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Dietary biochar as a feed additive for increasing livestock performance: A meta-analysis of *in vitro* and *in vivo* experiment

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Abstract: The present study aimed to evaluate the use of biochar as a feed additive on nutrient utilization and livestock performance by employing a meta-analysis method. Various *in vitro* and *in vivo* studies regarding the effects of biochar on rumen fermentation, nutrient disappearance, total gas, methane production, microbial population, feed intake, growth performance, blood constituents, nutrient digestibility and nitrogen retention were tabulated in a database. Data were analysed using the mixed model method in which the different studies were considered as random effects while the biochar addition was treated as a fixed effect. The addition of biochar reduced methane production (quadratic pattern; $P < 0.05$), but increased the total gas production ($P < 0.001$). Addition of biochar decreased (quadratic pattern; $P < 0.05$) volatile fatty acids and acetic acid in the *in vitro* rumen fermentation data. In the *in vivo* data, a reduction in feed conversion ratio (quadratic pattern; $P < 0.05$), as well as a reduction ($P < 0.05$) in the feed intake parameters of dry matter, organic matter, crude protein, and neutral detergent fibre were observed with biochar addition. Ammonia production and propionic acid tended to increase linearly ($P < 0.05$) with the biochar supplementation. The biochar supplementation increased ($P < 0.05$) the nutrient digestibility (dry matter, organic matter, crude protein, neutral detergent fibre) and nitrogen retention. In conclusion, biochar supplementation of ruminant diet modulates rumen fermentation by increasing propionic acid but decreasing methane emissions, and enhances livestock performance by increasing nutrient digestibility, growth performance as well as nitrogen retention.

Keywords: modelling; pyrolysis; rumen; supplement

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The demand for animal protein-based products is increasing along with the increasing number of the human population. In general, animal products contribute about a third of the human protein consumption, where ruminant-based products supply high protein products. An increase in supply and demand for food requires important changes to ensure food security. A nutrition sustainability strategy is needed to increase livestock performance efficiently so that it does not interfere with health and welfare or have to rely on the use of antibiotics, is beneficial to business sectors, and does not have a negative impact on the environment. Increasing the efficiency of feed for livestock production is a very important agricultural topic (Flachowsky et al. 2013), because 70% of the cost of livestock production input is used for feed (McGrath et al. 2018). Previously, antibiotics were widely used as feed additives since the compounds can increase the weight, growth, and performance of livestock (NRC 1999). However, if used continuously, antibiotics may cause resistance to microbes and have residues for livestock and humans (Marshall and Levy 2011), and these have led to the ban of their use as growth promoters. Due to the prohibition of using antibiotics, farmers have tried several alternatives, including active ingredients from plants (phytogenic), organic acids, probiotics, prebiotics, and zeolites to improve the health and performance of livestock (Papatsiros et al. 2013). More recently, plant extracts and plant bioactive compounds have gained attention to replace antibiotics since they are natural and considered to be safe for animals and environment. Furthermore, these natural compounds have been shown to mitigate enteric methane emissions from ruminants, i.e., essential oils (Calsamiglia et al. 2007), saponins (Holtshausen et al. 2009), and tannins (Jayanegara et al. 2015).

Another promising material as an alternative to antibiotics is biochar. Biochar is a carbonized material from the pyrolysis of biomass. This material is porous and has a large surface area, which allows it to absorb gases and carbon, binds to toxins, and provides a biofilm habitat for microbiota to proliferate (Hansen et al. 2012). Biochar may be used as a treatment of animal poisoning as well as for eliminating toxins (Naka et al. 2001). Biochar also holds electron-mediating properties in biological redox reactions (Yu et al. 2015) and provides benefits in the form of feed efficiency in ruminants and a reduction in greenhouse gas emissions (Leng et al. 2012a; Schmidt et al. 2019).

Although there have been a number of experiments investigating the effects of biochar on ruminant livestock both *in vitro* and *in vivo*, there has been no study to date that attempted to quantitatively summarize the results obtained. Therefore, the purpose of this study was to evaluate the use of biochar as a feed additive in relation to nutrient utilization and livestock performance by integrating data from various studies and to analyse them by using a meta-analysis method.

MATERIAL AND METHODS

Development of the database

A database was developed based on data from various published articles that reported the supplementation effects of biochar on nutrient utilization and ruminant livestock performance, both from *in vitro* and *in vivo* experiments. The articles were searched using a number of electronic databases such as Scopus, Science Direct and Google Scholar with the following keywords: "activated carbon", "biochar", "charcoal", "rumen", "methane", "growth performance", "feed intake", "nutrient digestibility", "blood constituents" and/or "nitrogen retention". Initially, a total of 51 articles were retrieved that comprised 20 *in vitro* and 31 *in vivo* papers. After further screening, 15 literature sources were excluded since they either were a review paper or did not report any additional levels of biochar. The final database consisted of 128 and 105 data points for the *in vitro* and *in vivo* experiments, respectively. The literature sources used in the database are presented in Table 1 for the *in vitro* experiments and Table 2 for the *in vivo* experiments.

The parameters included in this database were rumen fermentation products [pH, ammonia, total volatile fatty acids (VFA), acetic acid (C2), propionic acid (C3), n-butyric acid (C4), *iso*-butyric acid (*iso*-C4), n-valeric acid (C5), *iso*-valeric acid (*iso*-C5), the ratio of non-glucogenic to glucogenic acid, ratio of C2 to C3, *iso*-volatile fatty acid]; nutrient disappearances (dry matter, crude protein, neutral detergent fibre, acid detergent fibre); total gas and methane productions; protozoa population; growth performance (initial body weight,

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Table 1. Studies included in the meta-analysis of *in vitro* biochar addition

Reference	Pyrolisis (°C)	Source	Part	Level (g/kg DM substrate)	Method	Source of rumen	Substrate (% of DMB)		
							grass	legume	concentrate
Lee et al. (2002)	NA	*Quercus prinus*	wood	0–10	Tilly and Terry	NA	20.0	0.0	80.0
Leng et al. (2012a)	950	*Oryza sativa*	husk	0–50	Tilly and Terry	buffalo	0.0	0.0	93.0
Leng et al. (2012c)	950	*Oryza sativa*	husk	0–15	Tilly and Terry	beef cattle	0.0	26.5	70.0
Jiang et al. (2014)	NA	Bambusoideae	wood	0–10	Menke	dairy cattle	41.7	8.3	47.4
Pereira et al. (2014)	350 and 550	*Quercus prinus* and *Zea mays*	stover and wood	0–186	NA	cattle	100.0	0.0	0.0
Vongsamphanh et al. (2015)	950	*Oryza sativa*	husk	0–10	Tilly and Terry	cattle	0.0	27.5	70.0
Saleem et al. (2018)	500	*Quercus prinus*	wood	0–20	Rusitec	beef cattle	60.0	0.0	40.0
Hansen et al. (2012)	NA	commercial	gas form, straw, and wood	0–90	Menke	dairy cattle	50.0	0.0	50.0
Leng et al. (2013)	800	*Oryza sativa*	husk	0–10	Tilly and Terry	buffalo	0–16.7	11.3–17	0–16.7
Teoh et al. (2019)	650	deciduous	wood	0–72.8	Rusitec	dairy cattle	70.0	0.0	30.0
Vongsamphanh et al. (2015)	950	*Oryza sativa*	husk	0–10	Tilly and Terry	cattle	13.8	27.6	56.0
Phongphanith et al. (2016)	950	*Oryza sativa*	husk	0–10	Tilly and Terry	cattle	0.0	0.0	0.0
Cabeza et al. (2018)	550	*Brassica napus*, conifers, *Miscanthus sinensis*, *Oryza sativa*, and *Triticum aestivum*	husk, straw, and wood	0–116.7	Menke	beef cattle	100.0	0.0	0.0
Mirheidari et al. (2019a)	550	chicken manure and *Juglans* spp.	shell and waste	0–15	Fedorak	sheep	10.8	31.0	55.5
Saenab et al. (2018)	NA	Anacardium occidentale	shell	0–30	Theodorou	dairy cattle	NA	NA	NA

