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Effects of biochar and wood vinegar supplementation on growth performance, rumen parameters, and gas production in milk-fed Holstein calves

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Abstract This study investigated the effects of dietary biochar and wood vinegar supplementation, individually or in combination, on growth performance, skeletal measurements, rumen fermentation characteristics, and gas production parameters in milk-fed Holstein calves. Forty newborn male Holstein calves (38.6±3.4 kg) were randomly assigned to a completely randomized design with a 2×2 factorial arrangement from 3 days of age until weaning (66 days). The experimental treatments consisted of a control diet and the control diet supplemented with 0.5% biochar, 0.3% wood vinegar, or their combination. Body weight was recorded weekly, and starter intake was measured daily. Skeletal growth parameters were evaluated at 3, 6, and 9 weeks of age. At the end of the trial, rumen fluid was collected to evaluate ruminal fermentation characteristics, and gas production parameters were assessed using *in vitro* techniques. Neither the main effect of biochar nor the biochar × wood vinegar interaction affected growth performance parameters ($P>0.05$). The main effect of wood vinegar increased dry matter intake ($P<0.01$). The main effect of wood vinegar increased abdominal girth, withers height, and pin width at 3 weeks of age ($P<0.05$). At 6 weeks of age, the main effect of biochar increased abdominal girth, whereas the main effect of wood vinegar increased body length, abdominal girth, withers height, hip-to-pin distance, and pin width ($P<0.05$). At 9 weeks of age, the main effect of wood vinegar increased body length, chest girth, abdominal *girth*, hip-to-pin distance, and pin width ($P<0.05$). The main effect of biochar increased total volatile fatty acid (TVFA) concentration ($P=0.01$), whereas the main effect of wood vinegar increased TVFA concentration and acetate proportion and decreased butyrate proportion ($P<0.05$). The main effect of wood vinegar also increased gas production potential, gas production at 24 h, organic matter digestibility, metabolizable energy, and short-chain fatty acid production ($P<0.05$). Overall, wood vinegar showed greater potential than biochar as a feed additive, with stronger effects on feed intake, skeletal growth, ruminal fermentation, and gas production parameters in Holstein calves.

Keywords: biochar, calf, feed conversion ratio, volatile fatty acids, wood vinegar

