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Date palm seed-derived biochar as an environmentally sustainable feed supplement in cattle: impacts on gas production, methane emissions, fermentation parameters and performance predictions

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Abstract

In the context of climate change, the expanding cultivation of date palm *(Phoenix dactylifera)* results in substantial waste generation, posing environmental and pollution challenges. Valorization of this waste, particularly *phoenix dactylifera* seeds, presents a promising and eco-conscious strategy. This *in vitro* study innovatively evaluated the impacts of adding *phoenix dactylifera* seed-derived biochar (PSB) at concentrations of 0%, 1%, 2%, and 4% on gas production, methane (CH_4) emissions, nutrient degradability, fermentation parameters, and predicted cattle performance. The results indicated that 4% PSB significantly increased gas production at 12 h of incubation ($p < 0.05$), while all PSB inclusion levels significantly increased it at 24 h ($p < 0.05$). At 36 and 48 h, the 1% PSB treatment demonstrated the highest gas production compared to the control diet ($p < 0.05$). The addition of 1%, 2%, or 4% PSB significantly reduced CH_4 emissions when expressed per unit of dry matter (DM) by 50.0, 53.8, and 56.6, truly degraded dry matter (TDDM) by 50.5, 56.86, and 58.03%, and as a percentage of total gas production ($p < 0.01$) by 54.9, 55.2, and 58.9% compared to control, respectively. *In vitro* dry matter degradability (IVDMD, $p = 0.31$) and *in vitro* crude fiber degradability (IVCFD, $p = 0.33$) were non-significantly affect by biochar inclusion. Ammonia-nitrogen (NH_3-N) concentrations were significantly higher in the 4% PSB group compared to the other groups ($p < 0.01$). The addition of PSB (1%, 2%, or 4%) significantly improved TVFA (measured; mmol/L) and SCFA (predicted from 24-h gas production; mmol/200 mg DM), as well as ME, OMD, and NEL compared to the control diet ($p < 0.01$). Overall, PSB effectively reduced CH_4 production and enhanced nutrient degradability. These findings

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underscore the value of using *phoenix dactylifera* seed-derived biochar as a sustainable and eco-friendly resource to reduce the carbon footprint of livestock production.

Keywords *Phoenix dactylifera* Seed-Derived biochar, Carbon footprint, Nutrients degradability, Predicted cattle performance

Introduction

Developing cost-effective strategies to convert agricultural waste into valuable products offers a key solution to environmental pollution caused by waste accumulation. This approach also significantly benefits many agricultural and industrial sectors, including livestock, and aligns with the UN's sustainable development goals [1, 2]. The date-palm seed biochar (PSB; derived from *Phoenix dactylifera* L. seeds) is one of the oldest cultivated fruit trees, native to the arid regions of the Arabian Peninsula, the Middle East, and North Africa. *Phoenix Dactylifera* trees abundant in the Arabian region, produce substantial amounts of residual biomass [1]. This biomass is often disposed of or burned on farms, contributing to pollution in date-producing nations. While some countries utilize this residual biomass for cattle feed production, more sustainable and widespread applications are needed [3, 4]. Rising global temperatures and concerns about water scarcity, driven by climate change, have increased the cultivation of this plant. This is primarily due to its remarkable adaptability to changing climate conditions and its high drought tolerance [3]. Furthermore, this tree provides a valuable food source such as fruits, which are rich in nutrients, among other benefits.

Global *phoenix dactylifera* cultivation, with over 120 million trees, yields millions of tons of dates and substantial secondary products (midribs, leaves, stems, fronds, coir). Over 84 million trees are located in Egypt, Saudi Arabia, Iraq, Algeria, Morocco, Iran, Tunisia, and the UAE [5]. Occupying ~ 3% of global cultivated land, date palms generate an estimated 12 million metric tons of waste biomass annually [6]. This substantial palm seed waste remains largely underutilized due to a lack of cost-effective processing [3, 4]. Valorizing *phoenix dactylifera* seed waste can increase economic value and reduce environmental impact, promoting sustainability and an eco-friendly approach to *phoenix dactylifera* cultivation [4].

