



Potential of *Citrullus colocynthis* as herbal feed additive for ruminants

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ABSTRACT

To assess the potential of *Citrullus colocynthis* as herbal feed additive for ruminants, colocynth fruit as a whole, its seeds and peel-pulp were evaluated at various levels (0, 0.5, 1.0, 1.5, 2.0, 2.5, 4.0%) with total mixed ration (roughage to concentrate ratio: 65:35) as substrate in 3×7 factorial design by *in vitro* gas production technique. Phytochemical analysis revealed significant variation ($P<0.01$) in total phenols, non-tannin phenols, true tannins, saponins, flavonoids and vitamin C content among different parts of colocynth fruit. Irrespective of the part used, varying level of *Citrullus colocynthis* had significant effect on net gas production (NGP; ml/24/g), metabolizable energy (ME) availability, methanogenesis, digestibility of neutral detergent fiber (NDF) and true organic matter (TOM) in comparison to control; however the effect was pronounced at 1% level of supplementation. No significant difference in total volatile fatty acids (TVFA) production and acetate production was observed at varying levels of *Citrullus colocynthis* except at 4% level when total mixed ration (TMR) was used as substrate. Irrespective of its level, supplementation of *Citrullus colocynthis* fruit as a whole, seeds and peel-pulp significantly improved NGP ($P<0.001$) and ME ($P<0.01$) availability in comparison to control group whereas partitioning factor (PF; $P>0.578$), digestibility of NDF ($P>0.905$) and true OM ($P>0.228$) remained unaffected between different test groups. However, TVFA production, acetate, propionate and butyrate concentration was observed lower ($P<0.001$) in peel-pulp and seed supplemented group. From above results it can be concluded that supplementation of *Citrullus colocynthis* fruit at 1% level of substrate had potential to improve rumen fermentation characteristics and reduce methanogenesis, however *in vivo* assessment on ruminants need to be conducted to evaluate the persistency of effects along with health concerns.

Key words: *Citrullus colocynthis*, Feed additive, Methanogenesis, Rumen fermentation

Designing of nutritive strategies to modulate ruminal fermentation, to improve fibre utilization, to reduce methane emission and nitrogen excretion by the potential addition of distinct plants or extracts rich in secondary compounds to animal feeds (Rira *et al.* 2015, Hundal *et al.* 2019 a & b) is a hot topic among researchers. But effects of plant secondary metabolites (tannins, saponins, essential oils, flavonoids etc.) on methanogenesis and rumen function are variable and source dependent (Patra *et al.* 2011, Hundal *et al.* 2019a). Therefore, there is need to explore new herbal feed additives (HFA) to assess their potential.

The *Citrullus colocynthis*, which is commonly known as colocynth/bitter apple, belonging to the *Cucurbitaceae* family, is widely distributed in the Sahara and Arabian deserts, Sudan and Southern part of Asia including all over India, Pakistan and Southern Islands (Hussain *et al.* 2014).

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On cultivation, air dried fruit yield of 120 to 150 q/ha and a seed yield of 450 kg/ha can be obtained by better management practices (Yadav ND and Singh PM 1992). *Citrullus colocynthis* traditionally used as medicinal plant because of presence of a variety of bioactive components, grouped as glycosides, flavonoids, alkaloids, carbohydrates, fatty acids and essential oils (Sagar R and Dumka VK 2018) but its potential to be used as herbal feed additive in ruminants still remained un-explored. Therefore, keeping in view the above background, the present study was designed to assess the potential of *Citrullus colocynthis* fruit as a whole, its seeds and peel-pulp supplementation on rumen fermentation characteristics at different levels (0%, 0.5%, 1%, 1.5%, 2%, 2.5%, 4%) using conventional total mixed ration as substrate in *in vitro*.

MATERIALS AND METHODS

The study was carried out at Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Science University, Ludhiana (30°54'26" N, 75°48'16" E, 240 m above sea level) in 2019.