DMB = dry matter basis; NA = data not available

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Table 2. Studies included in the meta-analysis of *in vivo* biochar addition

Reference	Pyrolisis (°C)	Source	Part	Level (g/kg DM substrate)	Animal	Breed	Sex	Age (days)	Feed ingredients (% of DMB)		
									grass	legume	concentrate
Garillo et al. (1995)	NA	*Cocos nucifera*	shell	0–3	sheep	Suffolk	F	375	19.9–79.7	0.00	19.9–79.7
Garillo et al. (1994)	NA	*Cocos nucifera*	shell	0–6	goat	Tokara	NA	NA	19.88	0.00	79.52
Kim and Kim (2005)	NA	NA	NA	0–20	beef cattle	Hanwoo	M	NA	32–32.2	0.00	67.8–67.97
Cha and Lee (2005)	NA	NA	NA	0–10	goat	Heugyeomso jeongol	M	NA	20.00	0.00	80.00
Mirheidari et al. (2019b)	550	*Juglans* spp., *Pistacia vera*, and chicken manure	shell and waste	0–10	sheep	Kermanian	M	105	9.80	30.00	57.5–58
Tobioka and Garillo (1994)	NA	NA	NA	0–5	beef cattle	Akaushi	M	280–555	9–15	0.00	85–91
Van et al. (2006)	NA	Bambusoideae	wood	0–48.5	goat	Boer × Barbari, Barbari, and Barbari × Bachthao	M	120–195	24.5–26.2	33–42.8	26.19–38
Erickson et al. (2011)	NA	Commercial	NA	0–45.9	dairy cattle	Holstein	F	NA	59.9–74	0–4	18–36
Silivong and Preston (2015)	NA	*Oryza sativa*	husk	0–11.3	goat	Lao local goat	mix	195	15.00	85.00	0.00
Silivong et al. (2018)	950	*Oryza sativa*	husk	0–12.9	goat	Lao local goat	mix	165	0.16–0.47	87.7–88.4	10.04–10.08
Silivong and Preston (2016)	NA	*Oryza sativa*	husk	0–12	goat	Lao local goat	mix	165	0.00	89.62	9.16
Hang et al. (2019)	950	*Oryza sativa*	husk	0–12.9	goat	Bach Thao	M	120	0.00	95.17	3.56
Phongphanith and Preston (2016)	950	*Oryza sativa*	husk	0–5.9	beef cattle	Yellow	NA	630	26.21	14.12	56.74
Vongkhamchanh et al. (2018)	950	*Oryza sativa*	husk	0–10	beef cattle	Yellow	NA	NA	32.16	30.24	33.37
Hang et al. (2018)	950	*Oryza sativa*	husk	0–8.9	goat	Bach Thao	NA	NA	68–99.1	0–31.5	0.00
Leng et al. (2012b)	400	*Oryza sativa*	husk	0–3.8	beef cattle	Yellow	mix	NA	0.00	38.55	61.08
Phuong et al. (2019)	NA	*Oryza sativa*	husk	0–10	goat	Bach Thao	M	NA	0.00	95–99	0.00
Saroeun et al. (2018)	NA	*Oryza sativa*	husk	0–3.11	beef cattle	Yellow	M	480	33.1–36.7	2.5–25.7	25.7–35.6
Mirheidari et al. (2019a)	550	*Juglans* spp. and chicken manure	shell	0–15	sheep	NA	F	NA	10.8–11.1	31.00	55.5–55.7
Winders et al. (2019)	NA	Pinoideae	wood	0–30	beef cattle	NA	M	NA	15–71	0.00	22–78
Al-kindi et al. (2016)	NA	Bambusoideae	wood	0–30	goat	Boer	M	NA	50.50	0.00	49.50

DMB = dry matter basis; F = female; M = male; NA = data not available

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metabolizable energy, final body weight, average daily gain, average daily intake, feed conversion ratio); feed intake (dry matter, ash, organic matter, crude protein, neutral detergent fibre, acid detergent fibre, hemicellulose); blood constituents (packed cell volume, glutamic pyruvic transaminase, red blood cell, white blood cell, eosinophil, neutrophil, lymphocyte, monocyte); nitrogen utilization (nitrogen in faeces, nitrogen in urine, digested nitrogen, nitrogen retention, nitrogen retention to digested nitrogen ratio, faecal and urinary nitrogen ratio). Data for identical variables are translated to the same measurement units in the process of tabulating data into the database, which allowed further analysis.

Data analysis

The database was further processed in a statistical meta-analysis based on a mixed model methodology (St-Pierre 2001; Sauvant et al. 2008). Different experiments were grouped as random effects and the fixed effects were the dosage or different types of biochar. The following statistical model was used:

$$Y_{ij} = B_0 + B_1X_{ij} + B_2X_{ij}^2 + s_i + b_iX_{ij} + e_{ij} \quad (1)$$

where:

Y_{ij} – dependent variable;
B_0 – overall intercept across all studies (fixed effect);
B_1 – linear regression coefficient of Y on X (fixed effect);
B_2 – quadratic regression coefficient of Y on X (fixed effect);
X_{ij} – value of the continuous predictor variable;
s_i – random effect of study i;
b_i – random effect of study i on the regression coefficient of Y on X in study i;
e_{ij} – unexplained residual error.

When the respective quadratic regression model was not significant at $P < 0.05$, the corresponding linear regression mixed model was applied. Variable study was declared in the class statement since it does not contain any quantitative information. Model statistics used were P-value and Akaike information criteria (AIC). All statistical analyses were carried out using the R software v3.60 (https://www.R-project.org/).

