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Biochar as a Bioactive Feed Additive in Pig Diet

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Abstract

Biochar is a porous, carbonaceous pyrogenic substance produced by pyrolysis of biomass at a temperature ranging from 350 ˚C to 1000 ˚C, whereby organic materials are decomposed in a low-to-no oxygen environment. Evidence suggests charcoal use as a traditional feed additive in animal production dates back centuries, largely to treat livestock digestive issues. Modern biochar studies on supplementing farm animal diet with biochar have been reported to exert a multitude of benefits including improved animal health, digestibility, feed consumption, feed efficiency, growth performance, pathogens infestation, detoxification of contaminants, and reduced enteric methane production among many other benefits. The enhanced performance of farm animals with biochar supplementation is mainly because of its adsorption capacity, in addition to other benefits, towards mycotoxins, pesticides, and pathogens that would otherwise hinder the performance. However, the result is dependent on the characteristics of biochar such as porosity, specific surface area, pH, aromaticity and, type and abundance of functional groups on the surface. These characteristics, in turn, are determined by the feedstock characteristics and pyrolysis conditions. However, in addition to adsorption of contaminants, biochar can potentially adsorb feed nutrients and pharmaceuticals and shift the gut microbiome composition upon long term use, which pose a major limitation in the long-term use of biochar as feed additive.

The objective of this study was to investigate biochar, as a supplement to compound feed, regarding its influence on growth performance and some other wellbeing parameters including diarrhea, stress, and joint problems. A total of 84 pigs (42 control and 42 test pigs) with 90 days of age and an initial average body weight of 56.62kg were housed in 8 pens, 8-12 pigs a pen, 4 pens with control pigs, and the other four with test animals. Treatments were control diet (no supplementation of biochar) and test diet (0.5% of pine wood biochar fed separately from the slurry compound feed). Weight gain was scaled every 2/3 weeks and data was registered for all other measured wellbeing parameters during the 60-day feeding trial. Statistical analysis conducted at the end of the experiment did not reveal any significant difference in weight gain performance between the two treatments. Likewise, no such incidences of diarrhea and joint problems were observed to conclude the effect between the treatments during the course of the experiment. Regarding the tail bitten incidence as a measure of stress, one pig in each treatment group was identified as tail bitten, and thus, biochar was not found to have an effect. Unlike the considerable number of documented results indicating positive tendencies regarding the efficacy of biochar in enhancing weight gain performance, this study does not conform to previous findings on the subject.

Keywords: Biochar, pyrolysis, adsorption, porosity, specific surface area, feed additive, growth performance

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1. Introduction

While biochar is a new term coined in recent times, it is not a new substance. The concept being inspired by black matter in the Terra Preta de Indio soil of the Amazon (Bezerra et al., 2019), biochar has found its use in a multitude of the area including waste management, mitigation of climate change, and agricultural practices; soil application and livestock feeding as a bio-active supplement (Jeffery et al., 2017). In the last ten years, the production and application of biochar have gained increasing popularity. Biochar has been reported to offer a range of environmental benefits in the agroecosystem including, for instance, mitigation of the damaging effect of global warming and climate change, carbon sequestration and, improved soil quality and crop yield (Toth & Dou, 2016). In livestock farming, biochar as a regular feed supplement has been documented to improve growth performance, resistance to gut pathogens, blood profile, increase digestibility, and subsequent nutrient intake efficacy and thus, productivity (Man et al., 2021).

Biochar is a porous, carbonaceous pyrogenic substance produced by pyrolysis of biomass at a temperature ranging from 350 ˚C to 1000 ˚C, whereby organic materials are decomposed in a low-to-no oxygen environment (EBC, 2012). The characteristics of biochar are defined by its quality aspect, feedstocks used, pyrolysis temperature, and the end application (EBC, 2012). The present guidelines of EBC classify biochar according to its application into four distinct divisions: EBC-Feed, EBC-Agro, EBC-AgroOrganic and, EBC-Material. EBC-Feed certified biochar meets all the requirements of EU Feed Regulations and requires that feedstock used to be only biomass from the natural, untreated wood trunk while the requirement for carbon content is set at a minimum of 80% of dry matter. EBC- Agro, and EBC-AgroOrganic certified biochar meets all the requirements of EU Fertilizer Regulations. Likewise, EBC-Material certification requires confirmation of sustainable production of biochar that can be used in textiles, electronics, plastic, and construction materials(EBC, 2012).

An overwhelming number of studies were performed during the first decade of modern biochar research, the majority of which centered on the application of biochar in the soil (Schmidt et al., 2019). Soil application of biochar revealed average to astonishing results in the productivity of a multitude of crops (Jeffery et al., 2017). More recently, after the publication of several veterinary articles over the last century, a significant number of comprehensive research centered on farmed animals have been conducted and published since 2010, dealing primarily with the influence of biochar on animal wellbeing, feed conversion rate, pathogen infestation, and greenhouse gas emission (Schmidt et al., 2019). Today, attributable to its non-toxic and thus, feedable and even edible characteristics, biochar has become an extensively used bioactive feed supplement for different farmed animal species. Apart from mixing it with the feed or feeding separately, there has been a common practice of mixing biochar with the silage. Biochar, when combined with perennial ryegrass during ensiling, has been shown to inhibit butyric acid development and facilitate lactic acid bacteria thereby, shifting the process towards lactic acid fermentation. (Pereira et al., 2014).

The comprehensive research on biochar conducted during the 2020s can be categorized into three main subject areas based on the impacts identified: animal performance, controlling pathogens and binding toxins, and minimizing enteric methane production. The overwhelming majority of studies elucidate that charcoal would enhance several parameters, including feed consumption, digestibility, and consequent weight gain; meat and egg quality and, carcass characteristics; improved immune system function; detoxification of contaminants from feed and forage sources, as well as organic chemicals; and reduction in antibiotic residues and pathogens as well as reduced enteric methane emission (Toth & Dou, 2016). However, despite the recent increase in the number of investigations dedicated to these subjects in recent years, the vast majority of research on charcoal as a health-promoting and performance-enhancing feed supplement is still observational. A very few studies have looked into the mechanisms behind the reported outcomes, and there is still a knowledge gap regarding how charcoal therapies work (Schmidt et al., 2019).

2. Literature Review

2.1 Historical perspectives on the use of charcoal/biochar in animal feed

The use of charcoal for medicinal purposes can be traced back to ancient times in a variety of cultures around the world. An Egyptian papyrus of 1500BC has various kinds of charcoal inscribed on it, each suited to a specific ailment. Evidence suggests charcoal use, as a traditional feed additive in animal production, dates back centuries, largely to treat livestock digestive issues (EBC, 2012) while, human use of charcoal, on the other hand, as a remedy to digestive problems is also common. Cato the Elder (230-149BC) was one of the first to mention the importance of charcoal in animal health. The statement in his classic On Agriculture says: "If you have reason to fear sickness, give the oxen before they get sick the following remedy: 3 grains of salt, 3 laurel leaves, 3 pieces of charcoal, and 3 pints of wine" (Schmidt et al., 2019). Traditional farmers used charcoal to treat a variety of internal ailments all over the world. From the late 1800s to the early 1900s, feeding a daily dose of charcoal to animals was a common practice to improve their health and performance. Activated charcoal, a chemically treated charcoal with acids, inorganic salts, or gases such as carbon dioxide to enhance porous characteristics (Man et al., 2021), has been used to treat a variety of digestive problems in various species, including canine flatulence and colic in horses. It seemed to have never caused any damage and was mainly helpful. The charcoal was given to some animals, such as chickens and pigs, in its purest form; for others, it was mixed with some other ingredients: butter (cows), eggs (dogs), or meat (cats) (Schmidt et al., 2019). The use of charcoal in the treatment of poisoned patients dates back centuries. Even today, poisoned patients receive treatment with activated charcoal in combination with gastric emptying (Derlet & Albertson, 1986).

In the United States of America, in the 19th century and the early 20th century, charcoal was considered an important constituent of various 'cow tonics. The benefits of these various cow tonics were claimed to minimize digestive problems, stimulate appetite, and enhance milk production (Pennsylvania State University, 1967). Charcoal was thought to be a superior feed additive for rising milk butterfat content and was considered a vital component of the feed ration (Savage, 1922). Biochar products were used in American swine breeding since 1880 and later in 1940, they became an important ingredient in poultry feed (Totusek & Beeson, 1953).

2.2 Studies on biochar as a feed supplement in the last century

There had been numerous studies on the soil application of biochar in the last centuries. However, when it comes to the application of biochar in animal feeding, very few studies were conducted. At the turn of the 20th century, German veterinarians were already investigating and recommending the use of activated and non-activated biochar feed for animal health and welfare (Schmidt et al., 2019). The ability of activated biochar to reduce and adsorb pathogenic clostridial toxins from Clostridium tetani and Clostridium botulinum has been studied since 1915 (Schmidt et al., 2019). (Volkmann, 1935) concluded that supplementing biochar to the diet of pets with coccidiosis or coccidial infections reduced excreted oocysts effectively. This limited number of research, yet encouraging and optimistic results with the prospect of further investigation has prompted the researcher all around to conduct broad-spectrum research. Since the second decade of this century, extensive research has been conducted around the globe on the influence of biochar on the performance and wellbeing of farmed animals.

