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Effect of supplementing total mixed ration with ajwain (*Trachyspermum ammi*) oil on the performance of buffalo calves

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ABSTRACT

This study was taken up to assess the impact of different levels of ajwain oil (rich in essential oils) on the nutrient utilization by *in vitro* gas production technique. The best level of ajwain oil obtained was tested on the performance of buffalo calves. Ajwain oil was supplemented at 0.05, 0.1, 0.15 and 0.2% of total mixed ration (TMR; concentrate:green fodder:wheat straw in 40:48:12 ratio on DM basis). The results revealed that supplementation of incremental levels of ajwain oil to the TMR resulted in linear decrease in net gas production (NGP), digestibility of true OM and NDF; ME availability and methane production. Supplementing the TMR with ajwain oil beyond 0.05% resulted in significant depression in total and individual volatile fatty acids (VFAs) and acetate to propionate ratio. The protozoa numbers declined linearly with increase in level of ajwain oil supplementation. Hence 0.05% level of ajwain oil was selected for assessing its impact on performance of growing buffalo calves. In the first experiment, a 146-day feeding trial was conducted on 20 male buffalo calves divided into 2 groups, offered a TMR-1 (concentrate: green fodder: wheat straw ratio of 40:48:12 on DM basis) or TMR-1 supplemented with 0.05% ajwain oil. After completion of this trial, another 138-day feeding trial was conducted on 18 male buffalo calves divided into 2 groups and were offered a TMR-2 (concentrate: wheat straw ratio of 50:50 on DM basis) or TMR-2 supplemented with 0.05% ajwain oil. In the first growth trial, supplementing the control diet with ajwain oil did not show any impact on daily DM intake, digestibility of nutrients and N-retention, but in the second experiment the digestibility of all the nutrients, except that of crude protein was improved significantly in the ajwain oil supplemented group. The daily DM intake and N-retention was comparable in both the groups. The blood profile, urinary purine derivatives excretion and microbial biomass synthesis was not affected by ajwain oil supplementation during both the experiments. The average daily gain was comparable between control and ajwain oil supplemented group in both the feeding trials. It was concluded that supplementing ajwain oil to the TMR suppressed the *in vitro* methane production; improved the digestibility of nutrients in wheat straw based TMR supplemented with 0.05% ajwain oil, but did not show any significant beneficial effect on the performance of buffalo calves.

Key words: Ajwain oil, Blood profile, Buffalo calves, Growth, *In vitro*, Nutrient utilization, Protozoa numbers

Ajwain (*Trachyspermum ammi* L.) is an important medicinal, aromatic and spice plant. Its seeds possess number of phytochemical, pharmacological and therapeutic properties like antibacterial, antifungal, antiprotozoal, antioxidant, anti-inflammatory, anti-filarial, nematicidal, anthelmintic, hypotensive, analgesic and anti-nociceptive, antitussive and bronchodilatory, antihypertensive, antispasmodic, bronchodilator, diuretic and anti-lithiasis and hepatoprotective activities (Gilani *et al*. 2005, Murthy *et al*. 2009, Sabar 2010, Awadhesh *et al*. 2011, Kamal Jeet *et al*. 2012, Chatterjee *et al*. 2013, Hasan *et al*. 2016, Sarfraz *et al*. 2016, Chahal *et al*. 2017). Ajwain seeds generally contain 2.5–5% essential oil (EO), which is made up of thymol (87.75%) and carvacrol (11.17%) as major constituents and major non-phenolic components quantified were p-cymene (60.78%) and small proportions of γ-terpinene (22.26%), α-pinene, β-pinene and α-terpinene (Pruthi 1992, Nagalakshmi *et al.* 2000, Minija and Thoppil 2002, Singh *et al.* 2004, Raghavan 2007, Chahal *et al*. 2017). The composition of essential oil varied with season and geographical location.

Amongst the pure essential oils like cinnamaldehyde, carvacrol, carvone and limonene; only carvacrol or limonene supplementation beyond 1% level reduced the *in vitro* methane production, nutrient utilization and ME availability from the substrate significantly (Hundal *et al*. 2016). However, no information is available on the herbal feed additives containing essential oil on the enteric methane production and nutrient utilization in livestock. This study was therefore, taken up to assess the impact of different levels of ajwain oil on the nutrient utilization by *in vitro* gas production technique. The best level of ajwain oil obtained was then tested on the performance of buffalo calves.