RESULTS

Addition of biochar *in vitro* study

The effects of biochar doses on *in vitro* rumen fermentation, nutrient disappearance, total gas, methane production, and protozoa number are shown in Table 3. Biochar supplementation increased total gas production in a linear pattern ($P < 0.001$) but it reduced methane production in a quadratic pattern ($P < 0.05$). The supplementation of biochar did not affect pH, ammonia, n-valeric acid, and the ratio of non-glucogenic to glucogenic acid. The biochar supplementation decreased the total VFA by following a quadratic pattern ($P < 0.05$). Supplementation of biochar decreased the acetic acid (C2) with a quadratic pattern ($P < 0.01$), and simultaneously it increased the propionic acid (C3) by following a quadratic pattern as well ($P < 0.01$). Biochar supplementation tended to decrease n-butyric acid (C4) production ($P < 0.1$). Supplementation of biochar decreased the *iso*-volatile fatty acid with a quadratic pattern ($P < 0.01$). Not all nutrient disappearances were affected by biochar supplementation, except that the biochar increased that of NDF quadratically ($P < 0.01$). Total protozoa were not affected by biochar supplementation.

Addition of biochar *in vivo* study

The effects of biochar doses on growth performance, feed intake, rumen fermentation, blood constituents, nutrient digestibility and nitrogen utilization are shown in Table 4. Growth performance parameters were not generally influenced by biochar supplementation, but biochar reduced the feed conversion ratio by following a quadratic pattern ($P < 0.05$). Biochar supplementation linearly decreased the daily nutrient intake (dry matter, organic matter, crude protein, and NDF) ($P < 0.05$) but it did not cause any effects on total VFA concentration and blood-related parameters. Rumen fermentation products, i.e. ammonia, propionic acid, n-valeric acid linearly increased ($P < 0.05$) while the ratio of non-glucogenic to glucogenic acid decreased linearly ($P < 0.05$) by increasing the dose of biochar. The biochar supplementation did not decrease methane emissions in ruminants. Nutrient digestibility (dry matter, organic matter,

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Table 3. Regression equations on the influence of biochar doses on *in vitro* rumen fermentation, nutrient disappearance, total gas, methane production, and protozoa population

Parameter	Model	*n*	Parameter estimates				Model estimates			Interpretation		
			Int.	SE Int.	slope	SE slope	*P*-value	RMSE	AIC[a]	trend	*x*[b]	*y*[c]
Total gas and methane production												
Total gas (ml/g dry matter substrate)	L	128	168	18.4	0.454	0.125	0.000	1.52	1 460	Pos.		
Methane (ml/g dry matter substrate)	Q	118	34	4.13	−0.239 0.001 3	0.1 0.000 656	0.019 0.050	1.68	999	Min.	92.10	23
Rumen fermentation												
pH	L	49	6.5	0.13	0.000 3	0.000 9	0.719	1.35	43.8	Pos.		
Ammonia (mmol)	L	66	12.4	1.74	−0.003 3	0.009 4	0.730	1.13	407	Neg.		
Volatile fatty acid (mmol)	Q	69	70.7	7.4	−0.391 8 0.002 4	0.169 3 0.001 1	0.023 8 0.027 8	1.62	633	Min.	80.7	54.90
Acetic acid (C_2) (% of VFA)	Q	69	59.3	2.14	−0.133 0.001	0.049 1 0.000 3	0.009 0.002	1.53	470	Min.	64.10	55.00
Propionic acid (C_3) (% of VFA)	Q	69	25.2	1.28	0.088 −0.000 5	0.029 5 0.000 2	0.004 0.007	1.22	402	Max.	84.60	29.00
n-Butyric acid (C_4) (% of VFA)	L	49	13.5	2.13	−0.022 8	0.0117	0.057	1.28	311	Neg.		
iso-Butyric acid (*iso*-C_4) (% of VFA)	Q	30	8.59	2.10	−0.854 0.007 6	0.254 0.002 1	0.002 0.001	1.10	189	Min.	56.30	−15.5
n-Valeric acid (C_5) (% of VFA)	L	10	2.3	1.13	0.112	0.088 2	0.240	1.04	41.0	Pos.		
iso-Valeric acid (*iso*-C_5) (% of VFA)	L	21	3.3	1.59	0.084 9	0.031 9	0.016	1.53	108	Pos.		
Ratio of non-glucogenic to glucogenic acids	L	63	3.2	0.23	−0.001 5	0.001 3	0.251	1.67	140	Neg.		
Acetic acid and propionic ratio	Q	63	2.34	0.168	−0.010 2 0.000 063	0.003 8 0.000 024 2	0.008 0.011 6	1.55	120	Min.	80.90	1.93
Iso volatile fatty acid (% of VFA)	Q	41	8.11	1.6	−0.155 0.001 6	0.074 6 0.000 6	0.044 0.012	1.33	244	Min.	47.20	4.44
Nutrient disappearance												
Dry matter (% of fresh matter)	L	64	66.4	1.89	0.006 1	0.026 6	0.819	1.38	402	Pos.		
Crude protein (% of dry matter)	L	10	64.1	13.47	0.820	0.744	0.302	0.96	81.1	Pos.		

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Table 3 to be continued

Parameter	Model	*n*	Parameter estimates				Model estimates			Interpretation		
			Int.	SE Int.	slope	SE slope	*P*-value	RMSE	AIC[a]	trend	x^b	y^c
Neutral detergent fiber (% of dry matter)	Q	22	43.6	2.59	0.462 −0.005 8	0.147 0.001 6	0.005 0.002	1.34	145	Max.	39.60	52.80
Acid detergent fiber (% of dry matter)	L	10	40.1	6.2	0.106	0.342	0.766	1.02	68.6	Pos.		
Microbiology												
Protozoa (log cell/ml)	L	12	5.3	1.24	0.066 1	0.063 4	0.321	1.23	51.4	Pos.		

AIC = Akaike information criterion; Int. = intercept; L = linear; Max. = maximum; Min. = minimum; *n* = number of data; Neg. = negative; Pos. = positive; Q = quadratic; RMSE = root mean square errors; SE = standard error; VFA = volatile fatty acids

[a]AIC is an estimator of the relative quality of statistical models for a given set of data; [b]level (mg/kg of diet); [c]optimal value of response parameter

crude protein, NDF) and nitrogen retention increased by following quadratic patterns ($P < 0.01$), whereas the ratio of nitrogen retention to digested nitrogen linearly increased ($P < 0.05$) due to biochar supplementation.