2.3 Specific function of biochar as a feed supplement

2.3.1 Adsorption

For many centuries, charcoal has been used as an emergency remedy for animal poisoning. Prior to the modern research in the early 2010s on the application of biochar as a regular feed additive to the livestock diet, charcoal and, activated carbon was the primary veterinarian-recommended remedy for digestive issues and poisoning (Hagemann et al., 2018). Owing to its high adsorption ability, biochar has been used for several contaminants, including mycotoxins, plant toxins, pesticides, harmful metabolites, and pathogens.

(Fagbohungbe et al., 2017) has categorized the adsorption mechanism of biochar into three distinct steps. Firstly, the adsorbate settles on the biochar's surface during physical adsorption. Then follows surface precipitation and complexation step, where adsorbate deposits and forms layers on biochar’s surface. Finally, the adsorbate condenses into the pores of biochar during the pore filling step. For organic molecules, the major mechanisms of adsorption have been suggested to be hydrophobic interaction, hydrogen bonding, and van de Walls force of attraction, the latter induced by the presence and number of surface functional groups and the sorption generally described as electron donor-acceptor relationship between adsorbent and the organic adsorbate (Fagbohungbe et al., 2017), (Mattson et al., 1969). Likewise, the hydrophobic interaction has been associated with the adsorption of insoluble organic adsorbates (Moreno-Castilla, 2004). For the adsorption of heavy metals, electrostatic attraction, ion exchange and precipitation on biochar’s surface have been shown as adsorption mechanism (Fagbohungbe et al., 2017).

The adsorption potential of biochar for organic contaminants is associated with its carbon content, surface area, pH, pyrolysis temperature and, hydrophobicity of the pollutants (Oh & Seo, 2016). The sorption affinity of biochar has been shown to be facilitated by low pH, particularly below 7 (Tong et al., 2011) and (Oh & Seo, 2016). (Li et al., 2018) demonstrated that the sorption potential of biochar is higher when pyrolyzed at a temperature of 500˚C or higher. The adsorption capacity of biochar (41.2-42.4mg/g) for microcystin, a toxin produced by certain freshwater cyanobacteria, was exhibited to be at least comparable if not greater to that of commercially available adsorbents like mesoporous silica (5.99mg/g), activated charcoal (16.1-83.3mg/g) and carbon nanotubes (5.9mg/g).

2.3.2 Toxin adsorption

2.3.2.1 Adsorption of mycotoxin

Animal feed contamination with toxins originating from the environment, insects, and microbial activities affects 25% of the global feed production, resulting in serious maladies in farm animals. (Mézes et al., 2010), (Man et al., 2021). To overcome the problem and protect animals from potential fatality and death, activated carbon and its special polymers are being extensively used, in addition to the widely used aluminosilicates (Huwig et al., 2001). In this regard, the adsorption potential of biochar in addition to its health-promoting effect on animals potentially makes it a better choice as an adsorbent with additional benefits compared to commercially available adsorbents. Following are some of the studies on farmed animals regarding the impact of biochar on mycotoxin adsorption.

A. Cows

Regarding the aflatoxin content in milk, (Di Natale et al., 2009) demonstrated the highest toxin reduction capacity (greater than 90% reduction of the toxin in milk with 0.5g/kg aflatoxin in the diet)of biochar of all the natural and synthetic adsorbent feed supplements for dairy cows. The strong adsorption ability of the biochar and thus, a significant reduction in the aflatoxin content of milk, as explained by the authors in general, is attributable to the biochar's high specific surface area in conjunction with a desirable micropore size distribution, as well as aflatoxin's high affinity for the biochar's polyaromatic surface. Likewise, a slightly positive influence on milk composition concerning protein, lactose, and organic acids content was found.

Fungal contamination of silage is common during fermentation and storage, particularly when effective control and monitoring of the important parameters are not well executed. An investigation (Erickson et al., 2011) conducted to study the effect of biochar co-feeding (0, 20, and 40g a day) to fungal contaminated silage fed Holstein dairy cows demonstrated the high feed intake and enhanced digestibility of the NDF, hemicellulose, and crude protein, and higher milk fat content of the biochar supplemented cows compared to the controls. However, cows seem to prefer the non-contaminated quality silage to charcoal amended fungal contaminated silage and thus, concerns should be given to quality silage instead of mitigating the effect of contaminated silage with feed amendment as biochar. (Galvano et al., 1996) in an experiment added 2% of activated biochar in the aflatoxin-contaminated pelleted feed for dairy cows and found the feed concentration of the toxin reduced by 74% and milk toxin concentration reduced by 45%.

B. Pigs

In vitro experiments with pig's gastrointestinal digestive fluids demonstrated that activated biochar effectively adsorbs Fusarium toxins such as deoxynivalenol (67%), zearalenone (100%), and nivalenol (21%) (Avantaggiato et al., 2005). Contrary to this finding, (Jarczyk et al., 2008) showed no remarkable reduction in ochratoxin level in the blood serum, liver, kidney and, muscle tissue when 0.3% biochar was added to pig's diet. Likewise, even with the addition of a higher proportion of biochar (1%) to the diet of piglets, (Piva et al., 2005) found no effect on the adsorption of the toxin fumonisin.

C. Poultry

The activity of key liver enzymes, important for mitigating the damaging effect of mycotoxin on the liver, was found to substantially improve when biochar was applied at a regular dose of 0.02% of the bodyweight (Ademoyero & Dalvi, 1983). In Another study by (Dalvi & McGowan, 1984), it was demonstrated that the supplementation of 0.1% biochar to the aflatoxin-induced broiler feed (10ppm aflatoxin) mitigated the negative effect of aflatoxin on feed intake and weight gain. (Edrington et al., 1996) showed reduced aflatoxin M_1 concentration in the urine of surgically colostomized turkey poults compared to control when administered activated carbon concomitant with the aflatoxin F1. The reduced level of the aflatoxin F1 major metabolite Aflatoxin M1 in the urine of the poults signifies the higher level of adsorption of the parent mycotoxin in the poults compared to control. Higher the amount of adsorption, lower the amount left for metabolization. On the other hand, commercially available alumina adsorbent product administered in combination with biochar treatment was shown to result in significant reduction in aflatoxin B level in liver and blood serum of the growing chicken, contrary to the alumina products when administered alone, which resulted in considerable liver and blood level of aflatoxin (Kubena et al., 1990).

The dose of the biochar and the contamination level of mycotoxin in the diet are critical parameters to be considered when administering biochar to the animal. This has been reinforced by a recent poultry experiment (Bhatti et al., 2018), where the varying level of aflatoxin-contaminated (0.1, 0.2, and 0.6 mg kg^{-1}) poultry diet was supplemented with biochar (2.5g/kg and 5g/kg) and the finding demonstrated the reduced aflatoxin content in the bird's liver by varying degree (16%-72%) depending on the level of aflatoxin in the diet and the doses of biochar.

Apart from the dose and the contamination level, the characteristics of the biochar are an important factor in relation to its efficacy to adsorb the mycotoxin. The outcome of the majority of the in vivo feeding trials with biochar has not been found to coincide with each other in terms of the result, not even with the same dose and the contamination level. These differences in the result may be attributed to the different characteristics of the biochar owing to variation in the feedstock used and the production condition (e.g., pyrolysis temperature). The characteristic effect of biochar on toxin sorption has been demonstrated by (Kim et al., 2017) in an in vitro experiment with three different biochar (organic medicinal charcoal;0.25% of basal diet, pyroligneous charcoal;0.5% and coconut tree charcoal;0.5%) where, the adsorption capacity was shown to be 100%, 20%, and 10% respectively. The finding illustrates that some biochar has greater adsorption potential than others, which is defined by the specific characteristics of the biochar, and thus, considering specific biochar properties is important. This implies that systematic trials with biochar from different feedstocks and production conditions are important for the systematic characterization and classification of biochar with regard to mycotoxin adsorption.

Feed matrix has an important influence on the sorption capacity of biochar. This can be explained by the fact that the majority of the studies regarding in vitro sorption of biochar in aqueous solution have not been shown to match the corresponding in vivo trial results (Huwig et al., 2001), where not only mycotoxin but also a variety of organic molecules compete for the biochar's free adsorption surface. Hence, systematic interpretation of the in vitro test result must be carried out carefully in order to address the different matrix effects of the feed and that in the digestive tract. The feed matrix influence on biochar sorption potential has been demonstrated by (Jaynes et al., 2007) in the finding where activated carbon adsorbed 200g/kg aflatoxin in clear solution compared to 100 times lower adsorption in a cornmeal suspension. The varying pH and redox conditions can potentially make the matrix even more complicated in the digestive tract.

2.3.2.2 Adsorption of bacteria and their metabolites

Experiments with Clostridium tetani and Clostridium botulinum bacterial toxins, as well as diphtheria toxin concerning the biochar effect started as early as the 1910s (Schmidt et al., 2019) while considering the importance of biochar's quality and consistency as well as the possible effect of different charcoals on toxin adsorption. Modern biochar research has led to significant findings in relation to biochar's capacity to bind bacterial pathogens and their toxic metabolites. In a 10-day finishing pig experiment, (Kim et al., 2017) indicated that fecal E. coli counts in the manure of pig fed 0.25 percent active biochar or 0.50 percent coconut tree biochar is significantly lower than that of control without biochar, while the number of beneficial bacteria Lactobacillus in feces rose in all biochar treatments. Biochar, particularly when combined with wood vinegar, was able to regulate parasitic protozoan Cryptosporidium parvum infection in both in vitro and in vivo experiments with bovine calves (Watarai et al., 2008). In goats as well, (Van et al., 2006) indicated the possible minimization of the parasitic incidence including coccidia oocysts and tapeworms.