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MATERIALS AND METHODS

In vitro *studies:* Three buffalo calves fitted with permanent rumen fistulae were offered concentrate mixture (wheat 10, maize 20, mustard cake 15, cotton seed cake 10, soybean meal 5, rice bran 15, wheat bran 12, deoiled rice bran 5, full fat soya 5, mineral mixture 2 and common salt 1% each), green fodder and wheat straw in 40:48:12 ratio on DM basis as per ICAR (2013) feeding standard for dairy cattle. The rumen contents were collected before feeding at 0900 in a thermos flask flushed with CO_2 and maintained at 39º 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 resazurine (0.1%) were mixed in a Woulff flask (3 l) with magnetic stirrer in a water bath at 39º C (Menke *et al.* 1979, Menke and Steingass 1988). The mixture was continuously flushed with CO_2. Strained rumen liquor (SRL) was added to the buffer media in the ratio of 1:2. Glass syringes (100 ml; Haberle Labortechnik, Germany) containing 375±5 mg TMR supplemented with different levels of ajwain oil (0, 0.05, 0.10, 0.15 or 0.20%) and buffered rumen liquor were incubated in triplicate in a water bath at 39ºC and swirled every 60 min over a 24 h incubation period. If the volume of gas in the syringe exceeded 70 ml after 8 h the volume was recorded and the gas was expelled. After 24 h, the volume of gas produced in each syringe was recorded and the contents of syringes were transferred to spout less beaker, boiled with neutral detergent solution for assessing the true OM and NDF digestibility. Each *in vitro* gas production set was repeated to check any variation in the net gas production and other parameters.

Estimation of volatile fatty acid: After 24 h of incubation, a 5 ml aliquot of fluid from each syringe was mixed with 1 ml of 25% meta phosphoric acid and kept for 1 h at ambient temperature (Erwin *et al.* 1961). Thereafter, it was centrifuged at 5,500 rpm for 10 min and clear supernatant was collected and stored at 20º C until analyzed. The volatile fatty acids were estimated using Netchrom 9100 gas chromatograph equipped with glass column (packed with chromosorb 101) and flame ionization detector (Cottyn and Boucque 1968). Temperature of injection port, column and detector was set at 250, 175 and 270ºC, respectively. The flow rate of carrier gas (N) through the column was 15 ml/min; and the flow rate of H_2 and air through FID was 30 and 300 ml/min, respectively. Sample (2 ml) was injected through the injection port using a 10 ml Hamilton syringe. Individual VFA's of the samples were identified on the basis of their retention time and their concentration (mmol) and calculated by comparing the retention time as well as the peak area of standards after deducting the corresponding blank values.

Methane estimation: Methane produced during fermentation of the feeds was estimated using the equation based on VFA proportions as described by Widiawati and Thalib (2009).

In vivo *studies on buffalo calves:* In the first experiment, a 146 day feeding trial was conducted on 20 male buffalo calves (average BW 83.6±3.43 kg) divided into 2 equal groups. The calves were offered TMR-1 (concentrate: green fodder: wheat straw ratio of 40:48:12 on DM basis) or TMR-1 supplemented with 0.05% ajwain oil as per ICAR (2013) feeding standard. The animals were weighed at fortnightly interval and the feeding schedule was adjusted accordingly. At the termination of feeding trial a 7-day metabolism trial was conducted.

In the second experiment, a 138-day feeding trial was conducted on 18 male buffalo calves (average BW 161.88±5.69 kg) divided into 2 groups. The animals were offered a TMR-2 (concentrate: wheat straw ratio of 50:50 on DM basis) or TMR-2 supplemented with 0.05% ajwain oil as per ICAR (2013) feeding standard. The ingredient composition of the concentrate mixture in both the feeding trials was similar to the one used in the *in vitro* studies. The animals were weighed at fortnightly interval and the feeding schedule was adjusted accordingly. At the termination of feeding trial a 7-day metabolism trial was conducted.

Blood profile: The blood samples were taken from the jugular vein of all animals at 4 h post parandial on the last day of both the metabolic trials. The blood samples were collected in heparin; and in vials containing sodium fluoride and oxalate for blood glucose. The serum was separated and stored at 0°C till analyzed.