Sample preparation and analysis: Unripen but fully developed fruits of *Citrullus colocynthis* were hand-picked

and divided into three parts. One part manually cut into small pieces and places in a hot air oven (Narang Scientific works, New Delhi) at 65°C for drying. Seeds of fruits were embedded in white spongy pulp. After removal of pericarp by peeling from the second part, seeds were removed manually from pulp and similarly dried in the oven. From the third part of fruits, after removal of seed and juice, remained leftover (peel and pulp) was dried in oven. The dried and finely grounded feed and *Citrullus colocynthis* samples were analysed for bioactive components, proximate principles DM, CP, EE and total ash, N (AOAC 2007) and fibre fractions (Van Soest *et al.* 1991).

In vitro evaluation: The effect of supplementing whole colocynth fruit, its seed or peel-pulp at 0.5, 1.0, 1.5, 2.0, 2.5 and 4.0% of the diet having roughage to concentrate ratio 65:35 were carried out by *in vitro* gas production technique (Menke and Steingass 1988). Rumen fistulated male buffalo maintained on standard diet (65 parts roughage: 35 parts concentrate) as per ICAR (2013) was used as a donor for rumen liquor. The roughage portion was made up of wheat straw and green berseem in 70:30 ratio, while the conventional concentrate was made up of maize (15), wheat (15), de-oiled mustard cake (15), mustard cake (10), soybean meal (10), rice bran (15), de-oiled rice bran (16), urea (1), salt (1) and mineral mixture 2% each.

Rumen liquor was collected from fistulated animals maintained on conventional diet at the farm before feeding at 0900 h in a thermos flask flushed with CO₂ and maintained at 39°C. The rumen contents were blended for 2–3 min in a blender and strained through four-layers of muslin cloth. The solution, containing 960 ml distilled water, 0.16 ml micro-mineral solution, 660 ml bicarbonate buffer, 330 ml macro-mineral solution and 1.6 ml resazurin (0.1%) were mixed in a Woulff flask with magnetic stirrer in a water bath at 39°C. The mixture was continuously flushed with CO₂. Then strained rumen liquor was added to the buffer media in the ratio of 1:2. Different herbs were added to 100 ml calibrated glass syringes (Haberle Labortechnik, Germany) containing 375 mg complete feed (as percent over 375 mg) with buffered rumen fluid. Blank and sample of standard hay were run in triplicate with each set. If the volume of gas in the syringe exceeded 70 ml after 8 h the volume was recorded and the gas was expelled (Menke *et al.* 1979; Menke KH and Steingass H 1988). After 24 h, the volume of gas produced in each syringe was recorded and the content of syringes were transferred to spout-less beaker, boiled with neutral detergent solution for assessing the true OM and NDF digestibility. For methane estimation, 200 mg of substrate was incubated for 24 h with buffered rumen liquor in duplicate. After the stipulated period, total gas production was measured. A 50/50 mixture of CH₄ and CO₂ (Spancan; Spantech Products Ltd., England) was used as a standard for estimation of methane with Netchrom 9100 gas chromatograph. The VFAs (Cottyn BG and Boucque CV 1968) and ammonia (AOAC 2007) were estimated and ME (Menke *et al.* 1979) value be worked out.

Statistical analysis: The data were subjected to analysis

of variance (ANOVA) 3×7 factorial design by using SPSS (2012) software version 20.0, taking different part of *Citrullus colocynthis* as one factor and their level as second factor. The means were tested for significant difference by using Tukey's b test. The statistical model used was:

$$Y_{ijk} = \mu + P_i + L_j + IP \times L + e_{ijk}$$

where, Y_{ijk} , the k^{th} observation on the parameter; μ , population mean; P_i , Effect of i^{th} part of *Citrullus colocynthis* (whole fruit, seed or peel-pulp); L_j , Effect of j^{th} level (0.5%, 1%, 1.5%, 2%, 2.5%, 4%); $IP \times L$, Effect of i^{th} part of *Citrullus colocynthis* at j^{th} level; e_{ijk} , Error.

