revealed major differences in adsorption efficiency between different types of biochar.

Galvano et al. (1996b) have also studied the adsorption capacity for OTA and deoxynivalenol of 19 different activated carbons. The different types of activated biochar were able to absorb 0.80-99.86% of OTA and up to 98.93% of deoxynivalenol. The wide range of results once again reveals the importance of the systematic characterization and classification of biochar.

6.1.2 Adsorption of bacterial pathogens and their metabolites

Biochar is able to influence the ratio of different bacterial groups in the digestive tract and therefore also in the manure. According to Shirrmann (1984), biochar has an especially strong adsorbing capacity for gram-negative bacteria with high metabolic activity. An example of such a bacterium is E. coli., which can cause mild to severe illnesses in humans. And is usually transmitted to humans either by direct contact with contaminated species or through faecal contaminated water or food (Duffy et al. 2014). In a study from Korea, pigs were fed 0.25% activated biochar or 0.50% coconut tree biochar and the number of E. coli present in the manure substantially decreased and at the same time the number of beneficial bacteria Lactobacillus grew in 10 days of the trial (Kim et al. 2017). Biochar has also shown the ability to lower the spread of bacteria in water and soil by adding it to the manure. Gurtler et al. (2014) have researched the ability of biochar to inactivate E. coli O157: H7 (EHEC) when applied to soils. Twelve types of biochar were used, all of them significantly lowered the concentration of EHEC. The most effective were fast pyrolysis of barley and oak log feedstock, where after 4 weeks EHEC was untraceable in a cultivation-based assessment. The regular feeding of biochar could potentially prevent the spread and outbreak of pathogenic bacteria such as E. coli, but further research is necessary.

A concern appearing could be the risk of biochar negatively affecting the digestive tract microflora, when fed long-term. According to a study focusing on the adsorption capacity of biochar for verotoxin producing E. coli and gram-positive bacteria naturally occurring in the intestinal microflora (*Enterococcus faecalis, Bifidobacterium thermophilum, Lactobacillus acidophilus*), biochar did absorb healthy bacteria, but at a much lower rate compared to the pathogenic E. coli. It is possible that the pore size was more compatible with the size of the E. coli compared to the gram-positive bacteria (Naka et al. 2001). So far, research indicates that the impact of biochar depends on the cell envelope of the microorganism, where gram-negative bacteria are better sorbed compared to gram-positive bacteria, but the gram-stain is not the detrimental characteristic deciding if a bacterium is pathogenic or not. More research is needed for understanding the adsorptive characteristics of biochar on different bacterial group (Schmidt et al. 2019).

6.1.3 Adsorption of drugs

Treatment of intoxication or poisoning with activated charcoal has been a commonly used practice already for some time. Activated charcoal has an ability to absorb a wide range of substances with only a few known exceptions such as cyanide, alcohols, and metals (e. g. iron or lithium). Due to this characteristic charcoal can absorb toxic substances before the toxins are absorbed into the body, but it can also eliminate the toxin after systematic absorption.

Single doses of oral activated charcoal have shown to be effective in preventing the gastrointestinal adsorption of most drugs and toxins. Repeated doses improve the adsorption of toxicologically important agents, including many industrial and environmental intoxicants. The use of activated charcoal is usually more effective than gastric emptying, even though it does not have to be always. In case, the toxic substance has been ingested in a large amount or the substance has a low affinity to charcoal, then gastric emptying might be a better choice (Neuvonen & Olkkola 1988).

6.1.4 Adsorption of environmental toxins and pesticides

Due to biochar´s high absorption capabilities, it is becoming very commonly studied and used for remediation. Furthermore, biochar is showing to be an eco-friendly and sustainable bioadsorbent, which is also cost-effective compared to expensive activated carbons. Studies have revealed that the application of biochar is effective for the removal of important and potentially severe organic pollutants such as pesticides and antibiotics. Recently there have been advancements enabling modifications (physical, chemical, and biological) of biochar to tailor them for specific needs and therefore improving its surface properties and removal capabilities (Zhou et al. 2021).

Many pesticides, herbicides, insecticides are increasingly found in animal feed. A common herbicide that contaminates most of the feed produced from genetically modified maize, rapeseed, and soybean, called glyphosate is currently a significant issue. It is a crop desiccation herbicide, which has been banned e. g. in Germany, but its use is still allowed in many countries. To the negative effects of glyphosate belongs immobilization of magnesium and zinc and antibiotic activity assumingly causing or promoting chronic botulism. Biochar is able to adsorb glyphosate, the effectiveness increases with lower pH and also with high-temperature biochars, however, it has also been found that glyphosate competes with other ions for sorption, which can decrease the efficiency (Schmidt et al. 2019). Gerlach et al. (2014) has conducted a study with 380 dairy cows, which were fed with glyphosate contaminated silage with an addition of humic acids (120 g/day) or with a mixture of 200 g of biochar and 500 g of sauerkraut juice for 4 weeks. The results have shown significant reduction of glyphosate concentration in the urine of the studied cows.