However, although the in vitro experiments with 5g/ml biochar demonstrated the significant reduction of E.coli counts from $5.33 *10^6$ to below 800 (Naka et al., 2001), no binding of the bacteria by the same commercial activated biochar was identified in the gastrointestinal tract of sheep (Knutson et al., 2006). The authors speculated the finding as either competing agents or other digestive bacteria were occupying the biochar binding sites, or that the interval between pathogen infection and biochar administration was too long. The surface properties of bacteria, as suggested by (Abit et al., 2014), play an important role in the bacteria's binding to biochar, which indicates an important perception of bacteria-biochar interaction and thus, needs to be studied systematically.

2.3.2.3 Adsorption of drugs

In the 1980s, a slew of human medical studies on the use of activated carbon in the poisoning was released, shedding light on the use of biochar as feed additives, particularly for the treatment of feed poisoning (Schmidt et al., 2019). Most medications and various contaminants can be prevented from gastrointestinal uptake by the adsorption effect of activated carbon, which is normally believed to be more effective than emptying gastric contents (Neuvonen & Olkkola, 1988). The authors suggested that the elimination of toxic substances such as aspirin, dapsone, cardiac glycosides and, dextropropoxyphene, and many toxicologically efficient environmental and industrial substances can be enhanced by the repeated intake of activated charcoal, without any known serious side effects.

2.3.2.4 Adsorption of pesticides

The prevalence of insecticides, pesticides, and herbicides in plant feed ingredients is ubiquitous, especially in the developing countries with poor regulation and control over the limit on the application of such chemicals in the crops, which has affected not only the feed production and the target animals but also the humans via the food chain. The earlier studies of biochar with regards to pesticide adsorption in the 1970s reported that biochar can be successfully applied in cattle, goat, sheep diet to adsorb the pesticide residues in the digestive tract and excrete eventually (Wilson & Cook, 1970), while, similar feeding trial with chicken did not show any remarkable effect on eggs and tissue pesticides residue levels (Foster et al., 1972). The experiments conducted in Japan to investigate the affinity of organochlorine compounds like dibenzofuran, dibenzo-p-dioxin, and dioxin-like PCBs, all of which are environmental toxins, to biochar in a 0.5% biochar amended layers feed revealed reduced PCDDs/PCDFs, non-ortho PCBs, and mono-ortho PCBs concentration in laying hen tissue and eggs by over 90%, 80% and 50% respectively (Fujita et al., 2012). The extent of binding of various organochlorine toxins with the biochar has been demonstrated by (Kawashima et al., 2009) as dependent on the aromaticity of the molecule.

The herbicide glyphosate, which has been shown to affect the feed produced from genetically modified soybean, rapeseed, and maize (Schmidt et al., 2019) by immobilizing divalent cationic nutrients such as magnesium, zinc, copper, and iron in addition to promoting botulism (Shehata et al., 2013), a fatal illness caused by toxins produced by bacteria Clostridium botulinum, has been demonstrated to be adsorbed by biochar depending on its characteristic; low pH and high pyrolysis temperature favoring the sorption efficiency (Herath et al., 2016). However, adsorbed glyphosate was shown to remobilize in 0.1M monopotassium phosphate, indicating feed glyphosate adsorbed in the biochar could be remobilized in the digestive tract due to multiple ions potentially vying for sorption sites. The finding implies the necessity of further in vitro and in vivo research considering the feed matrix and the digestive tract matrix into account.

While the organochloride insecticide dieldrin level in fat in pigs dropped significantly on feeding biochar amended feed (Dobson et al., 1971), cows fed with 1kg of daily biochar supplement for 14 days did not show any reduction in the milk fat content of the insecticide (Fries et al., 1970). This, perhaps, indicates the essence of further studies on the specific animal dependent effect of biochar's adsorption potential, considering the fact that the ruminants have different digestive tract physiology and the digestive process to that of monogastric animals, and the digestive tract matrix plays an important role in determining the adsorption potential of biochar, as suggested by studies.

2.3.2.5 Attenuation of plant toxin

Tannin, a naturally occurring plant antinutrient that is partially beneficial but detrimental to ruminants, has been shown to reduce feed acceptability, digestibility, and therefore weight gain and performance in farm animals (Naumann et al., 2013). According to a study looking into the impact of biochar on alleviating the effect of tannin-rich forage, feeding 50-100g of biochar per kg of tannin-rich acacia leaf to goats was found to significantly enhance the digestion of crude protein and increase weight gain by 17% and thus, mitigate the adverse effect of the antinutrient (Van et al., 2006). However, supplementing 150g of the same biochar did not show any improvement on weight gain compared to control without biochar, indicating the necessity of dose-dependent study on biochar's potential to attenuate the adverse effect of tannin.

In another experiment, (Scott et al., 2000)reported that on feeding 0.5-1.5g of biochar each day, the sheep consumed 26.4% bitterweed (Hymenoxys odorata DC containing a toxic level of sesquiterpene lactones) of the overall feed with no symptoms of toxicosis, while they refused the bitterweed containing feed without biochar. An intriguing example of biochar's important role in detoxification of plant toxin comes from (McKenzie, 1991), who demonstrated that biochar can be used to effectively treat the poisoning effect of invasive flowering plant, Lantana camara, in his experiment with calves where 5 out of 6 calves treated with biochar survived the poisoning while untreated 5 out of 6 could not make it. Similar results were observed when treated with bentonite but, healing took 3 days longer on average than biochar treatment. Biochar was also shown to be significantly efficient in treating Yellow tulip poisoning in cattle and Oleander poisoning in lambs (Schmidt et al., 2019).

2.3.2 Reduction of enteric methane emission

Of the 81% of greenhouse gas (GHG) production from ruminants in the livestock sector (Hristov et al.), cattle enteric fermentation, an important segment of the environmental footprint of animal production, accounts for 90%, primarily through eructation and minimally via flatulence in the gastrointestinal tract (McAllister et al., 2015). This production of methane (200-500L/day in cattle), being 28 times more potent than CO_2 for global warming, has been forecasted to contribute to nearly 2% of total GHG emission in the next 50-100 years with this level of cattle production and, accounts for 2-12% loss of total energy intake, which, otherwise, could be used for growth and production (Johnson & Johnson, 1995).

Archaea, single-cell prokaryotes are critical in governing the production of methane in ruminants, the production rate being affected not only by the abundance but also the composition of the organism (Tapio et al., 2017). Hydrogen and carbon dioxide produced as end metabolites by the various ruminal microbial community; bacteria, ciliate protozoa, and anaerobic fungi are metabolized into methane by the archaea, where hydrogen serves as an electron donor for carbon dioxide to get reduced to methane. So, the primary strategy, apart from manipulating the ruminal microflora, level of feed intake, type of carbohydrate in the diet, and feed processing, to mitigate methane emission in ruminants would be preventing hydrogen from donating an electron to carbon dioxide i.e., creating alternative hydrogen sink to carbon dioxide, where hydrogen will be disposed, thereby preventing its metabolization by the archaea. Various compounds and feed supplements have been experimented with to study their effect in mitigating the enteric methane productions in ruminants, but the majority of the studies have shown inconsistent results between the in vivo and in vitro trials as well as exhibited variable results among studies (Lee & Beauchemin, 2014). One of the convincing studies on creating hydrogen sink for the reduction of enteric methane production was conducted by (van Zijderveld et al., 2010), the result demonstrating significant reduction of methanogenesis with the dietary supplement of 2.6% of the dry matter of nitrate and sulfate resulting in 32% and 16% reduction in methane production respectively, while 47% reduction was observed when both of the compounds was used. However, the toxicity of nitrates, methemoglobinemia, resulting from its reduction to nitrite and further accumulation in the blood, downplays its possibilities to be used as an alternative hydrogen sink. This implies the necessity of effective and non-toxic strategy to tackle methane production in livestock and, biochar, which has been shown to have a multitude of benefits in addition to its health-promoting and performance-boosting efficacy, can be coined as the non-toxic, reliable, and efficient approach to alleviating methanogenesis in livestock.

One of the remarkable shreds of evidence for the potential use of biochar as a feed supplement contributing to reduced methanogenesis in ruminants was demonstrated by (Leng et al., 2012) in the in vitro experiment using buffalo ruminal fluid and cassava root meal as the substrate. Production of methane was shown to be reduced by 12% when biochar was added at 1% of the substrate while washing off biochar with distilled water, washing reported to increase specific surface area and porosity (Boakye et al., 2019), further reduced methane production by 5% as compared to unwashed biochar. However, increasing the amount of biochar from 2-5% did not significantly affect the reduction in methane production. In the same study, the addition of 1% biochar and 50% of urea and nitrate each as non-protein nitrogen (NPN) source reduced the methane production by 40.5% and 1% biochar with 100% nitrate resulted in as high as 49% reduction. The significant decrease in methane production when nitrate was used as NPN source can be attributed to the higher affinity of nitrate to hydrogen as compared to that of carbon dioxide. Another mechanism, that can be implied regarding higher reduction in methane production with nitrate and biochar, could potentially be the oxidation of methane by denitrifying anaerobic methane-oxidizing bacteria using nitrate as the oxygen source (Schmidt et al., 2019). The authors further suggested that biochar can potentially enhance the denitrifying methane oxidation via redox-active electron mediating properties, which accounts for the additional effect of the combined use of nitrate and biochar as compared to when used individually.

