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Role of dose dependent *Escherichia coli* as ruminal anti-microflora agent to mitigate biogases production in prickly pear cactus flour based diet

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ABSTRACT

The present investigation was conducted to evaluate the effects of *Escherichia coli* against the ruminal microflora fermentation activities in the mitigation of CH_4 and CO_2 production as well as ruminal fermentation kinetics by substituting dietary corn grain with prickly pear cactus (PC) flour. Three total mixed PC rations were prepared (/kg DM): 0 g (Control), 75 g (PC75), and 150 g (PC150). Besides, *E. coli* was supplemented at four different levels (dose): 0, 10, 20, and 40 mg/g DM of substrates. The *in vitro* rumen GP, CH_4, and CO_2 were estimated to be affected due to various doses of *E. coli* up to 72 h of incubation. Asymptotic GP, fractional rate of GP, and lag time were influenced significantly ($P < .05$) in the presence of ration. However, *E. coli* doses showed minor impact on the rate of GP as well as lag time. The asymptotic CH_4 production was decreased linearly ($P = .005$) at the ration PC150. *E. coli* doses reduced the asymptotic CH_4 production at 10 and 20 mg/g DM. The asymptotic CO_2 production was linearly ($P < .001$) decreased by different levels of PC. The cubic ($P = .023$) effect of *E. coli* doses as well as significant ($P = .002$) ration × *E. coli* doses impact were reported on asymptotic CO_2 production. The fractional rate of GP was quadratically ($P < .05$) influenced by PC and *E. coli* doses. The rations, dose, and rations × *E. coli* dose interaction had no influence ($P > .05$) on lag time. In a nutshell, PC flour inclusion in diet has the potentiality to replace the existing conventional feedstuffs for ruminant. Most importantly, revealing the first report, PC flours along with *E. coli* supplementation at varied doses mitigated the ruminal biogases production. This was as consequence to the antimicrobial impacts of *E. coli* against ruminal microflora, and that could certainly be a promising approach in order to improve ruminant's diet constituents.

1. Introduction

There is tremendous production of inexpensive agricultural solid wastes during diversiform agricultural practices globally. These agricultural by-products are ample sources of nutrients enriched feed ingredients, thereby demonstrating the pivotal applications for ruminant's nutrition in terms of dietary energy. In spite of promising attributes, these carbohydrate-rich agro-feeds are not eco-friendly. Most importantly, the increasing populace of world is a major threat for the animals not only due to the shortage of conventional feed availability but also in terms of its cost. The high productivity as well as fast growth of ruminant depends on the high concentrate diets. At present, cereals viz. barley, wheat, and corn are commonly used for intensive ruminant production. But the continuous rise in the price of these grains as well as possibility to develop acidosis and laminitis at a high level due to the supplementation of these grains in ruminant's diet have compelled the worldwide researchers to look for their ideal and cost-effective alternatives [1].

The selection of unconventional feedstuffs with no food values to human would be a promising approach in this regard. However, those feed must be not only inexpensive but also be available throughout annual seasons. Most importantly, it should be noteworthy that biogases emission from livestock, particularly methane (CH_4) and carbon dioxide (CO_2) are major concern because the production of CO_2 and CH_4 from ruminants is a diet dependent mechanism [2].

According to the Food and Agriculture Organization of the United Nations (FAO), CH_4 is the major biogas emitted from enteric fermentation through digestive process of ruminants, and CH_4 emission from animal production sector is responsible for about 20% of all biogas emissions, while CO_2 accounts for about 9% [3]. However, the excessive excretion of nutrients, inefficient digestibility, and high CH_4 emission are the major constraints in ruminant farming, thereby

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representing a net loss of 2–12% of gross dietary energy [4]. The estimation of *in vitro* gas production (GP) allows the quantification of short chain fatty acid from substrate, the energetic value of feed, and the amount of substrate fermented [5].

Recently, *Opuntia* spp. have been identified as one of the most widely used low cost alternative feeds in semi-arid regions globally due to their adaptation to drastic conditions [6], thereby indicating their utilization as green fodder for ruminants. The chemical composition (g/kg dry matter [DM]) of spineless cactus pear species as reported by Costa et al. [7] is: 78.9 DM, 48.3 crude protein (CP), 10.6 ether extract (EE), 108.7 ash, 290.7 neutral detergent fiber (NDF), and 257.1 acid detergent fiber (ADF). Additionally, it is considered to be an ample source of energy with high DM digestibility coefficient due to the presence of high non-fibrous carbohydrates [8].

Previously, several ingredients of rations viz. sodium or calcium propionate [9], disodium malate or calcium malate [10], exogenous fibrolytic enzymes [11], and *Saccharomyces cerevisiae* [12] have been used for ruminants. But the reports on the determination of biogases emission due to prickly pear cactus (PC) supplementation in the presence of *E. coli* are probably not available. In addition to this, it was hypothesized that the ruminal contamination with *E. coli* will influence adversely the ruminal microflora and fermentation, resulting in an affected biogases production. In view of this, a significant attempt was undertaken to investigate the impact of replacing corn grains (CG) of diet with PC in the presence of *E. coli* at different levels on ruminal *in vitro* GP, CH_4, and CO_2 productions as well as fermentation kinetics.