DISCUSSION

Influence of biochar on rumen fermentation

Biochar supplementation increased total gas production *in vitro*. Biochar has been known as a good adsorption material because of its relationship with various types of molecules. An increase in gas production may indicate a stimulatory effect of biochar on rumen microbial activity and fermentation. Gas production during the fermentation process is an evaluation of the fibre digestion kinetics *in vitro* (Menke et al. 1979; Menke and Steingass 1988). On the other hand, biochar supplementation decreased *in vitro* methane production. Methane produced during fermentation causes a significant loss of energy for animals (from 2% to 12% of total energy intake) (Tapio et al. 2017). Biochar in the feed may act as an electron acceptor and reduce methane production in the rumen (Leng et al. 2012a, b). This porous biochar structure absorbs methane in the rumen. Pores are a habitat for several bacterial communities including methanogens and methanotrophs (Leng et al. 2012b). Methanogenic bacteria are bacteria that produce methane, while methanotrophic bacteria are bacteria that utilize methane. Mitsumori et al. (2014) reported that the existence of methanotrophic bacteria such as Proteobacteria in the rumen can utilize methane so that methane production is lower with the addition of biochar. There are several reasons that may explain the reduction of methane caused by biochar. Biochar supplementation reduces methane apparently due to the physical effect of biochar, which has multiple and uniform pores. Biochar has been used as a food additive in order to build new microbial habitats and can change biofilm activity in the rumen (Leng 2014). These pores function to absorb gases in the rumen, including methane. Simultaneously, there are methanotrophs around the rumen as methane users, which causes the methanogen population and methane gas production to decrease (Saenab et al. 2018). The ability of biochar to absorb methane and other gases produced in the rumen may be affected

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Table 4. Regression equations on the influence of biochar doses on growth performance, feed intake, rumen fermentation, blood constituents, nutrient digestibility, and nitrogen retention

Parameter	Model	*n*	Parameter estimates				Model estimates			Interpretation		
			Int.	SE Int.	slope	SE slope	*P*-value	RMSE	AIC[a]	trend	x^b	y^c
Feed intake												
Dry matter (g as-fed $BW^{0.75}$)	L	94	1 183	166.3	–3.16	1.54	0.045	1.85	1 349	Neg.		
Ash (g DM $BW^{0.75}$)	L	64	81.4	15.2	–0.069	0.105	0.514	1.82	591	Neg.		
Organic matter (g DM $BW^{0.75}$)	L	62	969	138.5	–3.82	1.76	0.036	1.63	877	Neg.		
Crude protein (g DM $BW^{0.75}$)	L	87	170	25.8	–0.575	0.251	0.026	1.68	927	Neg.		
Neutral detergent fibre (g DM $BW^{0.75}$)	L	56	489	78.3	–1.59	0.788	0.052	1.52	704	Neg.		
Acid detergent fibre (g DM $BW^{0.75}$)	L	56	248	47.3	–0.977	0.465	0.044	1.68	648	Neg.		
Hemicellulose (g DM $BW^{0.75}$)	L	42	252	45.6	–0.734	0.416	0.090	1.12	463	Neg.		
Rumen fermentation												
pH	L	29	6.6	0.12	0.000	0.003 0	0.962	1.02	7.28	Neg.		
Ammonia (mg/100ml)	L	33	24.4	3.27	0.223	0.093 8	0.030	1.06	220	Pos.		
Methane (ppm)	L	12	31.2	14.57	–0.195	0.222 4	0.410	0.91	97	Neg.		
Carbon dioxide (ppm)	L	12	1 071	340.23	–2.609	3.817 5	0.516	0.89	156	Neg.		
Ratio of methane and carbon dioxide	L	20	0.03	0.00	0.000	0.000 1	0.162	1.07	–108	Neg.		
Volatile fatty acid (mmol)	L	17	105	3.50	0.248	0.298	0.429	1.15	123	Pos.		
Acetic acid (C_2) (% of VFA)	L	25	67.8	1.83	–0.092	0.0562	0.126	1.23	135	Neg.		
Propionic acid (C_3) (% of VFA)	L	25	17.7	1.32	0.121	0.0398	0.010	0.79	120	Pos.		
n-Butyric acid (C_4) (% of VFA)	L	25	12.2	0.80	–0.085	0.058 3	0.171	1.01	117	Neg.		
iso-Butyric acid (*iso*-C_4) (% of VFA)	L	11	1.52	0.24	0.012	0.011 5	0.356	0.75	18.5	Pos.		
n-Valeric acid (C_5) (% of VFA)	L	11	1.91	0.56	0.072	0.020 5	0.017	0.81	31.0	Pos.		
iso-Valeric acid (*iso*-C_5) (% of VFA)	L	11	2.37	0.66	0.025	0.017 4	0.212	0.91	30.7	Pos.		
Ratio of non-glucogenic to glucogenic acids	L	25	5.35	0.62	–0.038	0.015 0	0.025	0.85	79.7	Neg.		
Ratio of acetic acid and propionic acid	L	25	5.44	0.35	0.022	0.016 7	0.21	1.01	68.9	Pos.		
Iso volatile fatty acid (% of VFA)	L	11	3.89	0.90	0.035	0.021 5	0.164	0.89	35.3	Pos.		
Blood constituents												
Red blood cell ($10^6/mm^3$)	L	11	8.94	0.37	–0.016	0.037 7	0.682	0.98	41.3	Neg.		
White blood cell ($10^3/mm^3$)	L	11	8.89	0.63	–0.004	0.062 4	0.947	0.97	50.5	Neg.		
Eosinofil (% of WBCC)	L	11	2.31	0.78	–0.005	0.078 6	0.949	1.13	54.6	Neg.		
Neutrophile (% of WBCC)	L	11	32.27	4.02	–0.082	0.282 8	0.784	0.95	80.3	Neg.		
Lymphocite (% of WBCC)	L	11	61.62	6.03	0.069	0.287 3	0.819	0.95	83.7	Pos.		

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Table 4 to be continued

Parameter	Model	*n*	Parameter estimates				Model estimates			Interpretation		
			Int.	SE Int.	slope	SE slope	*P*-value	RMSE	AIC[a]	trend	x^b	y^c
Monocite (% of WBCC)	L	11	3.96	1.82	0.060	0.029 7	0.101	0.81	51.4	Pos.		
Digestibility nutrient and nitrogen retention												
Dry matter (% of as-fed)	Q	50	65.76	1.25	0.563 −0.014	0.139 0.004	0.000 4 0.000 9	1.25	324	Max.	20.51	71.53
Organic matter (% of DM)	Q	35	66.48	1.83	0.453 −0.011 6	0.138 0.003 6	0.004 2 0.004 7	1.12	236	Max.	19.49	70.89
Crude protein (% of DM)	Q	40	61.75	3.84	0.699 −0.012 3	0.174 0.004 2	0.000 6 0.008 4	1.51	299	Max.	28.41	71.68
Nitrogen (% of DM)	L	11	74.87	3.47	0.135	0.199 1	0.527	0.92	76.1	Pos.		
Neutral detergent fibre (% of DM)	Q	37	54.97	2.22	0.406 −0.009 9	0.146 9 0.003 8	0.011 9 0.016 8	1.06	253	Max.	20.59	59.16
Acid detergent fibre (% of DM)	L	31	46.5	1.95	−0.042	0.073 7	0.575	1.43	205	Neg.		
Hemicellulose (% of DM)	L	10	45.7	4.89	0.043	0.398	0.918	0.80	68.0	Pos.		
Nitrogen in faces (g/kg $BW^{0.75}$)	L	23	0.50	0.05	−0.001	0.001 5	0.460	1.10	−12.4	Neg.		
Nitrogen in urine (g/kg $BW^{0.75}$)	L	23	0.42	0.06	−0.003	0.001 4	0.071	1.09	−10.2	Neg.		
Nitrogen digested (g/kg $BW^{0.75}$)	L	23	1.09	0.09	0.003	0.002 6	0.286	1.12	11.8	Pos.		
Nitrogen retention (g/kg $BW^{0.75}$)	Q	23	0.611	0.088	0.017 7 −0.000 3	0.005 8 −0.000 1	0.010 8 0.038 0	0.938	26.04	Max.	27.81	0.86
Ratio nitrogen retention and digested	L	23	0.60	0.06	0.004	0.001 5	0.021	1.13	−8.2	Pos.		
Ratio nitrogen faeces and urine	L	23	1.61	0.34	0.004	0.002 1	0.126	0.85	31.1	Pos.		
Initial BW (kg)	L	98	201	37.2	−0.09	0.186	0.631	1.93	1 053	Neg.		
Initial metabolizable BW (kg $BW^{0.75}$)	L	98	46.6	7.21	−0.013	0.029 5	0.662	2.00	715	Neg.		
Final BW (kg)	L	57	264	53.1	−0.165	0.483	0.735	1.29	648	Neg.		
Average daily gain (g/h/day)	L	59	459	94.2	0.673	7.28	0.927	1.97	868	Pos.		
Average daily intake (g/h/day as DM)	L	98	4.73	798	−15.9	7.36	0.035	1.84	1 711	Neg.		
Feed conversion ratio	Q	59	13.8	1.56	−1.091 0.063	0.443 0.028	0.019 0.035	1.88	413	Min.	8.71	9.06