The in vivo experiment that came from the same authors demonstrated the enteric methane reduction by 22% and 29% with biochar and nitrate respectively, and the reduction was observed to be 41% when biochar and nitrate were used in combination(Leng et al., 2012), the result of the in vitro study being somewhat coincident with the in vivo study. Besides a significant reduction in enteric methane production, the live weight of the biochar experimented cattle fed on cassava root chips and foliage was found to be increased by 25%, with the enhanced feed conversion rate. (Hansen et al., 2012) in their in vitro experiment showed the efficacy of biochar (9% of dry matter) in reduced methane production, where biochar addition was shown to cause lowered methane production from 11% to 17% without significant effect on dry matter degradation.

Apart from the above-mentioned mechanisms of biochar in reduced methane production, some other likely phenomenon could be suggested to have played the role. Biochar, which has been shown to support the growth of beneficial lactobacillus and inhibit the growth of pathogens like E. coli (Kim et al., 2017), can be hypothesized to have suppressed the growth of archaea and enhanced that of methanotrophs and other denitrifying anaerobic methane-oxidizing (DAMO) bacteria resulting in a different microbial community so the methanotrophs outcompete the archaea to cause a reduction in the methane production.

While (Leng et al., 2012) and (Hansen et al., 2012) produced a significant reduction in methane production in cattle with biochar addition, studies from many authors could not conclude such a hopeful effect of biochar on mitigating enteric methane emission; 9% reduction (Winders et al., 2019), 5% reduction(Cabeza et al., 2018), 7% reduction (Vongsamphanh et al., 2015). Some of the studies even concluded the non-significant effect of biochar on lowering methanogenesis. The inconsistent result of the various study suggests the essence of systematic study of biochar effect with the standardized characterization of the biochar products with respect to porosity, density, feedstock used, and the pyrolysis temperature among many other parameters likely to have an influence on the effect of biochar in mitigating enteric methane emission. It is too early to conclude the effect of biochar before establishing systematic standardization and further systematic investigations of the effect of different production parameters, biomass used and different ruminal fluid on enteric methane production. Better understanding of the likely mechanism is needed.

2.4 Physicochemical characteristics of biochar

Biochars have varying yield and characteristics determined by the properties of the starting feedstock used and the carbonization condition including temperature and residence time. Feedstock properties was shown to define biochar's total carbon content and mineral element while pH, surface area, and CEC (cation exchange capacity) have been demonstrated to be affected more by pyrolysis condition; high-temperature treatment favoring an increase in pH and surface area and low temperature contributing CEC increase (Zhao et al., 2013), (Mukherjee et al., 2011). The elemental composition of biochar consists of C, H, O, Ca, Mg, Zn, K, Na, Cu, Fe, etc., with carbon exceeding 60% of the total composition followed by hydrogen and oxygen (Tomczyk et al., 2020), (Chen et al., 2019). The content of carbon and mineral matter in biochar have been demonstrated to increase with increasing pyrolysis temperature (Fuertes et al., 2010) and the hydrogen and oxygen content, as well as oxygen containing functional groups, was shown to decrease with increasing pyrolysis temperature (Chen et al., 2008) (Ambaye et al., 2020). Reduced oxygen-containing functional groups have been shown to be associated with lower adsorption of toxic heavy metals like Pb (Inyang et al., 2011). Unlike other organic matters, the unique characteristic of biochar is the presence of an exceptionally higher proportion of aromatic carbon (Schmidt & Noack, 2000) with a condensed aromatic structure having amorphous carbon formed at lower carbonization temperature and turbostrstic carbon dominant at higher pyrolysis temperature (Keiluweit et al., 2010).

Biochar is generally characterized by its high proportion of surface functional groups. Pyrolysis temperature causes breakdown and rearrangement of chemical bonds into new functional groups which include carboxyl, carbonyl, and hydroxyl groups including lactone, phenol, quinine, ether, pyridine, pyridine, etc., where higher temperature contributes to the hydrophobicity of the biochar. (Chen et al., 2019), (Tomczyk et al., 2020). Most of the surface functional groups in biochar are alkaline and oxygenated hydrocarbons originating from the carbohydrate structure of cellulose and hemicellulose.

Attributed to the inorganic molecules in the form of carbonates and phosphates, biochar is alkaline in nature (Yuan et al., 2011). Biochar pH is a function of pyrolysis temperature. With increasing temperature, there is an increased proportion of volatilization of organic acids and the decomposition of acid functional groups such as carboxyl and phenolic hydroxyl which consequentially leads to higher pH (Al-Wabel et al., 2013), (Yuan et al., 2011).

Generally, the specific surface area of biochar is around 500m^2/g depending on the pyrolysis temperature (Chen et al., 2019), increased temperature favoring the rise in surface area (Tomczyk et al., 2020). Organic matter decomposition leading to micropore formation (Katyal et al., 2003) along with aromatic lignin core exposure at higher temperature (Chen et al., 2008) favoring further porous structure contributes to higher specific surface area at a higher temperature. However, the surface area has been shown to increase with the increasing temperature only up to certain critical limit and beyond the limit, surface area decreases with increased temperature potentially due to destruction of microporous structure and enlargement of micropores (Brown et al., 2006).

2.5 Essence of Biochar Characterization

In recent years, biochar has become an extensively exploited substance for diverse applications including waste management, climate change mitigation, soil amendment, water purification, and as a feed supplement. However, the application of biochar for any specific purpose is defined by its physical and chemical characteristics (Jamieson et al., 2014). The physical and chemical composition and characteristics of feedstocks used for the production of biochar are distinct and the pyrolysis condition (temperature, residence time, etc.) causes a further change in the properties, which gives biochar unique characteristics depending on these parameters (Kloss et al., 2012), (Zhao et al., 2013). The elemental composition, moisture content, the compositional difference in certain polysaccharides such as cellulose, hemicellulose, and lignin in the feedstocks, and the pyrolysis condition cause an immense difference in the properties of respective biochar such as porosity and specific surface area (Lua et al., 2004), (Kloss et al., 2012), which in turn determines its suitability for a particular application. For instance, biochar having high sorption capacity attributable to its high porosity and specific surface area could be employed as sorbents in water treatment plants and additives to feed and, those with high water holding capacity and mineral-rich biochar can find its use in soil amendment (Zhao et al., 2013). A careful selection of feedstock and pyrolysis condition is indispensable for optimizing biochar with desired characteristics for specific application. In order to attain the maximum benefits from biochar use, it is critical that the characteristics of different biochar from varying feedstocks and processing conditions are thoroughly investigated, and the substance systematically characterized to assess its suitability for a particular application.

2.4 Limitations of biochar application as a feed additive

2.4.1 Possible side effects

To our knowledge, the studies on the application of activated and non-activated biochar in animal feed have not shown any negative effects so far, even though some studies could not produce the expected positive result. Based on the reported works of literature on the application of biochar as a feed additive, biochar has the potential to positively influence the growth (Phongphanith & Preston, 2018) and feed conversion rate (Sivilai et al., 2018), but does not have any negative effects on animal health and performance. However, long-term supplementation of animal feed with biochar can potentially shift the microbiome composition in the gastrointestinal tract and potentially adsorb the feed nutrients and pharmaceuticals (Schmidt et al., 2019).

In addition to the adsorption of pathogens like E. coli, biochar was also demonstrated to have adsorbed beneficial microorganisms in the gut including lactobacillus acidophilus, Bifidobacterium thermophilium, and Enterococcus (Naka et al., 2001). Even though the adsorption efficiency for these microflorae was significantly lower compared to pathogens, there is still a possibility that this phenomenon may lead to the shifting of microflora in the gut. It has been suggested that attributed to the size of the cell envelope, gram-negative bacteria are well adsorbed to the biochar compared to gram-positive bacteria which are either not adsorbed at all or adsorbed to a lesser degree (Schmidt et al., 2019). While there is more to understand, via systematic investigations with diverse pathogenic and beneficial gut microflora, the mechanism of the specificity of microbial adsorption of biochar, there is a possibility that biochar with certain characteristics (surface area or pore size distribution) could be produced to favorably adsorb specific target microflora. Moreover, it may also be possible to influence the gut microbiome composition with beneficial bacteria by the use of biochar as an inoculum matrix for the purpose (Naka et al., 2001).

Biochar incorporation in the layer's feed was shown to result in lower fat-soluble vitamin concentration in the egg compared to control (Fujita et al., 2012), indicating adsorption of the vitamin in the feed with co-feeding biochar. The α-tocopherol and ɣ-tocopherol level in the eggs was found to be significantly reduced by as high as 40%. (Prasai et al., 2017) demonstrated a significant reduction ($p=0.001$) in yolk color score with the increasing addition of biochar (1%, 2%, and 4%), suggesting the adsorption of carotenoids in the feed. The fact that there is co-adsorption of fat-soluble vitamins with the incorporation of biochar in animal feed opens up a new perspective on subject necessitating a systematic research emphasizing liposoluble feed nutrients so as to develop and characterize biochar accordingly.