2. Materials and methods

2.1. Bacteria of interest

E. coli was obtained from Centro de investigación y Estudios Avanzados en Salud, Facultad de medicina veterinaria y Zootecnia Universidad Autónoma del Estado de México, which was isolated from the ruminant's fecal source. Approximately 150 g of faecal samples were collected, placed in sterile plastic bags, and brought to the laboratory. The fresh faecal samples were thoroughly mixed in the plastic bags and 1 g was taken for coliform count by membrane filtration technique. Briefly, 1 g sample was transferred into a 120 mL diluent bottle containing 99 mL of 0.31 mmol KH_2PO_4/L buffer solution, and shaken vigorously before serial dilution. Membranes were placed on m-TEC agar (m-TEC HiCrome™ Agar, Sigma Aldrich, Mexico) and incubated for 24 h at 37 °C in a water bath before counting colonies of *E. coli.* The bacteria (*i.e., E. coli*) was previously sub-cultured on eosin methylene blue (EMB) medium.

2.2. Substrates and treatments

Total three mixed rations were prepared where CG was replaced with PC at three levels (per kg DM): 0 g (Control), 75 g (PC75), and 150 g (PC150). The ingredient and chemical composition of the experimental diet are shown in Table 1 (adapted from [13]). *E. coli* was used at four supplemental levels: 0, 10, 20, and 40 mg/g DM of substrates.

2.3. In vitro fermentation

Rumen inoculum was obtained from a Brown Swiss cow (450 kg BW) fitted with a permanent rumen cannula and fed *ad libitum* a formulated total mixed ration of a commercial concentrate (PURINA®, Toluca, Mexico) and alfalfa hay in the ratio of 1:1 DM according to NRC [14]. The cow was offered fresh water *ad libitum* during collection phase. After that, the rumen contents were flushed with CO_2, mixed, and strained through four layers of cheese cloth into a flask with O_2-free headspace. Samples (0.5 g) of each ration were weighed into 120 mL serum bottles with appropriate addition of extract dose/g DM.

Table 1
Composition of the experimental diets [13].

	Control	PC75[a]	PC150[b]
Ingredients (g/kg DM)			
Oats straw	249	248	248
Steam rolled corn	250	175	100
Soybean hulls	250	250	250
Steam rolled barley	120	110	120
Wheat bran	30	30	30
Corn gluten feed	30	30	20
Prickly pear cactus	0	75	150
Molasses	70	80	80
Vitamins/minerals[c]	1	2	2
Chemical composition (g/kg DM)			
Organic matter	964	940	957
Crude protein	130	119	113
Neutral detergent fiber	356	428	340
Acid detergent fiber	121	130	122
Ether extract	24	22	23

[a] PC75, prickly pear cactus was included at 75 g/kg DM of total mixed ration.
[b] PC150, prickly pear cactus was included at 150 g/kg DM of total mixed ration.
[c] Contained: Vitamin A (12 000 000 IU), Vitamin D_3 (2 500 000 IU), Vitamin E (15 000 IU), Vitamin K (2.0 g), Vitamin B_1 (2.25 g), Vitamin B_2 (7.5 g), Vitamin B_6 (3.5 g), Vitamin B_{12} (20 mg), Pantothenic acid (12.5 g), Folic acid (1.5 g), Biotin (125 mg), Niacin (45 g), Fe (50 g), Zn (50 g), Mn (110 g), Cu (12 g), I (0.30 g), Se (200 mg), Co (0.20 g).

Consequently, 10 mL of particle free rumen fluid was added to each bottle, followed by 40 mL of the buffer solution of Goering and Van Soest [15], with no trypticase added.

Three incubation runs were performed in three weeks. Bottles with substrates plus three bottles as blanks (rumen fluid only) were incubated for 72 h. Once all bottles were filled, they were immediately closed with rubber stoppers, shaken, and placed in an incubator at 39 °C. The volume of GP was recorded at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 48, and 72 h using the pressure transducer technique (Extech instruments, Waltham, USA) of Theodorou et al. [16]. Both the CH_4 and CO_2 productions were recorded at 4, 8, 12, 16, 20, 24, 48, and 72 h of incubation using Gas-Pro detector (Gas Analyzer CROWCON Model Tetra3, Abingdon, UK). After 72 h of incubation, the fermentation was stopped by swirling the bottles in ice. Bottles were uncapped, pH was measured using a pH meter (Conductronic pH15, Puebla, Mexico), and then the contents of each bottle were filtered under vacuum through glass crucibles (coarse porosity no. 1, pore size 100 to 160 μm; Pyrex, Stone, UK) with a sintered filter in order to achieve the non-fermented residue for determining degraded substrate after drying overnight at 65 °C.

2.4. Estimation of gas kinetics

For the estimation of GP, CH_4, and CO_2 kinetics, recorded gas, CH_4, and CO_2 volumes (mL/g DM) were fitted using the NLIN procedure of SAS [17] as per the below mentioned model of France et al. [18].

$$y = b \times [1 - e^{-c\ (t-Lag)}] \quad (1)$$

where, y is the volume of GP, CH_4, or CO_2 at time t (h); b is the asymptotic GP, the asymptotic CH_4, or the asymptotic CO_2 (mL/g DM); c is the fractional rate of fermentation (1/h); and *Lag* (h) is the discrete lag time prior to when any gas, CH_4 or CO_2 is released.

