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The effect of biochar supplementation on feed utilization, milk production and methane emission in lactating dairy cows

Marie T. Dittmann[a,*], Cem Baki[a], Melissa Terranova[b], Sergej L. Amelchanka[b], Sébastien Dubois[c], Andrea Wiget[d], Florian Leiber[a], Hans-Martin Krause[d], Stefan Baumann[d]

[a] Department of Livestock Science, Research Institute of Organic Agriculture FiBL, Switzerland
[b] ETH Zurich, AgroVet-Strickhof, Lindau, Switzerland
[c] Feed Chemistry Research Group, Agroscope, Posieux, Switzerland
[d] Department of Soil Science, Research Institute of Organic Agriculture FiBL, Switzerland

A R T I C L E I N F O

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A B S T R A C T

There is an increasing trend in agriculture to use biochar (BC) as a means for carbon storage and soil improvement, and it has been suggested, that feeding BC to livestock can improve animal health and performance, and reduce enteric methane emissions. The aim of this study was to investigate if adding BC to a balanced ration of a group of lactating Holstein dairy cows had an effect on their milk yield and quality, methane emission, nutrient digestibility and health. In a crossover experiment lasting for two 36-day periods, eight cows received their basal ration with and without 1 % DM BC. During the last week of each period, the cows were kept in tie stalls where milk yield and composition, feed intake and nutrient digestibility, as well as blood parameters were quantified. On the last two days, methane emissions were measured in respiration chambers. The results indicate that there was no significant effect of the addition of BC on any of the measured variables. Voluntary intake was not affected by the addition of BC ($P=0.52$) and none of the cows exhibited health problems in response to the treatment. There was no increase in milk yield ($P>0.46$) or composition ($P>0.23$) and methane emissions were not significantly affected ($P>0.37$). While the beneficial effects of feeding BC to animals suffering from health problems or receiving a poor diet cannot be excluded, the data of this experiment indicate that BC is not an effective feed additive to improve performance or reduce methane emission in dairy cows.

1. Introduction

Photosynthesis in plants is one of the most effective processes to remove the greenhouse gas CO_2 from the atmosphere and bind it in the form of carbohydrates. Through pyrolysis, plant material can be transformed into biochar (BC), a stable carbon compound. By its amendment to agricultural soils BC can be considered as a carbon sink, effectively removing CO_2 from the atmosphere (Smith, 2016). The usage of BC in agriculture, e.g. via co-composting or slurry amendments is proposed to have positive effects on plant growth and soil health (Jeffery et al., 2017; Schmidt et al., 2021). To exploit BC's potentially beneficial properties and incorporate it into current

* Corresponding author.
E-mail address: mdittmann@gmx.ch (M.T. Dittmann).

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agricultural management practice, it has also been suggested to use BC as feed additive for livestock (Joseph et al., 2015). Feeding BC to animals as been suggested to have positive effects on their performance (Lao and Mbega, 2020), health (Man et al., 2021; Schmidt et al., 2019) and methane emissions (Winders et al.2019). It can also be considered a means to load BC surfaces with nutrients and to achieve an even distribution of BC in the manure or slurry, which is then applied to the soil (Joseph et al.2015).

In Switzerland, cattle make up the largest proportion of livestock (1.5 Mio heads, Bundesamt für Statistik, 2021) and introducing BC into their rations would thus create a steady flow of carbon to the soil via manure. In addition to the potential of BC to physically store carbon, the claim that the feeding of BC reduces the emission of enteric methane (CH_4) is currently propagated among agricultural stakeholders. However, peer-reviewed studies quantifying such effects in cattle are scarce and they report variable results: Feeding experiments with heifers (Terry et al., 2019b), steers (Sperber et al., 2022; Winders et al., 2019) and beef cows (Conlin et al., 2021) could not confirm a significant effect of the feeding of BC on CH_4 emissions. None of the studies where emissions were quantified in small ruminants report an effect of feeding BC on enteric CH_4 production (Lind et al., 2020; Silivong and Preston, 2015). Only the results from one experiment conducted with calves of a tropical cattle breed show a significant reduction in absolute methane emission in the animals fed BC (Leng et al., 2012). To our knowledge, there is no study reporting the effect of feeding BC on methane emissions from lactating dairy cows.