2.4.2 Harmful organic compounds in biochar

Depending on the products and the production method, biochar has been documented to constitute the increased availability of some hazardous organic compounds, particularly polychlorinated aromatic hydrocarbons (PAHs) and polychlorinated dibenzodioxins and furans (PCDD/DFs) generated during the process of pyrolysis and gasification (Lyu et al., 2016), which might pose threat to human and animal health (El-Naggar et al., 2019) because of their carcinogenic, mutagenic and teratogenic effects (Rehmann et al., 2008), The production condition, more specifically pyrolysis temperature and the residence time being the key factors influencing the level of these compounds, low temperature (<500°C) facilitates the high level of low molecular weight/high vapor pressure PAHs while, high temperature (>500°C) supports the production of a higher level of high molecular weight/ low vapor pressure PAHs (Brown et al., 2006). (Wang et al., 2017) reported the relationship between the concentration of PAHs in biochar and the residence time during pyrolysis suggesting slow pyrolysis with longer residence time produces a lower level of PAHs compared to fast pyrolysis with short residence time.

Regarding the level of PAHs and PCDD/DFs, European Union has set some limitations on the use of biochar as a feed additive and thus only biochar with a certain limit value of these organic compounds is allowed to be supplemented in feed. The following table represents the list of such compounds with their corresponding reference limit value at 88% dry matter:

Table 1:Harmful compounds in biochar

Organic compounds	Trigger value	Limit value
Polychlorinated dibenzodioxins (PCDDs)/Polychlorinated dibenzofurans (PCDFs)	0.5 ng TE/kg	0.75 ng TE/kg
Dioxin-like Polychlorinated biphenyl (WHO-PCB)	0.35 ng TE/kg	----
Non-dioxin-like Polychlorinated biphenyl (DIN-PCB)	-----	10 µg TE/kg
PCDD / PCDF + dl-PCB	1.25 ng TE/kg	----
PAH benzo[a]pyrene		25µg/kg

(Source: EBC, 2012)

A considerable number of literature on the application of biochar as a feed additive in the farm animal’s diet demonstrate a positive tendency towards different investigated parameters such as growth performance, feed efficiency, toxin adsorption, digestion, and enteric methane emission.

However, some studies have exhibited statistically non-significant results. Likewise, the negative effect of biochar application in regard to nutrient adsorption has also been reported. Generalizing in overall the results of the recent studies carried out, biochar could potentially be a potent feed additive to influence farm animal health and thus, performance positively.

3. Materials and method

3.1 Location of the experiment

The experiment was carried out in co-operation with Standard Bio in a pig farm run by local farmer Anna Kristin Foreborg in Bo, Telemark. The farm raises approximately 2100 pigs every year. The feeding trial was conducted for 60 days between October 2020 and December 2020.

3.2 Experimental design

A total of 84 ninety-days old pigs with an average weight of 56.62kg were used in the experiment. Out of the 84 pigs, 27 pigs were duroc breed while the rest of the pigs were Noroc breed (a mix of Duroc and Norsk Landsvin) pigs. However, it was not a part of the experimental design to have 27 Duroc breed pigs and the rest Noroc, it was just that the farm was delivered with 27 Duroc breed pigs out of approximately 600 pigs the farm received. The total number of pigs experimented with biochar was 42, which acted as test animals and the other 42 pigs acted as control animals. Out of 42 on-char pigs, 18 pigs fed biochar were Duroc breed while only 9 pigs from the control group were Duroc and the rest were Noroc breed pigs. The average weight of test animals was scaled to be 57.37kg whereas, the weight of control pigs averaged 55.87kg. The pigs were housed in 8 pens, 8 to 12 pigs a pen, 4 pens with the test animals while, the other four with the control pigs. The floor of pens was concrete made and heated with hot water underneath the floor using the heating pump. The pigs were showered once a day while the floor used to be washed every morning at 7:00.

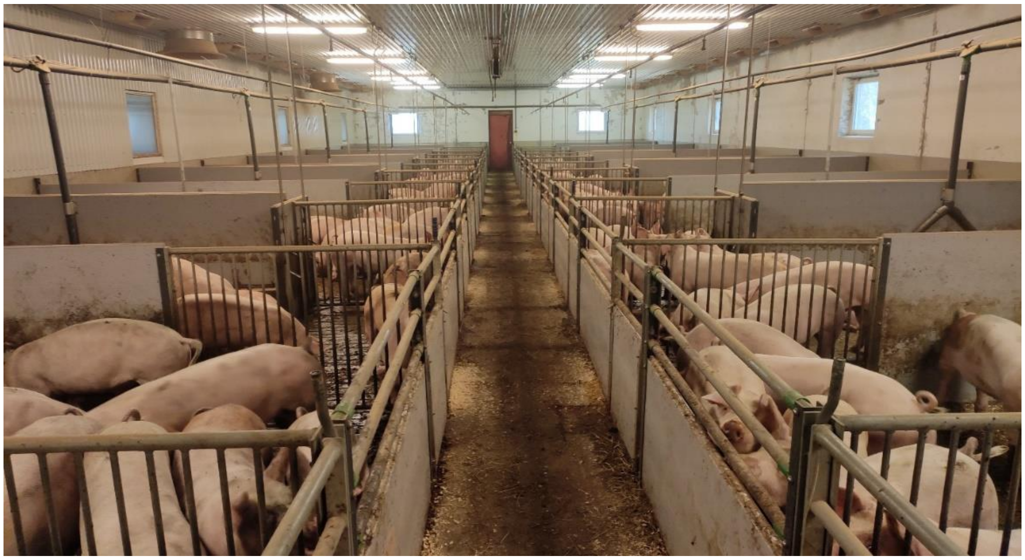

Figure 1: Pig farm in Bo, Telemark

3.3 Diet and diet composition

All the pigs were fed mix pellets (power feed for slaughter pigs) from Felleskjopet, with a feeding frequency of 3 times a day; 6:00 am, mid-day, and 9:00 pm. The feed was first blended with water in a large conical stainless-steel tank outside the farm and then fed in the form of slurry through a piping system installed in each of the pens, the pipes delivering the slurry feed into the feeding tray aligned longitudinally across the pen. The pigs (both control and test animals) received two different pellets format during the course of the experiment, namely ‘soft 125’ until the pigs gained an average live weight of around 80kg (for approximately the first three weeks in the farm) and then switched to ‘soft 115’ until the pigs left the farm for the slaughterhouse. ‘Soft 125’ was powered with 1.12 FUn (FUn is the net energy value of 1kg standardized feed for growing-finishing pigs, 1FUn=8.8MJ) while ‘soft 115’ had 1.10 FUn in it. The pigs had free access to fresh and clean drinking water with easy reach through an automatic water drinker tube.

Figure 2:Automatic water feeder tube used in the experiment

The soft format of feed used in the experiment (‘soft 115’ and ‘soft 125’) was designed to contribute homogenous wet feed and better pumpability at high dry matter content. The feed had high energy content and balanced protein and amino acid composition required for the slaughter pigs. The table below illustrates the amount of feed in feed unit (FUn) pigs received each day. The pigs were fed in groups of 8 to 12 pigs in a pen. So, the following table is an estimation of the amount of feed the pigs received assuming that each pig received an equal amount of feed every meal and every day.

Table 2*:* Daily feed intake throughout the experiment

Week	**FUn/pig/day**
1	2.53
2	2.7
3	2.88
4	3.05
5	3.23
6 until slaughter	3.4

The raw materials used for the formulation of feed include beet pulp, wheat bran, oat, animal fat, sugar cane molasses, corn grits, soymeal, and vegetable fat. Other ingredients used include salt, monosodium phosphate, amino acid mixes, magnesium oxide, and 100mg/kg of vitamin E. The following table shows the content of some of the nutrients in the feed.

Table 3: Nutrient composition of the diet

Nutrients	**Content (%)**
Crude protein	10.1
Crude fiber	14.4
Crude fat	6.6
Ash	6.7
Water	12.8
Calcium	0.66
Phosphorus	0.45
Sodium	0.63
Selenium	0.03 (mg/kg)
Lysine	0.62
Methionine	0.15

3.4 Biochar used in the experiment

The biochar used in the experiment was obtained from Standard Bio, a science-led research and development company specializing in high-quality biochar-based products. The production plant is located in Bo, Telemark, Norway. The state-of-the-art biorefinery system at Standard Bio involves the technology of a flameless combustion system with a rotating furnace to produce high-quality biochar.

