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INFLUENCE OF DIFFERENT TYPES AND AMOUNTS OF SPICES ON THEIR *IN VITRO* DEGRADABILITY, FERMENTATION, GAS AND METHANE PRODUCTION

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Abstract

This paper reports the *in vitro* degradability and fermentation of different amounts (0.25, 0.5 and 1 g 100 ml^{-1} of rumen fluid = RF) of five spices (cinnamon, cumin, coriander, turmeric and clove) alone or with rice straw during different incubation times. The main effects of spices were also considered for gas and methane production. Significant (P<0.001) differences were observed in *in vitro* dry matter degradability (IVD) and *in vitro* organic matter degradability (IVOMD) of spices and pH, ammonia and volatile fatty acids (VFA) of RF at 48 and 144 h. The IVD, IVOMD, ammonia and VFA were highest for cumin and lowest for cinnamon. IVD, IVOMD and pH were higher when smaller amount (0.25 g 100 ml^{-1}) of spices were used (P<0.001). All the spices increased IVD, IVOMD, pH, ammonia and VFA at longer incubation time. The pH was lowest in turmeric. While VFA were greater for the larger amount of all spices, ammonia was greater for the larger amounts of only cumin and coriander. The molar proportion of acetic acid was lowest for turmeric. The gas and methane production was higher for the low amounts of spices where total gas volume was highest in turmeric and lowest in cinnamon (P<0.05).

Keywords: Spices, degradability, ammonia, gas production, methane production.

Introduction

Essential oils and plant extracts have been of interest to animal researchers after the banning of antibiotics for their in-feed use to manipulate rumen function and animal production. Different plant extracts and secondary plant metabolites including saponins, tannin, anise oil, capsicum extract, clove oil, eucalyptus oil, garlic oil, origanum oil, peppermint oil, junipor oil, eugenol and cinnamaldehyde can alter ruminal microbial fermentation (Patra and Yu, 2012; Morsey *et al.,* 2012; Benchaar *et al.,* 2012). In general, at high doses of essential oils reduced ammonia nitrogen concentration and methane production (Benchaar *et al.*, 2008; Benchaar *et al.*, 2009). However, the long-term *in vitro* and *in vivo* studies suggested that benefits associated with essential oils diminish over time due to shifts in microbial populations or adaptation of individual microbial species to essential oils, for example (Benchaar *et al.*, 2008). Sometime some essential oils showed low potential for their use as a feed additive in dairy cow nutrition (Benchaar *et al.,* 2012). Some spices are high in tannin, saponins, essential oils and others are high in fatty acids. The extraction process of essential oils from different spices may increase the cost of animal feed and so the farmers from poor countries will not be able to use these spices economically. Conversely, the whole herbs or spices may contain some other useful components that might be more useful and may have more beneficial effects in the consortium than the essential oils or plant extracts alone on rumen fermentation (Khan and Chaudhry, 2010). Recently researchers (Chaudhry and Khan, 2012; Khan and Chaudhry, 2010; Jahani-Azizabadi *et al.*, 2010; Frankič *et al.,* 2009; Karami *et al.,* 2008) have used whole spices as supplements to modify rumen fermentation of various feed ingredients. However, it will be highly commendable to compare the *in vitro* degradability and fermentation profiles of various amounts of different spices before their use as additives to improve the utilization of various ruminant feeds. This paper includes two studies. In the first study five spices (cinnamon, cumin, coriander, turmeric and clove) were used alone in different amounts (0.25 g 100^{-1} ml, 0.5 g 100^{-1} ml, 1 g 100^{-1} ml) to observe their degradability and fermentation profiles. In the second study the main effects of spices were considered to evaluate the effect of spices on degradability and fermentation profile and to compare the gas and methane production by them.

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Materials and Methods

Feed Materials: Five spices were selected on the basis of their availability, cost and potential for use as feed additives. Here spices represented by cumin (Cm = *Cuminum cyminum*), coriander (Cr = *Coriandum sativum)*, clove (Cl = *Syzygium aromaticum*), cinnamon (Ci = *Cinnamomum cassia*) and turmeric (Tu = *Curcuma longa*) were collected from the local market of Newcastle upon Tyne, UK. The detailed chemical compositions of spices were carried out in this lab as shown in Table 1 and 2 using procedures as described by Chaudhry and Khan (2012) and Khan and Chaudhry (2010).

Table 1. Proximate composition (g kg^{-1} DM, unless stated otherwise) and fibre content, total sugars, starch, total phenolics, total tannins, condensed tannin and saponins of different spices (Modified from Khan and Chaudhry, 2010 and Chaudhry and Khan, 2012).