2.5. Statistical analyses

Data of each of the three runs within the same sample of each of the three individual samples of rations were averaged prior to statistical analysis. Mean values of each individual sample were used as the experimental unit. Results of *in vitro* GP and rumen fermentation parameters were analyzed as a factorial experiment using the PROC GLM option of SAS [17] as given below:

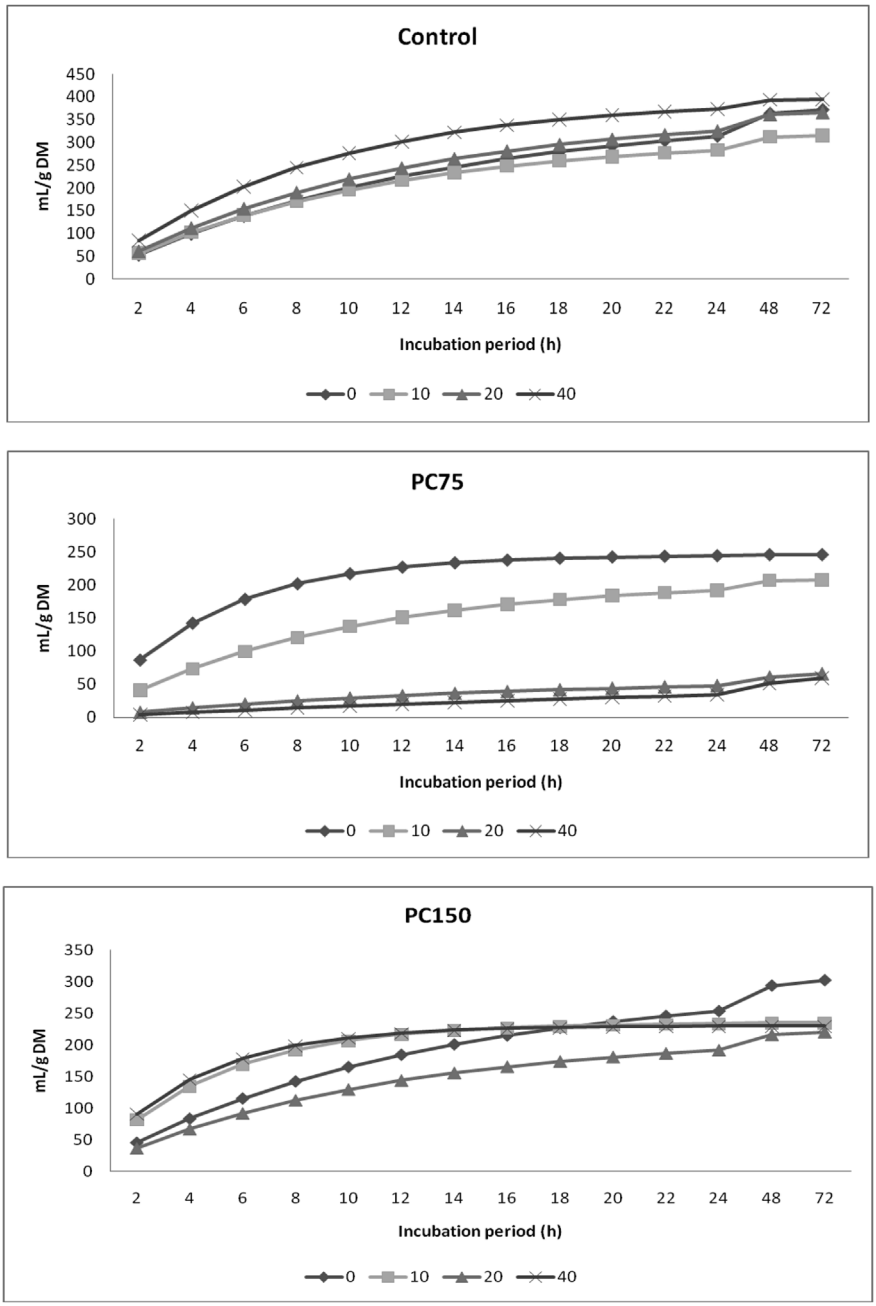


Fig. 1. Rumen gas production (GP, mL/g incubated DM) at three different levels of prickly pear cactus (PC) flour as affected by different levels of *E. coli* at 0, 10, 20 and 40 mg/g DM. Control - Corn grain (CG) was replaced with PC flour at 0 g/kg DM; PC75 - PC flour was included at 75 g/kg DM of total mixed ration; and PC150 - PC flour was included at 150 g/kg DM of total mixed ration.

$$Y_{ijk} = \mu + R_i + D_j + (R \times D)_{ij} + E_{ijk} \quad (2)$$

where, Y_{ijk} is every observation of the i^{th} ration type (R_i) with j^{th} *E.coli* dose (D_j); μ is the general mean; $(R \times D)_{ij}$ is the interaction between ration type and *E.coli* dose; E_{ijk} is the experimental error. Linear, quadratic, and cubic polynomial contrasts were used to examine responses of different rations with increasing levels of *E. coli*. Statistical significance was declared at $P < .05$.

3. Results

3.1. E. coli as anti-ruminal biogases production

The *in vitro* rumen gas production (GP, mL/g incubated DM) of three varied levels of prickly pear cactus (PC) flour as influenced by different doses of *E. coli* is depicted in Fig. 1. The GP was estimated to be affected in the presence of PC75 and PC150 rations at dose dependent manner of *E. coli* up to 72 h of incubation.