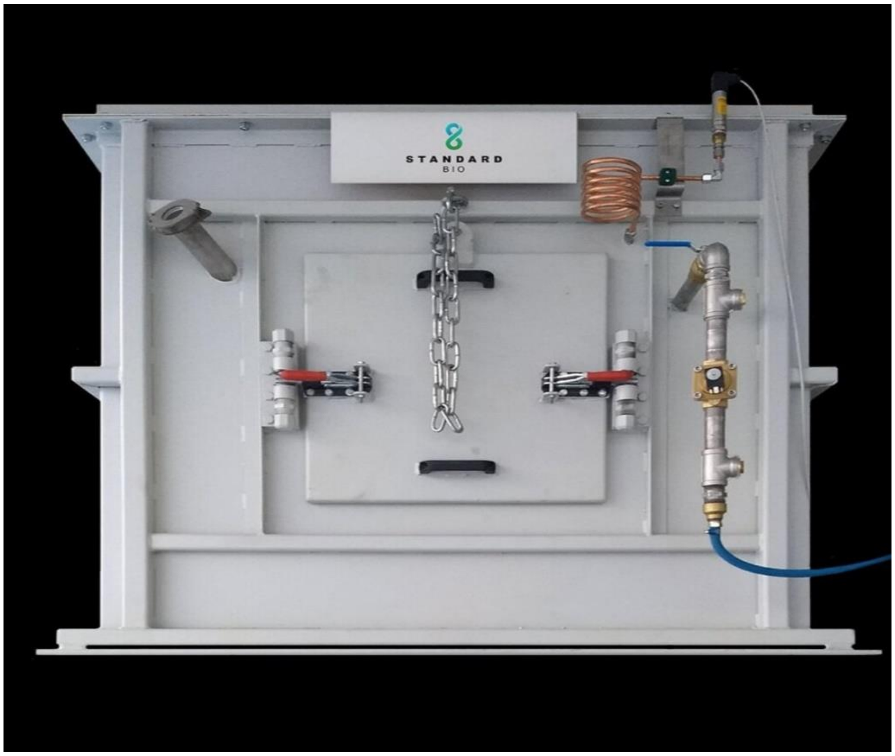

Figure 3:Furnace used to produce Up char

Source: Standard Bio

Certified wood (pine wood) was used as the feedstock material to produce biochar used in the experiment. The characteristics of biochar used have been listed as follows: (source: Standard Bio)

Table 4:Characteristics of biochar used in the experiment

Parameters	Measured value
Pyrolysis temperature	Above 700°C
Specific surface area	378m^2/g
Carbon content	90-93%

pH	7.5-8.3
Ash content (550°C)	1.9%
Moisture	28%
Bulk density	140kg/m^3
True density	1.5g/cm^3

3.5 Feeding of the supplementary biochar

The 42 test pigs were fed biochar each day early in the morning before the pigs were given the first feed of the day. The biochar was fed in its produced form (not pulverized) straight from the kiln. The pigs received approximately 0.5% biochar of the total amount of feed for the day. This accounted for approximately 10 liters of biochar (0.238 liters per pig assuming each pig received an equal amount of biochar) for the first three weeks and then the quantity was increased to 12 liters (0.285 liters per pig) afterward. The biochar was fed separately from the feed by spreading the biochar on the feeding tray across the pen.

Figure 4: The biochar from Standard Bio which was used in the feeding experiment

3.6 Harmful organic compounds content in the biochar used

The biochar is produced according to EBC (European Biochar Certificate) feed grade requirements meeting the guidelines for the EBC premium quality biochar to be used in animal feed. Followings are the list of the organic compounds (that can be harmful to animals) in biochar and their content per kg at 88% dry matter content.

Table 5: Harmful organic compounds content in the biochar used

Organic compounds	Amount in the biochar used
Polychlorinated dibenzodioxins (PCDDs)/Polychlorinated dibenzofurans (PCDFs)	0.35 ng TE/kg
Dioxin-like Polychlorinated biphenyl (WHO-PCB)	0.39 ng TE/kg
Non-dioxin-like Polychlorinated biphenyl (DIN-PCB)	0.26 µg TE/kg
PCDD / PCDF + dl-PCB	0.74 ng TE/kg

Source: EBC and Standardbio

3.7 Measured parameters and the methodology

3.7.1 Weight Gain

The pigs were scaled individually using a one decimal point electronic weighing balance. The test and control pigs, 42 each, were weighed every 2-3 weeks during the 3 months stay in the farm. Pigs were moved one by one to a flat metal platform load sensor connected electrically to the weighing indicator, Tru-test Ezi-weigh 5i weigh scale indicator. The device is manufactured by Auckland-based Agri-tech company Tru-Test.

Figure 5: weighing indicator used in the experiment

source: https://www.tannertrading.co.uk

3.7.2 Stress

Pigs were observed for the tail bitten incidences as a measure of stress. Both the control and test pigs were observed for any deep scars or wounds in the tail at the start of the experiment so that the wounds before the experiment do not interfere with the finding of the experiment in regard to stress measurement. The wound in the tail during the experiment that the farmer considers, from her personal judgment and experience, as tail bitten would be regarded as a case of tail biting and thus, counted as stress incidence. The number and frequency of such occurrences were registered during the entire experiment period.

3.7.3 Diarrhea

The pigs were observed for the consistency of the fecal matter as the animal defecates. Based on the personal judgment of the farmer, the animals defecating abnormally thinner and runny feces were considered as having diarrhea. The number, frequency, and duration of such occurrences were registered throughout the experiment duration.

3.7.4 Joint problems

Pigs were observed for any locomotive dysfunction, lameness, or visible swelling that the farmer, based on her experience and judgment, considers a joint problem. The number of such incidences was registered throughout the trial period. At the start of the experiment, pigs were observed for any of such occurrences so that they would not interfere with the finding of the experiment.

3.8 Data analysis

The analysis of variance of the relative weight gain of the two groups of pigs and among the pens was performed using an independent sample t-test at the confidence level of $p<0.05$. Mean value, standard deviation, and coefficient of variance was calculated using Microsoft Office Excel 15 and, illustrated using the graphical function of the same program.

4. Result

The initial average weight per pig of the 90-days old pigs when brought to the raising farm from the nearby breeding farm was scaled to be lower for the control group (55.87kg) in comparison to the test group (57.38kg). Among the on-char pens, the highest pen average initial weight was scaled to be 60.88kg while the lowest average initial weight was 55.50kg. Likewise, the control group pen average weight ranged from 54kg to 58kg.

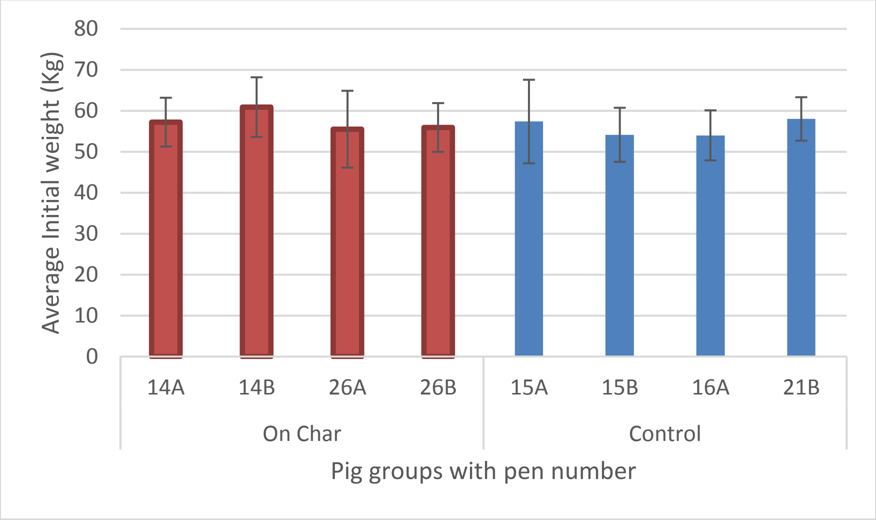

Figure 6:Average initial weight in each pen from two groups of pigs

The two groups of pigs (on-char and control) employed in the experiment showed varying degrees of weight gain over the period of the experiment. The weight gain among the on-char pen averaged from 47.55 kg to 55.91 kg while the average weight gain among the control pen ranged from 49.54kg to 60.43kg. In relation to weight gain performance between test and control animals, no significant difference (p=0.29) was found at 5% level of confidence. The coefficient of variance was calculated to be 21.76% and 20.51% for on-char and control groups, respectively.

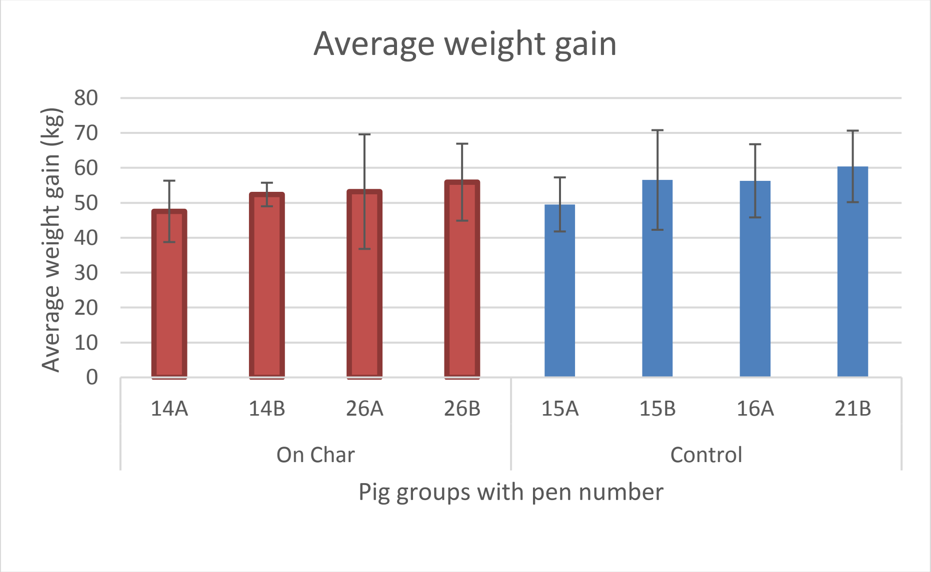

Figure 7: Average weight gain in each pen

Among the duroc breed between two groups, the highest weight gain performance from the test group for an individual animal was measured to be 60kg while the lowest was 34.5kg. On the other hand, the individual control duroc breed had weight gain performance between 37kg (lowest) and

58kg (highest). Likewise, the coefficient of variance of weight gain from the duroc breed was calculated to be 13.85% and 15.62% for the on char and control pigs respectively.