Besides the effect of BC on methane emission, its impact on performance and health has been investigated repeatedly in cattle. Erickson et al. (2011) reported that an addition of 20–40 g of BC per cow per day increased intake and digestibility of neutral detergent fiber (NDF) and crude protein (CP) in poor quality forage, but not in good quality forage. In fact, milk yield showed a slight drop with the addition of BC. An increased protein digestibility is not only beneficial with regard to animal efficiency but also because of reduced N pollution of the environment (Castillo et al., 2000). Optimizing the use of dietary N could thus be another benefit of feeding BC.

Leng et al. (2012) found a slight increase in daily gain and feed conversion in growing tropical cattle when BC was supplemented at 0.6 % dry matter (DM). In an experiment with beef heifers, the supplementation of 2 % BC had no significant effect on nutrient digestibility and nitrogen use (Terry et al., 2019). Sperber et al. (2022) even reported a negative effect of feeding BC on dry matter intake (DMI) and average daily gain (ADG) in growing beef cattle. None of the feeding experiments performed with cattle (Erickson et al., 2011; Winders et al., 2019; Leng et al., 2012; Terry et al., 2019; Sperber et al., 2022) reported any notable negative effects on animal health. As BC has been found to significantly reduce the availability of trace minerals to plants in the soil through binding them (Namgay et al., 2010), there is concern in the agricultural community, that a similar effect may also manifest when feeding BC to livestock. However, this hypothesis has never been tested in an animal trial.

Several feeding trials with BC have been carried out in small ruminant species. Van et al. (2006) found that the feeding of bamboo charcoal to goats increased digestibility of DM, organic matter (OM) as well as CP, and had no effect on NDF and acid detergent fiber (ADF) digestibility. These results could not be replicated in a study where goats where fed a coconut shell derived charcoal, which reported a decrease in the digestibility of NDF and ADF (Al-Kindi et al., 2017). Feeding three types of BC (based on walnut shells, pistachio by-product and chicken manure) lead to an increase in ADG and feed conversion rate (FCR), but only the supplementation of walnut shell-based BC significantly increased digestibility of DM, OM, CP and NDF (Mirheidari et al., 2020). A study with pine-based BC fed to lambs showed an increase in DM digestibility, but no effect on ADG, feed conversion rate (FCR), digestibility of NDF or CP, and a decrease in ADF digestibility (McAvoy et al., 2020). These inconsistent results may root in the fact that BCs, originating from different source materials, vary in their effect on digestive processes.

While there is an attempt to regulate the quality of BC used as animal feed through the European Biochar Certificate (EBC) (Schmidt et al., 2016) raw materials and production methods vary between manufacturers. Since the quality and properties of BC vary when different raw materials are used for production (Břendová et al., 2012) results of previous feeding trials may not be directly translated to other situations. For example, the BC used in previous feeding studies with cattle were produced from rice husks (Leng et al., 2012), American pine trees (Terry et al., 2019; Winders et al., 2019; Sperber et al., 2022), or unknown source (Erickson et al., 2011). Neither rice husks or American pine trees would be sources for EBC-certified BC in Europe. The aim of this study was to investigate the effect of feeding a locally produced EBC-certified BC to lactating dairy cows in a production system typical for central Europe.

Previous to the animal trial, an online survey was conducted on the use of BC among farmers in Switzerland (unpublished data). Of the 197 survey participants, 39 reported to use BC as a feed additive or as an oral treatment in case of illness in dairy cows. Of these, 6 participants reported to use BC to increase their animals' performance, 32 to treat or prevent diseases, 5 to increase feed quality, and 20 to reduce GHG emissions. The participants had the option to specify why they fed BC to their cattle. The answers included treatment of diarrhoea or improvement of digestion (7 participants), binding of toxins in the feed (2 participants), reducing somatic cell counts (SCC, 3 participants), a reduction in milk urea, generally better milk quality and healthier cows (1 participant each). One participant reported, that they observed higher cell counts when BC was used as a feed additive, which is why they stopped using it. Based on these reports and previous studies, the following hypotheses were investigated: Feeding BC i) reduces the production of enteric methane, ii) improves milk quality and nutrient digestibility, as well as iii) body condition and blood parameters related to animal health.

