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Determination of the Effects of Different Levels of *Prosopis farcta* Fruit Supplementation to Low-quality Roughages on Organic Matter Digestibility and Methane Formation using the *in vitro* Digestion Method

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ABSTRACT

Background: In arid regions and areas facing forage scarcity, *Prosopis species* have long been used in ruminant nutrition, yet their full potential has not been adequately assessed. Domesticated ruminants contribute approximately 15% of global methane emissions. Previously, antibiotics were routinely added to ruminant diets to reduce methane-associated energy losses and improve feed efficiency; however, sustainable alternative strategies are now needed.

Methods: In this study, widely used but nutritionally limited wheat straw and maize silage were supplemented with varying concentrations (2%, 4%, 6%, 8% and 10%) of *Prosopis farcta* fruit. Using the *in vitro* gas production technique and cattle rumen fluid, parameters, including total gas production, methane formation, ammonia nitrogen concentration, *in vitro* organic matter digestibility and metabolizable energy, were evaluated. Each treatment was conducted in quadruplicate under standard laboratory conditions.

Result: The lowest methane production was observed in the wheat straw treatment group at the control and 8% supplementation levels and in the maize silage at the control, 8% and 100% supplementation groups. The highest methane production occurred in the wheat straw treatment at 2%, 4%, 6%, 10% and 100% supplementation levels and in the maize silage at 6% and 10% supplementation. With respect to wheat straw, 2% and 6% of the straws significantly increased the amount of metabolizable energy, although methane production also increased considerably. Conversely, 8% supplementation did not increase the amount of metabolizable energy but did maintain methane production close to control levels (14.56% vs. 13.87%). In maize silage, 10% of the inclusion maximized the amount of metabolizable energy but caused excessive methane emissions. Inclusion levels of 4-6% in maize silage offer productivity benefits but pose environmental risks, whereas 8% supplementation provides a more sustainable balance.

Key words: Feed additives, *In vitro* digestibility, Metabolic energy, Methane emission, *Prosopis farcta.*

INTRODUCTION

Prosopis farcta (*P. farcta*) is a perennial shrub of the Fabaceae family that typically grows 0.3 to 1 meter in height and produces one to two dark brown pods per cluster (Pasiecznik *et al*., 2004). The genus *Prosopis* includes 44 species, such as *P. juliflora*, *P. velutina*, *P. glandulosa*, *P. laevigata*, *P. pallida* and *P. cineraria* (García-Andrade *et al*., 2013). The nutritional composition of *Prosopis* pods has been well documented, with crude protein ranging from 7-22%, fiber from 11-35%, fat from 1-6%, ash from 3-6% and carbohydrates from 30-75% (Choge *et al*., 2007). Notably, *P. juliflora* pods are richer in protein than leaves are and contain most of the essential amino acids (Eldaw, 2016). Furthermore, *Prosopis* seeds contain amino acids such as alanine, arginine, glutamic acid, lysine, methionine and trace amounts of tryptophan, along with fatty acids such as palmitic, oleic and linoleic acid (Robertson *et al*., 2011).

The utilization of *Prosopis species* in ruminant diets is attributed to their high carbohydrate and protein contents (Khobondo *et al*., 2019; Singh *et al*., 2020). The high energy, mineral and protein contents of these plants make them a preferred feed component for goats, sheep, camels and cattle (Mohamed *et al*., 2014; Pasiecznik *et al*., 2001). However, the shortage of quality forage remains a critical issue in livestock production (Paul *et al*., 2020). Although maize silage has been widely adopted, low-quality forages such as wheat straw continue to dominate ruminant diets. Straw-based rations, however, are associated with lower dietary nitrogen and higher enteric methane emissions (Blümmel *et al*., 2005), which may be mitigated through the inclusion of natural plant additives (Yurtseven *et al*., 2009). Ruminants are significant contributors to global

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methane emissions, with the rumen accounting for roughly one quarter of the total (Thorpe, 2009). Over the past 250 years, methane emissions have increased by approximately 149%, exacerbating the effects of climate change. Simultaneously, arid and semiarid lands now represent approximately 41% of the Earth's surface area and are sustained by more than one-third of the global population (Gutiérrez *et al*., 2018). Reducing methane emissions from livestock by 50% has thus become a priority for mitigating climate change (Ocko *et al*., 2021).