Spices	Ci	Cl	Cr	Cm	Tu
DM (g kg^{-1} fresh basis)	950	920	940	960	950
Crude Pprotein	74	102	106	223	86
Ash	43	85	65.6	80	69.4
Ether Exrtract	34	73	155	146	26
Neutral detergent fibre	450	280	540	550	380
Acid detergent fibre	540	250	380	240	300
Acid detergent Lignin	300	180	140	110	90
Total sugar	26	53	75	65	68
Starch	169	103	111	80	542
Total Phenol (TE)	51	214	11	19	22
Total tannin (TE)	36	109	2.8	4	11
Condensed tannin (CE)	30	NP	NP	NP	NP
Saponin (DE)	62	47	17	44	38

Ci = Cinnamon; Cl = Clove; Cr = Coriander; Cm = Cumin; Tu = Turmeric; TE = Tannic acid equivalent; CE = Catechin equivalent; DE = Diosgenin equivalent; NP= Not present;

Table 2. Mean mineral components (mg kg^{-1} DM) of different spices (Modified from Khan and Chaudhry, 2010).

	Ci	Cl	Cr	Cm	Tu
Ca	9630	9999	5970	8303	1539
K	3758	16403	15015	14180	24126
Mg	735	2794	2760	2700	2418
Na	360	2700	1275	2296	788
P	442	1135	3945	3969	2309
Cu	2.93	4.08	10.34	5.50	8.24
Co	2.11	1.97	1.76	1.78	0.68
Mn	196	612	27.9	50	22
Se	3.56	6.65	4.88	9.20	1.94
Zn	18.2	17.7	42.6	50.4	11.1

Ci = Cinnamon; Cl = Clove; Cr = Coriander; Cm = Cumin; Tu = Turmeric

Collection of rumen fluid from fistulated sheep: Rumen fluid (RF) was obtained from two fistulated sheep (Lleyn breed) with mean live-weight of 81 kg just before their morning feeding. Details method of collection of RF, management of sheep, preparation of buffered was as followed as Khan and Chaudhry, 2010.

***In vitro* degradability and fermentation profile of different amounts of spices:** This 5 × 3 × 2 factorial study examined the *in vitro* degradability and fermentation of five spices (turmeric, cinnamon, cumin, clove and coriander) by incubating each at three amounts (0.1, 0.2 and 0.4 g) in 40 ml of the buffered inoculum at two incubation times of 48 and 144 h using 50-ml polypropylene tubes. The tubes were purged with CO_2, sealed with rubber stoppers fitted with pressure release valves and incubated at 39°C in a water bath. After each incubation time, the tubes were submerged in an ice box to stop further fermentation. The pH was immediately measured before the liquid and residue were separated by centrifuging the tubes at 3000 rpm for 10 minutes. The supernatant of buffered RF was collected to determine the VFA and ammonia concentration. For the determination of VFA, 2 ml supernatants were collected to which 0.25 ml of deproteinising solution was mixed and the mixtures in tubes were then kept in a fridge until their analysis. For ammonia analysis, 2 ml of supernatant was acidified with 2 ml of 1 N HCl and kept in a fridge. The residues were washed with distilled water and used to determine dry matter (DM) and organic matter (OM) to estimate *in vitro* DM (IVD) and OM degradability (IVOMD), ammonia and VFA as described by Khan and Chaudhry (2010).

Total gas and methane production from different spices: Above mentioned five spices were used in a fixed amount (0.4 g) with 40 ml of the same concentrated buffered inoculum to observe the gas and methane production in the presence of different spices alone. Gas production from each incubation tube was measured using 30 ml glass syringes (Sanitex) for each treatment combination. Each syringe was attached to a 50-ml polypropylene tube containing feed sample, a spice and inoculum using transparent silicone tubing (bore size 4 mm, wall thickness 1.6 mm, Fisherbrand, UK) via plastic luer stopcocks (Male luer, Cole palmer, UK). These stopcocks were used to control the gas entry into these syringes by turning these on and off. Total gas production from each treatment combination was estimated by observing the displacement of piston in each syringe during the *in vitro* incubation of each spice at 6, 24, 48, 72, 96, 120 and 144 h. Methane was determined according to the method described by Upstill-Goddard *et al*. (1996) and Chaudhry and Khan (2012). A Completely Randomized Design (CRD) was used to assess the total gas volume for 0 to 144 h and methane production for 144 h only.

Statistical Analyses: The data from the first study were analyzed using General Linear Model of Minitab in a 5 × 3 factorial arrangement. The main effects of spices and amounts of spices in buffered RF and their interactions were considered for degradability, pH, ammonia production, total and individual VFA production. The data from the second study were analyzed using one way analysis of Minitab. The main effects of spices were considered for gas and methane production. Significant differences between means for each main effect and their interactions were compared using the Tukeys test at P<0.05.