Numerous experiments have investigated the effects of diets supplemented with *phoenix dactylifera* seeds on terrestrial [7, 8] and aquatic [9] animals. These diets may improve overall animal health and productivity, while also mitigating the environmental impact of *phoenix dactylifera* seed accumulation [5, 7]. However, the livestock sector promotes roughly 18% of global methane emissions, exacerbating climate change. Various strategies have been implemented to reduce these emissions while maintaining livestock productivity. One such approach involves the use of biochar incorporated into animal diets [10, 11]. Date-palm seed biochar (PSB), in particular, has shown promise as a sustainable adsorbent for environmental remediation [12, 13], offering a valuable application for agricultural waste and promoting a circular economy. The conversion of agricultural residues to biochar offers a promising pathway towards sustainable waste management and contributes to relevant development goals. Biochar's demonstrated stability in diverse environments [14], further supports its potential. Projections suggest that approximately 80% of global agricultural residues could be utilized for biochar production and/or energy generation by 2050 [15].

Biochar, a cost-effective soil amendment, finds widespread application in agriculture. Thermochemical conversion of agricultural residual biomass is the primary method for biochar production [16]. The physicochemical features of the resulting biochar, involving surface area, functional groups, and porosity, are contingent upon both the biomass feedstock and the specific pyrolysis parameters employed. *Phoenix dactylifera* seed-derived biochar has demonstrated efficacy in eliminating Cu^{2+} from aqueous liquids [17]. For example, supplementing animal feed with probiotic-inoculated biochar (50 g/kg DM) has been shown to enhance the apparent digestibility of dietary dry matter, improve microbial protein synthesis, and increase milk fat content without affecting milk yield [18]. Similarly, *phoenix dactylifera* seed-derived biochar has been reported to reduce gas emissions and promote growth in sheep [10]. The addition of biochar have significant effects on nutrient digestibility in sheep, affecting their growth and methane production [19]. Adding 4% and 6% of dry matter to in vitro ruminal samples from Holstein-Friesian steers significantly reduced methane production by 19.03% and 29.32%, respectively [20]. This also led to an increase in cumulative gas production by 10.1% and 12.7%, respectively, with no detrimental impacts on fermentation parameters [20]. However, other research indicates that the addition of biochar to dairy cow feed did not significantly impact milk yield, physiological parameters, or greenhouse gas emissions [11]. Given the inconsistent findings regarding the effects of biochar on greenhouse gas emissions and animal performance, this *in vitro* experiment was designed to investigate the influence of novel *phoenix dactylifera* seed-derived biochar (PSB) on gas production, methane emissions, nutrient degradability, fermentation parameters, and predicted cattle performance using *in vitro* technique.

Fig. 1 The steps of synthesized *phoenix dactylifera* seed-derived biochar

Table 1 Formulation and chemical constituents of the concentrate mixture, berseem hay, and basal diet dry-matter basis

Ingredients	Content (kg)
Yellow corn	287.50
Soybean meal	115.00
Wheat bran	95.00
Common salt	7.500
Limestone	12.50
Sodium bicarbonate	2.50
Mineral and vitamin mixture [a]	2.50
Berseem hay	500.00

Chemical composition (on DM basis)

Nutrient (%)	Concentrate mixture	Berseem hay	Basal diet [b]
Organic matter	96.02	92.00	94.01
Crude protein	17.44	12.50	14.97
Ether extract	3.27	2.30	2.79
Neutral detergent fiber	17.73	56.00	36.69
Acid detergent fiber	6.23	41.00	23.62
Ash	3.48	8.00	5.74
Crude fiber	5.28	30.00	17.64
Nitrogen free extract	70.03	47.20	58.62

[a]: Minerals and vitamins mixture contained: Copper 30000 mg, Iodine 800 mg, Selenium 300 mg, Iron 10000 mg, MgO 80000 mg, Zinc 100000 mg, Cobalt 400 mg, Vit. A 10000000 IU, Vit. D_3 2500000 IU, Vit. E 35000 IU, and $CaCO_3$to 3 Kg

[b]The basal diet was a total mixed ration containing 50% Berseem hay (*Trifolium alexandrinum*) and 50% concentrate mixture

Materials and methods

Biochar synthesis

Phoenix dactylifera seeds, collected from Linah farm, Monufia Governorate, Egypt were chopped and used as feedstock for biochar production. Pyrolysis was carried out in a muffle furnace (Nabertherm GmbH, Germany; L 9/11) at 550 °C for 2–3 h, under oxygen-depleted conditions to produce the biochar (Fig. 1). The resulting biochar was then ground using a cutting mill (Retsch SM100, Retsch GmbH, Haan, Germany) and sieved to 2.0 mm for uniformity [13]. The yield was stored in a dry environment until used in this experiment.