Introduction

The pre-weaning period is a crucial stage in dairy calf development, during which rapid gastrointestinal development and establishment of rumen microbial populations occur (Du et al., 2023). Although newborn calves are functionally monogastric, gradual rumen development and establishment of a stable microbial ecosystem are essential for efficient nutrient utilization, rumen fermentation capacity, and future productive performance (Leng, 2014). However, immature gastrointestinal function during this period increases susceptibility to digestive disorders, impaired nutrient utilization, and reduced growth performance (Lopez and Heinrichs, 2022). Therefore, nutritional strategies that promote rumen microbial development and improve fermentation efficiency may provide an effective approach to enhance calf performance and health (Azizi et al., 2024). Biochar has recently gained attention as a functional feed additive in animal nutrition due to its unique physicochemical characteristics and potential effects on rumen microbial ecosystems (Ayeneshet and Temesgen, 2025). Biochar is a carbon-rich material produced through pyrolysis of organic biomass under oxygen-limited conditions, and its properties are influenced by feedstock type, pyrolysis temperature, heating rate, and residence time (Lataf et al., 2022). Depending on production conditions, biochar contains stable carbon structures, minerals, ash components, and various oxygen-containing functional groups that contribute to its adsorption capacity and chemical activity (Amalina et al., 2022). The highly porous structure and large surface area of biochar provide suitable sites for microbial colonization, which may enhance microbial attachment, stimulate microbial activity, and improve ruminal fermentation processes (Kazemi et al., 2025). Previous studies reported that biochar supplementation can increase microbial activity, improve feed degradation, enhance nutrient utilization, and reduce energy losses associated with methane production (Leng, 2014; Han et al., 2018; Bagherpoor et al., 2023). Moreover, biochar may contribute to improved nitrogen utilization by retaining nitrogenous compounds and reducing ammonia losses (Nair et al., 2023). However, the effects of biochar on rumen fermentation characteristics depend on its physicochemical properties, including feedstock origin, surface area, porosity, and mineral composition (Tomczyk et al., 2020; Dong et al., 2025). Wood vinegar (WV), also known as pyroligneous acid, is another natural additive with potential applications in ruminant nutrition. It is a liquid by-product condensed during biomass pyrolysis and charcoal production under oxygen-limited conditions (Gama et al., 2024). Wood vinegar contains a complex mixture of organic acids, phenolic compounds, furans, ketones, alcohols, aldehydes, and other bioactive compounds, with its composition varying according to biomass source and production conditions (de Souza Araújo et al., 2018; Suresh et al., 2019). Organic acids present in wood vinegar, including acetic, formic, propionic, lactic, and butyric acids, may influence microbial fermentation by modifying the rumen environment and regulating microbial populations (Khan and Iqbal, 2016). Furthermore, phenolic compounds such as guaiacol and syringol possess antioxidant and antimicrobial properties that may contribute to maintaining microbial balance and improving nutrient utilization (Blavi Josa, et al., 2021). Previous research reported that wood vinegar supplementation can improve growth performance, nutrient digestibility, and microbial balance in livestock (Wang et al., 2012). Despite the potential benefits of biochar and wood vinegar, their effects on rumen function and productive responses in pre-weaned dairy calves remain unclear. Therefore, this study aimed to evaluate the effects of dietary biochar and wood vinegar supplementation on growth performance, rumen parameters, and gas production in milk-fed Holstein calves.

Materials and methods

Animals and experimental design

The experiment was conducted from March to May 2025 at Mahdasht Dairy Farm, Sari, Mazandaran Province, Iran. A total of 40 newborn male Holstein calves (initial body weight: 38.6±3.4 kg), born to multiparous cows, were enrolled in the study from 3 days of age until weaning (66 days). The calves were assigned to a completely randomized design with a 2×2 factorial arrangement. The experimental treatments were: 1) CON (basal starter diet), 2) BC (basal diet supplemented with 0.5% biochar), 3) WV (basal diet supplemented with 0.3% wood vinegar), and 4) BC+WV (basal diet supplemented with 0.5% biochar and 0.3% wood vinegar). Before the calves were housed, all pens were thoroughly cleaned, disinfected, and flame-sterilized. The starter diets were formulated to meet or exceed the nutrient requirements of Holstein calves according to the NRC (2001). Feed samples were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), ash, and organic matter (OM) according to AOAC (2005) procedures. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations were determined following the method of Van Soest et al. (1991). The ingredients and chemical composition of the experimental starter diets are presented in Table 1.

Agricultural biochar was produced from locally available rice husk collected from Mazandaran Province, Iran. The biomass was air-dried and subjected to slow pyrolysis under oxygen-limited conditions at approximately 550°C for 60 min. After cooling to room temperature, the biochar was ground and passed through a 2-mm sieve before being incorporated into the starter diet at the designated inclusion level. Prior to diet formulation, the physicochemical characteristics of the biochar were determined and are presented in Table 2. Moisture, ash, and total nitrogen contents were determined according to the standard procedures described by AOAC (2019; Methods 934.01, 942.05, and 984.13, respectively). Organic carbon content was analyzed using a CHNS elemental analyzer, and the C:N ratio was calculated based on the measured carbon and nitrogen concentrations. Biochar pH and electrical conductivity were determined in a 1:10 (w/v) biochar-to-deionized water suspension using a digital pH/EC meter. Bulk density, water-holding capacity, and particle size distribution were measured following standard procedures for solid carbonaceous materials and biochar characterization (ASTM International, 2018). Mineral elements (Ca, K, P, and Mg) were determined after acid digestion using an inductively coupled plasma optical emission spectrometer (ICP-OES; Optima 7300 DV, PerkinElmer Inc., Waltham, MA, USA).