2. Material and methods

2.1. Animals, diets, and study design

The experiment started in December 2022 and took place at the research facilities of AgroVet-Strickhof, Lindau, Switzerland. The study was designed as a cross-over experiment with two periods and eight multiparous Holstein dairy cows in mid-lactation. At the beginning of the study, the cows in the two groups were on average 170 ± 51 days in milk, in their 2nd to 6th lactation, 5.8 ± 1.8 years old, weighed 728 ± 36 kg, had a daily milk yield of 33.3 ± 3.3 kg (averaged over the week prior to study start). Based on the data collected prior to the beginning of the feeding trial, the cows were paired by milk yield and the members of each pair were distributed into one of two groups to achieve a similar average in days in milk (DIM) between groups.

In each experimental period, the groups received either of two diets: the standard basal mixed ration (BMR) or the same BMR with BC at a dosage of 1 % DM, which is in line with manufacturers recommendation. The BC used in this experiment was produced with a continuous auger pyrolysis system at temperatures of approx. 650°C (APD – Pflanzenkohle, APD Auen Pflege Dienst AG, Flaach, Switzerland). The BC was produced from wood chips (mixture of different soft- and hardwood) and certified according EBC-AgroOrganic and EBC-Feed (EBC 2012–2022). Biochar properties are shown in Table 1.

In addition to the BMR, all cows received a fixed amount of supplementary concentrate feed (UFA 243 and UFA 249, UFA, Sursee, Switzerland). The forage to concentrate ratio in the ingested diet was approximately 0.7:0.3 on a DM basis. The composition of the BMR is shown in Table 2.

The entire experiment lasted 72 days, which consisted of two 36-day periods. Each period consisted of 27 days of adaptation to the diet in loose housing, a 2-day adaptation period to the tie stalls, a 5-day collection period in tie stalls and two days spent in respiration chambers. Each cow underwent one period with and one period without BC in her diet.

2.2. Data recording and sampling

During the adaptation period, the experimental diet was offered in individually assigned feed troughs, which recorded the intake of feed. Feed intake (as fed) and milk yield were recorded on a daily basis. After the adaptation period, the cows were moved to tie stalls in the metabolic centre of AgroVet-Strickhof. On day 0, day 34 and day 70 cows were weighed on a livestock scale, BCS was assessed according to Isensee et al.(2014) and a blood sample was taken after the morning feeding from the coccygeal vein. All cows were fitted with smaxtTec boli (smaXtec animal care GmbH, Graz, Austria), which recorded the temperature in the reticulorumen every ten minutes throughout the duration of the entire experiment.

After a two-day adaptation to the tie-stalls, a 5-day collection period started. Cows were fed the BMR for ad libitum DMI, aiming for 10 % refusals. The feed was split into two portions which were fed after the morning and the evening milking. Refusals were removed, weighed and sampled daily before the fresh feed arrived. Supplementary concentrate feeds were offered together with the BMR on the feed table in the tie-stall or in the respiratory chamber troughs. Representative samples of the BMR were taken daily. At all times, cows had unrestricted access to water. Cows were milked twice daily at 5:30 and 16:30 and the milk of the individual cows was collected in buckets and weighed on a scale (ID2 Multirange, Mettler-Toledo, Greifensee, Switzerland). During each milking in the 5-day sampling period, milk samples were collected and preserved with Bronopol at 4 °C before being sent to the laboratory.

