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Effect of biochar or biochar and urea supplementation on feed intake, milk yield, feed conversion and methane production of dairy cows

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Abstract: Feed additives belong to a number of climate change mitigation strategies being sought to reduce methane emissions in ruminants. In this study, the effect of biochar or biochar and urea supplementation on dairy cow performance and methane production was assessed. Eighteen cows were used in a 3 × 3 latin square design with three feeding groups: control with no supplementation (CO), biochar supplementation (BC, 200 g/day) and biochar and urea supplementation (BC + U, 200 g/day biochar and 90 g/day urea). All cows were fed a forage mixture *ad libitum* and 5 kg of concentrates per day on average. Methane emissions were measured in respiration chambers. Biochar as well as biochar and urea supplementation did not affect total dry matter, energy and utilisable protein intake. However, lignin intake was higher in the BC group and crude protein intake was higher in the BC + U group compared to the CO group. Supplementation of feed additives did not affect milk production and milk composition, except for the higher milk urea content in the BC + U group. Feed conversion, diet digestibility and methane production were not affected by feeding strategy. In conclusion, biochar supplementation does not reduce methane emissions, but it does not negatively affect dairy cow performance.

Keywords: feed additives; methane reduction; digestibility; cattle; efficiency

Biochar is produced from plant biomass via pyrolysis and it has been used in soil amendment for decades. In recent years, biochar has also been used more and more in animal husbandry as an organic fertilizer supplement or as a feed additive (Schmidt et al. 2019). Biochar has been found

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to have several beneficial effects with regard to nutrient digestibility, performance, emissions and health when fed to cattle, sheep, goats, pigs, poultry and aquatic animals (Schmidt et al. 2016). However, studies examining the effects of biochar feeding on performance of ruminants, and especially dairy cows, are scarce. Studies that assessed the effect that biochar supplementation had on growing cattle daily gains produced contradictory results. For example, in a biochar feeding trial conducted by Leng et al. (2012a), biochar supplementation resulted in a daily gain increase of 25% in young cattle, however, in another study with steers it led to slightly lower daily gains (Kim and Kim 2005).

Besides increasing the performance, minimising the environmental impact of animal husbandry has become an important issue in recent years. An important source of greenhouse gases in agriculture is methane from ruminal fermentation, which accounts for about 5% of global greenhouse gas emissions (Nabuurs et al. 2022). To address this issue, significant research has been conducted on methane-reducing feeds and feed additives in the last 50 years (Beauchemin et al. 2020). One such feed additive that has gained attention in recent years is biochar. In an *in vitro* study conducted by Leng et al. (2012b), biochar supplementation (1.0% biochar in the ration) reduced methane production by up to 12.7%, while in another study conducted by the same authors, biochar supplementation of young cattle (0.6% biochar in the ration) resulted in a 24% reduction (Leng et al. 2012a). The authors hypothesized that the methane-reducing effect of biochar is due to its high specific surface area, which promotes the growth of methane oxidizing methanotrophs (Leng et al. 2012a, b). In a separate *in vitro* study conducted by Saleem et al. (2018), a 0.5% biochar inclusion rate resulted in reduced methane production and increased nutrient digestibility. However, in recent biochar supplementation *in vitro* (Teoh et al. 2019; Tamayao et al. 2021) and *in vivo* (Winders et al. 2019) studies, neither significant methane reduction nor feed digestibility effect was observed, regardless of the biochar inclusion rate. Furthermore, the simultaneous use of two or more feed additives should be considered in the reduction of methane production of ruminants. Rebelo et al. (2019) found that feeding urea reduced methane emissions in steers. This indicates that urea is a further potential methane-reducing feed additive. However, there is no information how the use of biochar and urea in the same ration affects methane production of ruminants.

It is clear that *in vitro* and *in vivo* biochar supplementation studies have resulted in contradictory results. Furthermore, we were not able to find any examples of dairy cow biochar supplementation feeding studies from the literature. This study aimed to assess the effect that biochar as well as biochar and urea supplementation has on dairy cow feed intake, milk yield, nutrient digestibility and methane emissions. We hypothesised that supplementation of biochar or biochar and urea does not affect performance and lowers methane emissions of dairy cows.