Prosopis species are rich in bioactive compounds such as saponins, alkaloids, tannins and oxalates, which may modulate rumen fermentation and reduce methane production (Shilwant *et al*., 2023). In particular, *P. farcta* contains high levels of palmitic acid methyl ester (~32.61%), a compound with known pharmaceutical value (Al-Waheeb, 2021). The species also exhibits anti-inflammatory, antimicrobial and antidiabetic properties (Sharifi-Rad *et al*., 2019; Meghwar and Dhanker, 2022) and is recognized for its high flavonoid content (Omidi *et al*., 2013). Flavonoids such as apigenin, quercetin and daidzein (Amarowicz and Pegg, 2008) and specifically luteolin, myricetin and quercetin in *Prosopis* (Young *et al*., 2017), contribute to its antioxidant activity (Jahromi *et al*., 2018). C-glycosyl flavonoids such as schaftoside and vitexin have also been associated with biological activity (Sharifi-Rad *et al*., 2019).

Furthermore, alkaloids from *Prosopis* seeds display antibacterial activity (Rahman *et al*., 2011) and a high tannin content in wood (up to 9%) enhances antimicrobial efficacy (Prabha *et al*., 2014). In the context of rising antibiotic resistance, these natural antimicrobial agents are of growing interest (Henciya *et al*., 2017). Tannins, particularly condensed tannins, can influence rumen microbial populations and reduce protein degradation by forming stable protein complexes (Ali *et al*., 2012). Hydrolysable tannins, while more absorbable and potentially toxic, also offer protective effects *in vitro* (Getachew *et al*., 2008). Essential oils and saponins have been shown to reduce ammonia levels and support ruminant performance by inhibiting proteolytic microbes (Yanza *et al*., 2024).

This study aimed to assess the feed value of *P. farcta*, a largely underutilized species and evaluate the effects of its bioactive compounds on methane production in ruminants. Given the ongoing decline in feed and water resources due to climate change, alternative feedstuffs such as *P. farcta* have become increasingly important. In this context, *P. farcta* fruit was added to wheat straw and maize silage at 2%, 4%, 6%, 8% and 10% inclusion levels, with a 100% *P. farcta* group included to evaluate its viability as a sole feed component and observe the effects at higher inclusion levels. This study employed an *in vitro* gas production technique to assess total gas, methane, ammonia nitrogen, *in vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME) levels.

MATERIALS AND METHODS

The study was conducted in 2014 at the *in vitro* digestion unit and laboratories of the Faculty of Veterinary Medicine, Harran University. Wheat straw and maize silage were obtained from the local market. *P. farcta* fruits were collected from wild plants naturally growing in the arid, hot climate of Şanlıurfa. Once fully ripe, the fruits were harvested, shade-dried and ground whole (including seeds) using a 1 mm mesh sieve (Simsek Laborteknik Ltd. Sti). Comprehensive chemical analyses were performed to determine the nutritional composition of *P. farcta* fruit, wheat straw and maize silage following official AOAC methods (AOAC 2005). Dry matter (DM) was measured by drying the samples at 105°C in a laboratory oven (Nuve FN 500) to a constant weight. The crude ash content was determined by incineration at 600°C in a muffle furnace (Elektro-Mag N1) and the organic matter (OM) content was calculated by subtracting the ash content from the DM. The crude protein (CP) content was determined by the Kjeldahl method, involving digestion, distillation and titration steps (Simsek Laborteknik Ltd. Sti). Fiber fractions, including neutral detergent fiber (NDF) and acid detergent fiber (ADF), were analyzed using the detergent system developed by Van Soest *et al*. (1991), employing Gooch crucibles with a porosity grade of 1. These analyses provided insights into cell wall composition and digestibility potential. As rumen fluid was collected from slaughtered animals at the abattoir, ethical approval was not needed. The chemical compositions of the feed samples are shown in Table 1.