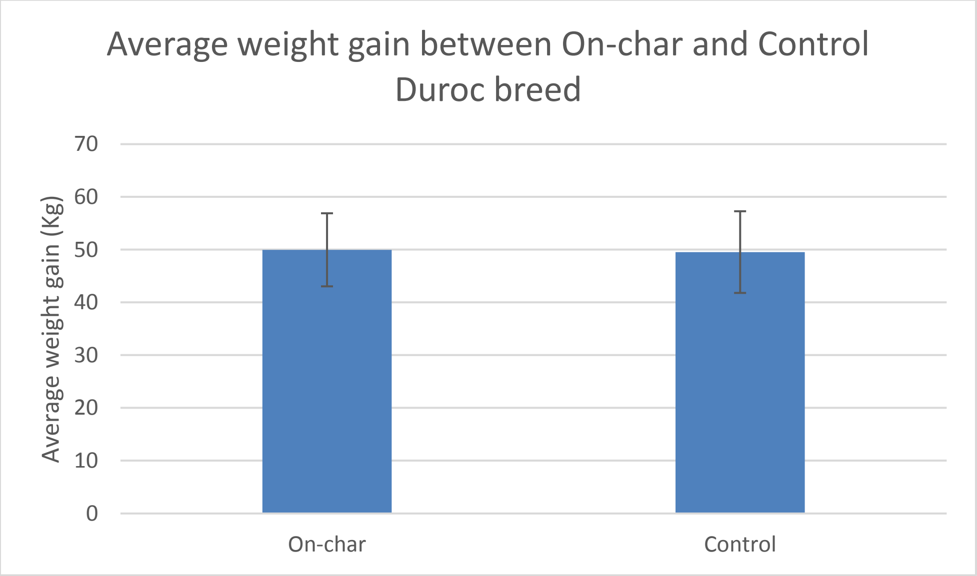

Figure 8:Average weight gain between on-char and control duroc breed

The average weight gain between on-char and control Noroc breed differed by only 3kg, control group having slightly higher average weight gain. The coefficient of variance between two, however, was found to differentiate by around 5%, on-char at 25.17% and control at 20.58%.

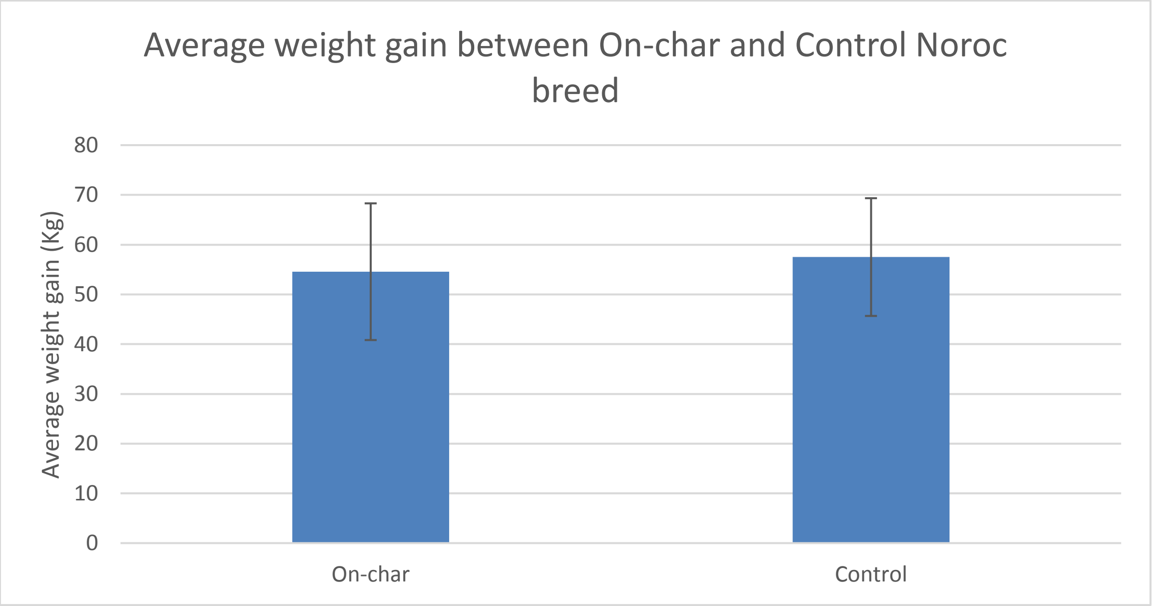

Figure 9:Average weight gain between on-char and control Noroc breed

Statistical analysis was also run to test if two breeds of on-char pigs show any difference in weight gain performance. The Noroc breed was found to have a higher average weight gain of about 5kg. The coefficient of variance, on the other hand, was calculated to be nearly twice as much for the Noroc breed.

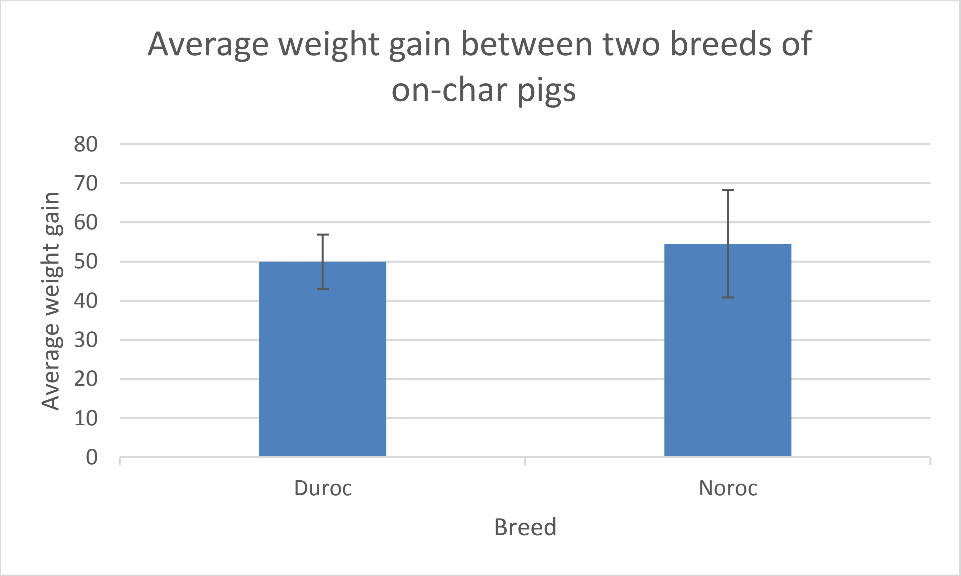

Figure 10: Average weight gain performance between two breeds of on-char pigs

Besides weight gain performance, the animals were also observed for the effect of charcoal on the general wellbeing of the animal. Three observed parameters of general health wellbeing, namely stress level, diarrhea, and joint disease incidences were chosen to study the effect of charcoal on the health performance of the pigs. In this experiment, biochar was not found to have any influence on the incidence of joint disease and diarrhea as no such incidences were observed in both the control and test group of animals. Hence, no data based on the observed symptoms of such occurrences could be collected on joint disease and diarrhea due to the complete lack of such incidences. However, regarding the stress level in pigs, only one incidence of tail biting as a measure of stress was observed in each group of pigs. Hence, due to the lack of a significant number of tail biting occurrences among the 84 experimented pigs, statistical analysis for the stress level was not deemed necessary. Overall, the hypothesis that biochar is effective in improving general well-being in terms of joint disease, diarrhea, and stress was not found to be demonstrable in this experiment.

Table 6: Incidences on wellbeing parameters

Wellbeing parameters	**Number of incidences**	
	On-char	**Control**
Diarrhea	0	0
Tail biting (Stress)	1	1
Joint disease	0	0

5. Discussion

Apart from the literature review of the application of biochar in animal feeding, the main objective of this thesis work was to test the hypothesis of whether biochar as a bioactive feed supplement is effective in enhancing the weight gain performance in pigs. Additionally, the efficacy of biochar in reducing stress, diarrhea, and joint problems in growing pigs was also considered as one of the agendas of the study.

With respect to weight gain performance, a comparison was made between the overall average weight gain of the control and test animals to find out whether there is any significant difference in weight gain between the two groups over the period of the experiment. Statistical analysis of the data demonstrated that the two groups of pigs did not show any significant difference (p=0.29) in weight gain at 5% level of confidence. The result of the experiment implies that the hypothesis of the study that feeding biochar serves to improve weight gain in growing pigs has not been proven in this study.