Results and Discussion

Results

***In vitro* degradability and fermentation profile of different amounts of spices:** Significant (P<0.001) differences were observed in IVD and IVOMD of spices at 48 and 144 h (Tables 3 and 4). The IVD and IVOMD were highest of cumin but lowest for cinnamon. IVD and IVOMD were higher when smaller amount (0.25 g 100 ml^{-1}) of spices were used (P<0.001). All the spices increased IVD and IVOMD at higher incubation time. The differences of IVD and IVOMD of coriander at two different incubation times were very low. On the other hand, the IVD and IVOMD increased greatly in turmeric at longer incubation time. The interaction between spices and amount of spices was also significant (P<0.001) for degradability for both 48 and 144 h of incubation. The difference of IVD with different amounts of spices was greater in clove and less in coriander. At 48 h the IVD of clove was greater than that of turmeric but at 144 h the IVD of turmeric was greater than clove.

Table 3. Mean IVD of different spices for their different amounts (g 100^{-1} ml) for 2 incubation hours.

IH	Spice amount	Ci	Cl	Cr	Cm	Tu	Mean
48	0.25	325	746	701	764	637	635
	0.50	276	636	693	795	511	582
	1.00	259	528	688	745	416	527
	Mean	287	637	694	768	521	
	SEM = 33.2	P for S < 0.001		P for C < 0.001		P for S × C < 0.001	
144	0.25	409	804	719	841	867	728
	0.50	389	795	714	813	840	710
	1.00	353	637	704	789	643	625
	Mean	384	745	712	814	783	
	SEM = 30.9	P for S < 0.001		P for C < 0.001		P for S × C < 0.001	

IVD = In vitro dry matter degradability; IH = Incubation hour; Ci = Cinnamon; Cl = Clove; Cr = Coriander; Cm = Cumin; Tu = Turmeric

Table 4. Mean IVOMD of different spices for their different amounts (g 100 ml^{-1}) for 2 incubation hours.

IH	Spice amount	Ci	Cl	Cr	Cm	Tu	Mean
48	0.25	302	659	648	770	563	588
	0.50	252	604	638	768	473	547
	1.00	225	519	636	742	453	515
	Mean	260	594	641	760	496	
	SEM = 32.1	P for S < 0.001		P for C < 0.001		P for S × C < 0.005	
144	0.25	399	784	684	805	823	699
	0.50	369	754	674	794	790	676
	1.00	312	627	666	786	697	618
	Mean	360	722	675	795	770	
	SEM = 30.7	P for S < 0.001		P for C < 0.002		P for S × C < 0.3	

IVOMD = In vitro organic matter degradability; IH = Incubation hour; Ci = Cinnamon; Cl = Clove; Cr = Coriander; Cm = Cumin; Tu = Turmeric

The value of pH also varied (P<0.001) with the type of spices and amount of spices used in the incubation. The pH was lowest in turmeric and highest in cumin at 48 h and in coriander at 144 h (Table 5). The pH was lower when higher amounts of spices were incubated. On an average, the pH was higher at 144 h than 48 h.

Table 5. Mean pH of different spices for their different amounts (g 100 ml^{-1}) for 2 incubation hours.

IH	Spice amount	Ci	Cl	Cr	Cm	Tu	Mean
48	0.25	7.57	7.38	7.50	7.58	7.39	7.48
	0.50	7.44	7.32	7.49	7.48	7.28	7.40
	1.00	7.33	7.22	7.35	7.38	7.17	7.29
	Mean	7.45	7.31	7.45	7.48	7.28	
	SEM = 0.023	P for S < 0.001		P for C < 0.001		P for S × C < 0.001	
144	0.25	7.91	7.80	7.90	7.88	7.67	7.83
	0.50	7.76	7.78	7.80	7.70	7.47	7.70
	1.00	7.63	7.55	7.74	7.60	7.32	7.57
	Mean	7.77	7.71	7.81	7.73	7.49	
	SEM = 0.033	P for S < 0.001		P for C < 0.001		P for S × C < 0.001	

Ci = Cinnamon; Cl = Clove; Cr = Coriander; Cm = Cumin; Tu = Turmeric

Table 6. Mean ammonia (mg l^{-1}) in rumen fluid after incubation with different spices in different amounts (g 100 ml^{-1}) at 48 and 144 h.

IH	Spice amount	Ci	Cl	Cr	Cm	Tu	Mean
48	0.25	40	55	129	146	36	81
	0.50	34	34	163	164	19	83
	1.00	4	20	183	173	11	78
	Mean	26	36	158	161	22	
	SEM = 12.4	P for S < 0.001		P for C < 0.331		P for S × C < 0.001	
144	0.25	88	96	164	186	94	126
	0.50	65	77	186	199	75	120
	1.00	36	35	198	204	9	96
	Mean	63	69	183	196	59	
	SEM = 12.4	P for S < 0.001		P for C < 0.001		P for S × C < 0.001	

Ci = Cinnamon; Cl = Clove; Cr = Coriander; Cm = Cumin; Tu = Turmeric

The ammonia in RF from donor sheep was 170 mg l^{-1} which was within the acceptable range to maintain rumen function. In a blank treatment, where no spices were used, ammonia was 66 mg l^{-1} of the buffered RF at 48 h and 86 mg l^{-1} at 144 h. Ammonia differed significantly (P<0.001) in the presence of different spices. Cumin and coriander produced higher ammonia compared to the other spices. The ammonia of RF incubating cinnamon, clove and turmeric were even lower than the blank sample at 48 h and the ammonia of RF incubating higher amount of these three spices were lower than that of the blank sample at 144 h. Ammonia were also affected by the amount of spices. Ammonia was greater for large amounts of cumin and coriander but smaller for the larger amounts of other spices. Ammonia was higher for longer incubation time for all spices (Table 6).