A total of 400 g of ground *P. farcta* fruit was extracted with 800 mL of 85% ethanol, homogenized at 10.000 rpm for 30 seconds (Isolab heavy duty homogenizer) and incubated in a 25°C shaking water bath for 24 hours. The extract was then centrifuged at 5,000 rpm for 15 minutes (M4815 PR), filtered (Whatman No. 1) and concentrated using a rotary evaporator at 40°C for 30 minutes (RE-2010). This process was repeated three times for maximum extraction. The final extract was stored at 4°C for further analysis (Sharifi-Rad *et al*., 2021).

Determination of total phenolic content (TPC)

The TPC was measured using the Folin-Ciocalteu method. The diluted extracts were mixed with 150 µL of Folin-Ciocalteu reagent and 450 µL of sodium carbonate, vortexed and kept in the dark for 30 minutes. The absorbance was read at 765 nm (Perkin Elmer Lambda 45 UV Vis) and the results are expressed as mg gallic acid equivalents (GAE)/g DM (Meyers *et al*., 2003).

Determination of total flavonoid content (TFC)

The TFC was determined by the aluminum chloride method (Chang *et al*., 2002). The reaction mixture containing the extract, methanol, aluminum chloride, potassium acetate and water was incubated for 40 minutes, after which the absorbance was read at 415 nm. The results are presented as mg quercetin equivalents (QE)/g DM.

Antioxidant activity

DPPH radical scavenging activity was assessed by mixing 0.1 mL of extract with 2.9 mL of 0.1 µM DPPH solution and incubating for 30 minutes in the dark. The absorbance was measured at 517 nm (Kulisic *et al*., 2004). The extract had 35.98 mg GAE/g TPC, 27.89 mg QE/g TFC and 33.78% DPPH inhibition.

Preparation of feed mixtures and formation of groups

The experimental groups were created by supplementing wheat straw and maize silage with *P. farcta* fruit at 0% (control), 2%, 4%, 6%, 8% and 10% inclusion levels (*e.g*., 10% group: 90 g of wheat straw + 10 g of *P. farcta* powder). A 100% *P. farcta* group (*P. farcta* Control) was added to evaluate its potential as a sole feed or to clarify outcomes in the case of unclear results from the mixed groups. In total, seven treatment groups were established. Approximately 0.2 g of each sample was placed in an *in vitro* gas production syringe in quadruplicate, after which the exact weights were recorded. *In vitro* gas production was conducted according to Menke *et al*. (1988).

Processing of rumen fluid and *in vitro* digestion

Rumen fluid was freshly collected from healthy cattle after slaughter at a local abattoir. Carbon dioxide was bubbled through the fluid, which was kept at 39°C in thermos flasks and transported to the laboratory. The solution was filtered through four layers of cheesecloth under a flow of carbon dioxide. Then, 500 mL of rumen fluid was mixed with 1000 mL of laboratory-prepared artificial saliva and carbon dioxide was continuously bubbled through the mixture for approximately 15 minutes. All procedures were conducted at 38°C.

***In vitro* incubation and gas measurement**

Approximately 0.2 g of each ground feed sample (1 mm sieve) was placed into glass syringes (200-220 mg capacity) and preincubated at 39°C. Then, 30 mL of the rumen fluid–artificial saliva mixture was added to each syringe. After removing air bubbles and recording the initial gas volume, the syringes were incubated in a custom water bath at 39°C for 24 hours. Gas volumes were recorded at the end of the incubation. All the treatments were tested in quadruplicate (n= 4). The results were subsequently used to calculate the ME and IVOMD. Methane and carbon dioxide levels in the fermentation gas were measured in real time with a methane analyzer (Sensors Analysentechnik GmbH and Co. KG, Berlin, Germany) connected to a networked computer. After incubation, the syringe contents were filtered through four layers of cheese cloth and the pH was measured (WTW 7310). The filtered fluid was frozen at -20°C for ammonia nitrogen analysis, which was performed using the Markham distillation method (Broderick and Kang, 1980).