RESULTS AND DISCUSSION

Chemical composition of different parts of Colocynth and substrate: The *Citrullus colocynthis* fruit contained 8.75% total ash, 17.15% crude protein (CP), 12.15% ether extract (EE), 29.4% cellulose, 55.3% neutral detergent fiber (NDF), 45.8% acid detergent fibre (ADF) and 15.7% acid detergent lignin (ADL) on dry matter basis whereas its peel-pulp and seed contained 16.0 and 2.35% total ash, 12.9 and 21.95% CP, 8.0 and 14.8% EE, 23.8 and 30.4% cellulose, 38.4 and 63.7% NDF and 32.2 and 45.3% ADF, respectively. Earlier studies observed that protein, fat and ash content varied from 13.19 to 26.86%, 14.48 to 24.62% and 2.00 to 4.46%, respectively in seeds of *Citrullus colocynthis* (Sadou *et al.* 2007) whereas, as per NRC (2006), the seed kernels contain about 50% oil, 30% protein, 10% carbohydrate, 4% ash and 3% fiber. The chemical analysis of total mixed ration (% DM) which was used as substrate for *in vitro* analysis had shown 21.45% CP, 3.35% EE, 20.5% cellulose, 57.4% NDF and 26.1% ADF content.

Phytochemical screening of different parts of *Colocynth* for bioactive components (Table 1) revealed that total phenols, non-tannin phenols, true tannins and aqueous saponin content were highest ($P < 0.001$) in *Citrullus colocynthis* fruit as a whole in comparison to peel-pulp and seed whereas peel-pulp of *Citrullus colocynthis* was rich ($P < 0.01$) in total flavonoids and vitamin C in comparison to seed however values remained at par with whole fruit. Methanolic extraction of whole fruit, peel-pulp and seed of *Citrullus colocynthis* revealed that methanol soluble saponin content remained comparable ($P > 0.05$) among different

Table 1. Active components in colocynth, % DM basis

Parameter	Whole	Peel-pulp	Seed	P value
Total phenolics***	1.15 ^a	1.91 ^c	1.27 ^b	0.000
Non tannin phenols***	0.35 ^b	0.53 ^c	0.16 ^a	0.000
True tannins***	0.79 ^a	1.37 ^c	1.11 ^b	0.000
Vitamin C**	0.076 ^b	0.078 ^b	0.014 ^a	0.005
Flavonoids**	0.15 ^b	0.14 ^b	0.11 ^a	0.003
Aq. saponin***	0.604 ^b	0.861 ^c	0.373 ^a	0.000
Meth. Saponin	5.20	5.63	5.33	0.292
Sugars***	3.49 ^b	5.40 ^c	1.17 ^a	0.000

Figures with different superscripts in a row differ significantly, ***($P < 0.001$), **($P < 0.01$).

parts. Earlier, Kumar *et al.* (2008) reported that seeds of *Citrullus colocynthis* contained 0.74% total phenols and 0.13% flavonoids on DM basis.

Level of Citrullus colocynthis supplementation and rumen fermentation characteristics, irrespective of its part: Results of the *in vitro* studies indicated that varying level of *Citrullus colocynthis* had significant effect on rumen fermentation characteristics when TMR was used as substrate (Table 2). The NGP (mL/24/g) was significantly ($P<0.001$) higher at 1% level of supplementation as compared to control whereas it remained comparable with 0.5 and 1.5% supplementation levels. The findings are in line with Singh *et al.* (2018) who reported higher ($P<0.05$) NGP on supplementation of tannin and saponin containing

herbs to total mixed ration at increasing levels. No difference in the digestibility of NDFD and true OM was observed at varying levels of *Citrullus colocynthis*, however depression in digestibility was observed at 4.0% level, which might be due to the cumulative effect of tannins, saponins and flavonoids or their interaction with rumen microbes at comparatively higher concentration. Contrary to present findings, Hundal *et al.* (2016a) observed reduced digestibility of NDF and true OM significantly ($P<0.01$) in the tannin supplemented groups as compared to control groups at all levels, but remained comparable within the tannin supplemented groups. Similarly, Patra *et al.* (2006) noted that addition of extract of plants containing tannin reduced DM and true OM digestibility by about 7% in