Table 7. Mean VFA concentration (mmol l^{-1}) in rumen fluid after incubation with different spices in different amounts (g 100 ml^{-1}) at 48 and 144 h.

IH	Spice amount	Ci	Cl	Cr	Cm	Tu	Mean
48	0.25	52	55	55	73	61	59
	0.50	39	62	67	91	66	65
	1.00	64	72	80	102	81	80
	Mean	52	63	67	89	69	
	SEM = 3.73	P for S < 0.001		P for C < 0.001		P for S × C < 0.09	
144	0.25	53	57	71	78	61	64
	0.50	43	67	91	95	86	76
	1.00	70	105	111	111	118	103
	Mean	55	76	91	95	88	
	SEM = 4.35	P for S < 0.001		P for C < 0.001		P for S × C < 0.004	

Ci = Cinnamon; Cl = Clove; Cr = Coriander; Cm = Cumin; Tu = Turmeric

Table 8. Molar proportion of acetic acid (mol 100 mol^{-1}) in rumen fluid after incubation with different spices in different amounts (g 100 ml^{-1}) at 48 and 144 h.

IH	Spice amount	Ci	Cl	Cr	Cm	Tu	Mean
48	0.25	70.4	66.0	66.4	67.6	63.3	66.7
	0.50	62.1	65.6	63.7	65.9	63.4	64.1
	1.00	61.9	65.9	61.1	64.5	60.9	62.9
	Mean	64.8	65.8	63.7	66.0	62.5	
	SEM = 0.58	P for S < 0.04		P for C < 0.001		P for S × C < 0.1	
144	0.25	71.4	67.3	69.9	66.4	66.1	68.2
	0.50	62.3	69.1	63.5	61.5	60.1	63.3
	1.00	58.0	71.0	63.0	60.7	59.4	62.4
	Mean	63.9	69.1	65.5	62.9	61.9	
	SEM = 0.92	P for S < 0.001		P for C < 0.02		P for S × C < 0.02	

Ci = Cinnamon; Cl = Clove; Cr = Coriander; Cm = Cumin; Tu = Turmeric

Table 9. Molar proportion of propionic acid (mol 100 mol^{-1}) in rumen fluid after incubation with different spices in different amounts (g 100 ml^{-1}) at 48 and 144 h.

IH	Spice amount	Ci	Cl	Cr	Cm	Tu	Mean
48	0.25	20.3	20.8	22.6	17.6	27.0	21.7
	0.50	27.7	22.9	22.6	21.5	21.5	23.2
	1.00	27.3	23.0	28.0	23.6	33.9	27.2
	Mean	25.1	22.2	24.4	20.9	27.5	
	SEM = 0.78	P for S < 0.001		P for C < 0.001		P for S × C < 0.09	
144	0.25	22.4	25.8	23.6	25.6	26.2	24.7
	0.50	31.0	25.2	30.4	29.8	32.6	29.8
	1.00	36.5	22.7	28.1	28.8	31.8	29.6
	Mean	30.0	24.6	27.4	28.1	30.2	
	SEM = 0.83	P for S < 0.001		P for C < 0.001		P for S × C < 0.004	

Ci = Cinnamon; Cl = Clove; Cr = Coriander; Cm = Cumin; Tu = Turmeric

VFA concentration varied significantly (P<0.001) in the presence of different spices being highest for cumin and lowest for cinnamon for both the incubation times (Table 7). Large amounts of spices also increased the VFA concentration in RF. The molar proportion of acetic and propionic acid also varied significantly (P<0.001) depending on the type of spices used. The molar proportion of acetic acid was highest for cumin at 48 h and clove at 144 h and lowest for turmeric at both incubation times (Table 8). The reverse result was found for the molar proportion of propionic acid (Table 9). Molar proportion of acetic acid was going down at higher amount of spices. On the other hand, molar proportion of butyric acid was highest for clove at 48 h and for coriander at 144 h and lowest for turmeric at 48 h and cinnamon at 144 h (Table 10). Finally, molar proportions of minor VFA were higher in coriander and cumin than other spices (Table 11).

Table 10. Molar proportion of butyric acid (mol 100 mol^{-1}) in rumen fluid after incubation with different spices in different amounts (g 100 ml^{-1}) at 48 and 144 h.