Table 1. Ingredients and chemical compounds of the experimental diets[1]

Item	CON	BC	WV	BC+WV
Feed ingredient (% M)				
Alfalfa hay, 15% CP	5.57	5.57	5.57	5.57
Wheat bran	1.00	1.00	1.00	1.00
Barley grain	13.22	13.22	13.22	13.22
Corn grain, ground	28.36	27.86	28.06	27.56
Soybean meal 44%	31.57	31.57	31.57	31.57
Soybean whole, roast	9.22	9.22	9.22	9.22
Canola meal solvent	4.76	4.76	4.76	4.76
Sodium bicarbonate	1.53	1.53	1.53	1.53
Magnesium oxide	0.51	0.51	0.51	0.51
Mineral and vitamin permix[2]	2.00	2.00	2.00	2.00
Calcium carbonate	0.76	0.76	0.76	0.76
Di-calcium phosphate	0.50	0.50	0.50	0.50
Salt white	0.50	0.50	0.50	0.50
Mycotoxin binder	0.50	0.50	0.50	0.50
Biochar	0	0.50	0	0.50
Wood vinegar	0	0	0.30	0.30
Chemical composition (% DM otherwise stated)				
Dry matter (% as fed)	90.69	90.61	90.60	90.51
Organic matter	93.45	93.39	93.44	93.38
Crude protein	21.69	21.64	21.68	21.63
Ether extract	3.88	3.85	3.87	3.84
Ash	6.55	6.61	6.56	6.63
Neutral detergent fiber	17.15	17.19	17.23	17.28
Acid detergent fiber	10.09	10.12	10.16	10.18
Non-fiber carbohydrate[3]	47.90	47.71	47.85	47.65
Calcium	0.50	0.51	0.50	0.51
Phosphorus	0.47	0.47	0.47	0.47
Metabolizable energy (Mcal/kg)	2.62	2.61	2.62	2.60

Values are means of five independent replicates per diet component. [1]Treatments containing: CON (control), BC (0.5% biochar), WV (0.3% wood vinegar), and BC+WV (0.5% biochar + 0.3% wood vinegar). [2]Mineral supplements: Calcium 195,000 mg; Phosphorus 90,000 mg; Magnesium 90,000 mg ؛Sodium 55000 mg; Zinc 3000 mg Iron 300 mg; Manganese 2000 mg; Cobalt 100 mg; Selenium 1 mg; Antioxidant 400 mg. Vitamin supplement: Vitamin A, 500,000 IU/ kg; Vitamin E, 100 mg/ kg; Vitamin D3 100,000 IU/ kg. [3]NFC = 100 − (%NDF + %Ash + %Fat + %CP) (NRC, 2001).

The wood vinegar used in this study was obtained from the pyrolysis of citrus wood residues collected from orchards in Mazandaran Province, Iran, and was characterized based on its physicochemical properties and bioactive compounds. The pH was measured using a calibrated digital pH meter (SevenCompact S220, Mettler Toledo, Switzerland), and total acidity was determined by titration with standardized sodium hydroxide solution and expressed as acetic acid equivalents (AOAC, 2019). The pH and total acidity values of the wood vinegar were 3.5 and 5.3% (w/v), respectively. Total phenolic and flavonoid contents were determined using the Folin–Ciocalteu and aluminum chloride colorimetric methods, respectively, and expressed as mg gallic acid equivalents (GAE)/g and mg quercetin equivalents (QE)/g of sample. The total phenolic and flavonoid contents were 10.61 and 2.80 mg/g, respectively (Chang et al., 2002).