Table 2. Effect of supplementation of different levels of *Citrullus colocynthis* fruit on *in-vitro* fermentation characteristics, irrespective of its part

Parameter	Control	Level of <i>Citrullus colocynthis</i> fruit (% DMB)						P value
		0.5	1.0	1.5	2.0	2.5	4.0	
<i>Fermentation parameters</i>								
NGP, ml/24 h/ g***	125.7 ^a	136.8 ^{ab}	142.9 ^b	138.6 ^{ab}	135.7 ^a	135.4 ^a	132.3 ^a	0.000
NDFD, %**	39.02 ^{ab}	38.6 ^{ab}	43.8 ^b	42.8 ^{ab}	40.91 ^{ab}	40.62 ^{ab}	37.72 ^a	0.005
TOMD, %**	63.2 ^{ab}	60.6 ^a	64.8 ^b	64.0 ^{ab}	62.8 ^{ab}	62.5 ^{ab}	60.4 ^a	0.006
PF, mg/ml	1.50	1.31	1.35	1.78	1.89	1.43	2.79	0.377
ME, MJ/kg DM***	7.53 ^a	7.81 ^{bc}	7.97 ^c	7.85 ^{bc}	7.86 ^{bc}	7.80 ^{bc}	7.68 ^{ab}	0.000
CH ₄ , % of NGP***	35.5 ^c	33.4 ^b	31.7 ^a	31.5 ^a	31.5 ^a	31.2 ^a	30.5 ^a	0.000
CH ₄ , ml/g DM***	44.5 ^{cd}	45.7 ^d	45.4 ^{cd}	43.7 ^{bc}	42.6 ^b	42.2 ^b	40.1 ^a	0.000
NH ₃ -N, mg/dl *	0.025 ^b	0.021 ^a	0.022 ^a	0.021 ^a	0.022 ^a	0.023 ^{ab}	0.0212 ^a	0.044
<i>Volatile fatty acid production, mM/dl</i>								
TVFA*	6.77 ^b	6.31 ^{ab}	6.56 ^{ab}	6.30 ^{ab}	6.12 ^{ab}	6.10 ^{ab}	5.91 ^a	0.016
Acetate*	4.48 ^b	4.12 ^{ab}	4.31 ^{ab}	4.18 ^{ab}	4.06 ^{ab}	4.11 ^{ab}	3.80 ^a	0.046
Propionate*	1.81 ^b	1.61 ^a	1.65 ^a	1.60 ^a	1.53 ^a	1.54 ^a	1.52 ^a	0.013
Butyrate	0.286	0.272	0.266	0.258	0.252	0.242	0.237	0.088
A:P	2.47	2.56	2.62	2.61	2.66	2.67	2.50	0.052

Figures with different superscripts in a row differ significantly, *** ($P<0.001$), ** ($P<0.01$), * ($P<0.05$).

Table 3. Effect of supplementation of different parts of *Citrullus colocynthis* fruit on *in vitro* fermentation characteristics, irrespective of its level and their interaction with level