IH	Spice amount	Ci	Cl	Cr	Cm	Tu	Mean
48	0.25	4.76	8.49	5.76	8.93	5.76	6.74
	0.50	4.78	6.82	6.82	5.93	4.20	5.71
	1.00	5.83	5.79	5.79	5.94	2.97	5.26
	Mean	5.12	7.03	6.12	6.93	4.31	
	SEM = 0.29	P for S < 0.001		P for C < 0.003		P for S × C < 0.008	
144	0.25	2.14	3.37	4.18	4.76	3.32	3.55
	0.50	2.48	3.54	5.32	4.87	3.94	4.03
	1.00	2.69	3.72	5.26	5.04	4.41	4.22
	Mean	2.44	3.54	4.92	4.89	3.89	
	SEM = 0.19	P for S < 0.001		P for C < 0.05		P for S × C < 0.8	

Ci = Cinnamon; Cl = Clove; Cr = Coriander; Cm = Cumin; Tu = Turmeric

Table 11. Molar proportion of minor VFA (mol 100 mol^{-1}) in rumen fluid after incubation with different spices in different amounts (g 100 ml^{-1}) at 48 and 144 h.

IH	Spice amount	Ci	Cl	Cr	Cm	Tu	Mean
48	0.25	4.52	4.73	5.24	5.95	3.98	4.88
	0.50	5.45	5.09	6.91	6.71	3.76	5.58
	1.00	4.98	4.91	7.21	6.05	2.19	5.07
	Mean	4.98	4.91	6.45	6.24	3.31	
	SEM = 0.29	P for S < 0.001		P for C < 0.003		P for S × C < 0.008	
144	0.25	4.06	3.48	4.27	4.24	4.36	4.08
	0.50	2.93	2.14	4.69	4.16	3.35	3.45
	1.00	2.87	1.66	3.93	5.45	3.73	3.53
	Mean	3.29	2.43	4.30	4.62	3.81	
	SEM = 0.19	P for S < 0.001		P for C < 0.05		P for S × C < 0.8	

Ci = Cinnamon; Cl = Clove; Cr = Coriander; Cm = Cumin; Tu = Turmeric

Total gas volume and methane production in the presence of different spices: The total volume of gas produced after incubation of different amounts of different spices is shown in Figs. 1, 2 and 3. Total gas volume varied with the type of spices, amounts of spices and the time of incubation. Per unit gas production was higher when low amounts of spices were used for incubation. Final gas volume was highest in turmeric, when turmeric was incubated at 0.25 and 1 g 100 ml^{-1} RF ($P<0.05$) and turmeric was second highest for gas production at 0.5 g 100 ml^{-1}. On the other hand, final gas volume was lowest in cinnamon at 0.25 and 1.00 g 100 ml^{-1}. Methane production unit^{-1} was higher when lower amount of spices were incubated (Fig. 4). Methane production was highest in turmeric followed by coriander and lowest in cumin though the value was not statistically significant. Concentration of methane was highest in coriander and lowest in cumin (Fig. 5). Methane concentration was higher when low amounts of spices were used as substrates with some exception. The differences of the value for the concentration of methane in different amount of spices were very low in case of cinnamon and the difference of the value was higher in clove.

Discussion

The main objective of the studies was to observe the degradability and fermentation profiles of spices alone as described in the introduction. Higher CP and mineral containing spices, cumin (Tables 1 and 2) showed higher IVD and IVOMD and lower CP and mineral containing spice, cinnamon had lower IVD and IVOMD. Recent studies on the effect of cumin on ruminal ecology has indicated greater digestibility of dry matter and organic matter in forages after inclusion of cumin with ruminal fluid *in vitro* (Khan and Chaudhry, 2010; Miri *et al.*, 2013; Chaudhry and Khan, 2012). In the present study, it was observed that coriander was degraded with short incubation time. Higher soluble sugars and lower total phenol and tannin might have lead coriander to degrade in shorter time. In contrast, turmeric took longer time for degradation. Khan and Chaudhry (2010) and Chowdhury (2012) also reported that turmeric containing forages showed higher degradation at longer incubation time. The result suggested that the chemical composition of turmeric (higher starch) delayed the action of microbes in ruminants. Further research can be considered on this aspect. Higher total phenol and tannins also reduced the IVD and IVOMD of clove in higher amount and due to lower total phenolics and tannins the difference of IVD and IVOMD of coriander was smaller when different amounts were incubated.