During the 5-day sampling period, cows were housed on rubber mats without bedding. Sampling of urine and faeces was done following the methods described Terranova et al. (2021): Faeces were collected in steel trays located below a grid at the rear end of the tie-stall. Urine was collected separately from faeces with urinals attached around the vulva of the cows. A hose attached to the urinals lead the urine into a container, and a subsample was diverted into a canister containing 30 g of 5 M sulfuric acid to prevent gaseous N losses. Faeces and urine were weighed and sampled once per day. A proportion of 1 % of the total faeces and 50 ml each of acidified and non-acidified urine were frozen at −20 °C. Faeces samples were later pooled to one sample per cow.

2.3. Respiration chamber measurements

Two open-circuit respiration chambers with a volume of 38 m^3 (no pollution Industrial Systems Ltd., Edinburgh, UK) were used to measure CH_4 production of the cows. The chambers were set to an ambient temperature of 12 °C, a relative humidity of 60 % and a negative air pressure of approximately 20 Pa. Airflow was set to 40 L/sec (extraction fan FPZ K05 Blower, FPZ Blower Technology, Concorezzo, Italy). Concentrations of CH_4, CO_2, and O_2 were measured in each chamber for 2 minutes at 10 min cycle for each chamber and fresh air (sequence: chambers 1, 2, 3, 4 and fresh air) using a gas analyser MGA 3500 (ADC Gas Analysis, Hertfordshire, UK). Before starting the measurement and every day after, the gas analyser was calibrated with pure N_2 for Zero level and a mixed gas

Table 1
Characteristics of the used biochar.

Parameter	Unit	Value
Bulk density (< 3 mm)	kg/m^3	209
Water holding capacity (< 2 mm)	%	341
Water content	Ma.-%	19.7
Ash content (550 °C)	Ma.-%	10.6
Carbon, organic	Ma.-%	83.5
H/C ratio (molar)		0.25
pH in CaCl2		8.6
Sum 8 EFSA-PAH	mg/kg	NA
Sum 16 EPA-PAH	mg/kg	2.2
Mesh analysis		
< 63 µm	Ma.-%	1
63 µm - 0.63 mm	Ma.-%	23
0.63–2 mm	Ma.-%	26
> 2 mm	Ma.-%	50

Table 2
Components and composition of the experimental diets. The basal mixed ration (BMR) served as control, while the treatment diet contained biochar.

Components	BMR without biochar	BMR with biochar
	In g / kg DM	In g / kg DM
Grass silage	549	544
Maize silage	155	154
Sugar-beet pulp	121	120
Hay	43	42
Commercial concentrates*	129	127
Salt (NaCl)	3	3
Biochar	0	10
Chemical composition		
Organic matter	893	893
Crude Ash	107	107
Neutral detergent fibre	427	428
Acid detergent fibre	266	275
Crude protein	148	147
Crude fat	33	31
Gross energy content (MJ/kg)	18.1	18.4

* Main components: soybean meal, triticale, extruded rape seed

(20.9 % O_2, 4'000 ppm CO_2, 800 ppm CH_4 in N_2 as carrier) for Span level. Cows were kept in the respiration chambers for 50 h (2 full days plus 2 h accounting for milking and cleaning in-between). During this time, the cows were milked twice daily and fresh feed was offered twice daily at the same times as during the collection period. Feed refusals were removed daily. Gas measurements generated during the times when people entered the chambers were excluded from the analysis. Total gas volumes produced by the cows where calculated based on the concentration of each gas in the incoming and exhaust air of the chamber as well as on the flow rate of the air through each chamber, which was corrected for standard temperature and pressure (STP). Gas concentrations were corrected according to recovery rates, which were quantified three times throughout the experiment (before, in the middle and straight after the experiment) by injecting pure CO_2 (99.995 %) and CH_4 (99.9995 %) via a mass flow controller (MC-5SLPM-RD/5 M, ALICAT Scientific, Tucson, United States). The mean recovery rate between the chambers was 103 % for CH_4 and 90 % for CO_2.