Table 2. Physicochemical characteristics of the agricultural biochar used in the experiment

Parameter	Unit	Value
Feedstock	-	Rice husk
Pyrolysis temperature	°C	550
Residence time	min	60
Moisture	%	5.4
Ash content	% DM	12.3
Organic carbon	% DM	78.5
Total nitrogen	% DM	0.72
C:N ratio	-	109
pH	-	9.4
Electrical conductivity	dS/m	1.2
Bulk density	kg/m³	230
Water holding capacity	%	265
Particle size	mm	<2
Calcium	%	1.8
Potassium	%	1.2
Phosphorus	%	0.54
Magnesium	%	0.31

Results

Growth performance

The effects of experimental treatments on growth performance in milk-fed Holstein calves are summarized in Table 3. No significant interaction between biochar and wood vinegar was detected for any of the measured variables (P>0.05). The main effect of biochar was not significant (P>0.05). The main effect of wood vinegar significantly increased dry matter intake (P<0.01), whereas the other growth performance traits were not affected (P>0.05).

Table 3. Effects of experimental treatments on growth performance in milk-fed Holstein calves

Item	Treatment				Main-effect means				SEM[4]	*P*-value		
	CON[1]	BC[2]	WV[3]	BC+WV	BC=0	BC=0.5	WV=0	WV=0.3		BC	WV	BC×WV[5]
FBW[6] (kg)	84.1	85.5	85.6	86.9	85.0	86.2	84.8	86.4	1.38	0.38	0.26	0.90
TWG[7] (kg)	46.1	46.8	47.0	47.8	46.5	47.3	46.5	47.4	0.77	0.32	0.24	0.92
ADG[8] (g/d)	614	624	626	637	620	630	619	631	10.35	0.32	0.24	0.92
DMI[9] (kg/d)	1.6	1.6	1.7	1.7	1.6	1.6	1.6^{b}	1.7^{a}	0.02	0.95	<0.01	0.75
FCR[10]	2.6	2.6	2.7	2.6	2.6	2.6	2.6	2.6	0.06	0.44	0.36	0.76

[a,b]: Within a row, means with different superscript letters differ significantly (P<0.05). Main-effect means are presented because the BC×WV interaction was not significant (P>0.05). [1]CON: Control; [2]BC: 0.5% biochar; [3]WV: 0.3% wood vinegar; [4]SEM: Standard error of the mean. [5]BC×WV: Interaction between biochar and wood vinegar; [6]FBW: Final body weight; [7]TWG: Total weight gain; [8]ADG: Average daily gain; [9]DMI: Dry matter intake; [10]FCR: Feed conversion ratio.

Skeletal traits

No significant biochar × wood vinegar interaction was detected for any of the measured parameters (P>0.05; Table 4). The main effect of biochar increased abdominal girth at 6 weeks of age (P=0.04). The main effect of wood vinegar significantly increased abdominal girth, withers height, and pin width at 3 weeks of age (P<0.05). At 6 weeks of age, the main effect of wood vinegar significantly increased body length, abdominal girth, withers height, hip-to-pin distance, and pin width (P<0.05). At 9 weeks of age, the main effect of wood vinegar significantly increased body length, chest girth, abdominal girth, hip-to-pin distance, and pin width (P<0.05).

Table 4. Effects of experimental treatments on skeletal traits of milk-fed Holstein calves