Analytical methods: The finely ground samples of feedstuffs (in triplicate), orts and faeces were analyzed for DM, CP and total ash (AOAC 2000), cellulose (Crampton and Maynard 1938) and other cell wall constituents like NDF, ADF, and ADL (Robertson and Van Soest 1981). Hemicellulose was determined by difference in NDF and ADF. The urine samples were analysed for total–N (AOAC, 2000), purine derivatives (PD); allantoin (Young and Conway 1942), and uric acid (Trivedi *et al.* 1978). Purines absorbed were calculated from the daily urinary PD excreted using the equation of Pimpa and Liang (2002). The microbial nitrogen synthesis was calculated from purines derivatives excretion (Chen and Gomes 1995). Serum samples were analysed for albumin (Doumas *et al.* 1971), urea (Evans 1968), triglycerides (McGowan *et al*. 1983) and cholesterol (Allain *et al.* 1974). The analysis was conducted on Erba (Mannheim) Chem 5X (Transasia). The serum collected with sodium fluoride and oxalate was used for assay of blood glucose (Trinder 1969). The data of *in vitro* and *in vivo* studies were analyzed by simple ANOVA (Snedecor and Cochran 1994) by using SPSS (2007) version 16.0 and the means were tested for the significant difference by Tukey's b-test.

RESULTS AND DISCUSSION

In vitro *gas production studies*: The results revealed that supplementation of graded levels of ajwain oil to the TMR resulted in linear decrease ($P<0.01$) in net gas production, digestibility of true OM and NDF; and ME availability and

methane production (Table 1). The decrease in these parameters could be due to suppressing effect of ajwain oil on the rumen microbes. Hundal *et al*. (2016) also reported that irrespective of the pure EO supplemented, the NGP, digestibility of NDF and TOM, total and individual VFA and methane production were depressed linearly with the increase in dose of EOs. Similar trend in reduction in methane production with depression in the digestibility of nutrients was observed, when peppermint or clove oil extract were added to the substrate (Patra *et al.* 2006, Agarwal *et al.* 2009). It is likely that the use of high doses of plant extracts and/or their secondary metabolites with antimicrobial activity decreased total microbial activity and diet fermentability (Cardozo *et al.* 2004).

The total volatile fatty acids production and acetate production was improved (P<0.01) when the control diet was supplemented with 0.05% ajwain oil, while the production of other VFAs was comparable in the 2 groups. Supplementing the TMR with ajwain oil beyond 0.05% resulted in significant depression in total, individual VFAs and acetate to propionate ratio (Table 2). Earlier studies revealed that higher doses of cinnamon oil and cinnamaldehyde decreased total VFA and ammonia-N concentrations, although cinnamaldehyde had stronger effects compared with cinnamon oil (Busquet *et al.* 2005, Patra and Yu 2012). The relative proportion of acetate was not affected by different levels of ajwain oil, except that it was improved (P<0.01) at 1.5% level of supplementation. The relative proportion of propionate and isobutyrate was depressed, while that of butyrate was improved and there was no effect on that of isovalerate and valerate proportion by supplementing the diet with ajwain oil. There was a significant decline in protozoa numbers in all the ajwain oil supplemented groups, confirming the earlier reports (Awadhesh *et al*. 2011, Hasan *et al*. 2016). The per cent decline in comparison to control TMR increased with the increase in level of ajwain oil supplementation.

Effect of ajwain oil on the performance of buffalo calves: The chemical composition of the total mixed ration fed to the buffalo calves in the first experiment was almost comparable with that of TMR fed during second experiment, except that TMR of the first experiment had numerically

Table 1. Effect of supplementing different levels of ajwain oil on the *in vitro* nutrient digestibility, ME availability and methane production from total mixed ration

Parameter	Control	Ajwain oil (%)				PSE	P value
		0.05	0.10	0.15	0.20		
NGP	186.89^{e}	173.11^{d}	82.89^{c}	73.11^{b}	34.00^{a}	15.94	0.002
TOMD (%)	61.98^{d}	48.13^{c}	35.51^{b}	35.71^{b}	33.02^{a}	3.64	0.007
NDFD (%)	35.95^{c}	12.86^{b}	12.38^{ab}	8.81^{a}	7.86^{a}	3.10	0.005
PF	1.91^{a}	2.06^{b}	4.46^{c}	4.92^{d}	10.79^{e}	1.07	0.000
ME (MJ/kg DM)	7.98^{e}	7.54^{d}	4.48^{c}	4.22^{b}	2.93^{a}	1.04	0.003
Methane (mM)	1.93^{d}	2.02^{e}	1.57^{c}	1.51^{b}	1.20^{a}	0.15	0.004