Calculation of IVOMD and ME

Gas production data were used to calculate IVOMD and ME using the following equations:

$$IVOMD\ (\%) = 14.88 + 0.889GP + 0.45CP + 0.0651CA$$

$$ME\ (MJ/kg\ DM) = 2.20 + 0.136GP + 0.057CP$$

Where;
GP = Gas production (mL) at 24 h.
CP = Crude protein (% DM).
CA = Crude ash (% DM).

The effects of *P. farcta* fruit supplementation on *in vitro* gas production, IVOMD, ME, carbon dioxide, methane, pH and ammonia nitrogen are summarized in Tables 2 and 3. The data were analyzed using one-way ANOVA in SPSS (SPSS 2010). Where significant effects were found, group means were compared using Duncan's multiple range test (Duncan 1955). Differences were considered at $P<0.05$. This statistical approach ensured robust evaluation of dietary treatment effects.

RESULTS AND DISCUSSION

The *P. farcta* fruit used in this study had a TPC of 35.98 mg GAE/g, a TFC of 27.89 mg QE/g and an antioxidant activity of 33.78%. These values were lower than those reported by Salari *et al*. (2019) (TPC: 366.21 mg GAE/g, TFC: 283.33 mg QE/g) but higher than the values found in *P. farcta* leaves by Sharifi-Rad *et al*. (2021) (TPC: 16.47 mg GAE/g, TFC: 0.21 mg QE/g). Similarly, Jahromi *et al*. (2018) reported phenolic and flavonoid contents of 61.55 mg GAE/g and 17 mg QE/g, respectively, in ethanolic fruit extracts of *P. farcta* fruit. Flavonoids are common polyphenolic secondary metabolites in plants (Panche *et al*., 2016) and phenolic compounds represent one of the most widespread metabolite groups (Prabha *et al*., 2014). Cardozo *et al*. (2010) also detected substantial levels of antinutritional factors, including saponins (317 mg/100 g), total phenols (640 mg/100 g), tannins (860 mg/100 g) and phytic acid (181 mg/100 g), in *Prosopis* pods.

The chemical compositions of the forage materials and *P. farcta* fruit are presented in Table 1. Since wheat straw is harvested after completing its vegetative phase, it has the highest levels of acid detergent fiber, neutral

Table 1: Crude nutrient content (%DM) of the forages used and *P. farcta* fruit.

	% DM	% OM	% CP	% ADF	% NDF	% Ash
Wheat straw	95.17	85.20	4.65	50.83	80.12	9.97
Corn silage	93.35	88.85	6.74	9.61	54.47	4.85
PFF	95.96	91.97	9.87	33.93	41.11	3.99

DM: Dry matter, OM: Organic matter, CP: Crude protein, ADF: Acid detergent fiber, NDF: Neutral detergent fiber, PFF: *P. farcta* fruit.

detergent fiber and ash. In contrast, *P. farcta* fruit had the highest OM (92.13%) and CP (9.87%) contents.

In this study, the effects of adding different amounts of *P. farcta* fruit to wheat straw on the total gas, methane, carbon dioxide, ammonia nitrogen, pH, IVOMD and ME are presented in Table 2.

