Bulletin of Environment, Pharmacology and Life Sciences
Online ISSN 2277-1808
Bull. Env. Pharmacol. Life Scien. Volume 1 [12] November 2012: 73- 77

Website: www.bepls.com

Original Article

Effect of sunflower oil Supplementation on *in vitro* Fermentation Patterns of Forage Based Diets for Ruminant

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ABSTRACT
The aim of present study was to investigation on influence of supplemented sunflower oil (SFO) in vitro fermentation of forage based mixed ration. Alfalfa forage and barley grain (as concentrated feed) were mixed to obtaining total mixed rations with 60 to 40% and 50 to 50% respectively for forage and concentrated portions. The SFO in the levels of 0, 2.5 or 5% of ration was added to mixed rations. Three fistulaed native bulls fed experimental rations twice daily for 15 days, and ruminal fluid was collected. Gas production of in vitro fermentation was measured as the volume of gas in the calibrated syringes and was recorded before incubation 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours after incubation. Total gas values were corrected for blank incubation which contained only rumen fluid. There was no any significant deference treatments (0, 2.5 or 5% SFO), whereas there was significant differences between two basal rations. The addition of vegetable oil to forage based ration didn't affect gas production parameters. It was concluded that, supplementation of sunflower oil to forage-based rations didn't affect gas production parameters, and in higher concentration may be inadvisable for ruminal fermentation.
Key words: *Chemical composition, in vitro gas production technique, fermentation, rumen.*

INTRODUCTION

Supplementation of fat to ruminant feeds may increase milk fat content and increase diets energy to match energy requirements of high producing animals [1]. For supplemental fat to be beneficial, it must not compromise rumen fermentation and, concomitantly, DMI (dry matter intake) and milk fat percentage. In other hand, feeding lipids has received attention as mechanism to methane emission [2] from ruminant, which is one of recent environmental concerns. C: 18 polyunsaturated fatty acids (includes sunflower oil) is a fat resource with potential to reduce methane production [3]. Various investigations for utilization of fat sources in ruminant ration had stated some beneficial or some detrimental effects. Plant oils, because of their unsaturated fatty acid contents can modify rumen fermentation based on fatty acid composition, origin and saturation [4]. *In vitro* and *in situ* investigations on some oil seeds or vegetable oils such as canola seed [5], flaxseed Oil [6], coconut oil [7], sunflower oil [8-9] had reported beneficial effects of these vegetable oils in ruminant feed.

Sunflower oil was considered as one of supplemented oil for hay based-diets in Chantaprasarn and Wanapat, [8]. The aim of present study was to investigation on influence of supplemented sunflower oil *in vitro* gas production of forage based-feed (50-60% alfalfa, 50-40% concentrated feed) for cattle.

MATERIALS AND METHODS

Experimental conditions and feeds

Commercial dietary SFO was obtained from vegetable oil industry. Alfalfa forage and barley grain (as concentrated feed) were mixed to obtain total mixed rations with 60 to 40% and 50 to 50% respectively for forage and concentrated portions. The SFO in the levels of 0, 2.5 or 5% of ration was added to experimental mixed ration via spraying to milled ration.

Chemical analysis of samples

Dry matter (DM) was determined by drying the samples at 105°C overnight and ash by igniting the samples in a muffle furnace at 550°C for 6 h. Nitrogen (N) content was measured by the Kjeldahl method [10] . Crude protein was calculated as N X 6.25. Acid detergent fiber (ADF) content and neutral detergent fiber (NDF) content of leaves were determined using the method described by Van Soest et al., [11] All chemical analyses were carried out in triplicate.

Statistical analysis: Data on apparent gas production parameters were subjected to one-way analysis of variance using the analysis of variation model ANOVA using SAS [12]. Multiple comparison tests used Duncan's Multiple-Range Test. All values were shown as standard error of difference between means (SEM).