Item (cm)	Treatment				Main-effect means				SEM[4]	*P*-value		
	CON[1]	BC[2]	WV[3]	BC+WV	BC=0	BC=0.5	WV=0	WV=0.3		BC	WV	BC×WV[5]
Week 3												
BL[6]	89.8	92.6	93.3	94.9	91.6	93.7	91.7	94.1	1.60	0.18	0.07	0.70
CG[7]	87.0	87.1	89.4	90.1	88.2	88.6	87.0	89.8	1.45	0.79	0.07	0.84
AG[8]	91.1	91.6	94.3	97.0	92.7	94.3	91.3^{b}	95.7^{a}	1.61	0.34	0.01	0.49
WH[9]	84.9	83.8	86.2	88.7	85.6	86.3	84.3^{b}	87.5^{a}	1.40	0.61	<0.05	0.20
HPD[10]	23.1	23.7	24.2	24.3	23.6	24.0	23.4	24.3	0.58	0.57	0.13	0.70
PW[11]	8.0	8.1	8.4	9.1	8.2	8.6	8.0^{b}	8.8^{a}	0.33	0.25	<0.05	0.41
Week 6												
BL	100.9	101.4	106.0	112.3	103.4	106.9	101.2^{b}	109.2^{a}	2.48	0.17	<0.01	0.25
CG	97.9	97.1	98.2	102.7	98.0	99.9	97.5	100.4	2.07	0.38	0.16	0.21
AG	102.9	103.8	105.6	110.9	104.2^{b}	107.3^{a}	103.3^{b}	108.2^{a}	1.52	0.04	<0.01	0.15
WH	89.9	89.8	92.0	98.2	94.9	94.0	89.8^{b}	95.1^{a}	1.65	0.07	<0.01	0.06
HPD	24.2	24.7	25.4	25.7	24.8	25.2	24.4^{b}	25.6^{a}	0.41	0.42	0.01	0.79
PW	8.9	8.6	9.1	9.3	9.0	8.9	8.7^{b}	9.2^{a}	0.23	0.81	<0.05	0.24
Week 9												
BL	110.9	111.4	114.8	116.8	112.8	114.1	111.2^{b}	115.8^{a}	1.84	0.49	0.01	0.69
CG	106.9	107.3	112.9	117.6	109.9	112.4	107.1^{b}	115.2^{a}	1.47	0.09	<0.01	0.16
AG	99.4	99.9	103.3	108.7	101.4	104.3	99.7^{b}	106.0^{a}	1.77	0.11	<0.01	0.17
WH	95.1	96.3	96.8	97.7	95.9	97.0	95.7	97.2	1.23	0.40	0.23	0.89
HPD	26.7	26.8	27.6	28.8	27.1	27.8	26.7^{b}	28.2^{a}	0.48	0.17	<0.01	0.25
PW	10.7	10.9	11.4	11.8	11.0	11.3	10.8^{b}	11.6^{a}	0.40	0.49	<0.05	0.89

[a,b]: Within a row, means with different superscript letters differ significantly (P<0.05). Main-effect means are presented because the BC×WV interaction was not significant (P>0.05). [1]CON: Control; [2]BC: 0.5% biochar; [3]WV: 0.3% wood vinegar; [4]SEM: Standard error of the mean. [5]BC×WV: Interaction between biochar and wood vinegar; [6]BL: Body length; [7]CG: Chest girth; [8]AG: Abdominal girth; [9]WH: Withers height; [10]HPD: Hip-to-pin distance; [11]PW: Pin width.

Rumen parameters

Ruminal fermentation parameters are presented in Table 5. No significant interaction between biochar and wood vinegar was observed for any of the measured variables (P>0.05). The main effect of biochar significantly increased TVFA concentration (P=0.01). Similarly, the main effect of wood vinegar increased TVFA concentration (P=0.04) and the molar proportion of acetate (P<0.01), while decreasing the molar proportion of butyrate (P=0.02).

Gas production parameters

The effects of the experimental treatments on *in vitro* gas production parameters are presented in Table 6. No significant interaction between biochar and wood vinegar was observed for any of the measured variables (P>0.05). The main effect of wood vinegar significantly increased gas production potential, gas production at 24 h, organic matter digestibility, metabolizable energy, and short-chain fatty acid production (P<0.05).