Parameters	Control	Part of <i>Citrullus colocynthis</i> fruit (% DM)			P value	Parts of <i>Colocynth</i> × Level
		Whole	Peel-pulp	Seed		
<i>Fermentation parameters</i>						
NGP, ml/24 h/g**	125.7 ^a	133.3 ^b	135.8 ^b	139.6 ^b	0.001	*
NDFD, %	39.02	40.8	41.5	40.5	0.905	NS
TOMD, %	63.1	63.1	63.2	61.3	0.228	NS
PF, mg/ml	1.50	2.24	1.60	1.54	0.578	NS
ME, MJ/kg/DM**	7.53 ^a	7.78 ^b	7.83 ^b	7.88 ^b	0.005	NS
CH ₄ , % of NGP***	35.5 ^b	31.6 ^a	32.2 ^a	31.0 ^a	0.000	NS
CH ₄ , ml/g DM***	44.5	43.8	43.4	43.06	0.616	NS
NH ₃ -N, mg/dl **	0.025 ^b	0.022 ^a	0.021 ^a	0.022 ^b	0.008	***
<i>Volatile fatty acid production, mM/dl</i>						
TVFA***	6.77 ^b	6.59 ^b	5.97 ^a	6.09 ^a	0.000	*
Acetate***	4.48 ^b	4.42 ^b	3.94 ^a	3.93	0.000	NS
Propionate***	1.81 ^c	1.67 ^b	1.52 ^a	1.54 ^a	0.000	NS
Butyrate***	0.285 ^b	0.274 ^b	0.234 ^a	2.55 ^{ab}	0.000	NS
A:P	2.47	2.65	2.60	2.56	0.092	NS

Figures with different superscripts in a row differ significantly, *** ($P<0.001$), ** ($P<0.01$).

comparison to control in *in vitro*. The difference may be attributed to the variability in type, dose and source of plant secondary metabolites used in two studies.

The availability of ME (MJ/Kg DM) followed the trend of NGP and reported ($P<0.001$) higher at 1.0% level of *Citrullus colocynthis* supplementation in TMR. In present study, the partitioning factor—an index for efficiency of utilization of organic matter remained statistically ($P>0.377$) comparable at all inclusion levels of herb and is supported by findings of Hundal *et al.* (2016 a&b).

Methane production (CH_4 , % NGP) which indicates loss of energy, reduced significantly ($P<0.001$) on supplementation of colocynth at all levels in comparison to control but remained at par among different treatment groups. Hundal *et al.* (2016a) had reported similar effects with increasing tannin level supplementation on methane production. Hariadi BT and Santoso B (2010) also reported that CH_4 production decreased with increasing concentration of total tannin in plants. The positive or negative effects of tannin on CH_4 production may vary depending on the amount of tannin. The condensed tannins reduce methane production from ruminants either indirectly through a reduction in fibre digestion, which decreases H_2 production or directly through inhibition of the growth of methanogens (Hundal *et al.* 2019a).

The $\text{NH}_3\text{-N}$ production was reduced ($P<0.05$) on inclusion of herbs (0.5–4%) comparable to control and the findings are in agreement with Hundal *et al.* (2016a) who also reported depressed $\text{NH}_3\text{-N}$ at 3 to 5% level of supplementation of tannins to the substrate. Irrespective of the part of colocynth supplementation, total VFA production and acetate were found at par among control and test levels however significant depression in both ($P<0.05$) was observed at 4.0% supplementation level.

Supplementation of Citrullus colocynthis fruit, seeds or peel-pulp and rumen fermentation characteristics, irrespective of its level: The supplementation of *Citrullus colocynthis* fruit or its parts led to significant increase in NGP ($P<0.001$) and ME ($P<0.01$) availability in comparison to control group. No significant effect of inclusion of colocynth with respect to PF ($P>0.578$), digestibility of NDF ($P>0.905$) and total OM ($P>0.228$) were observed among different treatment groups (Table 3). Supplementation of different parts of colocynth resulted in significant ($P<0.001$) depression in methane production from 9% (peel-pulp group) to 12.7% (seed group) as compared to control group. Earlier researchers opined that tannins and saponins content in plants inhibited ruminal protozoa and methanogens which might be related with decrease in methanogenesis in *in vitro* studies (Makkar *et al.* 1995, Hristov *et al.* 2003, Patra AK and Saxena J 2011). Moreover, Oskoueian *et al.* (2013) observed the inhibitory effect of flavonoids on total protozoa and methanogens as well as ruminal methane production. *Citrullus colocynthis* fruit, seeds and peel-pulp used in treatment groups contained varying concentration of tannins, saponins, flavonoids and vitamin C, which individually or interactively led to reduction in

methanogenesis in the present study.