Hour	0	6	24	48	72	96	120	144
SEM	0	1.7	2.3	3.5	3.8*	4.1*	4.5*	4.5*

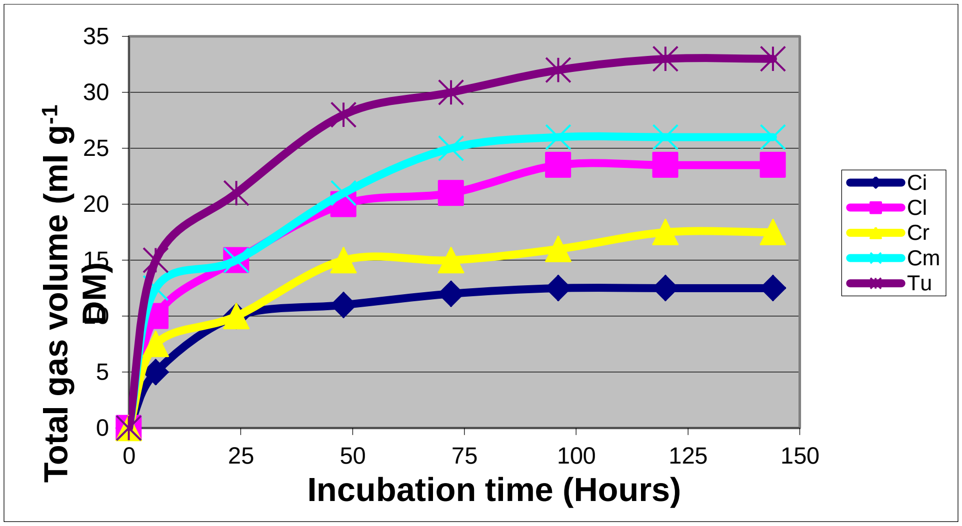

Fig. 1. Total gas volume produced by different spices at 0.25 g 100 ml^{-1} rumen fluid for up to 144 h. * is significant at $P<0.05$.

Hour	0	6	24	48	72	96	120	144
SEM	0	0.48	0.66	1.05	1.00*	0.90*	0.92*	0.93*

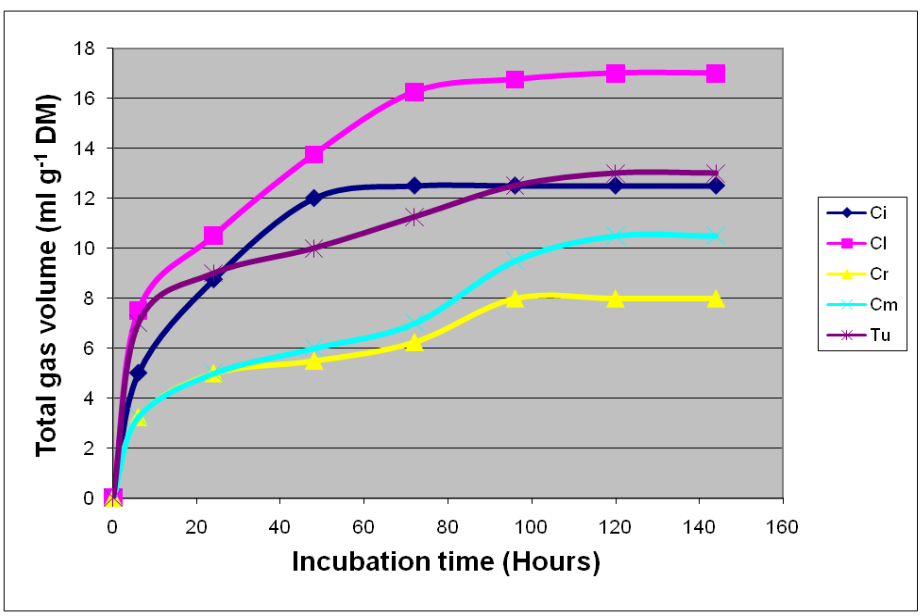

Fig. 2. Total gas volume produced by different spices at 0.5 g 100 ml^{-1} rumen fluid for up to 144 h.

Hour	0	6	24	48	72	96	120	144
SEM	0	0.40	0.43	0.68*	0.70*	0.70*	0.75*	0.76*

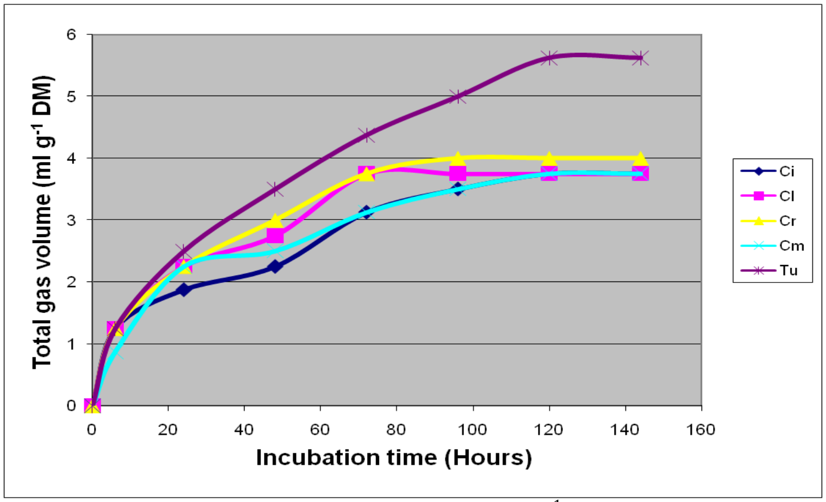

Fig. 3. Total gas volume produced by different spices at 1 g 100 ml^{-1} rumen fluid for up to 144 h. * is significant at $P<0.05$.