Fig. 2 shows the *in vitro* production of CH_4 (mL/g incubated DM) due to the dose dependent supplementation of *E. coli* in the presence of various levels of PC. The PC flour at 75 and 150 g/kg DM of total mixed ration exhibited significant reduction in CH_4 production in the presence of various doses of *E. coli* up to 72 h of incubation.

The dose dependent interaction of *E. coli* with varied rations of PC demonstrated effective mitigation of CO_2 up to 72 h of incubation. However, higher mitigation of CO_2 production was estimated at PC150 in the presence of concentration dependent inclusion of *E. coli* (Fig. 3).

3.2. E. coli and ruminal biogas kinetics and degradability

The values of pH were affected linearly ($P = .02$) and quadratically ($P = .024$) by the levels of PC, but it was not influenced significantly ($P > .05$) by the inclusion of *E. coli* (dose), and ration × *E. coli* dose interaction. Similarly, the different levels of ration had significantly

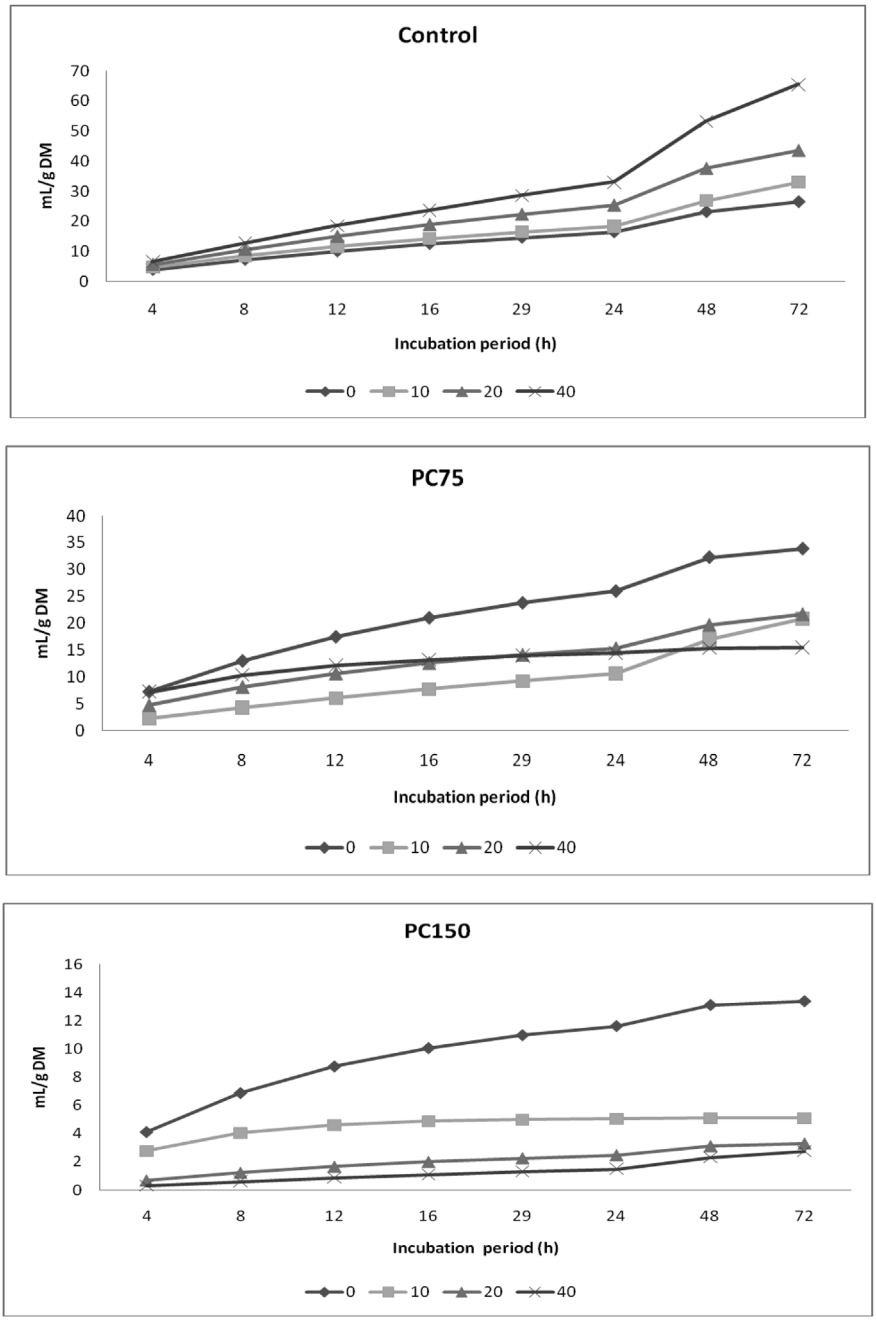


Fig. 2. Ruminal CH_4 production (mL/g incubated DM) at three different levels of PC flour as affected by different levels of *E. coli* at 0, 10, 20, and 40 mg/g DM. Control - Corn grain (CG) was replaced with PC flour at 0 g/kg DM; PC75 - PC flour was included at 75 g/kg DM of total mixed ration; and PC150–PC flour was included at 150 g/kg DM of total mixed ration.

