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Effect of vegetable seed oils on methane emission and fermentation of feed *in vitro**

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In ruminants, the vast majority of enteric methane (CH_4) production occurs in the reticulo-rumen. Reducing CH_4 production not only improves feed energy utilization, but also enhances the system efficiency. Mitigation of CH_4 emissions is receiving great attention, especially through dietary manipulations. The amount of methane produced in the rumen is affected by feed intake, other dietary factors like dietary fiber, starch, soluble sugars, dietary lipids, level of feeding, roughage to concentrate ratio, type of forage, stage of maturity of forage, rate of passage of digesta, efficiency of feed conversion, processing and supplementation (Blaxter and Clapperton 1965, Benchaar *et al*. 2001, Mills *et al*. 2001, Bakshi and Wadhwa 2009), plant secondary metabolites (Bakshi and Wadhwa 2010, 2012), feed additives (Wadhwa and Bakshi 2009), phytogenics (Bakshi and Wadhwa 2011) and ambient temperature (McAllister *et al*. 1996). Adding fat to the diet reduces CH_4 emission by decreasing organic matter fermentation in the rumen, reducing the activity of methanogens and protozoal numbers, and for lipids rich in unsaturated fatty acids, through hydrogenation of fatty acids (Maia *et al.* 2007, Beauchemin *et al*. 2008) consuming hydrogen in the process, which makes this process a potential alternate hydrogen sink. Fats and oils change the fermentation process in the rumen, producing more propionic acid and less methane. Since the effect of vegetable oils depends on diet, nature and level of supplementation, the present study was therefore planned to evaluate the effect of supplementation of vegetable oils at different levels on methane production, fermentation pattern and gas production *in vitro*.

The rumen contents were collected from 3 rumen fistulated male buffaloes in a thermos flask flushed with CO_2 and maintained at 39ºC. The rumen contents were blended for 2–3 min in a blender and strained through 4-layered muslin cloth and processed for *in vitro* gas production measurements as per standard procedure (Menke *et al*. 1979, Menke and Steingass 1988). About 375 mg of the ground sample of complete feed (oat fodder and concentrate mixture in 60: 40 ratio on DM basis) was incubated at 39ºC for 24 h in triplicate in 100 ml calibrated glass syringes with buffered rumen fluid for assessing the net gas production, digestibility of nutrients, volatile fatty acids (VFAs) production and ME availability. The set was repeated twice.

Methane was estimated by using the equation based on VFA proportions (Wolin 1960). VFA were estimated by using gas chromatograph equipped with a glass column (6 ft length and 1/8 inch diameter) packed with chromosorb 101 (Cottoyn and Boucque 1968). Samples were analysed for proximate (AOAC 2000) and cell wall components (Robertson and Van Soest 1981). Data were analysed by using 3×4 factorial design (Snedecor and Cochran 1994) by using SPSS (2007) Version 16 and the differences in means by using Tukey's b test.

The complete feed contained 13.5% CP and 2.8% EE. The carrot seed oil, rape seed oil and canola seed oil contained 61%, 12%, 13%; 60%, 28%, 12%; 61%, 27.8% and 10.6% mono unsaturated fatty acids, poly unsaturated fatty acids and saturated fatty acids, respectively. Supplementing the complete feed with different vegetable seed oils, irrespective of the level indicated that the NDF digestibility was highest ($P<0.05$) in rape seed oil as compared to carrot seed oil supplemented group. However, no difference was observed between rape seed and canola seed oil (Table 1). The other parameters like net gas production (NGP), true OM digestibility, partitioning factor and ME availability were comparable in all the vegetable oil supplemented groups. The fermentative CO_2 and methane production from complete feed was observed to be lowest ($P<0.05$) with rape seed oil supplementation.

The NGP, irrespective of source of oil, was highest ($P<0.05$) in the control group as compared to oil supplemented groups, but decreased linearly with the increase in level of oil supplementation. The digestibility of nutrients decreased with increase in levels of supplementation and this effect was more pronounced at

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Table 1. Effect of vegetable oils on net gas production, digestibility of nutrients and ME availability

Parameter	Vegetable seed oil			PSE	Level of oil, %				PSE
	Carrot	Rape	Canola		0	1	2	3	
NGP, ml/g DM/24h	182.89	182.56	183.33	0.37	180.67^{A}	185.56^{C}	183.33^{B}	182.15^{B}	0.45
NDFD, %	37.18^{a}	38.86^{b}	38.66^{ab}	0.19	45.54^{C}	36.57^{B}	34.63^{A}	36.20^{B}	0.24
TOMD, %	63.24	64.24	64.11	0.21	68.08^{C}	62.95^{B}	61.76^{A}	62.66^{B}	0.14
PF	1.98	1.98	1.98	0.01	2.01^{C}	1.94^{A}	1.97^{AB}	1.98^{B}	0.007
NH_3, mg/dl	0.029	0.030	0.030	-	0.030^{B}	0.030^{B}	0.029^{A}	0.030^{B}	-
FCO_2, mM	3.07^{a}	3.06^{a}	3.12^{b}	0.004	3.07^{B}	3.16^{D}	3.10^{C}	3.04^{A}	0.005
FCH_4, mM	2.06^{b}	2.03^{a}	2.06^{b}	0.01	1.981^{A}	2.111^{D}	2.083^{C}	2.022^{B}	0.01
ME, MJ/kg DM	8.30	8.29	8.31	0.01	8.25^{A}	8.36^{C}	8.31^{B}	8.28^{B}	0.01

NGP, net gas production; NDFD, neutral detergent fiber digestibility; TOMD, true OM digestibility; PF, partitioning factor; FCO_2, fermentative CO_2; FCH_4, fermentative methane; ME, metabolizable energy. Figures with different superscripts ^{a,b,c} for different oils and different superscripts ^{A,B,C,D} for different levels of oil in a row differ significantly, P<0.05.