In vitro gas production

Rumen fluids was obtained from four fistulated cattle (cross bred bulls), fed twice daily with a diet containing alfalfa and concentrate (two cattle for each experimental mixed ration). The samples were incubated in the rumen fluid in calibrated glass syringes following the procedures of Menke and Steingass [13] as follows. 0.200 g dry weight of the sample was weighed in triplicate into calibrated glass syringes of 100 ml in the absence. The syringes were pre-warmed at 39°C before injecting 30 ml rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39°C. The syringes were gently shaken 30 min after the start of incubation and every hour for the first 10 h of incubation. The gas production was measured as the volume of gas in the calibrated syringes and was recorded before incubation 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours after incubation. Total gas values were corrected for blank incubation which contained only rumen fluid. Cumulative gas production data were fitted to the model of Ørskov and McDonald [14].

$y= a + b (1-exp^{-ct})$

Whereas:

a = the gas production from the immediately soluble fraction (ml)

b = the gas production from the insoluble fraction (ml)

c = the gas production rate constant for the insoluble fraction (b)

t = incubation time (h)

y = gas produced at time 't'

The OMD (organic matter digestibility) of forages was calculated using equations of Abash et al., [15] as follows:

DOM %: 0.9042*GP+0.0492*CP+0.0387*CA+ 16.49

Whereas:

GP is 24 h net gas production (ml / 200 mg),

CP = Crude protein (%)

CA = Ash content (%)

ME (MJ/kg DM) content of forages was calculated using equations of Ismail Abash, et al , [15] as follows:

ME (MJ/kg DM): $0.136\times GP+0.0057\times CP+ 0.000286\times EE^2 +2.20$

NE_L (MJ/kg DM): $0.096\times GP+0.0038\times CP+ 0.000173\times EE^2 +0.54$

Whereas:

GP is 24 h net gas production (ml/200 mg),

CP = Crude protein (%)

EE = Ether Extract (%)

For determination of metabolizable energy (ME), net energy for lactation (NE_L) and digestibility of organic matter (DOM) in *in vitro* conditions, Menke and Steingass [13] equation was applied for gas production volume from a milligram of sample and turned it for 200 mg sample to 24h.

RESULTS

The chemical composition of ruminant diet is varied due to cultivar, climate, origin and etc. According to NRC report, crude protein rate of alfalfa is 13-20% based on harvesting time and 13.5% for barley grain. Also, the ADF and NDF concentration of alfalfa is 30-34% and 38-58% and for barley is 7 and 19 % respectively. But in present study these measure are estimated and presented as table1.

Table1. Chemical composition of experimental diet (%)

Compound / Ration	Dry matter	Crude protein	Ether extract	Ash	Acid detergent fiber	Neutral detergent fiber
TMR1	93.56	12.84	2	6.55	19.4	34
TMR2	93.76	14.32	1.2	9.50	21.2	36.6

-TMR: total mixed ration. TMR1 (50 to 50% for forage and concentrated feed), TMR2 (60 to 40% for forage and concentrated feed).

The gas production of Treatments shown in Table2. There are significant ($p<0.05$) differences between Treatments in volume of gas production in some incubation times.
The gas production parameters are shown in table 3. There are significant ($p<0.05$) differences between Treatments in estimated gas production parameters up to 12h. The gas production from the immediately soluble fraction (a), gas production from the insoluble fraction (b) and Potential gas production (a+b) were greater for TMR1 in compared with TMR2. Also, there was no any change for gas production parameters raised from addition of sunflower oil in deferent concentrations.
According to table 3, there was no any significant deference treatments (0, 2.5 or 5% SFO), whereas there was significant differences between ration (TMR1 and TMR2). Also there was not any significant difference for the gas production rate constant for the insoluble fraction, potential gas production, metabolizable energy, organic matter digestibility, and net energy between rations or treatments.