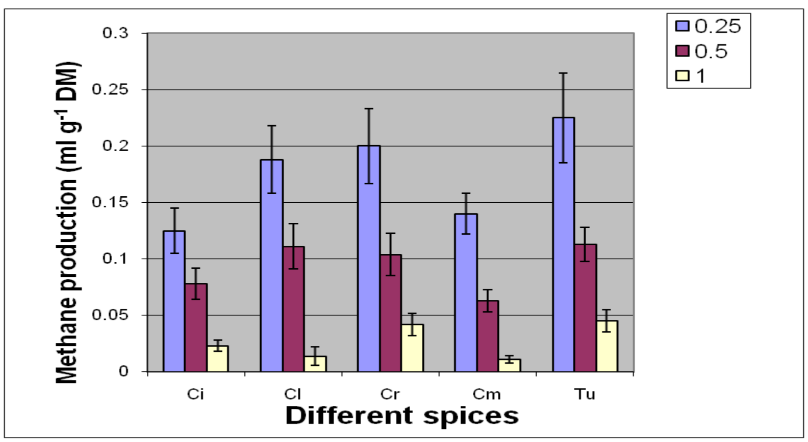

Fig. 4. Methane production of different spices at different amounts (g spices 100 ml^{-1}).

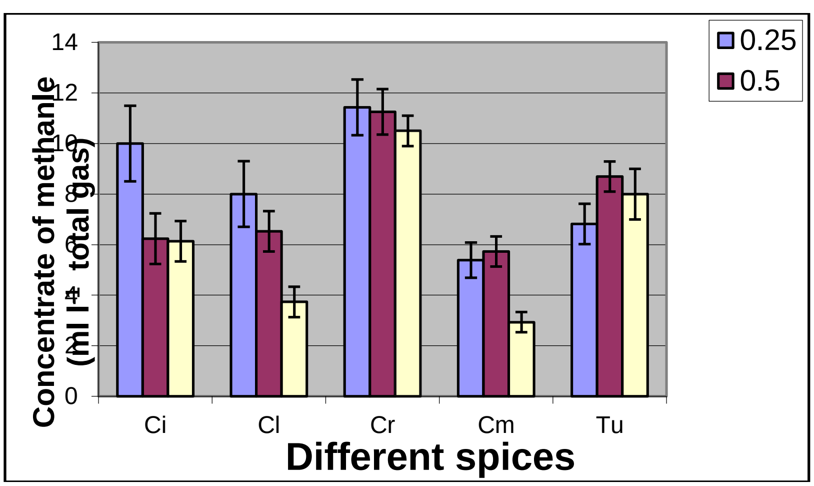

Fig. 5. Concentration of methane in total gas using different spices at different amounts (g spices 100 ml^{-1}).

Higher CP and EE of cumin (Table 1) might have increased the microbial activity and increased the IVD of cumin which resulted in its higher VFA concentration. Coriander was also higher in EE and CP contents than other spices but lower than cumin, this might have caused the value of VFA concentration in coriander very near to cumin. Higher amount of spices might have increased the availability of carbohydrates and other nutrients for the microbes that might have caused higher VFA concentration in the presence of higher amount of spices (Suarez *et al.* 2007). Higher IVD of turmeric at longer incubation time also might have helped turmeric to enhance VFA concentration at that time. Miri *et al*. (2015) observed no effect on molar proportion of VFA for cumin extract in goat.

In terms of ammonia, Khan and Chaudhry (2010) reported that the minimum requirement of CP in ruminant feed to maintain the normal ammonia should be 60 g kg^{-1}. Some of the spices (cinnamon, clove and turmeric) containing more than 70 g kg^{-1} CP reduced ammonia at higher amount which might be due to their higher tannin content. These tannins can precipitate protein (Min *et al.* 2003) and so can reduce ammonia in RF. Therefore, higher CP and lower tannin containing spices (cumin and coriander) produced greater ammonia and cinnamon, low CP or high tannin containing clove and turmeric might have produced lower ammonia.

Highest carbohydrate content in turmeric might have caused higher total gas volume. Highest saponin and lowest soluble sugar contents in cinnamon might have caused lowest final gas volume at higher incubation time as saponin can reduce total gas and methane production (Makkar *et al.* 1998). Lowest IVD of cinnamon also can cause lowest gas volume in cinnamon.

Largest amount of soluble sugars and lowest amount of total phenolics, tannins and saponins in coriander produced highest concentration of methane among the spices. Like gas production highest carbohydrate content in turmeric might also have caused highest methane production. As methane concentration was higher in coriander, it was second highest in coriander. Lowest carbohydrate content in cumin and high level of total phenolics, tannins and saponins in clove and cinnamon produced lowest amounts of methane. Miri *et al*. (2013) reported 15% lower methane emission for cumin seed supplemented groups than control for goats. When higher amount of spices were incubated, they also increased compounds like total phenolics, tannins and saponins, which might have caused lower concentration of methane.