Studies focusing on the adsorption of pesticides have been conducted already in the 1970s. An example can be a study from Wilson & Cook (1970), where they studied the effectiveness of activated charcoal in treating HEOD poisoning. HEOD is a major compound of dieldrin, which belongs to persistent organic pollutants (POPs) and was used as an organochlorine pesticide and later was banned. During the experiment goats, sheep and Jersey heifers were fed alfalfa hay. The HEOD was added to the rumen one hour after the activated carbon was added to the rumen as well and feces were collected at about 12-hour intervals. In all the tested subjects the concentration of HEOD excreted in the feces was several times higher compared to the control group. However, in a study from Fries et al. (1970) cattle have been fed with a concentrate containing dieldrin and DDT for several days. Two weeks after the last intake of the mentioned pesticides, the cattle were fed with silage mixed with activated carbon. The results have shown no significant effects of activated carbon on the rate of decline of pesticide concentration or on the milk and body fat concentration of the pesticides. It is assumed that activated carbon can absorb the pesticide only when present in the digestive tract, therefore it does not have an effect on the concentration of pesticides stored in the body fat tissue. When treating poisoning with activated carbon it is necessary to determine if the source of contamination is in the current feed supply or body fat stores from previous exposure.

6.2 Effects on general health and feed intake

Biochar or charcoal have been commonly used in humans and animals as a therapeutic treatment for several conditions such as feed poisoning, intoxication, etc (Nanda et al.2015). But there have not been many studies conducted for the use of biochar as a regular component of everyday feed. One of the reasons is that the feeding of biochar connects two fields that do not have much in common and that is the veterinary field and biochar research. This practice is rapidly spreading in countries such as Switzerland, Austria, and Germany (Kammann et al. 2017).

According to 27 scientific publications peer-reviewed by Schmidt et al. (2019), there have been neutral to positive results and most of the publication have shown improvement in one or more points listed below:

- Increase in feed intake,
- weight gain,
- increased feed efficiency,
- strengthening of the immune system,
- improvement of meat quality,
- improvement of stable hygiene and odour pollution,
- reduction of claw and feet diseases,
- reduction of veterinary costs.

There have not been found any toxic or negative side effects in any of the 27 scientific publications reviewed by Schmidt et al. (2019. Effects of using biochar as a feed additive were either positive or neutral.

A well-balanced animal diet contains several electron mediating substances. This is an issue in high energy livestock diets in intensive farming, which do not contain sufficient amount of these electron mediating compounds, therefore biochar can aid in this situation. Biochars, which are produced at temperatures above 700 °C become electrically conductive and become an electron mediator in biotic and abiotic redox reactions, therefore addition of biochar to feed can enable redox reactions to take place more smoothly and efficiently leading to higher feed efficiency. Furthermore, it is presumed that the buffering of redox potential and also the effect of electron shuttling between various species of microorganisms has a selective effect which alters the proportion of functional microbial groups in the rumen and negatively influences species living off metabolic products of the animal, which again could be an explanation for higher feed efficiency and improved animal health (Kammann et al. 2017).

The positive effects of biochar on increased feed efficiency may be also explained from the perspective of improving rumen fermentation. Biochar might have the ability to provide a habitat for ruminal microorganisms, leading to enhanced microbial growth efficiency through close and specific association of different species of microbes. The reason of this effect may be biochar´s large surface area created by its highly porous structure, which serves as a suitable space for microbial attachment and formation of biofilms. Many studies have focused this topic, but not all of them have shown significant effects of biochar on rumen fermentation. Mirheidari et al. (2020) have conducted a study focusing on the effects on nutrient digestibility, feed intake, ruminal fermentation parameters, microbial nitrogen supply (MNS), and growth performance when adding 1% walnut shell biochar, 1% pistachio by-product biochar, and 1.5% chicken manure biochar into the daily feed of 24 Kermanian ram lambs during a 90-day experimental period. Results have not shown any effects on the DMI, but with the addition of all types of biochar a significant increase in the average daily gain and feed conversion ration was observed. Furthermore, a study by Leng et al. (2012b) have recorded a 25 % higher weight gain when feeding 0.6% of rice hull-derived biochar to four cattle compared to a control group fed a diet without biochar during a 98 day time period.

Moreover, a recent meta-analysis has been published analysing the results of 15 *in vitro* and 21 *in vivo* studies, where the authors aimed to evaluation the effects of biochar as a feed supplement on nutrient utilization and livestock perfomance. Biochar supplementation was found to reduce methane production, lower the feed conversion ratio and increase propionic acid production in the rumen and NDF digestibility. Overall, they concluded that biochar has a potential to improve feed efficiency, animal health and livestock productivity, reduce nutrient loss, and greenhouse gases without any negative side effects (Qomariyah et al. 2023).