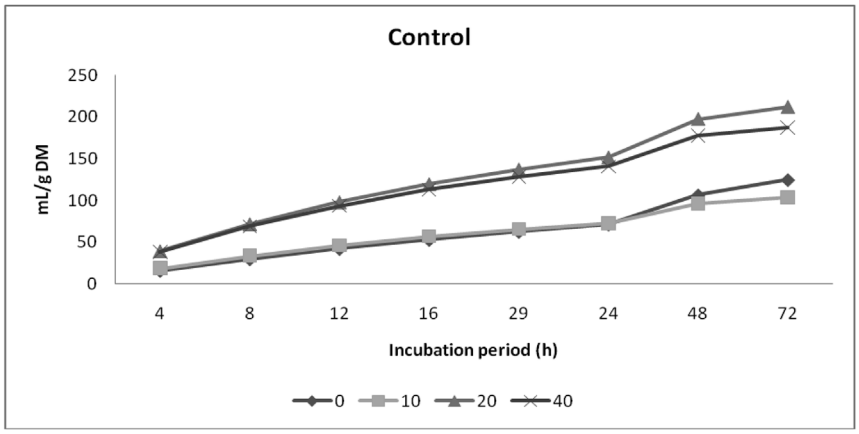
linear ($P = .007$) and quadratic ($P = .032$) effect on DMD values. Moreover, *E. coli* doses affected quadratically ($P = .039$) the DMD values (Table 2).

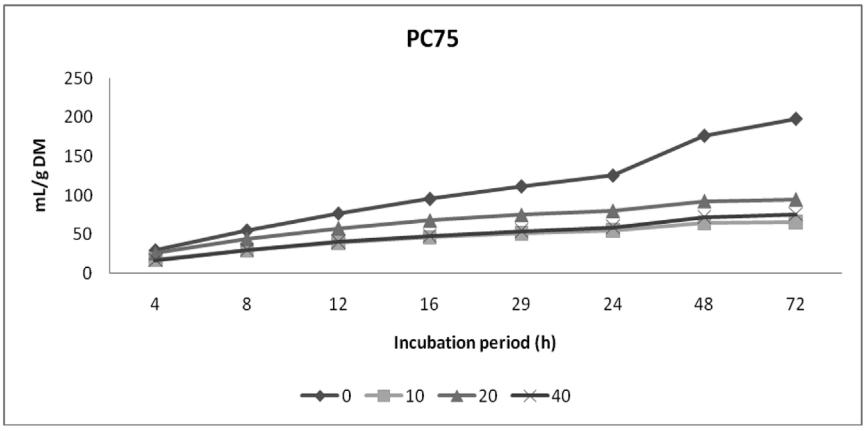
Asymptotic GP was affected linearly ($P < .001$) and quadratically ($P = .005$) due to the various levels of PC, but it was not influenced by the supplementation of *E. coli* doses as well as ration × dose. The different levels of PC revealed linear ($P < .001$) and quadratic ($P = .003$) impact on the fractional rate of GP, while *E. coli* doses had only linear ($P < .001$) and cubic ($P = .003$) effect on fractional rate of GP. Additionally, the ration × *E. coli* dose interaction influenced the rate of GP significantly. The lag time was affected significantly ($P < .05$) in the presence of rations, while quadratic ($P = .012$) influence of *E. coli* doses were reported on lag time (Table 2).

The asymptotic CH_4 production was decreased linearly ($P = .005$) at the ration PC150. The levels of *E. coli* affected the asymptotic CH_4 production with the lowest values being reported at the doses of 10 and 20 mg/g DM of the PC150 ration (Table 2). The ration PC150 showed the decrease (linear, $P < .05$) in CH_4 production (mL/g incubated DM and mL/g degraded DM) after 8, 24, and 48 h of incubation in the presence of various doses of *E. coli*. The proportional CH_4 production was linearly ($P = .01$) affected after 48 h of incubation. Both dose and ration × dose interaction had no significant ($P > .05$) influence on CH_4 production at all incubation periods (Table 3).

Fig. 3 depicts the *in vitro* rumen CO_2 (mL/g incubated DM) production at three different levels of PC as influenced by diverse doses of *E. coli*. The asymptotic CO_2 production was linearly ($P < .001$) affected by different levels of PC. The cubic ($P = .023$) effect of *E. coli* doses as well as significant ($P = .002$) ration × *E. coli* doses impact were reported on asymptotic CO_2 production. On the other hand, the quadric ($P < .05$) influence of PC and *E. coli* doses on the fractional rate of GP was observed. The rations, dose, and rations × dose interaction had no effect ($P > .05$) on lag time (Table 2).

The different levels of ration showed linear ($P < .05$) influence on CO_2 production (mL/g incubated DM and mL/g degraded DM) after 8,





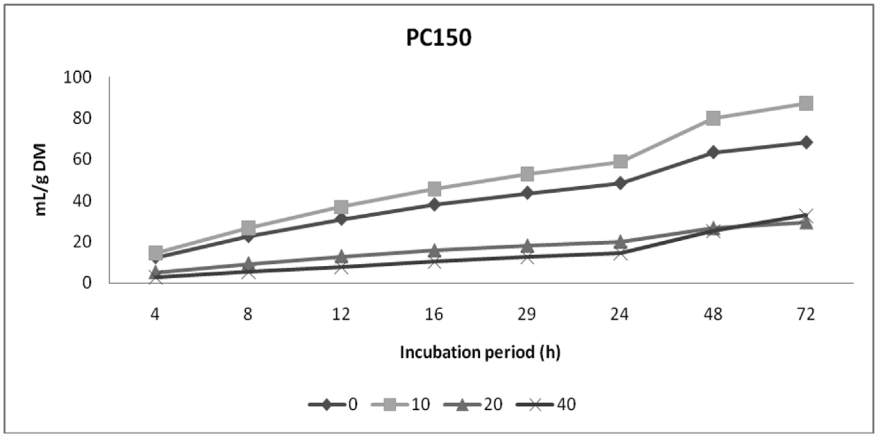


Fig. 3. Ruminal CO_2 production (mL/g incubated DM) at three different levels of PC flour as affected by different levels of *E. coli* at 0, 10, 20, and 40 mg/g DM. Control - CG was replaced with PC flour at 0 g/kg DM; PC75 - PC flour was included at 75 g/kg DM of total mixed ration; and PC150 - PC flour was included at 150 g/kg DM of total mixed ration.