2.4. Chemical analyses

Before analyses, feed and faeces samples were dried at 60 °C for 48 h. After being ground to pass a 1-mm screen (Brabender rotary mill; Brabender GmbH & Co. KG, Duisburg, Germany), feed and faeces samples previously dried were analysed for DM content by heating at 105 ℃ for 3 h followed by incineration at 550 °C until a stable mass was reached to determine the ash content according to ISO 5984_2002 (prepASH, Precisa Gravimetrics AG, Dietikon, Switzerland). OM was calculated as DM minus total ash. CP (N × 6.25) content of feed and faeces was determined by the Dumas method (ISO 16634–1:2008) using a LECO TruMac (Leco, Mönchengladbach, Germany). The content of total bound N in the urine was determined by a C-N-Analyzer (Multi N/C 2100S, Analytik Jena GmbH+Co. KG). Crude fibre content was determined gravimetrically (ISO 6865:2000) by incineration of residual ash after acid and alkaline digestions using a fibre analyser (Fibretherm Gerhardt FT-12, C. Gerhardt GmbH & Co. KG, Königswinter, Germany). The αNDF and ADF contents (ISO 16472:2006 for αNDF and ISO 13906:2008 for ADF) were analysed with the same fibre analyser (Fibretherm Gerhardt FT-12, C. Gerhardt GmbH & Co. KG, Königswinter, Germany) and were expressed without residual ash. Neutral detergent fibre (αNDF) was evaluated with heat stable amylase and sodium sulphite and expressed without residual ash after incineration at 600 °C for 3 h. ADL was analysed according to ISO 13906:2008. Fat content was extracted with petrol ether after an acid hydrolysis (ISO 6492:1999). Gross energy content was determinated by combustion in a calorimetric vessel under pure Oxygen condition using an adiabatic bomb calorimeter (ISO 9831:1998, AC600 Semi-Automatic Calorimeter, Leco Corporation, USA) The Bronopol-preserved milk was analysed for fat, protein, lactose, and urea concentrations with a MilkoScan FT6000 (Foss, Hillerød, Denmark) at Suisse-Lab (Zollikofen, Switzerland). Blood samples underwent chemical and histological analysis at IDEXX Diavet AG (Freienbach, Switzerland).

2.5. Calculations and data analysis

Digestibility (%) of the different nutrients was calculated as
(Nutrient intake - faecal nutrient output) / (nutrient intake) * 100

Nitrogen excretion was calculated by multiplying the concentration of N by the amount of DM faeces or the fresh weight of urine produced. These values were then set into relation with N intake, calculated as the N concentration in the feed multiplied by the daily DM. On some occasions, the urinals became leaky and some urine was lost. The urine volumes of these days were not taken into account when calculating the N excretion of the animals.

Based on the reticulorumen temperature recorded by smaXtec boli it was calculated, on how many days throughout the two 36-day experimental periods the temperature in the reticulorumen was elevated above 39.5 °C and 40.0 °C in at least three measurements for each cow. For blood values where reference ranges for healthy animals were given by the laboratory performing the analysis, it was calculated, how many animals had blood values outside of these thresholds. Absolute methane quantities were set into relation with the intakes of DM, OM, and NDF, as well as milk yield during the 5-day collection period previous to the respiration chamber measurements.

Data were analysed in R Studio (2022.07.2) using the packages lmer.test, psych and plyr. Linear mixed models were used to test the effect of the treatment on the outcome variables. In the models, treatment, period, and their interaction were added as a fixed factors and cow as a random factor. The interaction between period and treatment was removed from the model if it was not significant. P-values of the effect of treatment shown in Tables 1 to 4 are based on the inbuilt function of lmer.test, i.e. on t-tests using Satterthwaite's approximation for denominator degrees of freedom.

3. Results

The total daily intake of BC in the treatment group resulted in an average of 0.87 ± 0.04 % of the total DMI. This translates into an average intake of 234 ± 12 g BC (as fed) per animal per day. None of the variables related to animal performance (Table 3), animal health (Table 4), methane emission and N excretion (Table 5) showed any significant differences between the control and the BC treatment. In none of the models there was a significant interaction between period and treatment and the interaction was subsequently removed for the final models. There was a significant effect of experimental period on several variables: milk yield during the adaptation (P=0.018), feed conversion efficiency (FCE, P=0.017), and milk lactose content (P=0.004) decreased, while the contents of milk fat (P<0.001), protein (P=0.001), CP digestibility (P=0.009) and BCS (P=0.001) increased from the first to the second experimental period.