AIC = Akaike information criterion; BW = body weight; DM = dry matter; Int. = intercept; L = linear; Max. = maximum; Min. = minimum; *n* = number of data; Neg. = negative; Pos. = positive; Q = quadratic; RBCC = red blood cell count; RMSE = root mean square errors; SE = standard error; VFA = volatile fatty acids; WBCC =white blood cell count

[a]AIC is an estimator of the relative quality of statistical models for a given set of data; [b]level (mg/kg of diet); [c]optimal value of response parameter

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by the pore size. It was revealed that the addition of smaller-sized, powdered biochar had a greater reduction rate compared to larger granular biochar (Zhou et al. 2017). Besides, the decrease in methane could be related to the presence of secondary compounds in biochar such as phenolic compounds. It was reported that phenolic compounds could inhibit the growth of methanogens and some other bacteria, thereby reducing methane production (Jayanegara et al. 2015). In the pyrolysis process in the manufacture of biochar, lignin and cellulose will indeed be degraded but not completely so that there are still remaining phenolic compounds. What is lost are usually water-soluble phenolics (phenolic compounds that dissolve in water/polar solvents). In addition, it was shown by FTIR analysis that organic functional groups were still present in the biochar. These groups can also indicate the presence of phenolic compounds in the biochar which are still bound (Mierzwa-Hersztek et al. 2019).

The decrease in methane emissions may also be caused by the biochar pH. Alkaline pH activated charcoal (8.2–10.2) did not affect the *in vitro* production of methane (Hansen et al. 2012; Pereira et al. 2014), but acidic pH activated charcoal (4.8) caused a decline in methane (Saleem et al. 2018). The reduction of methane by biochar indicated that biochar supplementation could increase the efficiency of energy use in ruminants since methane production in the rumen fermentation caused lower energy utilization. According to Mukome et al. (2013), pyrolysis method and temperature are the key factors that influence the physical and chemical properties of biochar. However, the characteristics of biochar raw materials are easier to understand, for example, ash content is higher in biochar from wood compared to biochar from non-wood material. Meanwhile, on biochar from wood, its surface area correlates with the pyrolysis temperature; if the pyrolysis temperature is low, then the surface area is small. All biochar has common characteristics such as high pH (6.8–10.9) and a high C to N ratio (> 20). The relative proportion of the biochar component determines the overall chemical, physical, and biochar function (Brown 2009), which influences the application process, transportation, and impact on the environment (Downie et al. 2009).

Ammonia is the main nitrogen source for microbial protein synthesis in the rumen and the end product of dietary protein degradation by rumen microbes (Pisulewski et al. 1981). In the rumen, dietary proteins can be hydrolyzed and deaminated to form free peptides and ammonia by rumen microorganisms (Reynal et al. 2007). The high concentration of ammonia increases microbial protein synthesis in the rumen system because ammonia is a major precursor in microbial cell formation. There was no effect between biochar supplementation and ammonia concentration *in vitro*, which means that biochar did not inhibit protein degradation by proteolytic bacteria in the rumen. The pores in biochar may absorb ammonia that is produced in the rumen, but later on, the ammonia is then released slowly (Leng et al. 2012b). Different biochar sources can provide different responses to rumen fermentation due to differences in the structure and composition of the basic ingredients and the effectiveness of biochar. Digestion and metabolism of dietary protein can be inhibited by biochar so that free ammonia concentration will be reduced. The unpredicted NH_3 concentration after 24-hour incubation showed a decrease after the addition of biochar. Because *in vitro* incubation is a closed system, so there are two possible reasons. First, differences in NH_3 concentrations can be caused by a reduction in proteolysis and deamination of nitrogen constituents from the substrate, increased incorporation of NH_3 into microbial proteins, or even a combination of these two processes. The difference in energy supply for microbial growth (gas production or VFA) is small, so a reduction in proteolysis or deamination more likely seems to occur, but because there is no direct measurement, then it is speculative. The ability of biochar to adsorb NH_3 is inversely proportional to the temperature at which biochar is produced (an increase in pyrolysis temperature can reduce cation exchange capacity). The ability to absorb ammonia is also influenced by biomass source (Cabeza et al. 2018).

Biochar supplementation enhanced the molar proportion of propionic acid in the rumen. Propionate is the final fermentation product for different bacterial species, including the family Propionibacteriaceae (Chen et al. 2020). Hydrogen gas is produced by the acetic acid and butyric acid formation. On the other hand, propionate synthesis requires hydrogen. Supplementation of biochar can suppress methane production since the hy-

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drogen produced from the fermentation of carbohydrates is converted into propionate or used to hydrogenate unsaturated fatty acids or reduce nitrates. Propionic acid is the primary source of glucose for livestock for gluconeogenesis while acetic acid and butyric acid play a role in long-chain fatty acid synthesis (Morvay et al. 2011). The products from the degradation of branched-chained amino acids in the rumen fermentation are isobutyrate and isovalerate. Biochar increased isovalerate suggests that certain protein degradation of branched-chain amino acids could be induced. However, the stimulation may occur partially, and the degradation of amino acids to ammonia was not impaired because the production of ammonia in the rumen was not increased. It was reported that most ruminal cellulolytic microorganisms such as *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Fibrobacter succinogenes*, and *Butyrivibio fibrisolvens* require branched-chain volatile fatty acids for their growth (Shi et al. 1997). Ruminal microorganisms use these branched-chain volatile fatty acids (isobutyric, isovaleric and 2-methylbutyric acid) to synthesize branched-chain amino acids, i.e., valine, leucine, and isoleucine (Zhang et al. 2013). Therefore the addition of biochar would be beneficial for the growth of some cellulolytic bacteria in the rumen and, hence, it improves the digestibility of NDF. The increased disappearance of NDF suggests that biochar can contribute to the growth of a more efficient ruminal microbial population (Saleem et al. 2018). The addition of biochar to rumen fermentation products (*in vitro* study) had a positive effect with a maximal dose of 39.6 g/kg substrate.