24, and 48 h of incubation, while *E. coli* levels did not affect CO_2 production (mL/g incubated DM and mL/g degraded DM) after required periods of incubation. However, ration × dose interaction reported significant ($P < .05$) impact on CO_2 production (mL/g incubated DM and mL/g degraded DM) after 8, 24, and 48 h of incubation. On the other hand, proportional CO_2 production was observed to be unaffected ($P > .05$) by different levels of PC. However, various doses of *E. coli* were reported to depict linear ($P < .05$) influence on proportional CO_2 production after 8, 24, and 48 h (Table 4).

4. Discussion

In the last few years, significant attempts had been undertaken to utilize diversified nutrient enriched by-products in feed for ruminant's high productivity as well as environmental conservation. At present, the high costs of dietary energy sources for ruminant are drawing animal nutritionist's attention in quest of ideal alternatives of expensive feed supplements. It should also be noteworthy that in order to utilize unconventional feedstuffs as feed ingredients for ruminant, those by-products need to be not only highly nutritive to livestock but also non-nutritious to humans. Recently, calcium malate [10], organic acid salts [19], *Salix babylonica* extract [20], and browse tree leaves [21] were successfully implied in the feeding diets of livestock to investigate their biogas mitigation attributes. However, to the best of our knowledge, there is no study reported on the potent role of *E. coli* supplementation on the biogases mitigation from various concentrations of PC flour.

In general, the *in vitro* GP has been used as a significant trait of ruminal feed degradation. Higher GP corresponds to high digestibility, good fermentability, and promising protein production, thereby indicating a better availability of nutrients to rumen microbes [22]. In the present context, the *in vitro* GP was estimated to be significantly affected in the presence of PC rations at dose dependent manner of *E. coli*

Table 2
Ruminal total gas production (GP), methane (CH_4), and carbon dioxide (CO_2) kinetics of three different levels of prickly pear cactus (PC) as affected by different levels of *E. coli* (mg/g DM).

Ration[a]	*E. coli*	pH and degradability		GP (mL/g DM)[b]			CH_4 production (mL/g DM)[c]			CO_2 production (mL/g DM)[d]		
		pH	DMD	*b*	*c*	*Lag*	*b*	*c*	*Lag*	*b*	*c*	*Lag*
Control	0	5.68	543	373	0.078	1.11	34	0.029	9.9	149	0.029	4.08
	10	5.71	548	316	0.101	1.06	55	0.050	13.5	108	0.047	3.90
	20	6.04	572	366	0.093	1.06	50	0.032	10.7	219	0.052	4.14
	40	6.40	558	395	0.121	1.17	85	0.021	5.9	191	0.056	4.11
PC75	0	6.46	498	268	0.075	1.40	35	0.060	11.1	217	0.035	4.39
	10	6.40	537	229	0.092	1.62	30	0.019	14.2	66	0.073	3.58
	20	6.47	536	288	0.079	1.50	24	0.073	5.7	95	0.077	4.86
	40	6.49	497	230	0.122	1.30	15	0.240	8.3	77	0.062	5.08
PC150	0	6.65	507	305	0.085	1.03	13	0.096	12.0	71	0.050	4.72
	10	6.59	530	235	0.215	1.66	5	0.195	10.7	91	0.044	4.52
	20	5.98	524	221	0.092	1.64	3	0.060	9.8	33	0.041	4.83
	40	6.10	529	230	0.252	1.37	3	0.027	8.8	53	0.014	4.17
Pooled SEM[e]		0.212	15.9	27.4	0.0189	0.118	14.9	0.0465	11.93	20.0	0.0095	0.572
Ration effect												
Linear		0.020	0.007	< 0.001	< 0.001	0.006	0.005	0.086	0.972	< 0.001	0.219	0.237
Quadratic		0.024	0.032	0.005	0.003	0.013	0.591	0.254	0.346	0.956	0.005	0.638
E. coli effect												
Linear		0.677	0.525	0.489	< 0.001	0.691	0.597	0.499	0.423	0.160	0.749	0.634
Quadratic		0.415	0.039	0.201	0.333	0.012	0.732	0.729	0.603	0.080	0.020	0.970
Cubic		0.725	0.834	0.055	0.003	0.211	0.663	0.330	0.173	0.023	0.638	0.230
Ration × Dose		0.058	0.689	0.320	0.005	0.063	0.402	0.041	0.671	0.002	0.057	0.792

[a] PC75, prickly pear cactus was included at 75 g/kg DM of total mixed ration; PC150, prickly pear cactus was included at 150 g/kg DM of total mixed ration.
[b] *b* is the asymptotic GP (mL/g DM); *c* is the rate of GP (/h); *Lag* is the initial delay before GP begins (h).
[c] *b* is the asymptotic CH_4 production (mL/g DM); *c* is the rate of CH_4 production (/h); *L* is the initial delay before CH_4 production begins (h).
[d] *b* is the asymptotic CO_2 production (mL/g DM); *c* is the rate of CO_2 production (/h); *L* is the initial delay before CO_2 production begins (h).
[e] SEM standard error of the mean.

up to 72 h of incubation. In consonance with previous reports [12,23], the findings of the present study showed prominent availability of carbohydrate to the microorganisms, thereby affecting the GP after regular interval of time.