4. Discussion

The voluntary consumption of BC by ruminants is a prerequisite for using it as an effective feed additive. In this study, adding BC to the diet did not result in a lower DMI, which indicates good acceptance by the animals and is in line with previous studies on cattle and small ruminants (McAvoy et al., 2020; Mirheidari et al., 2020; Terry et al., 2019a; Winders et al., 2019). However, none of the investigated variables showed a statistically significant difference between the control and the BC treatment. The finding, that the feeding of BC results in a lower absolute CH_4 emission reported by Leng et al. (2012) could not be replicated. The lack of statistically significant difference between treatments lines up with previous studies investigating the effect of feeding BC on CH_4 emission in cattle (Sperber et al., 2022; Terry et al., 2019b; Winders et al., 2019) and other ruminants (Lind et al., 2020; Silivong and Preston, 2015).

The adaptation period to the diet in this study (28 d until the collection period and 33 days until gas measurements) was longer than in standard feeding trials. Therefore, it was impossible to capture a potential initial effect due to a change in diet in the data collection. Studies which report an effect of BC on CH_4 emissions (Leng et al., 2012) or nutrient digestibility (Erickson et al., 2011) had shorter adaptation periods of 21 d or 11 d, respectively. Winders et al. (2019), who reported a numerical (but not significant) decrease in absolute CH_4 emissions, performed gas measurements after 12 d of adaptation. A long-term study where beef cattle received 0.8 % BC in their diet over several months reported no effect on methane emissions (Sperber et al., 2022) and feeding BC to steers over a duration of 13 months had no effect on meat quality or blood parameters (Kim and Kim, 2005). It is therefore possible, that after an initial reaction to the new feed additive, the rumen adapts to BC after a few weeks. A reduction in the effectiveness of methane inhibitors over

Table 3
Feed intake, faeces and urine output, digestibility and milk performance (means +/- standard deviation; N=8) for the control and the biochar treatment. All values are based on the averages from the five-day collection period (except for DMI and milk yield during the four week adaptation period, as indicated in the table).

Outcome variable	Control	Biochar	P-value of treatment
DMI during adaptation (kg/d)	23.4 ± 1.2	23.8 ± 1.6	0.13
DMI (kg/d)	22.9 ± 1.3	22.8 ± 0.9	0.52
Faeces DM (kg /d)	6.9 ± 0.3	6.9 ± 0.4	0.58
Urine (kg / d)	32.0 ± 3.9	31.6 ± 2.0	0.80
Apparent digestibility (%) of			
Dry matter (DM)	72.3 ± 1.0	72.0 ± 0.9	0.20
Organic matter (OM)	76.5 ± 1.0	76.2 ± 0.7	0.31
Crude protein (CP)	72.5 ± 1.2	72.4 ± 0.8	0.73
Neutral detergent fibre (NDF)	68.2 ± 2.1	67.2 ± 1.9	0.16
Acid detergent fibre (ADF)	67.7 ± 2.5	67.6 ± 2.3	0.65
Milk parameters			
Milk yield during adaptation (kg/d)	31.7 ± 3.8	31.6 ± 6.1	0.87
Milk yield (kg/d)	31.2 ± 3.7	30.3 ± 6.1	0.46
Milk fat (g/100 g)	3.94 ± 0.50	4.04 ± 0.80	0.24
Milk protein (g/100 g)	3.61 ± 0.36	3.63 ± 0.48	0.32
Milk lactose (g/100 g)	4.77 ± 0.10	4.74 ± 0.21	0.31
Milk urea (mg/dl)	25.4 ± 3.8	26.8 ± 3.8	0.23
Feed conversion efficiency (kg milk/kg DMI)	1.36 ± 0.17	1.33 ± 0.27	0.46
Protein conversion efficiency (kg milk protein / kg CP intake)	0.25 ± 0.02	0.25 ± 0.03	0.67

Table 4
Animal condition and health parameters (means +/- standard deviation; N=8). Delta variables refer to the difference in the denoted variable since the start of the treatment.