MATERIAL AND METHODS

The measurement of diet digestibility with wethers (document number: BMBWF-66.019/0017-V/3b/2019) and of dairy cow methane emissions in the respiration chambers (document number: BMBWF-66.019/0019-V/3b/2019) were approved by the national authority (Austrian Federal Ministry of Education, Science and Research) according to §26 of the Law for Animal Experiments, Tierversuchsgesetz 2012-TVG.

Experimental design, housing and feeding

Overall, 18 dairy cows (12 Holstein Friesian and six dual-purpose Simmental) were used in a 3 × 3 latin square design to determine feed intake and milk production. The cows were divided into three groups of six animals, considering number of lactations, lactation stage, feed intake and milk yield. The average number of lactations was 2.6 (1/3 first-lactating cows), average days in milk (± standard deviation) were 110 ± 51 day, average live weight was 659 ± 56 kg, average dry matter (DM) intake was 19.4 ± 2.6 kg/day and average milk yield was 24.4 ± 3.4 kg/day at the beginning of the experiment. The three groups were offered different rations which varied only in the addition of the respective feed additives: control, no feed additive (feeding group CO); biochar, addition of 200 g/day biochar (BC); biochar and urea, addition of 200 g/day biochar and 90 g/day urea (BC + U). Feeding of 200 g biochar per day made up 1.04% of total daily ration, on average. Each experi-

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mental period lasted for five weeks with two weeks adaption phase and three weeks data recording phase. Therefore, the feed additive was changed between groups after five weeks, resulting in an overall experiment duration of 105 days.

The dairy cows were kept in a cubicle housing system with straw. The barn was equipped with a Calan Gate System, which allowed assessment of individual feed intake. The dairy cows were milked in an Automatic Milking System (AMS, Dairy Robot R9500, GEA, Düsseldorf, Germany) and milk samples were taken three times per week to analyse milk composition in a laboratory for milk analyses (Qualitätslabor Österreich, St. Michael, Austria). An automatic scale, which was part of the AMS, recorded live weight of cows after each milking event.

Fresh forage (mixture of 40% grass silage, 30% maize silage and 30% hay) was offered *ad libitum* twice a day at 05:00 and 14:00. Offered feed amount and residues were recorded for each feeding event. Concentrates were fed in three different ways: (I) 2 kg [fresh matter (FM)] of an energy-rich concentrate mixture (34% barley, 36% maize, 11% wheat, 11% sugar beet pulp, 7% wheat bran, 1% rapeseed oil) were fed by hand at the feeding bin. Feed additives were mixed in this concentrate mixture to ensure the intake of feed additives. Each 1 kg of concentrate mixture (plus additives) was fed twice a day at 06:00 and 15:00; (II) 2 kg (FM) of a commercial concentrate mixture (Kuhkorn PLUS Energie, Garant Tiernahrung GmbH, Pöchlarn, Austria) were provided in the AMS; (III) A milk yield-dependent amount of a concentrate mixture (24% barley, 25% maize, 8% wheat, 8% sugar beet pulp, 5% wheat bran, 15% soybean meal and 15% rapeseed meal) was fed at a concentrate feeder. The actual intake of yield-dependent concentrate was 1.41, 1.50 and 1.24 kg DM/day in the CO, BC and BC + U group. Additionally, a mineral mixture (RINDAMIN LE, H. Wilhelm Schaumann GmbH & Co. KG, Brunn am Gebirge, Austria), salt and calcium carbonate were fed at the concentrate feeder to meet the requirements of dairy cows.