However not all studies have shown such positive results. Teoh et al. (2019) have investigated changes in fermentation parameters, methane production and ruminal microbiota when using hardwood biochar (3.6 to 7.2% of dry matter substrate) as a feed supplement in an in vitro study using semi-continuous culture artificial rumen system called RUSITEC during a 15-day period. No effects of biochar supplementation were observed in regards to dry matter digestibility, pH, volatile fatty acids, effluent or total gas. What is more, Tamayao et al. (2021) have focused on the effects of biochars, which were produced with different post-pyrolysis treatments, on nutrient disappearence, rumen fermentation, microbial protein synthesis and rumen microbiota. The experiment was realized using the RUSITEC system and fed a barley silage-diet over a 15 day period. No changes were observed in any of the mentioned characteristics, therefore there way no improvement in ruminal fermentation.

Overall, biochar may have the potential to improve rumen fermentation and with that associated increased digestibility and feed efficiency. Nevertheless, the research shows different results and the mechanisms behind some processes are not yet understood, therefore more research is needed to fully understand the effects of biochar supplementation on rumen fermentation, digestibility and feed efficiency.

6.3 Alternative to antibiotics

Potentially, biochar could work as an alternative to substances such as antibiotics. Antibiotics are being widely used in animal husbandry and livestock to treat diseases, for prophylactic and metaphylactic purposes (prevention of infections) or as growth promotors (Mann et al. 2021). The use of antibiotics for the last two purposes mentioned are banned in the EU (European Medicines Agency 2022; European Commission 2005), but common in countries such as China, USA, Brazil, or India (Mann et al. 2021). The crucial problem, appearing with the extensive use of antibiotics often correlated with its misuse and overuse, is antibiotic resistance. Development of alternative antimicrobial agents is currently very important. One of the novel agents presently being studied are carbon and activated carbon-based nanomaterials, which have antimicrobial and unique physical-chemical properties and their easy availability, easy methods of production and economic viability make them attractive (Lakshmi et al. 2018). The application of biochar to animal feed as a feed supplement was built on the utilization of activated charcoal against digestive disorders in humans and animals (Man et al. 2021). Biochar has many benefits similar to antibiotics when used on a regular basis such as weight gain, strengthening of the immune system, etc.

7 Use of biochar against greenhouse gas emissions

The European union has set out a goal of becoming climate neutral by 2050 through the European Green Deal, therefore it needs to reduce its production of greenhouse gases (GHG) in many sectors including the agricultural sector. The production of methane through enteric fermentation takes up about 35 % of total agricultural greenhouse emissions in the European union (European Environment Agency 2022). There have been many efforts to lower methane production especially in cattle, where one cow can produce 200 to 500 l of methane daily, but unfortunately without much success. In the past few years however, there have been several studies showing reduced production of GHG when feeding a diet with 0.5 to 2 % of biochar (Schmidt et al. 2019).

The production of methane takes place in the rumen during the process of methanogenesis, where archaea convert microbial digestion products (H_2 and CO_2 or HCOOH) to methane. The reduction of formate (HCOOH) to H_2 and CO_2 requires six electrons and has several biochemical pathways. Several studies have attempted to find other electron acceptors, which would not change into methane and would be safe for the animal, but unsuccessfully (Schmidt et al. 2019).

In the past few years, research took place on the topic of biochar working as an electron acceptor and its use for lowering methane production. An example of such research can be an in vitro experiment which has taken place in Vietnam in 2012, where 0.5% and 1.0% biochar addition to the ruminal liquid lowered the production of methane by 10% and 12.7%. Higher percentage of biochar added did not reduce methane production further. This was done under a 2% presence of urea as a non-protein nitrogen source, when the urea was replaced by potassium nitrate the methane production was reduced by 49% (Leng et al. 2012a).

Furthermore, an in vivo study carried out by Leng et al. (2012b) found that a diet containing 0.6% of biochar reduced the methane production by 20%. The study tested 4 different diets with 12 cattle. The diets contained ordinary compound feed with the addition of biochar and nitrate, biochar and urea, only nitrate and only urea. The most effective diet was one containing both biochar and nitrate, which has reduced the methane production by 40% during the 98-day period. A diet with biochar and urea showed to be most effective when concerning live weight gain, where the weight increased by 25%. Interestingly, biochar and nitrate had a cumulative effect on the reduction of methane emissions, leading to the conclusion that both compounds have different mechanisms by which they reduce the emissions.

Yet, in an in vitro study conducted by Teoh et al. (2019) focusing on the effects of biochar on the reduction of enteric CH_4 emissions, results have not shown a significant reduction in the CH_4 production. The RUSITEC (rumen simulating technique) system was used with a control diet (oaten pasture, maize silage, concentrate) and a diet with a hard-wood biochar addition (3.6 and 7.2% of dry matter substrate) over a 15-day period. The higher dosage has shown a better ability to reduce the production of CH_4 compared to the lower dosage, however the difference in the biochar diet and the control diet was very low compared to the study mentioned in the previous paragraph. The variability in the results are proposed to be due to wide range of characteristics of the biochar such as the adsorptive property, particle size, electrical conductivity, etc. The results have also shown a decrease in the abundance of methanogens in the rumen of cows fed with a diet containing a higher dose of biochar, therefore the biochar may have a potential to decrease methane production through influencing the microbial composition.