Table 2. The gas production for different levels of SFO (ml/ 200 mg DM)

		Incubation times								
Ration	Treatment	2	4	6	8	12	24	48	72	96
TMR1	0 % SFO	10.12	23.99[a]	34.03[ab]	41.82[ab]	48.20[a]	58.10	66.43	68.46	70.02
	2.5% SFO	9.81	23.81[a]	34.19[ab]	42.22[a]	48.91[a]	58.96	66.98	68.70	69.48
	5% SFO	10.15	24.22[a]	34.14[ab]	41.95[ab]	48.67[a]	58.60	66.65	69.31	70.24
TMR2	0 % SFO	10.29	23.71[ab]	32.83[c]	39.85[c]	46.25[b]	55.38	63.57	65.91	67.16
	2.5% SFO	9.96	22.91[c]	31.69[d]	38.86[c]	45.40[b]	55.14	62.70	64.41	65.66
	5% SFO	10.02	23.98[abc]	32.84[c]	39.73[c]	45.53[b]	54.08	61.84	64.35	65.29
P value		0.49	0.006	0.017	0.014	0.018	0.063	0.24	0.39	0.198
SEM		0.28	0.22	0.32	0.34	0.45	0.66	0.81	0.87	0.77

Table 3. The estimated parameters from the gas production for supplemented SFO levels

Rations	Treatment	Estimated Parameters							
		a	b	|a|+b	C	OMD	ME	SCFA	NEL
TMR1	0 % SFO	1.84[a]	69.18[a]	71.02[a]	0.113	69.91	10.17	1.28	6.19
	2.5% SFO	3.42[a]	70.86[a]	74.29[a]	0.118	70.69	10.29	1.30	6.24
	5% SFO	1.95[a]	69.75[a]	71.71[a]	0.113	70.36	10.24	1.29	6.21
TMR2	0 % SFO	0.46[b]	64.98[b]	65.31[b]	0.110	67.61	9.81	1.22	5.91
	2.5% SFO	1.04[b]	64.51[b]	65.49[b]	0.110	67.42	9.78	1.21	5.88
	5% SFO	0.80[b]	63.9[b]	64.36[b]	0.115	66.46	9.64	1.19	5.78
	P value	0.0001	0.0001	0.0001	0.132	0.063	0.063	0.061	0.052
	SEM	0.64	0.64	2.01	0.002	0.60	0.09	0.011	0.069

a= the gas production froam the immediately soluble fraction (ml)
b=the gas production from the insoluble fraction (ml)
c = the gas production rate constant for the insoluble fraction (t)
a+b : Potential gas production,
ME : Metabolizable energy, (MJ/kg DM)
OMD : Organic matter digestibility (%)

NEL: Net Energy Lactation (MJ/kg DM)
SEM: standard error of the mean

DISCUSSION

In Vargas et al., [9] study, the fermentation pattern is affected by oil (SFO) supplementation for concentrate-based diets. Onetti et al., [16] in adverse to Vargas et al., [9] had reported that including fat in silage-based diets had negative effects on milk production and rumen fermentation pattern regardless of the source or level of supplemental fat. In Mohammadian-Tabrizi et al., [17] study, the addition of palm oil or hydrogenated tallow to wheat grains significantly decreased gas production during incubation.

It is clear that oil coating can make a barrier to microbial attachment to feed particles. Also, it can be toxic for some of ruminal microorganisms [18-20] especially for fibrolytic bacteria [20].

In present study, addition of vegetable oil to forage based TMR didn't affect gas production parameters, so present result are adverse to Vargas et al., [9]. Because their experimental rations were based on concentrates, but in present report, the diets were formulated based on forage or same proportion of these two compounds. Although, in our past study, the SFO supplementation has positive effect on gas production for concentrate-based diet [21]. It is suggested that, dietary coated with vegetable oils has not positive effect on forage- based diets, due to decreased attachment rate of ruminal microbes to feed. But the variability of feed ingredients in concentrate feeds can make a susceptible environment for microbial activity, facilities attachments, so coating with oil cannot make a barrier for microbial activities. It was concluded that, supplementation of sunflower oil to forage-based rations didn't affect gas production parameters, and in higher concentration may be inadvisable for ruminal fermentation.

ACKNOWLEDGMENT

The authors are thankful to Ilkhchi Branch-Islamic Azad University for their financial support of present project.

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