A considerable number of studies on biochar as a feed supplement have shown very positive results on weight gain improvement in farm animals. Bamboo charcoal (pyrolyzed at 700°C) fed to fattening pigs at 0.3% of feed dry matter was shown effective in improving weight gain by as much as 17.5% (Chu et al., 2013). (Sivilai et al., 2018) demonstrated that supplementing native Moo Lath pigs' diet with 1% rice husk biochar improves weight gain by a significant 20.1% (p=0.089). The authors have suggested that the sorption affinity of biochar towards toxins, providing sites for binding pathogens, in combination with biochar's ability to absorb pathogens, providing habitat for biofilm attachment have resulted in better body health and consequently, improved weight gain performance. The ensiled foliage (taro foliage and banana pseudo stem) used as the diet component in the experiment might contain mycotoxins produced pre- and post-harvest before acidification causes fermentation to cease during ensiling. Biochar's ability to bind the mycotoxin and perhaps, possible absorption of toxic calcium oxalate in taro leaves could have played the role. In vitro experiments using swine digestive fluids revealed that activated biochar effectively adsorbs Fusarium toxins such as deoxynivalenol by 67%, zeralenone by 100%, nivalenol by 21%, and ochratoxin A and deoxynivalenol by as high as 99.86 and 98.93% respectively (Avantaggiato et al., 2005), (Galvano et al., 1996). Besides, biochar combines with phenol in the gastrointestinal tract thereby preventing/reducing intoxication resulting from the interaction of tannin (highly prevalent in taro foliage) with phenolics. Additionally, biochar combines with tannin, thus preventing interference of hydrolysable tannin with enzyme function and protein digestion (Murdiati et al., 1991), which might result in enhanced weight gain. The feed used in our experiment was processed feed, with a possibly very low occurrence of such toxins, which potentially have resulted in no significant difference in weight gain. This can be further supported by the study that demonstrated that cows fed biochar and silage contaminated with mycotoxin deoxynivalenol exhibited enhanced digestibility of protein, NDF, and hemicellulose, a marker of improved weight gain performance, compared to those fed uncontaminated quality silage (Erickson et al., 2011). A similar study with the piglets experimented with wood vinegar absorbed biochar (1:4) and feeding on processed basal diet was not found to exhibit a significant difference in weight gain performance compared to the ones fed only the processed basal diet without biochar (Mekbungwan et al., 2004). While considerable studies have to be done to conclude whether biochar is more effective in improving weight gain in animals fed the foliage and forage or contaminated diet compared to that fed processed industrially manufactured pelleted feed, the possibility here is that biochar supplemented to the pathogen and toxin contaminated feed might result in more improved weight gain in farm animals than that supplemented to pelleted feed. The following table represents the data on the prevalence of three prominent mycotoxins in Norwegian feed:

Table 7: prevalent mycotoxins in Norwegian feed

Type of feed	**DON level (µg/kg) (recommended limit-8000 µg/kg)**	**HT2+T2 level (µg/kg) (Recommended limit-250 µg/kg)**
Pig feed	321	53
Poultry feed	360	80
Horse feed	377	43
Dog feed	408	29
Ruminant feed	1207	62

Source: Norwegian Veterinary Institute

While many charcoal experiments on pigs and other farm animals have shown improved weight gain performance, there are some studies that have shown biochar to be not as effective. In one of the studies, the daily weight gain between the piglets fed biochar incorporated feed and the other control group fed basal diet without incorporating biochar was not shown to exhibit significant difference statically (Kupper et al., 2015). Out of the total 28 studies on biochar experiments on different farm animals reviewed by (Schmidt et al., 2019), 61% of the data set revealed enhanced body weight gain induced by biochar while the rest did not show any significant difference. The reason why some trials have resulted in a positive response to charcoal in relation to body weight gain while some trials were just neutral (no significant difference between biochar and control treatment) could possibly be due to various factors including the basal diet used in the experiment as explained earlier, the experimental design and systemization of the research, the feedstock used for the production of biochar and the proportion administered (dosage), pyrolysis temperature and characteristics of biochar (specific surface area, pore size, number of pores per unit surface area, etc.).

Feedstock kind and pyrolysis temperature are the two major parameters defining the physiochemical properties of biochar including specific surface area, pore size, which thereupon determines the sorption potential of biochar (Tomczyk et al., 2020) (Schmidt et al., 2019). This implies that the variation in weight gain performance, attributed to the sorption capacity of biochar resulting in improved animal health, could potentially be due to the use of biochar differing in biomass used and pyrolysis condition (temperature, residence time). This highlights the importance of systematic characterization of biochar according to its application, through systematic trials using biochar of different feedstock kinds and pyrolysis conditions, when it comes to attaining the targeted objective of biochar in the animal production system. In one of the biochar experiments with pigs, (Kim et al., 2017) showed that for three different biochar supplemented at the same proportion of the basal diet, the aflatoxin absorption capacity was 10%, 20%, and 100% between the treatments with significantly different lactobacillus and E. coli count in the feces ($p<0.05$). This further demonstrates the significance of taking into account specific biochar properties in livestock farming.

The proportion of biochar supplemented to basal diet is an important parameter influencing the adsorption and weight gain performance. In their biochar experiment with cattle, (Leng et al., 2012) showed a 25% increase in live weight gain compared to control when biochar was supplemented at 0.6%. However, with a rather higher dosage (2% of basal diet) of biochar, (Kim & Kim, 2005)could not produce significantly improved weight gain in the cattle, demonstrating the importance of considering specific biochar dosage. The significance of dosage can further be supported by one of the pig experiments with biochar, where 0.3% supplementation of bamboo biochar produced significantly improved weight gain and feed conversion compared to 0.6% treatment (Chu et al., 2013). Furthermore, (Kana et al., 2011) in their biochar feeding trial with broiler chicken illustrated the importance of dosage as growth performance with biochar up to 0.6% was shown to be significantly higher compared to control, whereas higher dosage did not produce significant weight gain. The studies with varying proportions of biochar administered resulting in varying growth performance point out how different the response to different dosages of biochar on weight gain can be and, this indicates the necessity of further investigation on the dosage of biochar supplementation required to achieve optimal growth performance in production animals.

Proper research and a systematic experimental design are crucial in obtaining the result that can be reliable and replicable. In the current feeding trial, pigs were housed in pens in a group of 8 to 12. This kind of experimental design does not allow proper monitoring of the quantity of experimental feed supplement and the feed itself that each pig received. The possibility is strong that the pigs received varying amounts of biochar and the feed, which consequently leads to the deviation of the result from what it should be had the pigs been housed individually and provided an equal amount of biochar and the feed with required care, control, and monitoring. It is possible that the experimental design was insufficient to discriminate the effects of feeding biochar across the trial period.

In relation to weight gain performance among two breeds of the control and test group, the average weight gain performance between the on-char and control duroc breeds was found to be similar, while the control Noroc breed showed slightly better weight gain compared to the same breed of test animals. However, due to the lack of replications, analysis of variance could not be conducted. To my knowledge, no feeding trial experimenting with biochar on different breeds of the same animal or between two or more species of farm animals has been reported previously. Thus, a rather systematic experimental design would be required to study and conclude any findings on the breed-dependent effect of biochar on growth performance.

Regarding the stress measurement between the on-char and control groups, where tail biting incidence was taken as the only parameter of measured stress, both control and test groups exhibited a single case of such incidence. The finding of the experiment with regard to stress is difficult to consider fair and the data adequate to draw any justifiable conclusion and suggestions.

Tail bitten incidence alone is less likely to give a reliable measure of stress, which is associated with multifactorial causes like environmental, immunological, metabolic, etc. (Martínez-Miró et al., 2016). Stress can induce other aggressive behavior in pigs like fights, head knocks, levering other pigs, vocalizing, ear-biting, etc. Apart from the direct behavioral observation and automated behavior recognition video analysis used for the measurement of stress in pigs, different biomarkers are used for evaluation of stress, which is supposed to be ideal for a more accurate and reliable measure of the stress resulting from diverse causes (Martínez-Miró et al., 2016).

Concerning the diarrhea cases, no pigs from either of the groups showed any incidences of diarrhea irrespective of the treatment. Based on the personal judgment of the experienced farmer, neither of the 84 pigs over the period of the whole feeding trial had any symptoms that the farmer would consider as diarrheal incidence. Despite the result of the current study where the effect of biochar in relation to diarrhea control could not be demonstrated, biochar has a long history of usage as a treatment for gastrointestinal disorders including an antidote for poisoning and diarrhea. Studies related to the supplementation of biochar in animal feed aimed at finding its effect in reducing diarrhea are very limited. However, in one of the biochar trials with pigs, (Chu et al., 2013) found the reduced number of coliform bacteria in the feces of pigs fed 0.3% of bamboo biochar, suggesting the possibility that biochar may have an effect on inhibiting the proliferation of Escherichia coli, which is the dominant microorganism responsible for inducing diarrhea in the postweaning pigs (Sun & Kim, 2017). In another study with chicken, (Hien et al., 2018) also demonstrated a positive effect of rice husk biochar incorporated at 1% on the occurrence of E. coli. (Naka et al., 2001) showed complete adsorption of E. coli with 10mg of activated charcoal with maximum adsorption in five minutes and suggested that the high surface area and porous properties of biochar can be attributed to the adsorption of pathogens. Further investigations are needed to be conducted to conclude the proven effect of biochar in reducing diarrhea in farm animals and the mechanism behind this effect.

6. Conclusion

In this current experiment, biochar did not appear to significantly influence weight gain performance in pigs, nor it was shown to have any effect on other measured parameters. For all other observed parameters, a significant amount of data was not available to draw any conclusion while, regarding the weight gain performance, the result obtained contradicts the vast majority of results published earlier. However, with (myco)toxin and pathogens-laden basal diet where biochar can actually function what it is meant for, the result could potentially be expected to have a positive tendency towards growth performance. Furthermore, a more systematic and realistic condition with well-suited experimental design and proper monitoring in relation to intake of basal diet and test additive is highly recommended.

7. References

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