Adding *P. farcta* fruit to wheat straw had no significant effect on pH (P>0.05), but it significantly influenced total gas and carbon dioxide production (P<0.01), as well as methane, ammonia nitrogen, IVOMD and ME values (P< 0.001). Similarly, both the control and 8% inclusion groups had low levels of gas, methane and carbon dioxide, while 2% and 6% of the plants produced the most gas-suggesting that low doses may help overcome the nutritional limitations of wheat straw. The 8% reduction in gas and methane is likely due to the inhibition of microbial fermentation by phenolic compounds and tannins. Although *P. farcta* supplementation generally improved the ME and IVOMD in wheat straw and maize silage, it also led to higher methane emissions at most inclusion levels. With respect to wheat straw, 2% and 6% supplementation significantly increased the ME (7.79 and 7.52 MJ/kg DM, respectively, vs. 6.58 in the control) but nearly doubled the methane output. At the 8% level, the methane production was close to that of the control (14.56% vs. 13.87%), although the ME was relatively low (6.04 MJ/kg DM). These results highlight a trade-off: 4-6% of the population may maximize energy, while 8% of the population offers a more sustainable option by limiting methane emissions. Saponins are natural compounds known to regulate rumen fermentation and feed digestibility in ruminants (Baah *et al*., 2007). These bacteria can reduce methane production by suppressing methanogenic microorganisms (Zúñiga-Serrano *et al*., 2022) and exerting antimicrobial effects on bacteria, protozoa and methanogens (Cieslak *et al*., 2013). In the rumen, these bacteria also cause defaunation by disrupting protozoal membranes (Patra and Saxena, 2009). Extracts from *P. farcta* seeds and pods, particularly methanolic and ethanolic fruit pod extracts, have shown high phenolic content and significant antibacterial activity (Poudineh *et al*., 2015). Jahromi *et al*. (2018) identified 27 different compounds in *P. farcta* fruit oil, which together accounted for 97.3% of the total oil content. They also reported that the oil exhibited strong antimicrobial activity, with a minimum inhibitory concentration of 16/ µg/ml. The highest ammonia nitrogen concentrations were detected in the 6% and 10% *P. farcta* fruit groups. In contrast, the lowest level was recorded in the 100% group, which consisted solely of *P. farcta* fruit powder. This reduction is likely due to the inhibitory effects of tannins, saponins and essential oils on protein degradation.

The control group had the fourth lowest ammonia nitrogen level, which can be attributed to the low protein content of the wheat straw. The 2% and 4% *P. farcta* fruit groups displayed the second lowest levels, indicating that the ammonia-reducing effects of the compounds in *P. farcta* fruit begin to occur at these supplementation levels.

Table 2: Effect of adding different concentrations of *P. farcta* fruit to wheat straw on total gas (ml/g DM), methane (%), ammonia nitrogen (mg/dl), pH, IVOMD *in vitro* (%DM), ME (MJ/kg DM) and carbon dioxide formation (ml/g DM) *in vitro*.

Parameters	Control±SE	%2PFF±SE	%4PFF±SE	%6PFF±SE	%8PFF±SE	%10PFF±SE	%100PFF±SE	SEM	*P*
Total gas ml/g DM	152.17^{bc} ±1.48	196.28^{a}±12.29	164.67^{ab}±12.19	185.39^{a}±0.39	130.37^{c} ±12.55	180.95^{ab}±10.97	167.19^{ab}±11.76	8.80	*
% Methane	13.87^{b}±0.37	28.19^{a}±2.40	22.41^{a}±3.04	25.83^{a} ±0.66	14.56^{b} ±2.80	26.57^{a}±2.03	23.29^{a}±2.31	1.94	**
Carbon dioxide ml/g DM	87.88^{bc}±1.09	118.45^{a}±10.10	92.96^{ab}±9.14	109.65^{ab}±0.48	65.94^{c} ±9.88	104.62^{ab}±8.82	93.83^{ab}±9.67	0.24	*
Ammonia nitrogenmg/dl	9.43^{d}±0.72	19.34^{b}±0.24	20.34^{b}±0.36	22.88^{a} ±1.32	15.10^{c} ±0.20	24.38^{a}±0.57	5.85^{e}±0.02	0.49	**
pH	6.94±0.04	6.96±0.01	6.97±0.01	6.96±0.01	6.97±0.03	6.95±0.02	6.99±0.01	0.02	-
IVOMD % DM	44.35^{bc}±0.26	52.26^{a}±2.19	46.69^{ab}±2.17	50.44^{a}±0.07	40.72^{c} ±2.23	49.77^{ab}±1.95	50.05^{ab}±2.09	1.57	**
ME MJ/kg DM	6.58^{bc}±0.04	7.79^{a}±0.33	6.95^{ab}±0.33	7.52^{a}±0.01	6.04^{c} ±0.34	7.43^{ab}±0.30	7.79^{a} ±0.32	0.24	**

PFF: *P. farcta* fruit, DM: Dry matter, IVOMD: *In vitro* organic matter digestibility, ME: Metabolizable energy, SE: Standard error, SEM: Standard error of means. a, b, c. For each line, the mean values with different letters are significantly different (*: P<0.01; **: P<0.001; -: not significant).