Table 2. Effect of vegetable oils on *in-vitro* volatile fatty acid production (mM/dl) after 24 h incubation

Parameter	Vegetable seed oil			PSE	Level of oil, %				PSE
	Carrot	Rape	Canola		0	1	2	3	
TVFAs	5.78^{a}	5.92^{b}	6.0^{c}	0.01	5.87^{B}	6.04^{D}	5.94^{C}	5.76^{A}	0.009
Acetate (A)	4.06^{a}	4.10^{b}	4.12^{b}	0.01	3.98^{A}	4.21^{B}	4.18^{B}	4.00^{A}	0.012
Propionate (P)	0.95^{a}	1.07^{b}	1.09^{b}	0.007	1.11^{C}	1.04^{B}	1.02^{B}	0.97^{A}	0.008
Butyrate	0.54^{b}	0.50^{a}	0.54^{b}	0.002	0.53^{B}	0.53^{B}	0.50^{A}	0.53^{B}	0.002
Iso butyrate	0.036^{a}	0.067^{b}	0.069^{b}	0.063	0.067^{D}	0.060^{C}	0.049^{A}	0.054^{B}	0.001
Iso valerate	0.128^{c}	0.115^{a}	0.120^{b}	0.001	0.111^{A}	0.130^{D}	0.115^{B}	0.127^{C}	0.001
Valerate	0.064^{a}	0.076^{c}	0.071^{b}	0.07	0.062^{A}	0.072^{B}	0.075^{B}	0.072^{B}	0.002
A: P	4.31^{b}	3.84^{a}	3.79^{a}	0.03	3.59^{A}	4.07^{B}	4.09^{B}	4.16^{B}	0.04

TVFAs, total volatile fatty acids. Figures with different superscripts ^{a,b,c} for different oils and different superscripts ^{A,B,C,D} for different levels of oil in a row differ significantly, P<0.05.

2% level of supplementation. The partitioning factor decreased with increase in level of supplementation, in comparison to the unsupplemented feed. The fermentative methane production was higher (P<0.05) in oil supplemented than control group, but amongst the oil supplemented groups it was lowest (P<0.05) when oil was supplemented at 3% level. The availability of ME was improved (P<0.05) in all the oil supplemented groups as compared to control.

The VFAs, acetate, propionate and butyrate production, irrespective of level of vegetable oil was highest (P<0.05) in canola seed oil and lowest with carrot seed oil, except that of butyrate which was lowest in rape seed oil supplemented group, but A: P ratio followed the reverse trend (Table 2). TVFA and acetate levels were highest (P<0.05) at 1% level of supplementation, whereas propionate level was highest in control followed by 1% supplementation. The A: P ratio increased by 11.70% on an average compared to control. Machmüller *et al*. (2003) also reported that at supplementation of 50g/kg coconut oil, the molar proportions of propionate and isovalerate were reduced (P<0.01) and the molar proportion of butyrate (P<0.001) and A: P ratio were increased (P<0.05). On the contrary, Morales *et al.* (2012) obtained 28% decrease methane in 24 h *in vitro* incubations of rumen digesta with added 0.2g ricinoleic acid/l. There was no effect on the TVFA after 24 h as a result of ricinoleic acid addition (castor oil), but the molar proportions of acetate and butyrate were decreased.

SUMMARY

A study was conducted to assess the effect of carrot seed oil, canola seed oil and rape seed oil on rumen fermentation and methanogenesis *in-vitro*. The oils were supplemented to the complete feed (oat fodder and concentrate mixture in 60: 40 ratio) @ 1, 2 and 3% on DM basis, incubated for 24 h in 100 ml glass syringes. The digestibility of NDF varied significantly with highest in rapeseed and lowest in carrot seed, while OM digestibility and ME availability did not show any significant differences amongst the supplemented oils. The *in-vitro* methane production from complete feed supplemented with rape seed oil was observed to be the lowest. The TVFAs, acetate and propionate levels were highest in canola oil while A: P ratio was lowest.

TVFA and acetate levels were highest at 1% level of supplementation, whereas propionate level was highest in control followed by at 1% supplementation. The methane

production was significantly higher in oil supplemented groups as compared to control group, but amongst the oil supplemented groups it was significantly lowest when oil was supplemented at 3% level. Amongst the various oils evaluated for *in vitro* methane mitigation, the study conclusively revealed that the supplementation of diet with rape seed oil @ 2–3% on dry matter basis had an edge over other oils and levels.

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