Ruminal $\text{NH}_3\text{-N}$ concentration was reported lower ($P<0.01$) in *Citrullus colocynthis* fruit or peel-pulp supplemented group whereas values remained statistically analogous in control and seed supplemented group which may resulted from increase in incorporation of ammonia for synthesis of microbial protein. The microbial protein synthesis efficiency or better synchronization of nutrients in the presence of tannins improved microbial protein synthesis in rumen (Makkar *et al.* 1995).

TVFA production, acetate, propionate and butyrate concentration was observed lower ($P<0.001$) in peel-pulp and seed supplemented group in comparison to control and whole fruit supplemented group, however acetate to propionate ratio remained unaffected between different test groups (Table 3). The lower levels of TVFA and acetate in groups supplemented with peel-pulp and seed might be due to inhibitory effect on rumen microbes as colocynth has cucurbitacins which exhibited potential antibacterial activity (Hussain *et al.* 2014).

Interaction between level and different parts of Citrullus colocynthis fruit: Interactions between level $\times$ different parts of *Citrullus colocynthis* fruit w.r.t. fermentation characteristics are presented in Table 3. It was observed that interactions between different parts of *Citrullus colocynthis* fruit (fruit as a whole, seeds or peel-pulp) at different levels (0%, 0.5%, 1%, 1.5%, 2%, 2.5% or 4%) of supplementation had significant influence on NGP (ml/24 h/g), $\text{NH}_3\text{-N}$ (mg/dl) and TVFA (mM/dl) in *in vitro* evaluation. The findings are in line with Singh *et al.* (2018) who reported strong interaction between type of herb and its level w.r.t. to various fermentation characteristics, however contrary to the results of Singh *et al.* (2018), the interaction between herb and its level didn't influence ME availability, methane production, fermentation efficiency, digestibility of NDF and TOM in our study. The difference observed may be attributed to the type of herb, concentration of bioactive components and their interaction with each other or with rumen microbes during *in vitro* incubation.

From this study it is concluded that the addition of *Citrullus colocynthis* fruit had potential to improve rumen fermentation characteristics and *in vitro* methanogenesis however the best response with respect to the net gas production, digestibility of nutrients, methane production and ME availability from TMRs was observed at 1% level of supplementation but *in vivo* assessment on ruminants need to be conducted to evaluate the persistency of effects along with health concerns.

REFERENCES

- AOAC. 2007. Official Methods of Analysis. 18thedn. Association of Official Analytical Chemists, Arlington, VA.
- Cottyn B G and Boucque C V. 1968. Rapid methods for the gas chromatographic determination of volatile acids in rumen fluid. *Journal of Agriculture and Food Chemistry* **16**: 10–07.
- Hariadi B T and Santoso B. 2010. Evaluation of tropical plants containing tannin on *in vitro* methanogenesis and fermentation parameters using rumen fluid. *Journal of the Science of Food*