Outcome variable	Control	Biochar	P-value of treatment
Body weight (kg)	749 ± 50	736 ± 43	0.32
Delta Body weight (kg)	18.5 ± 44.2	1.5 ± 30.7	0.40
BCS+	2.4 ± 0.1	2.3 ± 0.1	0.67
Delta BCS+	0.1 ± 0.1	0.3 ± 0.1	0.41
Faeces DM (g/kg DM)	116 ± 12	118 ± 13	0.13
Somatic cell count in milk (1000 cells / ml)	105 ± 115	85 ± 66	0.56
Milkings* where SCC exceeded 100'000 cells per ml+	1.5 ± 3.0	2.25 ± 3.45	0.61
Days* with reticulorumen temperature >39.5 °C+	14 ± 3	17 ± 4	0.44
Days* with reticulorumen temperature >40.0 °C+	0 ± 0	0 ± 0	0.99
Blood parameters			
Glutamate dehydrogenase (U / l)	22 ± 9	22 ± 11	0.90
No. of animals with values outside reference range	7	5	
Gamma-glutamyltransferase (U / l)	23 ± 5	24 ± 8	0.67
No. of animals with values outside reference range	1	2	
Zinc (ug / l)	933 ± 198	992 ± 224	0.60
No. of animals with values outside reference range	1	2	
Selenium (ug / l)	99 ± 8	104 ± 11	0.29
No. of animals with values outside reference range	0	0	
Copper (ug / dl)	85 ± 14	86 ± 18	0.88
No. of animals with values outside reference range	0	0	

+For these variables, the numbers indicate median and standard error.
*Values refer to the number of days or milkings averaged over the eight cows per treatment

Table 5
Enteric CH_4 production and Nitrogen excretion. Excretions are based on the average of the five-day collection period. (means +/- standard deviation; N=8).

Outcome variable	Control	Biochar	P-value of treatment
CH_4 in g day^{-1}	512 ± 26	503 ± 37	0.37
CH_4 g kg^{-1} DMI	22.4 ± 1.5	22.1 ± 1.6	0.56
CH_4 g kg^{-1} OMi	23.2 ±1.3	22.8 ± 1.9	0.54
CH_4 g kg^{-1} dOM	30.3 ± 1.7	30.0 ± 2.4	0.69
CH_4 g kg^{-1} NDFi	55.6 ± 3.2	54.9 ± 4.5	0.51
CH_4 g kg^{-1} dNDF	81.5 ± 4.1	81.6 ± 5.4	0.93
CH_4 g kg^{-1} milk	16.6 ± 1.6	17.3 ± 4.0	0.55
N excretion faeces (% N intake)	27.5 ± 1.2	27.6 ± 0.8	0.73
N excretion urine (% N intake)	26.2 ± 5.0	27.5 ± 5.1	0.44
Total excreted N via faeces & urine (%NI)	53.7 ± 5.5	55.1 ± 5.1	0.44

time is a general concern as long-term data are lacking (Hristov, 2023).

The effect of treatment period, which is representative for an advance in lactation duration, a change in day length and climatic conditions, as well as the effect of adaptation to the measurement period in the tie stalls, had a stronger effect on the assessed variables than the addition of BC. This is a common finding in change-over designs, but compared to continuous trials, variation between cows is reduced in such experimental designs which increases the precision in detecting small to moderate effects of diet (Huhtanen and Hetta, 2012). With progressing lactation, the cows in this study showed a lower milk yield during the adaptation period, a lower FCE and a higher BCS, indicating that the conversion of feed to milk was shifted to the conversion of feed to body reserves.