Table 5. Effects of experimental treatments on ruminal parameters of milk-fed Holstein calves

Item	Treatment				Main-effect means				SEM[4]	*P*-value		
	CON[1]	BC[2]	WV[3]	BC+WV	BC=0	BC=0.5	WV=0	WV=0.3		BC	WV	BC×WV[5]
pH	6.9	6.3	6.5	6.5	6.5	6.4	6.5	6.5	0.11	0.30	0.84	0.37
NH_3-N (mM)	8.0	8.3	8.3	8.4	8.1	8.3	8.1	8.3	0.38	0.54	0.66	0.74
TVFA[6] (mM)	122.8	134.6	131.5	143.8	127.1^{b}	139.2^{a}	128.7^{b}	137.6^{a}	4.05	0.01	<0.05	0.95
<u>% of TVFA</u>												
Acetate	51.5	53.6	55.3	54.6	53.4	54.1	52.5^{b}	55.0^{a}	0.74	0.36	<0.01	0.09
Propionate	33.0	30.5	30.5	32.3	31.8	31.4	31.8	31.4	1.35	0.79	0.78	0.14
Butyrate	10.1	9.3	8.6	7.4	9.4	8.3	9.7^{a}	8.0^{b}	0.67	0.14	<0.05	0.82
Valerate	3.9	4.8	4.3	4.4	4.0	4.6	4.3	4.3	0.56	0.36	0.96	0.45
Isovalerate	1.5	1.8	1.3	1.3	1.4	1.6	1.6	1.3	0.22	0.47	0.16	0.43
A/P[7]	1.6	1.8	1.8	1.7	1.7	1.7	1.7	1.8	0.08	0.62	0.30	0.09

^{a,b}: Within a row, means with different superscript letters differ significantly (P<0.05). Main-effect means are presented because the BC×WV interaction was not significant (P>0.05). [1]CON: Control; [2]BC: 0.5% biochar; [3]WV: 0.3% wood vinegar; [4]SEM: Standard error of the mean. [5]BC×WV: Interaction between biochar and wood vinegar; [6]TVFA: Total volatile fatty acids; [7]A/P: Acetate/propionate ratio.

Table 6. Effects of experimental treatments on gas production during *in vitro* incubation

Item	Treatment				Main-effect means				SEM[4]	*P*-value		
	CON[1]	BC[2]	WV[3]	BC+WV	BC=0	BC=0.5	WV=0	WV=0.3		BC	WV	BC×WV[5]
GPP[6] (mL)	35.0	35.6	36.6	37.2	35.8	36.4	35.3^{b}	36.9^{a}	0.65	0.36	<0.05	0.99
GPR[7] (mL/h)	0.062	0.061	0.065	0.067	0.063	0.064	0.062	0.066	0.005	0.84	0.46	0.79
GP24[8] (mL)	25.5	26.6	27.8	28.5	26.7	27.6	26.1^{b}	28.2^{a}	0.48	0.09	<0.01	0.61
GP96[9] (mL)	35.4	36.0	38.1	37.7	36.8	36.9	35.7	37.9	1.03	0.90	0.05	0.63
OMD[10] (%)	45.0	46.4	47.2	48.2	46.2	47.3	45.8^{b}	47.7^{a}	0.60	0.08	<0.01	0.74
ME[11] (MJ/kg)	6.6	6.8	6.9	7.0	6.7	6.9	6.7^{b}	7.0^{a}	0.08	0.08	<0.01	0.73
SCFA[12] (mM)	0.56	0.58	0.61	0.62	0.59	0.60	0.57^{b}	0.62^{a}	0.01	0.09	<0.01	0.61

^{a,b}: Within a row, means with different superscript letters differ significantly (P<0.05). Main-effect means are presented because the BC×WV interaction was not significant (P>0.05). [1]CON: Control; [2]BC: 0.5% biochar; [3]WV: 0.3% wood vinegar; [4]SEM: Standard error of the mean. [5]BC×WV: Interaction between biochar and wood vinegar; [6]GP: Gas production potential; [7]GPR: Gas production rate; [8]GP24: Gas production at 24 hours; [9]GP96: Gas production at 96 hours; [10]OMD: Organic matter digestibility; [11]ME: Metabolizable energy; [12]SCFA: Short-chain fatty acids.