Feed analysis

Samples for DM determination and nutrient analysis were collected daily for forage and weekly for concentrates. For nutrient analysis, samples of forage and concentrates were pooled per period. The nutrient analysis was carried out using methods published by VDLUFA (2012); DM: method 3.1; crude protein (CP): 4.1.2; ether extract (EE): 5.1.1; crude fibre (CF): 6.1.1; crude ash: 8.1; neutral detergent fibre assayed with heat stable amylase and expressed exclusive of residual ash (NDF): 6.5.1; acid detergent fibre expressed exclusive of residual ash (ADF): 6.5.2; acid detergent lignin (ADL): 6.5.3; enzyme soluble organic matter (ESOM): 6.6.1; HCl-insoluble ash: 8.2. Energy content of forage mixture was examined by a digestibility trial with wethers according to GfE (1991), using four wethers per diet. The average age of the wethers was 4.3 years, and average live weight 80 kg. Wethers were fed 1 kg DM forage mixture per day, which was supplemented with 20 g mineral supplement and 4 g salt per day (on FM basis). This ration corresponded approximately to the maintenance requirements of the animals. The whole trial lasted for 19 days with a 2-week adaptation period and a 5-day sampling period. The amounts of feed intake and faeces were recorded daily. Feed and faeces were analysed once during the sampling period using a pooled sample and the same methods like described above. Additionally, the nitrogen (N) content of faeces was determined in fresh material to prevent N losses during the drying process by VDLUFA method 4.1.1 (VDLUFA 2012). Content of metabolisable energy (ME) and net energy for lactation (NEL) in forage mixture was calculated based on the results of the digestibility trial (GfE 2001), while an equation based on ESOM was used to determine the ME and NEL content of the concentrates (GfE 2009). Content of utilisable crude protein [uCP, equation 9 on page 44 in GfE (2001)] and ruminal nitrogen balance [RNB = (CP – uCP)/6.25] of diets were calculated using equations published by GfE (2001). Table 1 gives an overview on nutrient composition of experimental feeds.

The biochar used in this experiment was produced from pure ash wood by Biochar-Nergy GmbH (Gabersdorf, Austria). The wood was chopped, sieved (< 1 cm) and dried to a water content of approx. 10%. A pyrolysis temperature of 500 °C at least was maintained for a minimum of 10 min, ensuring pyrogenic degradation of any organic micropollutants present. Table 2 presents parameters which have to be examined to get a specific

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Table 1. Nutrient composition and energy content of experimental feeds

Parameter	Forage mixture	Concentrates				
		YDC	AMS	T-CO	T-BC	T-BC + U
Dry matter (g/kg FM)	456	908	862	873	881	878
Crude protein (g/kg DM)	138	203	160	115	99.3	242
Ether extract (g/kg DM)	23.0	30.0	45.9	39.8	30.4	31.6
Neutral detergent fibre (g/kg DM)	483	224	164	216	233	223
Acid detergent fibre (g/kg DM)	311	102	66.1	78.3	116	101
Acid detergent lignin (g/kg DM)	38.2	22.8	18.8	14.8	50.4	38.6
Hemicellulose (g/kg DM)	172	122	97.9	138	117	122
Cellulose (g/kg DM)	273	79.2	47.3	63.5	65.8	62.2
Non-fibre carbohydrates (g/kg DM)	254	498	576	596	594	462
Organic matter (g/kg DM)	897	955	946	967	956	959
Ash (g/kg DM)	103	44.6	54.3	33.2	43.5	41.2
Metabolisable energy (MJ/kg DM)	9.30	12.59	13.25	13.59	13.01	13.61
Net energy for lactation (MJ/kg DM)	5.48	7.84	8.38	8.67	8.25	8.62

AMS = concentrate fed in the automatic milking system; DM = dry matter; FM = fresh matter; T-BC = trial-concentrate biochar; T-BC + U = trial-concentrate biochar + urea; T-CO = trial-concentrate control; YDC = yield-dependent concentrate fed via the concentrate feeder

certification for biochar as animal feed according to the European Biochar Certificate. The biochar used in this experiment met all threshold values.

Table 2. Quality parameters of biochar used in this experiment (laboratory report by BEST-Bioenergy and Sustainable Technologies GmbH, Graz, Austria)

Parameter	Value	Threshold
Dry matter (g/kg FM)	788	
Ash (g/kg DM)	154	
Carbon (g/kg DM)	763	
pH of the eludate	10.1	
Specific surface area (m²/g)	295	
Heavy metals		
Arsen (mg/kg DM)	0.97	< 2
Cadmium (mg/kg DM)	0.04	< 1
Mercury (mg/kg DM)	0.02	< 0.1
Lead (mg/kg DM)	2.9	< 10
Polyaromatic hydrocarbons (PAH)		
Sum PAK 16 (mg/kg FM)	< 3	< 4
Benzo-a-pyren (mg/kg FM)	< 0.025	< 0.025
Dioxine		
Upperbound [ng/kg I-TEQ (WHO 2005)]	0.31	< 0.75
Mediumbound [ng/kg I-TEQ (WHO 2005)]	0.16	
Lowerbound [ng/kg I-TEQ (WHO 2005)]	0.005	