Diet and chemical analysis

The present study was carried out in the Laboratory of Animal Nutrition, Animal Production Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt during the period spring season (February to April 2025). The basal diet for *in vitro* incubations was composed of 50% berseem hay (*Trifolium alexandrinum*) , and 50% concentrate mixture. The biochar was added to the concentrate mixture during the diet formulation. This dried diet was used for chemical analysis and *in vitro* gas production studies, the chemical composition of the diet is provided in Table 1. Following the methods based on the reports of AOAC [21], the samples were analyzed for dry matter (DM), ash, organic matter (OM), crude protein (CP) and ether extract (EE). While the neutral detergent fiber (NDF) of samples was analyzed by using the method of [22].

In vitro incubations

According to the technique described in [23], ruminal liquid samples were collected from five slaughtered animals from a crossbreed Holstein and a local Egyptian breed at the local slaughterhouse in Zagazig, Sharkia Governorate, Egypt. These animals ranged in weight from 450 to 550 kg and were between 12 and 14 months old. Animals were fed on *ad libitum* a ration based on 50% forage (berseem hay) and 50% concentrate. Rumen fluids were quickly transferred to the research laboratory in a pre-warmed (39 °C) isolation flask and stored under anaerobic conditions until used. The rumen liquid was filtered using four layers of cheesecloth, then incubated in a water bath at 39 °C and soaked with CO_2 until inoculation.

The buffered incubation media (MB9) has NaCl (2.8 g/L), $CaCl_2$ (0.1 g/L), $MgSO_4.7H_2O$ (0.1 g/L), Na_2HPO_4 (6 g/L) and $KH_2PO_4.H_2O$ (2 g/L). The MB9 media pH was adjusted to 6.8, and to maintain anaerobic conditions the CO_2 was flushed for 30 min [24]. The MB9 media was mixed with the rumen fluid at a 2:1 ratio (v/v). The incubation glass tubes that contain 200 mg of the diet and probiotic strain at various levels were injected with thirty millimeters of mixed ruminal fluid, closed rapidly with a gas-release rubber stopper connected with a tri-way valve and a measured plastic syringe for measuring gas production. The gas production volume was assessed through incubation times 3, 6, 12, 24, 36, and 48 h, and a blank tube was used to adjust the total gas volume [25]. Each run has four blank bottles (without substrate) and six bottles for each treatment.

At the end of incubation and after recording the final gas volume the methane emission was estimated by using NaOH (10 M) according to Fievez et al. [26], and the methane intensity (CH_4 mL/TDDM, CH_4 mL/TDOM, CH_4 percentage from total gas) was calculated at 48 h of incubation.

Estimation of pH, ammonia-N, volatile fatty acids concentration, partitioning factor, and true nutrient degradation

At the end of in vitro incubation, a digital pH meter was used to measure the ruminal pH immediately. Following the 48-hour incubation period, the contents of three incubation tubes from each treatment group were utilized to assess in vitro dry matter degradability (IVDMD). Each tube received 30 mL of neutral detergent solution, after which the mixture was thoroughly homogenized, refluxed at 105 °C for 3 h, filtered using pre-weighed Gooch crucibles, and then dried at 105 °C for another 3 h [27]. The remaining residue was subsequently weighed to calculate IVDMD, following the method described by Blümmel et al. [27].

Thereafter, *in vitro* crude fiber degradability (IVCFD) was determined in accordance to AOAC [21] procedures. The contents of another three tubes of each treatment were used to determine the concentration of NH_3-N and total volatile fatty acids (TVFA). TVFA values were established using the steam distillation method, according to Warner [28]. TVFA values denote the measured concentration in the incubation medium determined by steam-distillation/titration and are expressed as mmol/L. The ruminal NH_3-N concentration was measured by the technique according to Conway [29]. The partitioning factor (PF) was estimated as the ratio of OM (mg) degradability to gas production volume (in mL after 24 h) [27].