Tannins form complexes with proteins, reducing ruminal degradation and increasing protein flow to the intestines (Patra and Saxena, 2011). Hydrolyzable tannins limit rumen proteolysis by binding to bacteria and proteins (Zhao *et al*., 2023) and suppressing protozoal growth, lowering ammonia nitrogen levels (Santoso *et al*., 2007). Essential oils can also reduce nitrous oxide and ammonia nitrogen emissions in dairy cattle (Carrazco *et al*., 2020). Güler *et al*. (2019) reported that the addition of B. lactis to wheat straw reduced total gas production, methane formation, carbon dioxide levels and IVOMD, whereas supplementation with *S. boulardii* increased methane production. Yucca schidigera extract (25-50 g/day) reduces ruminal volatile fatty acid levels (Guyader *et al*., 2017) and saponins-whether from plants or extracts-have been shown to lower ammonia nitrogen concentrations (Hu *et al*., 2005) and nitrogen excretion (Jayanegara *et al*., 2019). In lambs, the ammonia nitrogen concentration in *P. laevigata* pods increases at 250-500 g/kg (Pena-Avelino *et al*., 2016), suggesting the safe use of up to 500 g/kg ammonia. Yanza *et al*. (2024) reported that saponins up to 40 g/kg DM had no negative effect on intake or palatability. Ramirez-Lozano *et al*., (2017) studied four diets in rumen-fistulated sheep. The control diet (*Medicago sativa* only) showed the highest CP digestibility and a positive nitrogen balance (P<0.05). Diets with *Senegalia greggii* and especially *P. juliflora* resulted in lower nitrogen intake and negative nitrogen balance, with *P. juliflora* showing the poorest performance. *M. sativa* was the most effective for protein use and nitrogen retention.

The effects of *P. farcta* fruit supplementation on *in vitro* total gas (ml/g DM), methane (%), ammonia nitrogen (%), pH, IVOMD (% DM), ME (MJ/kg DM) and carbon dioxide (ml/g DM) in corn silage are presented in Table 3. In this study, significant differences in pH were observed among the maize silage groups, unlike in the wheat straw groups (P< 0.001). The highest pH (6.99) occurred in the group with 100% *P. farcta* fruit, likely due to fermentation-inhibiting compounds in the fruit. The naturally acidic nature of the maize silage may have also contributed to this variation. *P. farcta* inclusion significantly affected the total gas, methane, carbon dioxide, ammonia nitrogen, pH, IVOMD and ME values (P<0.001). As shown in Table 3, total gas production was lower in the control group (4%, 8% and 100%), while it increased at 2% and 6%, peaking at 10%. These results suggest that maize silage deficiencies started to be addressed at 2% and were best corrected at 10%. However, the low gas output in the 100% group may reflect the inhibition of microbial fermentation by excess bioactive compounds. Therefore, using *P. farcta* fruit at 6-8% inclusion appears to offer a more balanced and practical supplementation approach than using it as a sole feed source.

Soltan *et al*. (2012) suggested that the methane-reducing effect of *P. juliflora* leaves may result not only from tannins but also from other bioactive compounds. Khan *et al*. (2010) reported moderate levels of indole alkaloids in the leaves, which are potentially responsible for reduced gas output. Despite low tannin levels (1.0 g/kg DM), *P. juliflora*

Table 3: Effect of adding different concentrations of *P. farcta* fruit to corn silage on total gas (ml/g DM), methane (%), ammonia nitrogen (mg/dl), pH, IVOMD *in vitro* (%DM), ME (MJ/kg DM) and carbon dioxide formation (ml/g DM) *in vitro*.