- and Agriculture **90**(3): 456–61.
- Hristov A N, Ivan M, Neill L and McAllister T A. 2003. Evaluation of several potential bioactive agents for reducing protozoal activity *in vitro*. *Animal Feed Science and Technology* **105**: 163–84.
- Hundal J S, Singh I, Wadhwa M, Singh C, Uppal C and Kaur G. 2019a. Effect of *Punica granatum* and *Tecomella undulata* supplementation on nutrient utilization, enteric methane emission and growth performance of Murrah male buffaloes. *Journal of Animal and Feed Science* **28**: 110–19.
- Hundal J S, Wadhwa M and Bakshi M P S. 2016a. Effect of supplementing essential oils on the *in vitro* methane production and digestibility of wheat straw. *Journal of Research and Animal Nutrition* **1**: 1–14.
- Hundal J S, Wadhwa M and Bakshi M P S. 2016b. Methane production potential of tannins and their impact on digestibility of nutrients. *Animal Nutrition and Feed Technology* **16**: 505–13.
- Hundal J S, Wadhwa M and Bakshi M P S. 2019b. Herbal feed additives containing essential oil: 1. Impact on the nutritional worth of complete feed *in vitro*. *Tropical Animal Health and Production* <https://doi.org/10.1007/s11250-019-01887-1>
- Hussain A I, Rathore H A, Sattar M Z, Chatha S A, Sarker S D and Gilani A H. 2014. *Citrullus colocynthis* (L.) Schrad (bitter apple fruit): A review of its phytochemistry pharmacology traditional uses and nutritional potential. *Journal of Ethno Pharmacology* **155**(1): 54–66.
- ICAR. 2013. *Nutrient Requirements of Cattle and Buffalo*. Indian Council of Agricultural Research, New Delhi, India.
- Kumar S, Kumar D, Manjush A, Saroha K, Singh N and Vashishta B. 2008. Antioxidant and free radical scavenging potential of *Citrullus colocynthis* (L.) Schrad. methanolic fruit extract. *Acta Pharmaceutica* **58**: 215–20.
- Makkar H P S, Blummel M and Becker K. 1995. *In vitro* effects and interactions between tannins and saponins and fate of tannins in the rumen. *Journal of Science Food and Agriculture* **69**: 481–93.
- Menke K H and Steingass H. 1988. Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. *Animal Research and Development* **28**: 7–55.
- Menke K H, Raab L, Salweski A, Steingass H, Fritz D and Scheider W. 1979. The estimation of digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor *in vitro*. *The Journal of Agriculture Science* **93**: 217–22.
- NRC. 2006. Lost crops of Africa: volume II: vegetables. The National Academies Press Washington DC.
- Oskoueian E, Abdullah N and Oskoueian A. 2013. Effects of flavonoids on rumen fermentation activity, methane production and microbial population. *Biomedical Research International* **8**: 1–8.
- Patra A K and Saxena J. 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *Journal of the Science of Food and Agriculture* **91**: 24–37.
- Patra A K, Kamra D N and Agarwal N. 2006. Effect of plant extracts on *in vitro* methanogenesis, enzyme activities and fermentation of feed in rumen liquor of buffalo. *Animal Feed Science and Technology* **128**: 276–91.
- Patra A K, Kamra D N, Bhar R, Kumar R and Agarwal N. 2011. Effect of *Terminalia chebula* and *Allium sativum* on *in vivo* methane emission by sheep. *Journal of Animal Physiology and Animal Nutrition* **95**: 187–91.
- Rira M, Chentli A, Boufenerab S and Boussebouaa H. 2015. Effects of plants containing secondary metabolites on ruminale methanogenesis of sheep *in vitro*. *Energy Procedia* **74**: 15–24.
- Sadou H, Sabo H, Alma M M, Saadou M and Leger C L. 2007. Chemical content of the seeds and physico-chemical characteristic of the seed oils from *Citrullus colocynthis*, *Coccinia grandis*, *Cucumis metuliferus* and *Cucumis prophetarum* of Niger. *Bulletin of the Chemical Society of Ethiopia* **21**(3): 323–30.
- Sagar R and Dumka V K. 2018. A Brief Review on Bitter Apple-Pharmacological Activities. *International Journal of Current Microbiology and Applied Sciences* **7**: 281–87.
- Singh I, Hundal J S, Wadhwa M and Lamba J S. 2018. Assessment of potential of some tannins and saponins containing herbs on digestibility of nutrients, fermentation kinetics and enteric methane production under different feeding systems: An *in vitro* study. *Indian Journal of Animal Science* **88**(4): 443–52.
- SPSS. 2012. Statistical Packages for Social Sciences. Version 20, SPSS Inc., Illinois, USA.
- Van Soest P J, Robertson J B and Lewis B A. 1991. Methods of dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**: 3583–97.
- Yadav N D and Singh P M. 1992. Tumba and Mateera cultivation in the Indian Arid Zone. Central Arid Zone Research Institute Jodhpur, Rajasthan, India.