Calculations

The equation of Menke and Steingass [30] was applied to compute The metabolizable energy (ME, MJ/kg DM) and net energy of lactation (NEL, MJ/kg DM)

$$\mathrm{NEL}\ \left(\frac{\mathrm{MJ}}{\mathrm{kg}}\mathrm{DM}\right) = (0.115\times\ \mathrm{GP}) +\ (0.0054\times\ \mathrm{CP}) +\ (0.014\times\ \mathrm{EE}) -\ (0.0054\times\ \mathrm{CA}) -\ 0.36$$

$$\mathrm{ME}\ \left(\frac{\mathrm{MJ}}{\mathrm{kg}}\mathrm{DM}\right) = (0.157x\ GP) + (0.0084x\ CP) + (0.022x\ EE) - (0.0081xCA) + 1.06$$

Where.

GP = net gas production (ml/0.2 g DM) at 24 h of incubation;

EE = ether extract; CP = crude protein; CA = crude ash.

Short-chain fatty acid concentrations (SCFA) were estimated corresponding to Getachew et l [31]. as:

$$\mathrm{SCFA}\ \left(\frac{\mathrm{mmol}}{200}\mathrm{mg\ DM}\right) = (0.022XGP) - 0.00425$$

Where GP is the 24-hour net gas production (mL/200 mg DM).

SCFA denotes the predicted short-chain fatty acids yield from 24-h gas production (Getachew et al.), expressed as mmol per 200 mg DM.

Microbial CP biomass construction was estimated, giving to Blümmel et al. [27] as follows:

$$\mathrm{MCP}\ \left(\frac{\mathrm{mg}}{\mathrm{g}}\mathrm{DM}\right) = \mathrm{DMD\ (mg)} - \left(\mathrm{gas,\ mLX\ 2.2.}\frac{\mathrm{mg}}{\mathrm{mL}}\right)$$

Where: 2.2 mg/mL is a stoichiometric factor that states mg of C, H, and O planned to produce SCFA gas associated with production of 1 mL of gas.

Table 2 Effect of *Phoenix dactylifera* seed-derived Biochar dosage (0, 1, 2 and 4%) on gas production (mL/g DM, 48-hour incubation)

Item	Treatment				SEM	*P*-value
	PSB0	PSB1	PSB2	PSB24		
3 h	18.33	16.25	22.92	25.00	1.76	0.280
6 h	48.33	38.75	42.08	44.17	1.69	0.250
12 h	68.75^{b}	70.42^{b}	73.33^{ab}	83.33^{a}	2.00	0.030
24 h	87.92^{b}	110.42^{a}	107.92^{a}	105.42^{a}	2.16	0.001
36 h	108.75^{b}	125.83^{a}	115.83^{ab}	118.33^{ab}	2.24	0.050
48 h	116.25^{b}	129.58^{a}	120.00^{ab}	122.92^{ab}	2.09	0.040

$^{a–b}$ Means in the same column bearing different letters differ significantly ($p < 0.05$); SEM indicates the standard error of the mean. Basal diet supplemented with 0 (PSB0), 1 (PSB1), 2 (PSB2) or 4% (PSB4) *phoenix dactylifera* seed-derived biochar

Table 3 Effect of *Phoenix dactylifera* seed-derived Biochar dosage (0, 1, 2 and 4%) on methane emission ($n = 6$ replicates/treatment; 28 replicates/run)

	Treatment				SEM	*P*-value
	PSB0	PSB1	PSB2	PSB24		
Methane emission						
mL/1 g DM	50.00 ^{a}	25.00 ^{b}	23.08 ^{b}	21.67 ^{b}	2.59	0.001
mL/1 g TDDM	76.5 ^{a}	37.8 ^{b}	33.0 ^{b}	32.1 ^{b}	4.08	0.001
% of total gas	43.1 ^{a}	19.4 ^{b}	19.3 ^{b}	17.7 ^{b}	2.34	0.001

$^{a–b}$ Means in the same row bearing different letters differ significantly ($p < 0.05$); SEM indicates the standard error of the mean; TDDM, total dry matter degradability. Basal diet supplemented with 0 (PSB0), 1 (PSB1), 2 (PSB2) or 4% (PSB4) *phoenix dactylifera* seed-derived biochar

Menke et al. [32] equation was used to calculate the in vitro organic matter digestibility.