In the present study, pH and DMD values were affected linearly and quadratically by the levels of PC. However, the dose dependent supplementation of *E. coli* had no significant effect on pH. Moreover, *E. coli* doses affected quadratically the DMD values. According to Elghandour et al. [19], a significant reduction in pH level was observed after supplementing varied doses of PC due to rapid fermentation of the available carbohydrate contents of PC. Further, the authors reported decline in DMD with increasing levels of PC. In view of the present findings and previous reports, further investigation certainly needs to be studied because *in vitro* DMD was expected to increase with supplementation

Table 3
Proportional of methane (CH_4) production as a percent of total gas production (GP) at three different levels of prickly pear cactus (PC) as affected by different levels of *E. coli* (mg/g DM).

Ration[a]	*E. coli*	CH_4 production								
		mL/g incubated DM			mL/g degraded DM			Proportional CH_4 production		
		8 h	24 h	48 h	8 h	24 h	48 h	8 h	24 h	48 h
Control	0	7.3	16.5	23.3	13.6	30.7	43.3	4.0	5.1	6.4
	10	8.6	18.4	27.0	15.5	33.2	48.4	5.0	7.1	10.0
	20	10.6	25.3	37.6	18.8	44.9	66.4	5.6	7.6	10.0
	40	12.9	33.1	53.3	23.0	59.1	95.0	5.1	8.6	13.1
PC75	0	13.0	26.0	32.3	23.0	46.0	57.2	6.6	6.6	6.7
	10	4.2	10.6	16.9	7.2	18.2	29.0	2.0	4.4	7.0
	20	8.1	15.3	19.7	14.2	26.8	34.3	3.7	5.5	6.9
	40	10.3	14.4	15.4	17.8	24.9	26.5	7.1	7.8	8.2
PC150	0	6.9	11.6	13.1	11.9	20.1	22.7	3.5	5.0	5.6
	10	4.0	5.1	5.1	7.3	9.1	9.2	3.1	2.4	2.3
	20	0.6	2.5	3.1	2.3	4.7	5.9	4.9	4.8	4.8
	40	2.4	1.5	2.3	1.2	2.9	4.5	5.0	4.9	4.8
Pooled SEM[b]		2.42	5.38	8.16	4.26	9.33	13.95	1.48	1.88	2.54
Ration effect										
Linear		0.002	0.005	0.003	0.002	0.003	0.002	0.457	0.055	0.010
Quadratic		0.134	0.493	0.928	0.165	0.564	0.984	0.761	0.738	0.955
E. coli effect										
Linear		0.898	0.977	0.679	0.859	0.997	0.686	0.199	0.208	0.206
Quadratic		0.137	0.241	0.388	0.134	0.220	0.348	0.360	0.549	0.855
Cubic		0.343	0.327	0.491	0.345	0.314	0.467	0.304	0.479	0.891
Ration × Dose		0.179	0.212	0.195	0.191	0.212	0.182	0.457	0.876	0.806

[a] PC75, prickly pear cactus was included at 75 g/kg DM of total mixed ration; PC150, prickly pear cactus was included at 150 g/kg DM of total mixed ration.
[b] SEM standard error of the mean.

Table 4
Proportional of carbon dioxide (CO_2) production as a percent of total gas production (GP) at three different levels of prickly pear cactus (PC) as affected by different levels of *E. coli* (mg/g DM).

Ration[a]	*E. coli*	CO_2 production								
		mL/g incubated DM			mL/g degraded DM			Proportional CO_2 production		
		8 h	24 h	48 h	8 h	24 h	48 h	8 h	24 h	48 h
Control	0	30	71	106	55	132	198	16	22	29
	10	34	73	96	61	131	173	20	28	35
	20	72	151	197	127	269	350	38	46	53
	40	69	141	178	124	252	318	28	37	44
PC75	0	55	126	177	98	223	313	29	32	35
	10	30	55	64	51	94	110	13	21	25
	20	44	80	92	77	139	161	20	28	32
	40	30	59	73	52	103	126	20	30	36
PC150	0	23	48	64	39	84	110	12	21	27
	10	27	59	80	48	106	144	21	28	35
	20	10	20	27	18	38	51	29	35	40
	40	5	15	25	10	28	49	45	48	53
Pooled SEM[b]		10.2	18.6	21.4	17.9	32.5	36.9	7.5	7.3	6.8
Ration effect										
Linear		0.004	0.001	< 0.001	0.003	< 0.001	< 0.001	0.870	0.979	0.816
Quadratic		0.344	0.520	0.721	0.423	0.636	0.879	0.250	0.246	0.088
E. coli effect										
Linear		0.835	0.816	0.438	0.848	0.798	0.415	0.041	0.023	0.013
Quadratic		0.706	0.931	0.510	0.720	0.899	0.461	0.765	0.607	0.624
Cubic		0.205	0.140	0.081	0.193	0.129	0.071	0.249	0.316	0.326
Ration × Dose		0.036	0.014	0.005	0.036	0.013	0.005	0.176	0.397	0.395

[a] PC75, prickly pear cactus was included at 75 g/kg DM of total mixed ration; PC150, prickly pear cactus was included at 150 g/kg DM of total mixed ration.
[b] SEM standard error of the mean.

level, since PC appears to be more degraded than CG. This is based on increasing rate of GP as the level of PC along with *E. coli* increases in the diets.