Similar results were observed in a study by Tamayao et al. (2021), who have also used the RUSITEC system over a 15 day period using biochars with different post-pyrolysis treatments. No effects on total gas and methane production have been observed.

Another in vivo study by Khoa et al. (2018) has tested a diet containing biochar and green tea by-products rich in tannins. Tannins are naturally occurring polyphenols synthetised by plants during their secondary metabolism as means of protection against pathogens, insects, and vertebrates. We can divide tannins into two groups: hydrolysable and condensed tannins. The hydrolysable tannins are potentially toxic to animals, but ruminants are able to adjust within a certain exposure. However, condensed tannins have shown the ability to improve animal health such as prevention of bloating and increased live weight (Addisu 2016). Lately there have been several studies investigating the effect of tannins on methane production. During the experiment in the group fed with 5% green tea by-product (1.25% tannin) and 1% biochar a 7% decrease of production of methane has been recorded without any effects on the animal's performance.

As mentioned, biochar has a very porous structure, which enables it to not only adsorb toxins, but also gases in soil and, therefore it might be able to adsorb gases produced in the rumen as well. But it is not very probable that the small doses of biochar would be able to adsorb such big volumes of methane in the rumen. It is also believed that biochar influences the microbiota in the rumen. Biochar has a mitigating effect on GHG emissions in soil, where it supports the methanotrophic bacteria, which oxidize CH_4. In soils amended by biochar, the ratio of methanotrophs and methanogens increase in favour of methanotrophs. The presence of methanotrophs in the rumen is still debatable, but if they are present, they might be influenced by biochar in the rumen as well. The most important microbes that are responsible for the production of methane in the rumen are methanogens and there is a possibility that biochar has an inhibitory effect on these bacteria. It is possible that biochar changes the microbiome in the rumen, but further research needs to be conducted to understand the mechanisms (Terry et al. 2019).

One more explanation connected to the mitigating effect of methane production in ruminants can be biochar´s function of an electron mediator in redox reaction mentioned in the previous chapter. Due to this property, biochar is able to improve feed efficiency, which is also connected to the production of methane. Methane is a form of energy that the ruminant cannot use, therefore it ends up as lost energy, thus with better feed efficiency there is less energy lost and less methane produced (Schmidt et al. 2019).

8 Administration and feed control

All biochars used as a feed additive in animal feed need to be analysed and controlled of all relevant parameters. A voluntary industry standard is currently functioning in Europe carrying the name European Biochar Certificate (EBC). This certification is mandatory for any biochar sold for agriculture in Switzerland. Several other countries have aligned their regulations according to the European Biochar Certificate guidelines (European Biochar Certificate 2022). The analysis of biochar for the use as a feed additive should take place at an accredited laboratory focused on biochar and feed analytics. Parameters such as the content of heavy metals, PCBs, PAHs, fluor, etc. should be analysed according to the EBC guidelines. And biochar should be produced through pyrolysis with temperatures above 500 °C for at least 10 minutes to ensure the pyrogenic degradation of organic micropollutants such as pharmaceuticals and mycotoxins. It is also required that the processing and administration of biochar should always be in a moist state to prevent formation of dust (European Biochar Foundation 2022).

The tested and approved biochar can be then added to any common animal feed or also to water. As was shown in the articles cited in this thesis, biochar was often added to feed mixture in combination with another component such as sauerkraut juice, wood vinegar, nitrate, tannins, etc. The combination of biochar and another component was to enhance the effect of the feed supplement and it opens a lot of space for further research to create effective feed supplements for specific needs of animals and also for specific animal species (Schmidt et al. 2019).

9 Materials and methods

9.1 Animal care

The experiment was conducted in compliance with the laws and regulations of both Europe and the Czech Republic. The protocol for the experiment was reviewed and approved by the Institutional Animal Care and Use Committee of the Institute of Animal Science in Prague. It was carried out at the experimental farm in Netluky, Prague, which is part of the Institute of Animal Science in the Czech Republic.

9.2 Biochar

The experimental biochar was derived from softwood (spruce) using a twin-fire gasifier via a two-step process. Firstly, the wood underwent pyrolysis at 500–600°C for a holding time of 3 –6 hours. Secondly, the volatile matter of the biomass was partially oxidized at around 900°C, and then the auto-activation of biochar followed using a combination of water vapour and carbon dioxide. The activation process was carried out gradually, with temperatures decreasing from 900°C to 750°C over a period of 1 hour. This production method yielded high-temperature biochar with elevated carbon content, ash, specific surface area, porosity, and increased pH and total alkalinity, as detailed in Table 1.

According to ISO 18134-3 (ISO, 2015a), dry matter (DM) content was determined using a 10 g sample and getting it to a constant weight under a temperature of 105 °C. And according to ISO 18122 (ISO, 2015b), for the ash content determination a temperature of 550 °C has been used. The elemental composition of biochar has been analyzed through several methods. In the case of the content of C, H, O, N and S an elemental analyzer (Flash EA 1112, Thermo Scientific, Waltham, MA, USA) in CHNS/O configuration was used. In the case of the content of other macronutrients (Ca, P, K, Mg) and micronutrients (Zn, Cu) an atomic absorption spectroscopy (AAS), atomic emission spectroscopy (AES), and inductively coupled plasma-optical emission spectrometry (ICP-OES) was used. Prior to analysis, digestion of the biochar sample in a mixture of HNO_3/HCl at 200 °C for 1 h in a high-pressure microwave oven (CEM Mars 5, CEM Corp., Matthews, NC, USA) has taken place.