Degradability of spices, pH, ammonia and VFA concentration varied when different amounts of spices were used for the *in vitro* incubations, however the ranking of spices for IVD, pH, ammonia and VFA concentration were not affected by the amount of spices used for incubation. Gas and methane production depended on chemical structure of spices. The chemical composition also might have an effect on different types of microbes that might have affected degradability, fermentation profile and gas and methane production. Different types of microbial count for spices can be considered for the future research.

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References

AFRC. 1993. Energy and protein requirements of ruminants. CAB International, Wallingford, Oxon OX10 8DE, UK. 175.

Benchaar C, Calsamiglia S, Chaves A V, Fraser G R, Colombatto D, McAllister T A and Beauchemin K A. 2008. A review of plant-derived essential oils in ruminant nutrition and production. Anim. Feed Sci. Technol. 145:209-228.

Benchaar C, Hristov A N and Greathead H. 2009. Essential oils as feed additives in ruminant nutrition, In: T, S. (Ed.), Phytogenics in Animal Nutrition, Nottingham University Press, Nottingham, UK.

Benchaar C, Lettat A, Hassanat F, Yang W Z, Forster R J, Petit H V and Chouinard P Y. 2012. Eugenol for dairy cows fed low or high concentrate diets: Effects on digestion, ruminal fermentation characteristics, rumen microbial populations and milk fatty acid profile, Anim. Feed Sci. Technol. 178:139 -150.

Chaudhry. A S and Khan M M H. 2012. Impacts of different spices on *in vitro* rumen dry matter disappearance, fermentation and methane of wheat or ryegrass hay based substrates. Livest. Sci. 146:84-90.

Chowdhury. 2012. Effects of spices supplementation to reduce the ammonia nitrogen loss in ruminants. MS thesis, Dept of Biochemistry and Chemistry, Sylhet Agricultural University.

Demeyer D and Henderickx H K. 1967. The effect of C18 unsaturated fatty acids on methane production *in vitro* by rumen bacteria. Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism.137:484–497.

Frankič T, Voljč M, Salobir J and Rezar V. 2009. Use of herbs and spices and their extracts in animal nutrition. Acta Agric. Slovenica. 94:95–102.

Jahani-Azizabadi H, Danesh Mesgaran1 M, Vakili A R, Rezayazdi K and Heravi Mousavi A R. 2010. Effect of various Iranian native medicinal herbs or spices on *in vitro* ruminal disappearance of lucerne hay. British Society of Animal Science (BSAS)**,** Annual Conference 2010, Belfast 12-14 April, 2010, 250p.

Karami M, Alimon A R, Sazili A Q and Goh Y M. 2008. Effects of source of oil and dietary supplementation of antioxidants on growth and performance of fattening goats, The 13th Animal Science Congress of the Asian-Australasian Association of Animal Production, September 22 ~ 26, 2008, Hanoi, Vietnam, 33p.

Khan M M H and Chaudhry A S. 2010. Chemical composition of selected forages and spices and the effect of these spices on *in vitro* rumen degradability of some forages. Asian-Aust. J. Anim. Sci. 23: 889-900.

Makkar H P S, Sen S, Blummel M and Becker K. 1998. Effects of Fractions Containing Saponins from Yucca schidigera, Quillaja saponaria, and Acacia auriculoformis on Rumen Fermentation. J. Agric. Food Chem. 46:4324-4328.

McDougall E I. 1948. Studies on ruminant saliva. The compositionand output of sheep's saliva. Institute of animal Pathology, University of Cambridge. pp. 99-100.

Min B R, Barry T N, Attwood G T and McNabb W C. 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. Anim. Feed Sci. Technol. 106:3-19.

Miri V H, Tyagi A K, Ebrahimi S H and Mohini M. 2013. Effect of cumin (Cuminum cyminum) seed extract on milk fatty acid profile and methane emission in lactating goat. Small Ruminant Research. 113:66-72.

Miri V H, Ebrahimi S H and Tyagi A K. 2015. The effect of cumin (*Cuminum cyminum*) seed extract on the inhibition of PUFA biohydrogenation in the rumen of lactating goats via changes in the activity of rumen bacteria and linoleate isomerase enzyme. Small Ruminant Research. 125:56-63.

Morsy T A, Kholif S M, Matloup O H, Abdo M M and El-Shafie M H. 2012. Impact of anise, clove and juniper oils as feed additives on the productive performance of lactating goats. International J. Dairy Sci. 7:20-28.

Patra A K and Zhongtang Yu. 2012. Effects of essential oils on methane production and fermentation by, and abundance and diversity of, rumen microbial populations. Appl. Environ. Microbiol. 78: 4271.

Suarez B J, Van Reenen C G, Stockhofe N, Dijkstra J and Gerrits W J J. 2007. Effect of Roughage Source and Roughage to Concentrate Ratio on Animal Performance and Rumen Development in Veal Calves. J. Dairy Sci. 90:2390-2403