DM = dry matter; FM = fresh matter; I-TEQ = international toxic equivalent of dioxins

Measurement of methane emissions in respiration chambers

Measurement of methane emissions of dairy cows was carried out in two respiration chambers which were identical and controlled individually. These airtight facilities were ventilated by a controlled ventilation system. Air from inside the chambers was steadily pumped out by a vacuum pump and fresh air was flowing into the chamber by a pressure gradient. The volume of the air pumped out of the chamber and the concentration of methane were recorded every 12 minutes. The gas volume was measured by a volume meter (vane wheel flow sensor ZS25, Höntzsch, Waiblingen, Germany) and the methane concentration by a photoacoustic gas analyser (Photoacoustic Gas Monitor Innova 1412i, LumaSense, Frankfurt/Main, Germany). The chamber air was steadily conditioned to 20 °C and 60% relative humidity by an air conditioning system.

Each cow was kept in the respiration chamber for one measurement cycle (4 days) during the whole experiment, as this is the maximum duration according the animal experiment approval. Therefore, only one ration per cow could be tested in the respiration measurements resulting in methane emission data from six cows for each ration (CO, BC and BC + U). In each period, two cows per

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group were chosen for respiration measurements. The decision which cow is measured in which period was made based on days in milk to achieve a similar average stage of lactation during methane recording in all three experimental groups. During the first two days in the chamber, cows were adapted to the new environment and the last two days were used for data recording. A staff member entered the respiration chambers twice a day (05:30 and 16:00) to feed and milk the cows and to check their well-being. Dry matter intake (DMI) of forage and concentrates, milk yield and milk ingredients (fat, protein, lactose and urea) were recorded daily and cows were weighed before and after the respiration measurements. Furthermore, faecal samples of cows were collected during the stay in the respiration chamber and analysed by the same methods as described for feed analysis. Based on the concentration of nutrients and HCl-insoluble ash in feeds and faeces, apparent digestibility was calculated (Kirchgessner et al. 2008).

Before starting the experiment, the tightness of the chambers was tested and calibration results were used as correction factors in data analysis. Furthermore, volume meter data and gas concentration data were corrected to standard temperature, air humidity and air pressure. Based on these corrected data, methane production of the cows in a measurement interval (12 min) was calculated. Methane production data during feeding and milking phases, which were falsified by the opening of chamber doors, were corrected by linear interpolation using the statistical program Statgraphics Centurion XVII (2015 Statpoint Technologies, Inc., Warrenton, VA, USA). To calculate the daily methane production of cows, amounts of methane production in each measurement interval between 4:30 a.m. on the respective day and 4:30 a.m. on the following day were summed up.

Statistical analysis

Data were checked with the statistical software Statgraphics Centurion XVII. Statistical analysis was carried out using Statistical Analysis Software (SAS Institute Inc., Cary, NC, USA). Data on feed intake and milk production were analysed using Proc MIXED and the fixed effects group (CO, BC, BC + U), period of the trial, week within a period and lactation number and the covariates days in milk and live weight. Measurements on the same cow (nested in a feeding group) in consecutive weeks were considered as repeated measurements in the model. Furthermore, cow (nested in a feeding group) was considered as random effect. The model for the analysis of methane production and nutrient digestibility consisted of the fixed effects group and lactation number and the covariates days in milk, daily dry matter intake and concentrate proportion in the ration. In this analysis, week of measurement was considered as random effect and measurement on the same cow (nested in a feeding group and a respiration chamber) on consecutive days as repeated measurement. Interactions were not considered in statistical analysis as they were not significant. Multiple comparisons of LSMeans were carried out using the Tukey test. Differences between feeding groups were considered to be significant, if statistical analysis resulted in P-values < 0.05.