To determine fraction distribution, 200 g of biochar were sieved for 10 minutes using a vibratory sieve shaker (Analysette 3 Pro, Fritsch, Idar-Oberstein, Germany) using test sieves with mesh sizes of 0.5, 2, and 5 mm (Retsch, Haan, Germany). The fraction distribution was calculated as the weight ratio of the fraction to the input 200 g of biochar. Analyses to determine the surface area, specific surface area of mesopores, total pore volume, micropores volume, and intrusion volume of biochar followed and were performed according to Moško et al. (2021) method. At first before the analyses, adsorbed moisture has been released through drying under a deep vacuum (180 °C, 12 hours, < 1 Pa). Nitrogen physisorption measurements were taken at cryogenic conditions (77.35 K) using ASAP 2020 and ASAP 2050 automated volumetric gas adsorption instruments (Micromeritics Instrument Corp., Norcross, GA, USA). To determine pH, biochar samples were mixed with water at a 1:10 sample:water (w/v) ratio, left for 1 hour in a rotator (Multi Bio RS-24, Biosan, Riga, Latvia), and then measured using a pH meter (inoLab pH/Cond Level 1, SenTix 41 electrode, WTW, Weilheim, Germany). Following Fidel et al. (2017), total alkalinity was determined through a reaction with HCl, followed by back titration.

In spite of the fact that the biochar used in this experiment has not been officially certified, it does pass the requirements of the EBC for feed-grade biochar taking into account the content of carbon, heavy metals, polycyclic aromatic hydrocarbons, and other organic pollutants and the degree of carbonization–*H/C*$_{org}$. Primarily, the fractions of biochar that passed through the 2 mm test screens were used for feeding with the aim to reach an even distribution in the diet mixture.

Table 1: Characterization and chemical composition (g/kg DM unless stated otherwise) of biochar added to the TMR of the experimental group of dairy cows.

Parameters	**Biochar**
Characterization	
Feedstock	Soft wood – spruce
Carbonization conditions	max 900 °C; up to 7 hours
Surface area (S_{BET}, m^2/g)	412
Specific surface area of mesopores, (S_{meso} m^2/g)	91
Total pore volume, (V_{tot} mm^3_{liq}/g)	239
Micropores volume, (V_{micro} mm^3_{liq}/g)	165
pH	9.9
Chemical composition	
Dry matter (g/kg)	977
Ash	34
C	929
H	7
O	19
N	11
S	0
P	0.6
K	3.4
Ca	6.1
Mg	1.4
Zn	19.7
Cu	7.5

9.3 Experimental design, cows, and diets

The trial included 34 healthy Holstein dairy cows (initial average BW of 704 ± 80 kg, 85 ± 27 DIM, 46 ± 6 kg milk yield, and 3 ± 1 parity) on their first (eight cows) or second and higher lactation (26 cows). The dairy cows have been housed in the experimental barn where they were divided into two balanced groups (according to current milk yield, day in lactation, parity and weight). The control group (CTL) was fed with the total mixed ration (TMR), the experimental group (BIO) was fed with the same ration with the addition of biochar (1.1% of the DM of the ration, at an intake of 22 kg DM this means about 240 g biochar per day). Biochar has been added to the concentrate mixture. TMR was available to the cows *ad libitum* and was freshly prepared and delivered twice a day at around 0400 and 1600 h. The feeding troughs were filled up with a shovel at least five times per day. The TMR composition and biochar characteristics are described in Table 2 and

. The total duration of the experiment was 60 days. This period consists of two experimental periods (cross-over design), each lasting for 30 days (30 days adaptation + 10 days sampling). In the first period, one group was fed the control and the other the experimental diet, and in the second period the diets were swapped. The cows were housed in a free-stall barn with free access to water and milked twice a day at around 0530 and 1630 h.

Table 2. Components and chemical composition (g/kg of DM unless otherwise stated) of the fed TMR.

Item	**Amount**
Components	g/kg
Forage and liquid feed	708
Corn silage	246
Lucerne silage	141
Ensiled corn cobs with leaves (LKS)	138
Energie MG[a]	115
Brewer’s grain	68
Concentrate mixture	292
Wheat	108
Rapeseed meal	101
Barley	43
Vitamin and mineral mix[b]	27
C16[c]	9
Sodium bicarbonate	4
Chemical composition	g/kg DM
Dry matter (g/kg as fed)	421.3
Organic matter	922.5
Crude protein	162.8

Ether extract	27.3
Starch	332.2
NDF	341.3
ADF	173.8
NFC[d]	391.1
Net energy for lactation (MJ/kg of DM)	7.0

[a] Mix of molasses and glycerol at a 1:1 ratio (Commodity Trading, s. r. o., Olomouc, Czech Republic).