(OMD %) as

$$\mathrm{OMD}\ (\%) = 14.88 + (0.889\mathrm{xGP}) + (0.045\mathrm{xCP}) + (0.061\mathrm{xXA})$$

Where XA = Ash (%).

Data analysis

The statistical analysis of the in vitro findings was achieved using the general linear model procedure (GLM) using SPSS 21 (Chicago, IL) software. Data normality was assessed using the Shapiro-Wilk and Kolmogorov-Smirnov tests. All results are presented as mean ± pooled standard error (SE).

The following is the statistical method that has been applied:

$$\mathrm{Yijk} = \mu + \mathrm{Tij} + \mathrm{eijk}$$

where Y_{ij}, observation; μ, observed mean; T_i, effect of treatments; e_{ij}, experimental random error. The significant differences in mean were analyzed by Duncan's multiple comparison test at $p < 0.05$.

Results

Effects of PSB on gas production

The addition of *phoenix dactylifera* seed biochar (PSB) at levels of 1, 2, and 4% did not significantly affect gas production during the first 6 h of incubation (Table 2). At 12 h, gas production significantly increased ($p < 0.05$) in the PSB groups compared to the control. The PSB2 group exhibited intermediate gas production at 12 h compared to the other groups. All PSB-supplemented groups showed significantly higher gas production at 24 h ($p < 0.01$). Likewise, at 36 and 48 h, where the PSB1 group significantly improved gas production ($p \leq 0.05$, $p = 0.04$, respectively) compared to PSB0 group. However, there were no significant differences in gas production between the 2% and 4% biochar palm seed treatments at 36 and 48 h ($p > 0.05$) and other groups. Overall, adding 1% *phoenix dactylifera* seed-derived biochar may enhance in vitro gas production, potentially benefiting cattle performance.

Effects of PSB on methane emission

The total methane emission was measured at the end of the treatment period (48 h, Table 3). The addition of 1, 2, or 4% PSB significantly reduced methane emissions when expressed as dry matter (DM), total digestible dry matter (TDDM), and percentage of total gas production ($p < 0.01$). The addition of 1%, 2%, or 4% PSB significantly reduced CH_4 emissions when expressed per unit of dry matter (DM) by 50.0, 53.8, and 56.6, TDDM by 50.5, 56.86, and 58.03%, and as a percentage of total gas production ($p < 0.01$) by 54.9, 55.2, and 58.9% compared to control, respectively.

Effects of PSB on nutrient degradability and fermentation parameters

Impacts of *phoenix dactylifera* seed-derived biochar supplementation on cattle diets had no significant impact on in vitro dry matter degradability (IVDMD, $p = 0.31$) and in vitro crude fiber degradability (IVCFD, $p = 0.33$), as shown in Table 4. Ammonia levels were lower in the

Table 4 Effect of *Phoenix dactylifera* seed-derived Biochar dosage (0, 1, 2 and 4%) on nutrient degradability and fermentation parameters

	Treatment				**SEM**	***P*-value**
	PSB0	**PSB1**	**PSB2**	**PSB24**		
Degradability						
IVDMD (%)	65.33	66.17	70.00	67.50	0.91	0.31
IVCFD (%)	38.83	42.36	45.07	43.65	1.20	0.33
Fermentation parameter						
Ammonia-N (mg/100 ml)	48.16 ^{a}	45.92 ^{a}	47.04 ^{a}	41.44 ^{b}	0.87	< 0.01
TVFA (mmol/L)	192.33 ^{b}	216.67 ^{a}	235.00 ^{a}	236.00 ^{a}	6.08	< 0.01
pH	5.62	5.67	5.65	5.74	0.02	0.35

$^{a-b}$ Means in the same row exhibiting separate letters differ significantly ($P < 0.05$); SEM shows the standard error of the mean; TVFA, total volatile fatty acids (measured; mmol/L); Ammonia-N (mg/100 mL); IVDMD, in vitro dry matter degradability (%); IVCFD, in vitro crude fiber degradability (%). Basal diet supplemented with 0 (PSB0), 1 (PSB1), 2 (PSB2) or 4% (PSB4) *phoenix dactylifera* seed-derived biochar

Table 5 Effect of *Phoenix dactylifera* seed-derived Biochar dosage (0, 1, 2 and 4%) on predictive performance