The asymptotic GP is used to predict feed intake. In the present investigation, the asymptotic GP was affected significantly ($P < .05$) due to the various levels of PC, but it was not influenced by the supplementation of *E. coli* doses as well as ration × dose. In fact, the lack of *E. coli* dose effect on asymptotic GP depicts that the rumen modulator did not improve the availability of carbohydrate. The different levels of PC revealed linear ($P < .001$) and quadratic ($P = .003$) effect on the fractional rate of GP, while *E. coli* doses had only linear ($P < .001$) and cubic ($P = .003$) effect on fractional rate of GP. The linear enhancement in the fractional rate of GP with increased level of PC in the presence of varied doses of *E. coli* is indicator of enhanced degradability or fermentability of the rations. In a nutshell, PC promotes microbial growth, colonization, and degradation. The growth of microorganisms and accessibility of the feed to microbial enzymes are reflected by the rate at which various chemical constituents are degraded. Since fractional rate of GP is positively correlated with feed intake [24], higher level of PC ration in the presence of *E. coli* would likely enhance feed intake and performance of ruminants. This is because performance is correlated with feed intake, which is a better indicator of nutritional value of feed than apparent digestibility [25].

The discrete lag time prior to GP was improved from the control ration to the supplemented rations. Lower lag phase of the control ration relative to the PC supplemented based rations reveals the faster microbial adaptation to the ration and its availability to provide a greater proportion of nutrients [26]. Higher lag time of *E. coli* dose is an indication of delayed microbial degradation. In this context, rumen modulator like higher doses of *E. coli* might have delayed the adaptation of rumen microbes to the substrate. In contrary to our results, Rodriguez et al. [12] observed reduced lag time due to the supplementation of microbial additives. The variation in our results in a comparison with previous report might be due to the types of additives, physiological status of rumen fluid donor, substrate used, composition and quantity of rumen modulator applied, and methods implied [27].

Detrimental biogases viz. CO_2 and CH_4 are mainly produced inside the rumen during ruminal fermentation process. The present study clearly revealed that the inclusion of PC in the presence of *E. coli* mitigated CH_4 and CO_2 production, thereby determining the eco-friendly role of rations. This might be due to the presence of increased fibres content and reduced non-structural carbohydrates in the rations. It may also be because of the antagonistic role of *E. coli* on ruminanl microflora that caused decreased fermentation capability of diets, thereby resulting reduced emission of CH_4 and CO_2 after a regular time period. A significant increase in the PC level of the ration linearly decreased the asymptotic CH_4 production. It was expected that the pronounced effect in GP with PC inclusion should be accompanied by a promising reduction in CH_4 emission. On the other side, PC rations and *E. coli* doses had no significant impact on lag time and rate of CH_4 production. However, ration type × *E. coli* dose interaction affected the rate of CH_4 production significantly. In contrary to our results, Elghandour et al. [19] observed that replacing CG with PC as ration in the presence of varied doses of organic acid salts (OAS) increased CH_4 production. The variation in the findings of present study from earlier report might be attributed to the types of doses used. In the present investigation, *E. coli* was implied at diversified doses, whose mechanism of action during fermentation differed from the other previously supplemented doses containing different constituents.

Further, in this context, the reduction in the aymptotic CO_2 production at various PC rations in the presence of *E. coli* doses was observed to be against the reports of Elghandour et al. [19] who demonstrated increment in the CO_2 level at various PC rations in the presence of OAS doses. Additionally, in accordance to our findings, the rate of CO_2 production and lag time were not much influenced due to rations and *E. coli* doses. It is suggested that the increasing cell wall content may adversely affect the microbial metabolism, thereby causing reduced CO_2 emission.

Ration type as well as *E. coli* showed lack of effect on proportional *in vitro* CH_4 production at different incubation period. In fact, further study is undoubtedly essential to investigate the cause of non-significant effect of ration and *E. coli* doses on proportional CH_4

production. On the other hand, *E. coli* doses revealed linear impact on proportional *in vitro* CO_2 production, thereby revealing the potentiality of PC ration along with *E. coli* doses to mitigate the emission of CO_2.

5. Conclusion

The findings of the present study clearly revealed the potential antimicrobial influence of *E. coli* against the methanogenic ruminal microflora to mitigate the biogases (CH_4 and CO_2) production by ruminants. The study suggests the potential fermentation efficacy and fermentation profile of PC that can be undoubtedly included in concentrate ration as an alternative to conventional energy sources. The dietary inclusion of 150 g PC/kg DM along with varied tested doses of *E. coli* may be used as rumen modifier in terms of reducing animal's ruminal gases (CH_4 and CO_2) production. Further studies certainly need to be conducted in order to investigate the efficacy of PC in the presence of *E. coli* under *in vivo* trials for paving an improved feeding path for livestock.

Conflicts of interest

None declared.

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