Discussion

Growth performance and skeletal traits

The lack of improvement in growth performance with biochar supplementation suggests that the biochar used did not induce changes in nutrient availability in pre-weaned calves. Although biochar may modify microbial habitats, nutrient retention, and fermentation efficiency, its effects vary with feedstock source, pyrolysis conditions, physicochemical properties, and inclusion level (Teoh et al., 2019; Terry et al., 2019). Growth responses are inconsistent, with some studies reporting improved nutrient utilization, whereas others found no effects on body weight gain or feed efficiency (Winders et al., 2019; Lind et al., 2024). This variability may reflect the limited degradability of biochar, whose effects involve indirect modulation of the gastrointestinal environment rather than supplying fermentable nutrients (Teoh et al., 2019; Ni et al., 2024). Its porous structure and high surface area may provide microbial attachment sites (Leng, 2014). However, such changes do not consistently improve productivity despite effects on microbial interactions or fermentation characteristics (Teoh et al., 2019; Tamayao et al., 2021; Ni et al., 2024). In contrast, the main effect of wood vinegar increased DMI and improved skeletal development, suggesting its potential to support structural growth during the pre-weaning period. The increase in feed intake may be related to its bioactive constituents, particularly organic acids and phenolic compounds, which can modulate microbial activity, fermentation, and gastrointestinal function (Suresh et al., 2019; Gama et al., 2024). Organic acids may alter ruminal fermentation pathways and volatile fatty acid production, whereas phenolic compounds may regulate microbial populations through antimicrobial and antioxidant mechanisms (O'Reilly et al., 2021; Gama et al., 2024; Melo et al., 2025). The skeletal response may reflect improved nutrient utilization and substrate availability for tissue development. These bioactive compounds can enhance nutrient digestibility, microbial balance, and digestive efficiency, thereby supporting growth (Wang et al., 2012; Zarghi, 2018). Enhanced microbial activity and microbial protein synthesis may also contribute to nutrient supply for tissue accretion during the pre-weaning period (Putri et al., 2021; Santos et al., 2021).

Rumen parameters

The increased TVFA concentration in response to biochar supplementation suggests enhanced microbial fermentation without major changes in the relative distribution of fermentation end products (Leng, 2014). Although biochar is not a fermentable substrate, its porous structure and high surface area may facilitate microbial attachment and interactions with feed particles, while its potential electron-mediator activity may support microbial metabolic efficiency (Teoh et al., 2019). The unchanged individual VFA proportions and acetate-to-propionate ratio indicate that biochar primarily affected fermentation extent rather than end-product distribution. Similar responses have been reported, with biochar effects varying according to its physicochemical properties and dietary conditions (Saleem et al., 2018; Ni et al., 2024). The lack of effects on ruminal pH and NH3-N suggests that the increased TVFA occurred without substantial changes in pH or nitrogen metabolism. Ruminal pH is regulated by VFA production and absorption and the buffering capacity of ruminal contents, whereas variability in NH3-N responses may reflect differences in biochar source, production conditions, and dietary protein characteristics (Cabeza et al., 2018; Qomariyah et al., 2021). Thus, biochar effects may depend on interactions between its properties and the rumen environment (Teoh et al., 2019; Terry et al., 2019). Similarly, the main effect of wood vinegar supplementation increased TVFA concentration, suggesting greater fermentation activity. This response may reflect the combined effects of its organic acid constituents, with acetic acid contributing to acetate availability and other acids potentially influencing fermentation pathways (O'Reilly et al., 2021). Phenolic compounds may also modulate microbial populations through their antimicrobial properties (Suresh et al., 2019; Gama et al., 2024). The increased acetate proportion may reflect shifts in fermentation pathways, whereas the reduced butyrate proportion may indicate selective modulation of butyrate-producing populations or pathways (Suresh et al., 2019; O'Reilly et al., 2021). Similar responses have been reported for wood vinegar, particularly increased acetate without consistent changes in other fermentation characteristics (O'Reilly et al., 2021). Overall, both additives increased TVFA, whereas wood vinegar additionally altered specific fermentation end products (Teoh et al., 2019; Gama et al., 2024).