	Treatment				**SEM**	***P*-value**
	PSB0	**PSB1**	**PSB2**	**PSB24**		
Predictive value						
SCFA (mmol/200 mg DM)	0.39 ^{b}	0.49 ^{a}	0.47 ^{a}	0.46 ^{a}	0.01	0.001
ME (MJ/Kg DM)	3.96 ^{b}	4.67 ^{a}	4.59 ^{a}	4.51 ^{a}	0.07	0.001
NEL (MJ/Kg DM)	1.75 ^{b}	2.27 ^{a}	2.21 ^{a}	2.15 ^{a}	0.05	0.001
OMD (%)	37.62 ^{b}	41.62 ^{a}	41.18 ^{a}	40.73 ^{a}	0.38	0.001
MCP (mg/g DM)	600.90^{c}	607.77^{c}	648.67^{a}	622.57^{b}	8.93	0.025
PF (mg TDOM/mL gas)	1.56 ^{b}	1.69^{ab}	1.76 ^{a}	1.71 ^{a}	0.03	0.040

$^{a-b}$ Means in the same row exhibit diverse letters differ significantly ($p < 0.05$); *SEM* specifies the standard error of the mean, short-chain fatty acids *SCFA*, short-chain fatty acids predicted from 24-h gas production (mmol/200 mg DM), metabolizable energy *ME,* net energy lactation *NEL,* microbial crude protein production *MCP,* organic matter degradability *OMD*, partitioning factor *PF*. Basal diet supplemented with 0 PSB0), 1 (PSB1), 2 (PSB2) or 4% (PSB4) *phoenix dactylifera* seed-derived biochar

4% date palm seed biochar group compared to the other treated and free-PSB diets ($p < 0.01$). The 1% and 2% groups showed similar results for Ammonia-N levels compared to the control diet ($p > 0.05$). It is interesting to note that the addition of PSB at 1, 2 and 4% significantly improved the total volatile fatty acids (TVFA) by 12.65%, 22.18%, 22.7%, respectively compared to the control diet ($p < 0.01$). The dietary presence of PSB did not notably affect the pH values as a fermentation marker ($p > 0.05$), but there was a slight improvement.

Effects of PSB on predictive performance

The predictive values of certain performance measurements may provide indicators of how feed additives will affect animal productivity. The addition of date palm seed biochar at 1, 2, or 4% significantly improved SCFA concentrations, ME, NEL, and OMD compared to the untreated control ($p < 0.01$, Table 5). MCP production was highest in the 2% date palm seed biochar treatment, followed by the 4% treatment ($p < 0.01$). The 1% treatment did not significantly affect MCP production compared to the control ($p > 0.05$). Dietary supplementation with 2% or 4% date palm seed biochar significantly increased the partitioning factor (PF) at 72 h of incubation compared to the PSB0 and PSB1 groups. The 1% *phoenix dactylifera* seed-derived biochar treatment exhibited intermediate PE values ($p > 0.05$) compared to the other treatments.

Discussion

Ruminants contribute significantly to global warming through the production of substantial amounts of greenhouse gases. For decades, researchers have explored various mitigation strategies, including the use of phytochemicals, organic acids, and probiotics. Concurrently, *phoenix dactylifera* cultivation generates large quantities of byproducts, such as *phoenix dactylifera* seeds, which have been used in animal feed without adverse effects on performance or productivity. These substantial quantities of byproducts represent a significant environmental and economic challenge. Therefore, valorizing these byproducts to help mitigate greenhouse gas emissions in animals, without negatively impacting performance, is a crucial step towards sustainable and environmentally sound byproduct utilization. This study found that adding *phoenix dactylifera* seed-derived biochar (PSB) improved gas production, reduced methane emissions, enhanced nutrient degradability, improved fermentation parameters, and enhanced predictive performance (SCFA, NEL, OMD, MCP, and OMD), and potentially benefiting cattle performance.