RESULTS AND DISCUSSION

Feed intake, milk production and feed conversion

The DM and nutrient intake from forage was not influenced by biochar or biochar and urea supplementation (Table 3). Furthermore, DM, ME, NEL and uCP intake from the concentrate was also on a similar level in all three groups. However, urea supplementation led to a significantly higher CP intake and a lower NFC intake from concentrates in the BC + U group. The high CP intake from the concentrate also resulted in the higher total CP intake. Therefore, RNB of the BC + U group was markedly positive (38.7 g/day) compared to the nearly balanced rations of the CO (3.89 g/day) and BC (3.22 g/day) groups. Adding biochar increased NDF, ADF and ADL intake from the concentrate and total ADL intake, but not total NDF and ADF intake.

Our results are in accordance with earlier studies on steers (Winders et al. 2019), beef heifers (Terry et al. 2019) and sheep (Lind et al. 2020), which did not find any effect of biochar supplementation on feed intake either. Feed intake is regulated by physical (rumen fill) and physiological mechanisms (feeling of satiety) in the metabolism of cows. The rumen fill and the capacity of feed intake de-

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Table 3. Feed intake of dairy cows fed a control ration (CO) or a ration supplemented with biochar (BC) or biochar and urea (BC + U)

Parameter	Ration			*P*-value	RSD
	CO	BC	BC + U		
Forage intake					
Dry matter (kg/day)	14.2	14.2	14.3	0.994	0.6
Crude protein (g/day)	1 926	1 941	1 946	0.961	151
Neutral detergent fibre (g/day)	6 889	6 890	6 907	0.997	313
Acid detergent fibre (g/day)	4 380	4 418	4 415	0.972	271
Acid detergent lignin (g/day)	537	540	542	0.974	35
Non-fibre carbohydrates (g/day)	3 661	3 621	3 643	0.944	450
Metabolisable energy (MJ/day)	132	133	133	0.995	6
Net energy for lactation (MJ/day)	78.0	78.1	78.3	0.995	3.6
Utilisable crude protein (g/day)	1 777	1 783	1 787	0.990	81
Concentrate intake					
Dry matter (kg/day)	4.82	5.07	4.93	0.264	0.49
Crude protein (g/day)	737[b]	744[b]	993[a]	< 0.001	101
Neutral detergent fibre (g/day)	951[b]	1 042[a]	991[ab]	0.016	108
Acid detergent fibre (g/day)	385[c]	482[a]	437[b]	< 0.001	45
Acid detergent lignin (g/day)	88[c]	161[a]	137[b]	< 0.001	11
Non-fibre carbohydrates (g/day)	2 648[a]	2 791[a]	2 459[b]	< 0.001	244
Metabolisable energy (MJ/day)	62.3	64.6	64.0	0.462	6.2
Net energy for lactation (MJ/day)	39.4	40.8	40.4	0.500	3.8
Utilisable crude protein (g/day)	860	884	911	0.227	93
Total feed intake					
Dry matter (kg/day)	19.0	19.3	19.2	0.909	0.6
Crude protein (g/day)	2 661[b]	2 683[b]	2 934[a]	0.009	152
Neutral detergent fibre (g/day)	7 838	7 926	7 889	0.954	296
Acid detergent fibre (g/day)	4 764	4 898	4 848	0.760	263
Acid detergent lignin (g/day)	625[b]	701[a]	678[ab]	0.006	34
Non-fibre carbohydrates (g/day)	6 310	6 411	6 103	0.270	576
Metabolisable energy (MJ/day)	194.6	196.9	196.5	0.930	6.4
Net energy for lactation (MJ/day)	117.3	118.6	118.4	0.934	3.9
Utilisable crude protein (g/day)	2 635	2 663	2 690	0.819	84
Ruminal nitrogen balance (g/day)	3.89[b]	3.22[b]	38.7[a]	< 0.001	13.7

RSD = residual standard deviation

[a–c]Means bearing different superscripts within a row differ at $P < 0.05$

pend on the degradation rate and the passage rate of the feed in the rumen (Mertens 1994; Gruber et al. 2001). Therefore, similar feed intake in all groups indicates that biochar as well as biochar and urea supplementation did not significantly influence ruminal digestion processes.