Parameters	Control±SE	%2PFF±SE	%4PFF±SE	%6PFF±SE	%8PFF±SE	%10PFF±SE	%100PFF±SE	SEM	*P*
Total gas ml/g DM	229.96^{c}±7.78	280.79^{b}±7.74	234.83^{c}±18.32	293.97^{ab}±13.40	214.47^{c}±17.97	332.51^{a}±22.65	167.19^{d} ±11.76	14.23	**
% Methane	$25.85d^{e}$±0.96	45.31^{bc}±1.97	36.83^{cd}±4.45	48.30^{ab}±3.43	30.20^{de}±5.27	58.50^{a}±4.91	23.29^{e}±2.31	3.33	**
Carbon dioxide ml/g DM	153.15^{cd}±7.72	184.53^{bc}±5.67	147.19^{d}±14.02	194.53^{ab}±10.44	133.79^{d}±13.10	223.51^{a}±17.62	93.83^{e}±9.67	11.18	**
Ammonia nitrogen mg/dl	7.30^{d}±0.34	19.94^{c}±0.66	20.78^{bc}±0.06	21.38^{b}±0.29	21.32^{b}±0.02	24.40^{a}±0.30	5.85^{e}±0.02	0.24	**
pH	6.84^{d}±0.01	6.96^{ab}±0.02	6.90^{c}±0.02	6.91^{c}±0.01	6.92^{bc}±0.01	6.91^{c}±0.01	6.99^{a}±0.01	0.01	**
IVOMD % DM	59.23^{c}±1.38	68.31^{b}±1.38	60.17^{c}±3.26	70.73^{ab}±2.38	56.63^{cd}±3.20	77.67^{a}±4.03	50.05^{d}±2.09	2.53	**
ME MJ/kg DM	8.97^{cd}±0.21	10.36^{b}±0.21	9.12^{c}±0.50	10.73^{ab}±0.36	8.58^{cd}±0.49	11.80^{a}±0.62	7.79^{d}±0.32	0.39	**

PFF: *P. farcta* fruit, DM: Dry matter, IVOMD: *In vitro* organic matter digestibility, ME: Metabolizable energy, SE: Standard error, SEM: standard error of means. a, b, c, d, e. For each line, the mean values with different letters are significantly different (**: *P*<0.001).

leaves were associated with lower methane emissions (P<0.05), indicating the presence of additional inhibitory metabolites. Dos Santos *et al*. (2013) demonstrated that chloroformic extracts of *P. juliflora* pods, which are rich in juliprosopine, prosoflorine and juliprosine, suppressed gas production similarly to monensin after 36 h. Melesse *et al*. (2019) reported higher ME and IVOMD in *P. juliflora* pods than in other legumes, despite elevated gas production. Saad *et al*. (2017) observed moderate antimicrobial activity in *P. farcta* aerial parts, particularly in n-hexane and methylene chloride extracts. Güler *et al*. (2019) reported that probiotic supplementation (including *Lactobacillus rhamnosus, Bifidobacterium lactis* and *Saccharomyces boulardii*) to maize silage had no significant effect on methane or carbon dioxide concentrations. Saponin-rich diets have been linked to improved fermentation and health and reduced methane emissions (Baheg *et al*., 2017).