Gas production in the rumen is affected by the dietary composition and type; and bacterial community, and microbial protein synthesis of the volatile fatty acids (VFA) produced during fermentation. The present study found that adding 1% PSB may enhance the gas production in cattle. Our results indicate that adding PSB significantly reduced methane emissions in in vitro trials by 50%, 53.84%, and 56.66% with the addition of 1%, 2%, and 3% PSB, respectively. Biochar in enteric fermentation promotes the reaction of hydrogen ions (H^+) with nitrogen to produce ammonium (NH^4) instead of methane. Normally, these hydrogen ions would react with carbon dioxide to form methane [33]. The presence of biochar in sheep diets led to a substantial reduction in methane CH_4 gas emissions decreasing them by 65.58%–78.39% [10]. This finding is consistent with another study, which reported that adding biochar to dairy farm manure significantly reduced methane emissions by 58% [34]. Furthermore, our data aligns with previous research showing that sheep fed an inoculated biochar group had a significant 9% reduction in methane production (kg of DM) compared to the control group [18]. These results support existing studies that indicate biochar supplementation in animal diets can significantly lower methane emissions by 8.8–12.9% without negatively affecting rumen fermentation or dry matter intake (DMI) [35]. There is conflicting evidence regarding the effect of biochar on methane emissions in cattle. The addition of 0.6% biochar to cattle diets has been shown to result in an approximate 40% reduction in CH4 emissions [36]. Moreover, a separate study found that biochar had no effects on milk yield or methane emissions in cows [37]. Other study found that a 0.6% biochar diet enhanced the intake of Laos yellow cattle and exhibited up to a 24% decrease in CH_4 levels, and a 40% decrease when the biochar was combined with potassium Nitrate at 6% [38]. Additionally [39], found no difference in methane production regardless of the diet. Likewise [40], did not discover any reduction in CH_4 yield in steers on finishing regimes, but reported a 10% CH_4 decrease in steers fed growing diets when 0.8% biochar was augmented. Conversely, Leng et al. [38] stated that up to 24% decrease in CH_4 levels were exhibited in biochar (0.6%) was enhanced to the intake of Laos yellow cattle and 40% decrease when biochar was merged with potassium Nitrate at 6%. Research on palm seed-derived biochar is limited, with only one study showing positive effects on growth, nutrient degradability, and overall sheep health [10]. These conflicting findings warrant further investigation through larger in vivo experiments to better understand the variables influencing biochar's efficacy.

Volatile fatty acids (VFAs), produced by rumen fermentation, significantly impact ruminant production and product composition. This is largely due to their role as the primary energy source for ruminants, providing approximately 75% of their metabolizable energy. Dietary biochar had varying effects on rumen fermentation parameters. Dry matter degradability, CFD, and pH were not significantly affected. While ammonia levels only improved with the 4% biochar inclusion, all biochar-supplemented diets resulted in higher TVFA concentrations compared to the control. TVFA is essential for production and many physiological processes in the body. Recently, Biochar supplementation in ruminant diets did not significantly affect rumen fermentation, performance, or methane emissions in dairy cows [11].

Variations in biochar properties (including parent material, dose, and composition), the basal diet, and the animals studied (class and physiological stage) likely explain the inconsistent and often contradictory results seen in in vivo studies examining biochar's effect on ruminant methane production. Adding 1, 2, or 4% biochar significantly increased SCFAs concentrations, MP, ME, NEL, and OMD degradability. These results suggest that *phoenix dactylifera* seed-derived biochar, particularly at 2% and 4% inclusion rates, can positively impact animal productivity. In our in vitro experiment, *phoenix dactylifera* biochar significantly increased SCFA concentrations, suggesting improved productive traits. Short-chain fatty acids (SCFAs) are crucial energy sources for supporting optimal growth, production, and reproductive performance in animals [41]. In our study, the addition of biochar had no significant effect on nutrient digestibility using an in vitro technique. These results are inconsistent with findings from another study [19], which reported that the addition of biochar did have significant effects on nutrient digestibility in sheep, affecting their growth and methane production. Adding 4% and 6% of dry matter to in vitro ruminal samples from Holstein-Friesian steers significantly reduced methane production by 19.03% and 29.32%, respectively [20]. This also led to an increase in cumulative gas production by 10.1% and 12.7%, respectively, with no detrimental impacts on fermentation parameters [20].