The supplementation of biochar or biochar and urea did not affect milk production and fat, protein and lactose content of milk (Table 4). Therefore, our results contradict findings of a German survey in which farmers stated that biochar supplementation increased the protein and fat content of milk (Schmidt et al. 2019). However, the supplementation of 90 g urea per day resulted in a significant increase of milk urea content from 18.9 (CO) and 18.2 (BC) to 24.6 mg/100 ml (BC + U). Such high urea concentrations in milk are critical as milk urea content is highly correlated with urine urea con-

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Table 4. Milk production, nutrient balance and feed conversion of dairy cows fed a control ration (CO) or a ration supplemented with biochar (BC) or biochar and urea (BC + U)

Parameter	Ration			*P*-value	RSD
	CO	BC	BC + U		
Milk production					
Milk production (kg/day)	21.5	21.6	21.6	0.984	1.4
ECM (kg/day)	22.0	22.2	22.3	0.966	2.7
Fat content (%)	4.19	4.25	4.24	0.836	0.20
Protein content (%)	3.58	3.53	3.55	0.626	0.06
Lactose content (%)	4.75	4.74	4.75	0.955	0.08
Urea content (mg/100 ml)	18.9[b]	18.2[b]	24.6[a]	< 0.001	5.1
Nutrient balance					
NEL balance (MJ/day)	6.07	6.46	6.25	0.994	5.51
NEL balance (%)	105.5	106.3	105.5	0.948	5.1
uCP balance (g/day)	261	297	317	0.654	73
uCP balance (%)	111.2	113.2	114.0	0.513	3.0
Feed conversion					
Dry matter (kg DM/kg ECM)	0.868	0.880	0.870	0.907	0.050
NEL (MJ NEL/kg ECM)	5.36	5.42	5.38	0.929	0.46
uCP (g uCP/kg ECM)	120	121	122	0.893	7

ECM = energy-corrected milk production; NEL = net energy for lactation; RSD = residual standard deviation; uCP = utilizable crude protein

[a,b]Means bearing different superscripts within a row differ at $P < 0.05$

tent. High urinal urea excretion can significantly enhance ammonia emissions from slurry as shown by previous research (Burgos et al. 2010; Powell et al. 2011; van Duinkerken et al. 2011).

The ration did not influence any parameter of nutrient balance or feed conversion, which further confirms that the biochar inclusion in dairy cow diets had no impact on dairy cow metabolism or productivity. This is in contrast with results presented by Leng et al. (2012a), who found that feed conversion in young cattle was improved by biochar supplementation. However, as the diet composition used in the study by Leng et al. (2012a) (root chips and foliage as fibre-rich feed source) was markedly different from this study, the differences in study outcomes could be an indicator that the biochar effect is impacted by the type and quality of fibre-rich feeds or forages provided to the cattle (Teoh et al. 2019).

Diet digestibility and methane production

Diet digestibility was not influenced by the ration type (Table 5). However, supplementation of urea led to a slight increase in OM, NDF and ADF digestibility, which could be due to a higher supply of rumen microbes with nitrogen. Our results correspond well to outcomes of earlier studies which did not find any effect of biochar supplementation on diet digestibility in ruminants (Calvelo Pereira et al. 2014; Teoh et al. 2019; Terry et al. 2019; Winders et al. 2019; Tamayao et al. 2021). However, biochar supplementation altered the concentration of volatile fatty acids and ammonia in the rumen fluid compared to a control treatment in an experiment with heifers (Terry et al. 2019) and in *in vitro* studies (Calvelo Pereira et al. 2014; Saleem et al. 2018). Furthermore, Terry et al. (2019) found a reduction of protozoa counts in rumen, but concentrations of methanogenic bacteria were not influenced by the supplementation of biochar indicating a low potential of biochar in a reduction of methane emissions from ruminants.

In fact, the supplementation of biochar or biochar and urea did not significantly affect daily methane production (g/day), methane yield (g/kg DMI) and methane intensity (g/kg energy-corrected milk production) in our experiment (Table 5). Compared to CO group, methane production and

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Table 5. Diet digestibility and methane production of dairy cows fed a control ration (CO) or a ration supplemented with biochar (BC) or biochar and urea (BC + U)[1]