Gas production parameters

The lack of significant effects of biochar supplementation on gas production parameters, OMD, ME, and SCFA production indicates that the applied biochar did not substantially modify ruminal fermentative capacity (Teoh et al., 2019). Although biochar may enhance fermentation through microbial attachment, its porous structure, and potential electron-transfer mechanisms, its effects depend largely on feedstock origin, pyrolysis conditions, surface characteristics, and inclusion level (Tomczyk et al., 2020; Ni et al., 2024). The limited response observed herein is consistent with previous findings showing variable or negligible effects of biochar on gas production, digestibility, VFA profile, and microbial activity, emphasizing the influence of biochar characteristics and dietary conditions (Teoh et al., 2019; Tamayao et al., 2021). Thus, the lack of response may reflect limited interactions between the physicochemical properties of the applied biochar and substrates available for microbial fermentation (Terry et al., 2019; Lind et al., 2024). In contrast, the main effect of wood vinegar supplementation improved several indicators of fermentative efficiency, suggesting enhanced utilization of available substrates. These responses may be associated with organic acids and bioactive compounds in wood vinegar, which can modulate microbial activity and substrate degradation (Suresh et al., 2019; O'Reilly et al., 2021; Gama et al., 2024). Acetic acid, a major organic acid in wood vinegar, may influence ruminal fermentation by modifying the local acid–base environment and acetate availability, whereas phenolic compounds such as guaiacol may modulate microbial populations and fermentation activity (Suresh et al., 2019; O'Reilly et al., 2021). The increase in SCFA production is consistent with evidence that bioactive compounds can enhance fermentation metabolite production (Emmanuel et al., 2025). Likewise, greater OMD and ME with wood vinegar may indicate more efficient utilization of dietary organic matter, potentially through improved microbial access to fermentable substrates and stimulation of fibrolytic activity (Putri et al., 2021; Santos et al., 2021). Although phenolic compounds can exert antimicrobial effects at high concentrations, their relatively low intake may have favored microbial modulation rather than inhibition of fermentation (Al Rharad et al., 2025). The unchanged gas production rate further suggests that wood vinegar affected the extent of substrate fermentation without substantially altering fermentation kinetics. The absence of a significant biochar × wood vinegar interaction indicates that the effects of each additive were independent of the other. Biochar may primarily influence the ruminal environment through physicochemical mechanisms, whereas wood vinegar may exert more direct effects through its organic acids and phenolic constituents (Teoh et al., 2019; Gama et al., 2024).

Conclusion

In conclusion, the main effect of wood vinegar supplementation improved DMI, several indicators of skeletal development, and ruminal fermentation, as evidenced by increases in TVFA concentration, acetate proportion, gas production potential, gas production at 24 h, OMD, ME, and SCFA production. Biochar had limited effects on growth and skeletal development but increased TVFA concentration, suggesting a modest enhancement in ruminal fermentation. No significant biochar × wood vinegar interactions were detected for any of the measured parameters, indicating that the effects of each additive were not significantly modified by the inclusion of the other. Overall, wood vinegar showed greater potential than biochar for supporting feed intake, structural development, and ruminal fermentation in pre-weaned Holstein calves, whereas the effects of biochar may depend more strongly on its physicochemical characteristics and application conditions.

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Ethics Statement

All calves were managed according to established farm protocols for feeding, housing, and animal care. All animal handling and sample collection procedures were conducted under veterinary supervision, with appropriate measures taken to safeguard animal welfare and minimize discomfort.

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Conflict of Interest

The authors declare that they have no financial or personal relationships with individuals or organizations that could inappropriately influence or bias the content of this manuscript.

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