The connection between diet and SCFAs helps keep a healthy balance of microbes in the gut. It does this by supporting a variety of microbes and a healthy gut lining in normal animals, and by boosting the gut's defenses when the gut is more acidic [42]. *Phoenix dactylifera* biochar supplementation in sheep diets significantly improved growth rate, likely due to enhanced nutrient degradability and improved fermentation characteristics [10]. The observed improvement could be due to the biochar's influence on feed passage within the gastrointestinal tract, potentially enhancing digestibility and reducing pathogenic bacteria populations. Another explanation, this action could be attributed to biochar's ability to increase the abundance of the *Ruminococcaceae* and

Lachnospiraceae families in the gut microbiome of Holstein steers [43]. These two bacteria families are known to play a key role in the digestion of fiber and other complex carbohydrates. A study by [18] reported that dietary biochar inclusion improved microbial protein supply (MCP) in sheep. Incorporation of biochar can improve the MCP in sheep based on in vitro studies [44] and in vivo [10, 18, 45]. We suggest that the improved MCP with biochar supplementation reflects its ability to promote the abundance of fiber-digesting bacteria in the rumen and increase the availability of fermentable nutrients essential for microbial protein synthesis. Microbial protein is essential for ruminant productivity, providing 60–85% of absorbed amino acids. Thus, optimizing its production is key to meeting animal nutritional needs and the human demand for high-quality animal protein. Biochar supplementation did not affect daily dry matter intake, milk yield, or FCR in sheep [18]. This study offers insight into the potential of novel *phoenix dactylifera* seed-derived biochar for mitigating methane emissions from livestock. Using artificial rumen model, the addition of 0.3 g of DM biochar to diet significantly decreased CO_2 production and methane production [46]. A new systematic review found that adding biochar to dairy cow diets improved live body weight gain from 94.3 kg to 96.6 kg [47]. Additionally, adding walnut shell biochar to sheep diets enhanced the total tract digestibility of organic matter, dry matter, crude protein, and neutral detergent fiber [45]. The study also found that lambs fed either walnut shell or pistachio by-product biochar had greater ruminal ammonia-N (NH3-N) levels than the control group [45]. However, a study by [37] was stated that adding with a 4% maximum presence level of biochar on dairy cows' feed significantly augmented methane production from 322 to 348 g/day. It suggests improvements in nutrient degradability, fermentation parameters, and productive performance. However, the study has limitations. First, it examined only one species (cattle). Including additional species would provide more comprehensive data on the precise mechanisms of biochar's influence on methane production. Second, future in vivo studies are needed to optimize biochar dosage for methane mitigation and ultimately contribute to reducing livestock-related global warming.

Conclusion

According to in vitro results, adding date palm seed-derived biochar (PSB, 1%) improved gas production, reduced methane emissions, improved fermentation parameters (ammonia-N and TVFA concentration), and enhanced predictive performance values (SCFA, ME, NEL, OMD and MOP). These findings suggest potential improvements in the sustainability and environmental impact of date palm production and livestock feeding. While the results show promise for reducing methane emissions using *in vitro* evaluation techniques, further in vivo studies are needed to confirm these findings in various ruminant species, with different PSB inclusion rates, and over the long term. Additionally, incorporating advanced technologies such as nutrigenomics and proteomics could provide new insights for future perspectives.

Abbreviations

PSB	Date palm seed -derived biochar
DM	Dry matter
TDDM	Total digestible dry matter
SCFA	Short-chain fatty acid
ME	Metabolizable energy
NEL	Net energy for lactation
OMD	Organic matter degradability
OM	Organic matter
CP	Crude protein
EE	Ether extract
NDF	Neutral detergent fiber
PF	Partitioning factor

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Authors' contributions

Sameh Abdelnour, El-Sayed Hemdan Eissa and Mohamed Abdelkreem; designed the experiment, analysis the data, writing and reviewing the manuscript. Mohamed Medhat performed experiments, management of animals, and laboratory analysis. Hesham S. Ghazzawy, Mohamed Shawky El Syed, Abdullah Sheikh and Nashi K. Alqahtani supervised the experiment, writing the first draft. Hassan M. Ali-Dinar, Ehab El-Haroun and Roshmon Thomas Mathew; analyzed the data, writing and reviewing the manuscript. All authors read and approved the final version of the manuscript.

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Data availability

The data supporting the findings of this study will be made available upon reasonable request to the corresponding author.

Declarations

Ethics approval and consent to participate

All experimental procedures and animal handling were reviewed and approved under approval NO. ZU-IACUC/2/F/25/2023 by Animal Use in Research Committee (IACUC), Zagazig University, Egypt. In this experiment, all efforts were made to decrease the animal suffering in compliance with the ARRIVE guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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