[b] Vitamin and mineral mix composition (per kg): 403, 100 IU vitamin A, 73,494 IU vitamin D_3, 1,200 mg vitamin E, 133 g Ca, 33 g P, 52 g Na, 40 g Mg, 630 mg Cu, 4,855 mg Mn, 3,160 mg Zn, 18 mg Se, 53 mg I, 21 mg Co.

[c] Palmitic acid (≥ 98%; LodeStar™, Berg + Schmidt Malaysia Sdn. Bhd., Selangor, Malaysia).

[d] NFC (non-fiber carbohydrates) = 1000 – (aNDFom g/kg + crude protein g/kg + ether extract g/kg + ash g/kg).

9.4 Sampling and analysis

The cows were fed using a roughage intake control (RIC) system (Hokofarm Group BV, Marknesse, The Netherlands), which is able to record the daily feed intake of each cow. This is possible due to ear tags with a unique radio frequency, which enable the cow to access the feed through the transponder-controlled gates and recognizes it. The through records the weight of the consumed feed (to the nearest 0.1 kg) and the start and end time of each visit (to the nearest 1 s). Remainder of the feed was removed and replaced with fresh TMR during morning milking (between 05:00 and 06:00). The two groups of cows (17 cows each) were kept separately in two identical parts of one barn with 10 electronic feeding throughs available for each group. The DMI data were obtained by correcting the feed intake for the DM content of the feed.

Each week feed samples were collected and composited by period. The analysis of composite samples was done according to AOAC International (2005) for the following values: DM content (method 934.01), crude protein (method 954.01), crude fat (method 920.39), and ash (method 942.05). The heat-stable amylase was utilized to determine the Neutral detergent fibre (NDF), which was expressed without residual ash (Mertens 2002). Whereas the acid detergent fibre (ADF) was measured following the method 973.18 of AOAC International (2000). The starch content was determined using the Ewers method ISO 10520, by means of polarimetric analysis (ISO 1997).

An electronic livestock scale (AfiWeigh scale; Afimilk Ltd., Kibbutz Afikim, Israel) placed in the common exit alley of the milking parlour was used to measure body weight twice daily after each milking, while milk yield was recorded (AfiMilk MPC Milk Metre, Afimilk Ltd., Kibbutz Afikim, Israel) daily at the cow level. Sum of both the morning and evening milking represents the daily milk yield. Only data collected during the final 10 days of each period were used for statistical analysis, although feed intake, body weight, and milk yield were monitored throughout the experiment. Samples of milk were collected from each cow during morning and evening milkings on days 22 and 29 of each experimental period, and they were pooled according to individual milk yield before being analysed for milk fat, protein, lactose, and urea concentrations by infra-red spectroscopy (Foss FT2, MilkoScan, Foss Electric, Hillerod, Denmark).

In order to gather daily rumination data for individual cows, a rumination monitoring system (Vitalimetr 5 P, Farmtec a. s., Jistebnice, Czech Republic) was affixed to each cow. This system comprised of a collar 3-axis accelerometer sensor (62 × 53 × 35 mm; 200 g) placed beneath the neck, a data logger with built-in data analysis, and software for handling electronic data (Farmsoft, Farmtec a. s., Jistebnice, Czech Republic). A standard algorithm to detect a specific rumination pattern was used by the data logger and it produced hourly summaries, which was then combined into one-day summaries (24 hourly summaries). As a result, 10 one-day summaries for each cow in each period were obtained and the data was then averaged per cow and period. After these steps, statistical analysis was conducted.

9.5 Calculations

Fat-corrected milk (FCM; 4% of fat) yield was calculated based on Nutrient Requirement of Dairy Cattle (2001) as shown below:

$$4\%\ \text{FCM (kg/d)} = 0.4 \times \text{milk yield (kg/d)} + 15 \times \text{fat yield (kg/d)}$$

Energy-corrected milk (ECM) yield was calculated in line with Sjaunja et al. (1991) as shown below:

$$\text{ECM (kg/d)} = \text{milk yield (kg/d)} \times (383 \times \text{fat } [\%] + 242 \times \text{protein } [\%] + 165.4 \times \text{lactose } [\%] + 20.7)\ /3{,}140$$

The collected data of DMI, milk yield, BW and rumination data (10 days) and milk composition data (2 days) were averaged per cow and period. The resulting values underwent statistical analysis with the statistical software package SAS (SAS Enterprise Guide version 6.1, SAS Institute Inc., Cary, USA) using PROC MIXED according to the following model:

$$Y_{ijkl} = \mu + G_i + C(G)_{ij} + P_k + T_l + e_{ijkl}$$

The values are described below:

- Y_{ijkl} = dependent variable
- μ = overall mean
- G_i = the group effect
- $C(G)_{ij}$ = effect of the cow within the group
- P_k = period effect
- T_l = treatment effect
- e_{ijkl} = residual error

All effects were fixed, except the group (G_i) and cow within group ($C(G)_{ij}$) effect. The results are presented as least squares means. Statistical differences of p-value less than 0.05 was regarded as significant. Trends are discussed also at p-value between 0.05 and 0.10.