The feeding of BC in this study was investigated in a system representative for intensive dairy farming in Switzerland, which is characterised by high milk yields, comparatively high amounts of concentrate feed and typical conventional dairy breeds. In this setting, with a limited number of cows in mid lactation, the feeding of BC had neither negative nor positive effects on the assessed variables. The results of this experiment may not be representative for the effect of BC in extensive systems. A previous study performed with dairy cows could demonstrate, that the effect of activated carbon was only detectable, when cows were on a poor quality diet (Erickson et al., 2011). It appears that BC is effective in binding toxins in the digestive tract of ruminants (summarized in Schmidt et al., 2019) and there are anecdotal records of a reduction in SCC and hoof problems, as well as a decrease in diarrhea symptoms when feeding BC (Gerlach and Schmidt, 2012). Furthermore, BC may have a different effect in beef cattle, young or sick animals. Several studies report a positive effect of BC on weight gain in cattle (summarized in Man et al., 2021) and it may be effective in treating Cryptosporidiosis in calves (Watarai and Koiwa, 2008). Two health related variables in this study were elevated in some animals: SCC and serum levels of glutamate dehydrogenase, the latter indicative of liver damage. In these animals, feeding BC did not result in an obvious improvement of the values. The blood samples taken in this study cannot depict variation within or between days, but they were representative for a veterinary check-up to determine serious health problems. Based on the blood samples, there was no indication for an under-supplementation with trace elements. However, animals were on a well formulated dairy ration and thus probably over-supplemented with the essential nutrients and minerals. The risk that these cows would enter a state of deficiency was probably low, even if certain components bind to the BC and are excreted with it. Overall, despite the lack of effect of BC on health-related variables assessed in this study, charcoal may be effective to treat or prevent specific health problems in cattle and there was no indication of negative side effects.

Feeding studies with BC in cattle were not only carried out with BC of different origins, but also different with dosages. Erickson et al. (2011) detected an effect on digestibility with a very low dosage of BC (0.1–0.2 % DM) and Leng et al. (2012) found a reduction of CH_4 on a diet with 0.6 % BC. Two studies assessing the effect of different dosages of BC (0.8 and 3 % in Winders et al., 2019 and 0.5 %, 1 % and 2 % in Terry et al., 2019) found no differences between dosages. The dosage of 0.9 % DMI in this study is therefore in line with other feeding trials. When determining the effective dosage of BC as a feed additive, several aspects need to be considered: the more BC is included in the ration, the higher the potential for carbon storage, but the lower the effective intake of digestible nutrients as the BC displaces a comparatively large volume of feed. For example, the 230 g of BC fed to the cows in this study corresponded to an effective volume of 1 L. At higher dosages, it can be expected that BC has a negative impact on the nutrient supply of the animal as it is in effect an indigestible component of the diet. Another aspect of BC supplementation that could impact its effect is its frequency. A constant supply of BC may have a different effect on the rumen than the occasional supplementation of high dosages. In summary, the cause for the variation in results between studies assessing the effect of BC as a feed additive remains to be investigated.

5. Conclusion

In a group of healthy, high performing dairy cows fed a balanced diet, the feeding of 1 % BC in the TMR had no significant effect on CH_4 emission, digestive efficiency, milk yield or animal health. Along with other publications in this field, the results indicate that BC is no effective feed supplement to reduce CH_4 emissions from cattle. Despite exposure to rather high BC doses no negative effect on animal health and performance was observed. Although it cannot be excluded that feeding BC to ruminants with health problems or receiving a poor diet may have positive effects, it remains questionable, if feeding BC on a large scale to cattle is worth the logistical and financial efforts it encloses.

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Ethical statement

This trial was approved by the veterinary office of the canton of Zurich (national license number: 35060).

CRediT authorship contribution statement

Marie T. Dittmann: Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Cem Baki:** Methodology, Investigation, Data curation. **Melissa Terranova:** Writing – review & editing, Resources, Methodology, Investigation. **Sergej L. Amelchanka:** Writing – review & editing, Validation, Data curation. **Sébastien Dubois:** Writing – review & editing, Investigation. **Andrea Wiget:** Writing – review & editing, Investigation. **Florian Leiber:** Writing – review & editing, Supervision. **Hans-Martin Krause:** Writing – review & editing, Resources, Funding acquisition. **Stefan Baumann:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

None.

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