Influence of biochar on livestock performance and nutrient utilization

The present meta-analysis summarized that adding biochar decreases nutrient intake, apparently due to the bitter taste of the material. This could be attributed to the role of biochar supplementation: it had an influence on the digestibility of dry matter, organic matter, crude protein, neutral detergent fibre, nitrogen retention, and nitrogen retention to digested nitrogen ratio at a certain maximum point, then the effect will decrease. The high yield of dry matter digestibility is thought to be due to the high availability of N and carbon framework in the feed which can optimize microbial growth so that more feed is degraded. Biochar raw material and particle size are important factors to determine the effectiveness of biochar as a feed additive to changes in feed digestibility. Leng (2014) stated that biochars can enhance some rumen microbial populations by offering strong areas of the surface where microorganisms can effectively move and enhance the efficiency of the production of ATP, increasing the digestibility of feed and digestion efficiency. A positive effect of biochar addition was found on N retention, no effect of biochar on nitrogen digestibility was observed. The retention of N in the ruminant is determined by the amount of energy supply and N in the network. The amount of energy supply for ruminants comes from the production of VFA in the rumen, while the N supply comes from the flow of rumen microbial N and the ruminal bypass feed protein (Storm and Orskov 1983). Biochar addition can affect and alter the bioavailability of N and other nutrients (Taghizadeh-Toosi et al. 2012). N retention and N retention to digested nitrogen ratio were both improved by supplementation of biochar. Presumably the increase of protein digestibility with addition of biochar, required by microorganisms for efficient rumen digestion, increases feed efficiency. Degradation of ruminal protein is affected by pH of the rumen and predominant microbial population in the rumen.

There was no influence on average daily gain but biochar increased feed efficiency. Although the addition of biochar can improve feed efficiency, it is necessary to consider the palatability of feed containing a high level of biochar in *in vivo* experiments. The use of biochar to improve livestock performance in pigs has been carried out since the 1880s and in poultry since the 1940s (Totusek and Beeson 1953). The use of biochar as a food additive has not been reported to cause any negative effects (Kammann et al. 2017). Biochar is able to increase good bacteria in the digestive tract, thereby increasing feed efficiency. Van et al. (2006) attributed the increases in digestibility to the ability of charcoal to adsorb contaminants and tannins, prevent them from accessing the intestines and hinder the excretion of enzymes, resulting in better digestion. Naka et al. (2001) stated that the use of biochar increased the adsorption capacity of harmful bacteria in the livestock digestive

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tract. In addition, it increased the ratio of beneficial bacteria to harmful bacteria. In other words, the transport of biochar as a matrix of beneficial bacteria significantly increased the intestinal flora in the digestive tract. Biochar supplementation quadratically improved ($P < 0.050$) FCR. The addition of biochar to decrease FCR (*in vivo* study) had a positive effect with a maximal dose of 8.71 g/kg substrate. Improvement in nutrient digestibility caused by biochar addition to the diet would increase nutrient retention and FCR. The biochar surface contributes to an increase in the population of methanotrophic relatives for methanogenic microbes, so as to reduce methane production leading to improved feed efficiency (Leng et al. 2012a, b). Increased efficiency leads to an increase in the propionate to acetate ratio.

CONCLUSION

This meta-analysis study found a consistent effect of biochar addition between *in vitro* and *in vivo* experiments by increasing propionic acid production in the rumen and NDF digestibility. The addition of biochar to rumen fermentation products (*in vitro* study) and a decrease in FCR (*in vivo* study) had a positive effect with a maximal dose of 39.6 g/kg substrate and 8.71 g/kg substrate. The use of biochar as a feed additive has the potential to improve animal health, feed efficiency, and livestock productivity, reduce nutrient loss, and greenhouse gases. The most important finding is that there was no significant negative effect on animal health in the publications reviewed. It cannot be denied that, although there are many scientific publications, further research is needed to uncover the mechanisms observed and to optimize biochar-based feed products.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Al-kindi A, Dickhoefer U, Schlecht E, Sundrum A, Schiborra A. Effects of quebracho tannin extract (Schinopsis balansae Engl.) and activated charcoal on nitrogen balance, rumen microbial protein synthesis and faecal composition of growing Boer goats. Arch Anim Nutr. 2016 Jul 3;70(4):307-21.

Brown R. Biochar production technology. In: Lehmann J, Joseph S, editors. Biochar for environmental management: Science and technology. London: Earthscan; 2009. p. 127-46.

Cabeza I, Waterhouse T, Sohi S, Rooke JA. Effect of biochar produced from different biomass sources and at different process temperatures on methane production and ammonia concentrations in vitro. Anim Feed Sci Technol. 2018 Mar 1;237:1-7.

Calsamiglia S, Busquet M, Cardozo PW, Castillejos L, Ferret A. Invited review: Essential oils as modifiers of rumen microbial fermentation. J Dairy Sci. 2007 Jun;90 (6):2580-95.

Cha SW, Lee SK. Effects of activated carbon and charcoal on the nutrients utilization and ruminal fermentation characteristics in goat. J Agric Sci. 2005 Dec;32 (2):197-203.

Chen J, Harstad OM, McAllister T, Dorsch P, Holo H. Propionic acid bacteria enhance ruminal feed degradation and reduce methane production in vitro. Acta Agric Scand A Anim Sci. 2020 Jul 2;69(3):169-75.

Downie A, Crosky A, Munroe P. Physical properties of biochar. In: Lehmann J, Joseph S, editors. Biochar for enviromental management: Science and technology. London: Earthscan; 2009. p. 13-32.

Erickson PS, Whitehouse NL, Dunn ML. Activated carbon supplementation of dairy cow diets: Effects on apparent total-tract nutrient digestibility and taste preference. Prof Anim Sci. 2011 Oct 1;27(5):428-34.

Flachowsky G, Gruen M, Meyer U. Feed-efficient ruminant production: Opportunities and challenges. J Anim Feed Sci. 2013 Sep 2;22(3):177-87.

Garillo EP, Pradhan R, Tobioko H. Effects of activated charcoal on ruminal characteristics and blood profiles in mature goats. West Japan J Anim Sci. 1994;37:85-9.

Garillo EP, Pradhan R, Tobioko H. Effect of activated carbon on growth, ruminal characteristics, blood profiles and feed digestibility in sheep. Asian-Australas J Anim Sci. 1995 Feb;8(1):43-55.