Meena *et al*. (2017) noted that increasing concentrates in *P. cineraria*-based rations elevated ammonia nitrogen, VFAs and protozoan populations (P<0.05). In the present study, ammonia nitrogen levels peaked at 10% *P. farcta* fruit supplementation and decreased progressively in the 6%, 8% and 100% groups, suggesting that its bioactive compounds may inhibit ammonia nitrogen formation and promote bypass proteins. Gül *et al*. (2017) reported that black cumin and its oil increased gas production (P<0.05) without affecting methane, carbon dioxide, ammonia nitrogen or pH, whereas thyme and thyme oil significantly influenced all gas parameters. According to Table 3, the highest IVOMD and ME values were observed in the group supplemented with 10% *P. farcta* fruit (P<0.001), indicating that nutritional deficiencies in maize silage were partially addressed at 2-6% and optimally corrected at 10%. In contrast, the 100% *P. farcta* treatment had the lowest values, likely due to the inhibitory effects of saponins and tannins on microbial activity. The similarity of this group to the control group highlights the poor nutritional value of unsupplemented silage. These results suggest that *P. farcta* is most effective at 8-10% inclusion. While the 10% group achieved the highest ME (11.80 MJ/kg DM) and IVOMD (77.67%), it also had the highest methane production (58.50%). The 8% group produced lower MEs (8.58 MJ/kg DM) but maintained methane levels close to those of the control group (30.20% vs. 25.85%). Despite the low methane concentration, the 100% treatment offered limited nutritional benefit, indicating that the silage is unsuitable as a sole feed. Overall, 6-8% inclusion of these materials appears to be optimal for improving efficiency while minimizing methane emissions. Gül *et al.* (2017) reported that supplementing corn silage with black cumin and its oil affected IVOMD and ME values (*P*<0.05), with reductions of 0.92% in black cumin and increases of 0.3% in black cumin oil, likely due to enhanced microbial activity. Similarly, thyme and thyme oil influenced IVOMD and ME, with the lowest and highest values observed at 8.6% and 0.15%, respectively. Meena *et al.* (2017) reported that increasing concentrate levels elevated gas, methane and ME levels (*P*<0.05), while high-tannin diets reduced methane and improved IVOMD. These results help to explain why the nutrients present in *P. farcta* fruit support microbial activity and gas production in the rumen environment to a certain extent, as they compensate for the nutritional deficiencies inherent in maize silage. According to the IPCC (2022), 81% of agricultural nitrous oxide emissions arise from the sector itself, with 46% linked to ruminant excreta. Phytochemicals may reduce such emissions by accumulating natural nitrification inhibitors in urine (Totty *et al*., 2013). Effective feeding strategies can modulate nitrogen metabolism, decreasing both urinary nitrogen and atmospheric nitrous oxide (Stewart *et al*., 2019).

While *P. farcta* fruit supplementation has the potential to improve rumen fermentation and reduce emissions, limitations must be acknowledged. As the study was conducted *in vitro*, the results may not reflect *in vivo* rumen dynamics (Baah *et al*., 2007; Jahromi *et al*., 2018) and practical application should be approached with caution. Additionally, the phytochemical composition of *P. farcta* may vary seasonally and environmentally (Salari *et al*., 2019; Sharifi-Rad *et al*., 2021), affecting consistency. Nevertheless, its nutrient profile and emission-reducing properties (Sharifi-Rad *et al*., 2021) support its use as a potential feedstuff (Meghwar and Dhanker, 2022). Future *in vivo* studies are needed to evaluate animal performance, health and environmental outcomes. Moreover, the synergistic effects of other additives or probiotics warrant investigation (Güler *et al*., 2019).

CONCLUSION

The literature shows that different fractions of *Prosopis species* can affect digestibility, performance, rumen fermentation, blood metabolites and nitrogen utilization in ruminants, with effects depending on the species, dose and origin. Owing to their rich bioactive content, *Prosopis species* may modulate rumen fermentation and improve metabolic responses. In the wheat straw groups, 2% and 6% *P. farcta* supplementation significantly increased ME but also increased methane emissions. The 8% treatment did not enhance ME but did keep methane close to control levels (14.56% vs 13.87%), suggesting that it may offer a more sustainable balance than 4-6%, which, while efficient and poses environmental concerns. The 100% *P. farcta* treatment group had greater IVOMD and ME than did the control group, indicating that the former had greater nutritional potential than did the latter. In maize silage, 10% supplementation maximized ME and methane output. Although 8% reduced methane to near-control levels, it failed to improve ME. Thus, while 4-6% may be optimal for energy, methane remains a concern. The 100% *P. farcta* group exhibited low methane and moderate digestibility, but its low energy value limits its use as a sole feed.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are

responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

As rumen fluid was collected from slaughtered animals at the abattoir, ethical approval was not needed.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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