10 Results

The results focusing on feed intake and rumination parameters are shown in Table 3. DMI had a tendency to decrease ($p < 0.080$) in the BIO. The feeding time ($p < 0.356$), through visits ($p < 0.111$), feeding rate ($p < 0.627$), rumination time per day ($p < 0.397$) and rumination time per kg of DMI ($p < 0.906$) did not differ.

Table 3: Feed intake and rumination of the control (CTL) and biochar-fed group (BIO) of cows.

	Diet		**SEM**	**P-value**
Item	**CTL**	**BIO**		
Dry matter intake (kg/d)	21.00	20.61	0.4746	0.080
Feeding time (min/d)	216	222	14.62	0.356
Trough visits (visits/d)	60.6	58.9	3.14	0.111
Feeding rate (g/min)	150.6	149.3	8.933	0.627
Rumination (min/d)	454	445	14.94	0.397
Rumination (min/kg of DMI)	21.8	21.7	0.7821	0.906

The results regarding the milk production are presented in Table 4. The milk yield ($p < 0.001$) was lower in the BIO and the same can be said in the case of the 4 % FCM ($p < 0.030$) and ECM ($p < 0.008$). The feed efficiency described as milk/DMI ($p < 0.031$) decreased in the BIO, whereas the feed efficiency expressed as ECM/DMI ($p < 0.164$) remained unaffected.

The milk composition was similar in both diets, when looking at the proportion of fat ($p < 0.683$), protein ($p < 0.813$), casein ($p < 0.769$) and lactose ($p < 0.283$). However, a decrease in the daily production of protein ($p < 0.001$), casein ($p < 0.001$) and lactose ($p < 0.001$) was observed in the BIO. The daily production of fat ($p < 0.243$) did not differ among the two groups.

Table 4: Milk production of the control (CTL) and biochar-fed group (BIO) of cows.

	Diet		**SEM**	**P-value**
Yield	**CTL**	**BIO**		
Milk (kg/d)	44.06	42.30	1.5033	0.001
4% FCM[2] (kg/d)	35.79	34.52	1.1873	0.030
ECM[3] (kg/d)	37.14	35.75	1.1129	0.008
Feed efficiency				
Milk/DMI	2.10	2.05	0.060	0.031
ECM/DMI	1.76	1.73	0.032	0.164

Milk component				
Fat (%)	2.73	2.76	0.1054	0.683
Fat (kg/d)	1.21	1.17	0.0570	0.243
Protein (%)	3.05	3.06	0.0401	0.813
Protein (kg/d)	1.34	1.29	0.0351	0.001
Casein (%)	2.34	2.34	0.0360	0.769
Casein (kg/d)	1.03	0.98	0.0267	0.001
Lactose (%)	5.06	5.04	0.0343	0.283
Lactose (kg/d)	2.23	2.14	0.0899	0.001

[1] Diet: 25% conventionally processed corn silage, 25% corn shredlage.

[2] Fat-corrected milk (with 4% fat) yield calculated using this formula: 4% FCM (kg/d) = 0.4 × milk yield (kg/d) + 15 × fat yield (kg/d) (NRC, 2001).

[3] Energy-corrected milk yield calculated using this formula: ECM (kg/d) = milk yield (kg/d) × (383 × fat [%] + 242 × protein [%] + 165.4 × lactose [%] + 20.7)/3,140 (Sjaunja et al., 1991).

11 Discussion

11.1 Feed intake and rumination

In this study, the addition of biochar to the cow feed did not have a statistically significant effect on the feeding and rumination parameters. Only a minor decrease was observed in the DMI in the BIO. The same tendency was recorded by Qomariyah et al. (2023). Many other studies have observed no changes in the DMI of biochar-fed cows compared to the control group. For example, Leng et al. (2012b) reported no change in DMI while adding biochar (0.6% of DM) to the diet of "Yellow cattle". Winders et al. (2019) observed no effect of two doses of biochar on DMI of growing and finishing steers. And the same can be stated in a study by Terry et al. (2020) with a biochar dose of 0.5%, 1% and 2% of DM diet of feedlot steers. The decline in DMI can be ascribed to the exceptional adsorbent quality of biochar (Schmidt et al. 2019). Its extensive surface area enables it to adsorb and bind specific nutrients, which renders them indigestible and unabsorbable by the animal. This process might decrease the digestibility of feed and subsequently contribute to a reduction in feed intake.The effects of biochar on palatability, due to certain compounds or simply due to the altered texture of the feed, could be also taken into consideration (Qomariyah et al. 2023).

The Holstein cows in this study have been ruminating for 449 min/d on average. This rumination time is in consistency with previous studies with values such as 450 min/d (Clemént et al. 2014) or 436 min/d (White et al. 2017). The rumination time did not change with the addition of biochar to the diet in both units of min/d or min/kg of DMI. This value is strongly affected by NDF intake, hardness of the feed, particle size of the diet, the indigestibility of fibre and complex interactions between the mentioned factors (Beauchemin 2018). As biochar is an inert material, it does not have a high impact on the above-mentioned factors, therefore it might be the reason due to why no changes were observed.