Hang LTT, Preston TR, Leng RA, Ba NX. Effect of biochar and water spinach on feed intake, digestibility and N-retention in goats fed urea-treated cassava stems. Livest Res Rural Dev. 2018 May;30(5): 93.

https://doi.org/10.17221/124/2022-CJAS

Hang LTT, Preston TR, Ba NX, Dung DV. Effect of biochar on growth and methane emissions of goats fed fresh cassava foliage. Livest Res Rural Dev. 2019 May;31(5): 67.

Hansen HH, Storm IMLD, Sell AM. Effect of biochar on in vitro rumen methane production. Acta Agric Scand Sect A Anim Sci. 2012 Apr;62(4):305-9.

Holtshausen L, Chaves AV, Beauchemin KA, McGinn SM, McAllister TA, Odongo NE, Cheeke PR, Benchaar C. Feeding saponin-containing Yucca schidigera and Quillaja saponaria to decrease enteric methane production in dairy cows. J Dairy Sci. 2009 Jun;92(6):2809-21.

Jayanegara A, Goel G, Makkar H, Becker K. Divergence between purified hydrolysable and condensed tannin effects on methane emission, rumen fermentation and microbial population in vitro. Anim Feed Sci Tech. 2015 Nov 1;209:60-8.

Jiang YH, Wang P, Yang HJ, Chen Y. The efficacy of bamboo charcoal in comparison with smectite to reduce the detrimental effect of aflatoxin B1 on in vitro rumen fermentation of a hay-rich feed mixture. Toxins. 2014 Jul 10;6(7):2008-23.

Kammann C, Ippolito J, Hagemann N, Borchard N, Cayuela ML, Estavillo JM, Fuertes-Mendizabal T, Jeffery S, Kern J, Novak J, Rasse D, Saarnio S, Schmidt H-P, Spokas K, Wrage-Mönnig N. Biochar as a tool to reduce the agricultural greenhouse-gas burdenknowns, unknowns and future research needs. J Environ Eng Landsc Manag. 2017 Jun;25(2):114-39.

Kim BK, Kim YJ. Effects of feeding charcoal powder and vitamin A on growth performance, serum profile and carcass characteristics of fattening Hanwoo steers. J Anim Sci Technol. 2005 Apr 30;47(2):233-42.

Lee SK, Cha SW, Kim SK. Effects of activated carbon and charcoal on in vitro nutrient disappearances and ruminal fermentation characteristics. J Agri Sci. 2002 Dec 31;29(2):35-42.

Leng RA. Interactions between microbial consortia in biofilms: A paradigm shift in rumen microbial ecology and enteric methane mitigation. Anim Prod Sci. 2014 Feb;54:519-43.

Leng RA, Inthapanya S, Preston TR. Biochar lowers net methane production from rumen fluid in vitro. Livest Res Rural Dev. 2012a Jun;24(6): 13.

Leng RA, Preston TR, Inthapanya S. Biochar reduces enteric methane and improves growth and feed conversion in local "Yellow" cattle fed cassava root chips and fresh cassava foliage. Livest Res Rural Dev. 2012b Nov;24(11): 8 p.

Leng RA, Inthapanya S, Preston TR. Methane production is reduced in an in vitro incubation when the rumen fluid is taken from cattle that previously received biochar in their diet. Livest Res Rural Dev. 2012c Nov 6;24(11): 211.

Leng RA, Inthapanya S, Preston T. All biochars are not equal in lowering methane production in in vitro rumen incubations. Livest Res Rural Dev. 2013 Jun 2;25(6): 106.

Marshall BM, Levy SB. Food animals and antimicrobials: Impacts on human health. Clin Microbiol Rev. 2011 Oct;24(4):718-33.

McGrath J, Duval SM, Tamassia LFM, Kindermann M, Stemmler RT, de Gouvea VN, Acedo TS, Immig I, Williams SN, Celi P. Nutritional strategies in ruminants: A lifetime approach. Res Vet Sci. 2018 Feb 1;116:28-39.

Menke KH, Steingass H. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim Res Dev. 1988; 28:7-55.

Menke KH, Raab L, Salewski A, Steingass H, Fritz D, Schneider W. The estimation of the digestibility and metabolisable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor in vitro. J Agric Sci. 1979;93:217-22.

Mierzwa-Hersztek M, Gondek K, Nawrocka A, Pinkowska H, Bajda T, Stanek-Tarkowska J, Szostek M. FT-IR analysis and the content of phenolic compounds in exogenous organic matter produced from plant biomass. J Elem. 2019;24(3):879-96.

Mirheidari A, Torbatinejad NM, Shakeri P, Mokhtarpour A. Effects of walnut shell and chicken manure biochar on in vitro fermentation and in vivo nutrient digestibility and performance of dairy ewes. Trop Anim Health Prod. 2019a Nov;51(8):2153-60.

Mirheidari A, Torbatinejad NM, Shakeri P, Mokhtarpour A. Effects of biochar produced from different biomass sources on digestibility, ruminal fermentation, microbial protein synthesis and growth performance of male lambs. Small Rumin Res. 2019b Dec 19;183: 6 p.

Mitsumori M, Enishi O, Shinkai T, Higuchi K, Kobayashi Y, Takenaka A, Nagashima K, Mochizuki M, Kobayashi Y. Effect of cashew nut shell liquid on metabolic hdyrogen flow on bovine rumen fermentation. J Anim Sci. 2014 Mar;85(3):227-32.

Morvay Y, Bannink A, France J, Kebreab E, Dijkstra J. Evaluation of models to predict the stoichiometry of volatile fatty acid profiles in rumen fluid of lactating Holstein cows. J Dairy Sci. 2011 Jun;94(6):3063-80.

Mukome FND, Zhang X, Silva LCR, Six J, Parikh SJ. Use of chemical and physical characteristics to investigate trends in biochar feedstocks. J Agric Food Chem. 2013 Jan; 61(9):2196-204.

Naka K, Watarai S, Tana, Inoue K, Kodama Y, Oguma K, Yasuda T, Kodama H. Adsorption effect of activated charcoal on enterohemorrhagic Escherichia coli. J Vet Med Sci. 2001 Mar;63(3):281-5.

NRC – National Research Council. The use of drugs in food animals: Benefits and risks. Washington DC: The National Academy Press; 1999. 276 p.

https://doi.org/10.17221/124/2022-CJAS

Papatsiros VG, Katsoulos PD, Koutoulis KC, Karatzia M, Dedousi A, Christodoulopoulos G. Alternatives to antibiotics for farm animals. CAB Rev. 2013 Apr;8: 15 p.

Pereira RC, MuetzeL S, Arbestain MC, Bishop P, Hina K, Hedley M. Assessment of the influence of biochar on rumen and silage fermentation: A laboratory-scale experiment. Anim Feed Sci Tech. 2014 Oct 1;196:22-31.

Phongphanith S, Preston T. Effect of rice-wine distillers' by product and biochar on growth performance and methane emissions in local "Yellow" cattle fed ensiled cassava root, urea, cassava foliage and rice straw. Livest Res Rural Dev. 2016 Oct;28(10): 178.