Parameter	Ration			*P*-value	RSD
	CO	BC	BC + U		
Diet digestibility					
Organic matter (%)	74.6	74.9	76.4	0.305	1.4
Crude protein (%)	66.0	64.2	69.0	0.232	3.0
Neutral detergent fibre (%)	66.3	66.2	69.0	0.121	1.4
Acid detergent fibre (%)	63.9	61.9	65.3	0.324	2.2
Non-fibre carbohydrates (%)	89.9	90.9	90.8	0.463	1.3
Methane production					
Methane production (g/day)	322	348	371	0.210	5
Methane yield (g/kg DMI)	18.0	19.6	20.8	0.221	0.3
Methane intensity (g/kg ECM)	19.2	16.8	17.2	0.580	0.8

DMI = dry matter intake; ECM = energy-corrected milk production; RSD = residual standard deviation
[1]Average days in milk, DMI and ECM of cows used for the measurement of diet digestibility and methane production were 194, 16.9 and 16.0 in CO group, 178, 17.6 and 22.5 in BC group and 199, 18.9 and 21.7 in BC + U group

methane yield increased numerically while methane intensity decreased slightly when adding biochar or biochar and urea to the diet. However, the numerical differences in methane intensity are likely due to the lower energy-corrected milk yield of CO group cows that were used for the measurements in the respiration chamber. Like in our study, biochar supplementation did not significantly affect methane production by steers (Winders et al. 2019), heifers (Terry et al. 2019), and lambs (Lind et al. 2020) as well as in an *in vitro* experiment (Teoh et al. 2019). Winders et al. (2019) and Terry et al. (2019) even tested different biochar inclusion rates of up to 3% daily feed intake. Therefore, it is likely that increasing the biochar supplementation percentage used in this study would not result in a different result of methane reduction from that being observed. This is further supported by the *in vitro* study conducted by Saleem et al. (2018), who observed the lowest methane production at a biochar inclusion rate of 0.5% compared to 0%, 1% and 2%. However, in their study the biochar inclusion resulted in methane production being significantly reduced (25.3%). This reduction in methane production is comparable with the results of Leng et al. (2012a), who found 24.3% lower methane production ($P = 0.066$) in young cattle fed a diet supplemented with biochar compared to those fed the control diet.

Although the results of this study and several previous studies show no methane reduction effect, the outcome of the two above-mentioned studies (Leng et al 2012a; Saleem et al. 2018) shows the opposite. It has to be mentioned that biochar is an umbrella term for a lot of different products made from different feedstocks and produced under different conditions. While the biochar used in this study was produced from ash, other studies used biochar produced from pine (Calvelo Pereira et al. 2014; Saleem et al. 2018), spruce (Tamayao et al. 2021), corn stover (Calvelo Pereira et al. 2014), eucalyptus (Teoh et al. 2019), straw (Hansen et al. 2013) and rice husks (Leng et al. 2013). In an *in vitro* study, different biochars had different effects on ruminal methane production. Biochar from rice husks significantly reduced methane production, while activated charcoal did not show any marked effect (Leng et al. 2013). In addition to the feedstock used, the production process, especially pyrolysis temperature, can influence the characteristics of the biochar produced (Weber and Quicker 2018). Calvelo Perreira et al. (2014) produced biochar at 350 °C and 550 °C pyrolysis temperature and found significantly lower total gas production and a tendency for lower methane production when biochar produced at 550 °C was used. Some of the results mentioned above indicate that there might be types of biochar that could have the potential to lower methane emissions from ruminal fermentation. Therefore, more research is needed to assess the effect that the parameters of feedstocks and production processes have on biochar ability to reduce methane emissions in ruminants.

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CONCLUSION

Supplementing cattle rations with 200 g biochar per day did not affect feed intake, milk production, milk ingredients, nutrient digestibility, feed conversion and methane production of dairy cows. The only significant difference was the higher ADL intake of cows fed the ration supplemented with biochar compared to those fed the control ration. Supplementation of biochar and urea led to higher total CP intake, higher RNB and higher milk urea content while all other parameters were not affected by the feeding of these additives. In conclusion, supplementation of biochar or biochar and urea has no effects on the performance and methane emissions of dairy cows, but supplementation of biochar and urea could stimulate ammonia emissions due to the higher urea excretion of the cows. Although this research lacks in finding a methane-reducing effect, it cannot be finally concluded that biochar has no methane-reducing effect as it can be produced from different feedstocks and in different ways. Therefore, further research should focus on studying the effect of feedstock and production process parameters on the methane reduction potential of biochar.

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Conflict of interest

The authors declare no conflict of interest.

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