11.2 Milk production

To the best of author´s knowledge, this might be the first experiment, which focuses on effects of biochar supplementation on milk production in high-producing dairy cows. There have been a few studies which have instead used activated carbon or focused on a different animal species (Mirheidari et al. 2019; Erickson et al. 2021; Al-Azzawi et al. 2021). According to the results in this study, the addition of biochar to the diet caused a significant decrease in milk yield ($p < 0.001$). A similar reduction in milk production after feeding biochar was also observed by Erickson et al. (2011). However, the difference found by these authors was not statistically significant. This may have been due to the small number of animals in the study, with the authors including only six dairy cows in the study. Conversely, a study by Al-Azzawi et al. (2021) focused not only on changes in milk production observing a herd of 180 dairy cows. They have recorded a statistically insignificant increase in the daily milk yield ($p = 0.171$). The differences in these studies might be due to the varied stages of lactation of the studied cows. In our study the average day of lactation at the beginning of the experiment was 85 days, therefore the cows went from early to mid-lactation throughout the experiment, compared to the six cows, which were in their late-lactation (Erickson et al. 2011). In the study by Al-Azzawi et al. (2021) the stages of lactation is not specified and in relation to the high number of cows it probably varied. It is possible that the milk yield was influenced by the inert character of biochar, meaning it dilutes the energy content of the feed, therefore the cow needs to intake a larger volume of feed to reach the same energy intake as in the control diet (Cabeza et al. 2018). This effect can be especially important during the early phase of lactation, where the cow is not able to intake enough energy to meet high energy demands (Hutjens 2002) leading to negative effects on the milk production. Furthermore, the stage of lactation might have further influenced the milk production, due to the natural decrease in production going from early to mid-lactation. Results may also vary due to the differences in biochar and activated carbons.

In correlation to the milk yield, the feed efficiency (milk/DMI) was also reduced by the supplementation of biochar. According to the review by Schmidt et al. (2019), biochar has shown in several cases an increase in the feed conversion ratio, but none of the studies focused on milk production. A decrease in the feed efficiency (milk/DMI) can be also noted in the study by Erickson et al. (2011), although again without statistical importance due to the low number of cows. Conversely, the feed efficiency calculated as ECM/DMI was not affected. It is possible, that the reduced milk yield and with it the feed efficiency can be attributed to dilution of feed ration by biochar addition. Other factors such as the phase of lactation of the cows or the specific types of biochar used might have had an impact as well. Nevertheless, the assessment of these factors is beyond the scope of this thesis.

Furthermore, the proportion of observed milk components (fat, protein, casein, lactose) did not change. Only the daily production of protein, casein and lactose have lowered, which is in correlation with the lowered milk production. According to Erickson et al. (2011), the inclusion of activated carbon to the diet did not affect the milk composition except the fat content, where an increase was observed. A significant increase in fat and protein content was observed in the study by Azzawi et al. (2021), where they assume it might be caused by the alteration in rumen fermentation and biohydrogenation resulted from the supplementation of biochar. This thesis did not focus enough on the changes in rumination to offer a relevant comparison.

12 Conclusion

We hypothesized that biochar in the diet of dairy cows would not decrease DMI, eating intensity, and rumination time. This was confirmed throughout this experiment, where no changes were observed in the eating intensity and rumination time in cows with biochar supplementation. However, a tendency of lower DMI was recorded, which might have occurred due to the highly adsorbent nature of biochar, which can make certain nutrients unavailable for digestion. Additionally, a decrease in the daily milk yield, including the yield of 4% FCM and ECM was shown, which has also led to a lowered feed efficiency. The milk composition was not affected.

So far, there have been just very few studies which focused on the effects of biochar on the milk production of dairy cows. More research is needed to understand how biochar influences milk production and if it could be an effective feed additive in the dairy industry.

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14 List of abbreviations and symbols

AAS – atomic absorption spectroscopy
ADE – Amazonian dark earths
ADF – acid detergent fibre
AES – atomic emission spectroscopy
AF – aflatoxin
AOAC – Association of Official Agricultural Chemists
BIO – biochar-fed group
BW – body weight
CTL – control goup
DIM – day in milk
DM – dry matter
DMI – dry matter intake
EBC – European Biochar Certificate
ECM – energy-corrected milk
EHEC – E. coli O157: H7
FB – fumonisin
FCM – fat-corrected milk
GHG – greenhouse gases
ICP-OES – inductively coupled plasma-optical emission spectrometry
ISO – International Organization for Standardization
MNS – microbial nitrogen supply
NDF – neutral detergent fibre
OTA – ochratoxin A
PAH – polycyclic aromatic hydrocarbon
PCB – polychlorinated biphenyl
RIC – roughage intake control
TC – trichothecene
TMR – total mixed ration
ZEN – zearalenone