NGP, Net gas production (ml/24h/gDM); NDFD, neutral detergent fiber digestibility; TOMD, true OM digestibility; PF, partitioning factor; ME, metabolizable energy. Figures with different superscripts^{a,b,c} in a row differ significantly; PSE, pooled standard error.

Table 2. Effect of supplementing different levels of ajwain oil on *in vitro* volatile fatty acid production (mM/day) from total mixed ration.

Parameter	Control	Ajwain oil (%)				PSE	P value
		0.05	0.10	0.15	0.20		
TVFA	6.27^{c}	6.50^{d}	4.60^{b}	4.67^{b}	3.68^{a}	0.36	0.007
Acetate (A)	4.38^{c}	4.58^{d}	3.28^{b}	3.38^{b}	2.64^{a}	0.24	0.001
Propionate (P)	1.19^{c}	1.19^{c}	0.59^{b}	0.55^{ab}	0.51^{a}	0.11	0.003
Isobutyrate	0.069^{c}	0.071^{c}	0.043^{b}	0.034^{b}	0.020^{a}	0.007	0.006
Butyrate	0.518^{b}	0.540^{b}	0.596^{c}	0.616^{c}	0.428^{a}	0.022	0.001
Isovalerate	0.057^{c}	0.058^{c}	0.044^{b}	0.041^{b}	0.031^{a}	0.003	0.001
Valerate	0.062^{c}	0.064^{c}	0.046^{b}	0.045^{b}	0.034^{a}	0.004	0.003
A:P	3.68^{a}	3.83^{a}	5.54^{bc}	6.16^{c}	5.18^{b}	0.51	0.002
		Relative proportion (%)					
Acetate	69.79^{a}	70.38^{ab}	71.25^{ab}	72.48^{b}	71.66^{ab}	0.54	0.037
Propionate	18.95^{c}	18.36^{c}	12.88^{ab}	11.77^{a}	13.84^{b}	1.50	0.000
Isobutyrate	1.11^{c}	1.08^{c}	0.94^{bc}	0.72^{ab}	0.55^{a}	0.07	0.009
Butyrate	8.26^{a}	8.30^{a}	12.96^{c}	13.19^{c}	11.64^{b}	1.15	0.005
Isovalerate	0.91	0.90	0.96	0.88	0.84	-	0.506
Valerate	0.98	0.98	1.01	0.96	0.92	-	0.058

TVFA, Total volatile fatty acids. Figures with different superscripts^{a,b,c} in a row differ significantly; PSE, pooled standard error.

higher CP, NDF and hemicelluloses than that of TMR fed during second experiment (Table 3).

In the first growth trial, supplementing the control diet with ajwain oil did not show any significant impact on daily DM intake and digestibility of nutrients (Table 4), confirming the earlier reports that essential oil/blend of essential oils did not affect the digestibility of nutrients (Sallam *et al*. 2009, Santos *et al.* 2010). In the second experiment supplementing the control diet with ajwain oil improved the digestibility of all the nutrients significantly, except that of crude protein which was comparable between two groups. The daily DM intake was comparable in both the groups. Similar results on daily DM intake have been reported earlier also, when the diet of lamb or sheep was supplemented with essential oils either individually or in different combinations (Lin *et al.* 2013, Ma *et al*. 2015). The digestibility of nutrients clearly indicated that ajwain oil had positive effect in high roughage diet. The daily N–intake, total N–excretion and N–retention was comparable in control group with that of ajwain oil supplemented group in both the experiments.

Supplementing the green fodder based diet (experiment 1) as well as wheat straw based diet (experiment 2) with ajwain oil did not have any adverse effect on the blood profile of buffalo calves, except that cholesterol and calcium levels were improved significantly in the second experiment (Table 5). Plasma concentrations of glucose, cholesterol, triglyceride, urea-N, β-hydroxybutyrate, alanine aminotransferase and aspartate aminotransferase were not changed by supplementing the diet with thyme and cinnamon essential oils in calves (Vakili *et al.* 2013). The level of all the parameters of blood profile was within the normal physiological range.