Phongphanith S, Preston TR, Leng RA. Effect of water spinach (Ipomoea aquatica) and cassava leaf meal (Manihot esculenta Crantz) with or without biochar on methane production in an in vitro rumen incubation using ensiled or dried cassava root meal as source of carbohydrate. Livest Res Rural Dev. 2016 Jun 2;28(6): 112.

Phuong LTB, Preston TR, Van NH, Dung DV. Effect of additives (brewer's grains and biochar) and cassava variety (sweet versus bitter) on nitrogen retention, thiocyanate excretion and methane production by Bach Thao goats. Livest Res Rural Dev. 2019 Jan 1;31(1): 1.

Pisulewski PM, Okorie AU, Buttery PJ, Haresign W, Lewis D. Ammonia concentration and protein synthesis in the rumen. J Sci Food Agric. 1981 Aug;32(8):759-66.

Reynal SM, Ipharraguerre IR, Lineiro M, Brito AF, Broderick GA, Clark JH. Omasal flow of soluble proteins, peptides and free amino acids in dairy cows fed diets supplemented with proteins of varying ruminal degradabilities. J Dairy Sci. 2007 Apr;90(4):1887-903.

Saenab A, Wiryawan K, Retnani Y, Wina E. Manipulation of rumen fermentation by bioindustrial products of cashew nut shell (Anacardium occidentale) to reduce methane production. JITV. 2018 May;23(2):61-70.

Saleem AM, Ribeiro GOJr, Yang WZ, Ran T, Beauchemin KA, McGeough EJ, Ominski KH, Okine EK, McAllister TA. Effect of engineered biocarbon on rumen fermentation, microbial protein synthesis, and methane production in an artificial rumen (RUSITEC) fed a high forage diet. J Anim Sci. 2018 Jul;96(8):3121-30.

Saroeun K, Preston TR, Leng RA. Rice distillers' by product and molasses urea blocks containing biochar improved the growth performance of local Yellow cattle fed ensiled cassava roots, cassava foliage and rice straw. Livest Res Rural Dev. 2018 Sep 3;30(9): 162.

Sauvant D, Schmidely P, Daudin JJ, St-Pierre NR. Meta-analyses of experimental data in animal nutrition. Animal. 2008 Aug;2(8):1203-14.

Schmidt HP, Hagemann N, Draper K, Kammann C. The use of biochar in animal feeding. PeerJ. 2019 Jul 31;7: 54 p.

Shi Y, Odt CH, Weimer PJ. Competition for cellulose among three predominant ruminal cellulolytic bacteria under substrate-excess and substrate-limited conditions. Appl Environ Microbiol. 1997 Feb;63(2):734-42.

Silivong P, Preston TR. Growth performance of goats was improved when a basal diet of foliage of Bauhinia acuminata was supplemented with water spinach and biochar. Livest Res Rural Dev. 2015 Mar 3;27(3): 58.

Silivong P, Preston TR. Supplements of water spinach (Ipomoea aquatica) and biochar improved feed intake, digestibility, N retention and growth performance of goats fed foliage of Bauhinia acuminata as the basal diet. Livest Res Rural Dev. 2016 May 1;28(5): 98.

Silivong P, Preston TR, Van NH, Hai DT. Brewers' grains (5% of diet DM) increases the digestibility, nitrogen retention and growth performance of goats fed a basal diet of Bauhinia accuminata and foliage from cassava (Manihot esculenta Crantz) or water spinach (Ipomoea aquatica). Livest Res Rural Dev. 2018 Mar 1;30(3): 55.

St-Pierre NR. Invited review: Integrating quantitative findings from multiple studies using mixed model methodology. J Dairy Sci. 2001 Apr;84(4):741-55.

Storm EA, Orskov ER. The nutritive value of rumen micro-organisms in ruminant. 1. Large-scale isolation and chemical composition of rumen microorganisms. Br J Nutr. 1983 Sep;50(2):463-70.

Taghizadeh-Toosi A, Clough TJ, Sherlock RR, Condron LM. Biochar adsorbed ammonia is bioavailable. Plant Soil. 2012 Jul;350(1):57-69.

Tapio I, Snelling TJ, Strozzi F, Wallace RJ. The ruminal microbiome associated with methane emissions from ruminant livestock. J Anim Sci Biotechnol. 2017 Jan 19; 8(7):1-11.

Teoh R, Caro E, Holman DB, Joseph S, Meale SJ, Chaves AV. Effects of hardwood biochar on methane production, fermentation characteristics, and the rumen microbiota using rumen simulation. Front Microbiol. 2019 Jul 10;10: 13.

Tobioka H, Garillo E. Growth performance of Japanese brown cattle fed consentrate-based diets fortified with activated charcoal. West Japan J Anim Sci. 1994 May;37:48-53.

Totusek R, Beeson WM. The nutritive value of wood charcoal for pigs. J Anim Sci. 1953 May;12(2):271-81.

Van DTT, Mui NT, Ledin I. Effect of method of processing foliage of Acacia mangium and inclusion of bamboo charcoal in the diet on performance of growing goats. Anim Feed Sci Tech. 2006 Nov;130(3-4):242-56.

Vongkhamchanh B, Inthapanya S, Preston TR. Methane production in an in vitro rumen fermentation is reduced when the carbohydrate substrate is fresh rather than ensiled or dried cassava root, and when biochar is added to the substrate. Livest Res Rural Dev. 2015 Oct 1;27(10): 208.

https://doi.org/10.17221/124/2022-CJAS

Vongkhamchanh B, Preston TR, Leng RA, An LV, Hai DT. Effect of biochar on growth performance of local "Yellow" cattle fed ensiled cassava roots, fresh brewers' grains and rice straw. Livest Res Rural Dev. 2018 Sep 3;30: 4 p.

Vongsamphanh P, Napasirth V, Inthapanya S, Preston TR. Effect of biochar and leaves from sweet or bitter cassava on gas and methane production in an in vitro rumen incubation using cassava root pulp as source of energy. Livest Res Rural Dev. 2015 Apr 1;27(4): 72.

Winders TM, Jolly-Breithaupt ML, Wilson HC, MacDonald JC, Erickson GE, Watson AK. Evaluation of the effects of biochar on diet digestibility and methane production from growing and finishing steers. Transl Anim Sci. 2019 Feb;3(2):775-83.

Yu L, Yuan Y, Tang J, Wang Y, Zhou S. Biochar as an electron shuttle for reductive dechlorination of pentachlorophenol by Geobacter sulfurreducens. Sci Rep. 2015 Nov 23; 5(1): 10 p.

Zhang HL, Chen Y, Xu XL, Yang YX. Effects of branched-chain amino acids on in vitro ruminal fermentation of wheat straw. Asian-Australas J Anim Sci. 2013 Apr; 26(4):523-8.

Zhou GW, Yang XR, Marshall CW, Li H, Zheng BX, Yan Y, Su JQ, Zhu YG. Biochar addition increases the rates of dissimilatory iron reduction and methanogenesis in ferrihydrite enrichments. Front Microbiol. 2017 Apr 6;8: 14 p.

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