The effect of ajwain oil supplementation on the urinary excretion of purine derivatives revealed that during experiment 1 the uric acid excretion in the urine of calves was depressed significantly in ajwain supplemented group, while allantoin excretion was comparable in both the groups (Table 6). But in the second experiment both uric acid and allantoin excretion was depressed significantly in both the groups. However, the total purine derivatives excretion was not affected by ajwain oil supplementation during both the

Table 3. Chemical composition of feedstuffs

Parameter	Ash	OM	CP	NDF	ADF	Cellulose	HC
Experiment 1							
Concentrate mixture	8.3	91.7	19.75	41.6	22.9	13.7	18.7
Green fodder	8.08	91.93	10.04	75.9	47.25	37.4	28.65
Wheat straw	7.78	92.23	3.33	82.4	57.9	44.2	24.5
TMR-1	8.13	91.87	13.12	62.96	38.79	28.74	24.17
Experiment 2							
Concentrate mixture	8.7	91.3	19.9	42.5	22.55	11.8	19.95
Wheat straw	7.65	92.35	3.11	80.6	57.2	44.7	23.4
TMR-2	8.18	91.83	11.51	61.55	39.88	28.25	21.68

TMR-1, Concentrate: green fodder: wheat straw (in 40:48:12 ratio on DM basis); TMR-2, Concentrate: wheat straw (in 50:50 ratio on DM basis)

Table 4. Effect of supplementing ajwain oil to the total mixed ration on the digestibility of nutrients in buffalo calves

Parameter	Experiment 1		PSE	P value	Experiment 2		PSE	P value
	TMR-1	TMR-1 + AO			TMR-2	TMR-2 + AO		
DMI (kg/d)	4.28	4.28	–	1.00	5.74	5.74	–	1.00
Digestibility of nutrients (%)								
DM	57.60	58.31	1.45	0.745	48.14^{a}	60.93^{b}	2.65	0.002
OM	60.34	61.26	1.35	0.667	51.47^{a}	63.74^{b}	2.54	0.002
CP	75.57	75.59	1.55	0.981	70.49	71.36	1.36	0.666
NDF	49.31	49.79	1.13	0.852	36.71^{a}	52.47^{b}	3.16	0.000
ADF	43.91	45.53	1.50	0.627	31.90^{a}	49.28^{b}	4.0	0.012
Cellulose	78.37	79.00	–	0.646	48.29^{a}	60.15^{b}	2.48	0.002
HC	58.00	56.64	1.15	0.435	45.45^{a}	58.28^{b}	3.19	0.029
Nitrogen retention (g/day)								
N–Intake	93.32	93.32	–	1.00	108.09	108.09	1.73	1.00
Faecal–N	22.80	22.77	1.5	0.981	32.01	31.00	1.39	0.747
Urinary–N	25.43	24.74	1.23	0.803	41.15	41.06	1.12	0.974
N–Retained	45.09	45.80	1.08	0.770	34.95	36.03	1.01	0.628

TMR-1, Concentrate: green fodder: wheat straw (in 40:48:12 ratio on DM basis); TMR-2, Concentrate: wheat straw (in 50:50 ratio on DM basis); AO, Ajwain oil; Figures with different superscripts^{a,b,c} in a row differ significantly; PSE, pooled standard error.

Table 5. Effect of supplementing ajwain oil to the total mixed ration on the blood profile (mg/dl) of buffalo calves

Parameter	Experiment 1		PSE	P value	Experiment 2		PSE	P value
	TMR-1	TMR-1 + AO			TMR-2	TMR-2 + AO		
Glucose	73.50	68.50	2.16	0.277	62.25	64.6	2.74	0.244
TP	6.55	6.60	0.10	0.829	5.72	6.45	0.22	0.104
ALB	2.95	2.70	0.09	0.182	2.97	2.72	0.09	0.204
GLB	3.6	3.9	0.13	0.289	3.5	3.72	0.35	0.776
A:G	0.82	0.70	–	0.202	0.80	0.74	–	0.849
Cholesterol	58.25	72.00	3.81	0.062	60.93[a]	76.09[b]	3.52	0.014
TG	23.95	24.69	3.14	0.920	27.18	21.26	6.06	0.661
Ca	9.05	8.72	0.15	0.303	6.75[a]	10.42[b]	0.87	0.018
P	8.62	8.85	0.24	0.681	8.45	8.25	0.33	0.787
BUN	19.25	20.00	0.75	0.656	19.75	21.75	0.86	0.276

TMR-1, Concentrate: green fodder: wheat straw (in 40:48:12 ratio on DM basis); TMR-2, Concentrate: wheat straw (in 50:50 ratio on DM basis); AO, ajwain oil; TP, total protein; ALB, albumin; GLB, globulin; A:G, albumin: globulin ratio; TG, triglycerides; Ca, calcium; P, phosphorus; BUN, blood urea nitrogen. Figures with different superscripts[a,b,c] in a row differ significantly; PSE, pooled standard error.

Table 6. Effect of supplementing ajwain oil to the total mixed ration on the urinary excretion of purine derivatives in buffalo calves

Parameters	Experiment 1		PSE	P value	Experiment 2		PSE	P value
	TMR-1	TMR-1+AO			TMR-2	TMR-2+AO		
UA (mM/d)	1.00[b]	0.49[a]	–	0.021	0.55[b]	0.14[a]	-	0.025
ALL (mM/d)	19.85	18.88	1.70	0.798	37.57[b]	31.90[a]	5.22	0.626
PD (mM/d)	20.86	19.37	1.70	0.699	38.81	32.20	5.27	0.604
UA (%)	5.11[b]	2.56[a]	0.95	0.048	1.56[b]	0.46[a]	0.78	0.039
ALL (%)	94.89[a]	97.44[b]	0.95	0.048	98.44[a]	99.54[b]	0.78	0.039
PA (mM/d)	86.49	75.59	14.02	0.728	212.87	162.49	43.80	0.605
MNS (g/d)	62.88	54.96	10.20	0.728	154.77	118.13	31.84	0.605

TMR-1, Concentrate: green fodder: wheat straw (in 40:48:12 ratio on DM basis); TMR-2, Concentrate: wheat straw (in 50:50 ratio on DM basis); AO, ajwain oil; UA, uric acid; All, allantoin; PD, purine derivatives; PA, purines absorbed; MNS, microbial nitrogen synthesized. Figures with different superscripts[a,b,c] in a row differ significantly; PSE, pooled standard error.

Table 7. Effect of supplementing ajwain oil to the total mixed ration on the performance of buffalo calves

Parameters	Experiment 1		PSE	P value	Experiment 2		PSE	P value
	TMR-1	TMR-1 + AO			TMR-2	TMR-2 + AO		
Initial BW (kg)	83.11	84.11	3.43	0.889	162.25	161.51	5.69	0.950
Final BW (kg)	155.67	157.22	5.50	0.892	242.85	246.55	7.01	0.801
Gain in BW (kg)	72.56	73.11	2.46	0.914	80.59	85.04	2.05	0.292
ADG (g)	496.96	500.76	16.84	0.914	584.0	616.21	14.87	0.292

TMR-1, Concentrate: green fodder: wheat straw (in 40:48:12 ratio on DM basis); TMR-2, Concentrate: wheat straw (in 50:50 ratio on DM basis); AO, ajwain oil; BW, body weight; ADG, average daily gain; PSE, pooled standard error.

experiments. The uric acid excretion expressed as percent of total purines excretion was higher in unsupplemented control diet as compared to that observed in ajwain oil supplemented group. Reverse trend ($P<0.01$) was observed in allantoin expressed as percent of total purines excreted in both the experiments. The purines absorbed and microbial biomass synthesized was depressed slightly in ajwain supplemented group as compared to those fed unsupplemented control diet in both the experiments. But the differences were statistically nonsignificant.

The average daily gain in weight was numerically higher in the ajwain oil supplemented group as compared to control diet in both the experiments (Table 7). The effect of ajwain oil on ADG was more pronounced in the wheat straw based diet (experiment 2) than that of green fodder based diet (experiment 1). However, the differences were statistically nonsignificant in both the experiments. It was concluded that supplementing ajwain oil to the TMR suppressed the *in vitro* methane production; improved the digestibility of nutrients in wheat straw based TMR supplemented with 0.05% ajwain oil, but did not show any significant beneficial effect on the performance of buffalo calves.

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