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Influence of sunflower oil supplementation on *in vitro* gas production of mixed ration for ruminants

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ABSTRACT

The aim of present study was to investigation on influence of supplemented sunflower oil (SFO) in vitro gas production of mixed ration (concentrated feed + forage) for ruminants. The SFO in the levels of 0, 2.5 or 5% of ration was added to experimental mixed ration via spraying to milled ration. Three native bulls were fistulated and fed with experimental ration twice daily for 15 days and ruminal fluid was collected. 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. For determination of metabolizable energy (ME), net energy for lactation (NEL) and digestibility of organic matter (DOM) in in vitro conditions, an equation was applied for gas production volume from a milligram of sample and turned it for 200 mg sample to 24h. With inclusion of 5% SFO, significant decreases (p<0.05) in gas production volume and both of soluble (a) or insoluble (b) fractions were observed. It was concluded dietary inclusion of 5% sunflower oil may cause considerable decreases in in vitro gas production parameters and energy indices of mixed ration (40%: forage and 60% concentrated feed) for ruminant.

Key words: Chemical composition, *in vitro* Gas production, Metabolizable energy and Net energy for lactation.

INTRODUCTION

Dietary supplementation of sunflower oil (SFO) has beneficial effect; Beauchemin et al., [1] and Machmüller et al., [2] had stated that SFO in ruminant ration can prevent to methane emission from rumen, without any considerable negative effect on rumen pH, fatty acid production in rumen, milk yield or milk composition in cattle. 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 [3]. Digestibility of fatty acids may be varied via dry matter intake, According to Getachew [4] report, application of gas production bio-technique with activity of cellulose and hemicelluloses fermentation can estimate ruminal microbial activity and shows rate and level of substrate digestion or fermentation in rumen. In this regard, Menke et al., [5] and Menke and Steingass [6] had establish *in vitro* gas production bio-technique for evaluate nutritional value of forages and degradability of dry matter via indirect method with produced CO2 during fermentation phase. The aim of present study was to investigation on influence of supplemented sunflower oil in vitro gas production of mixed ration (concentrated feed + forage) for cattle.

MATERIALS AND METHODS

Experimental conditions and feeds

Commercial dietary SFO was obtained. Alfalfa forage and barley grain (as concentrated feed) were mixed to obtain total mixed ration with 40 to 60% 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.

Animals: Three native bulls were fistulated and fed with experimental ration twice daily for 15 days and ruminal fluid was collected.

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 [7] . 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., [8]. 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 [9]. 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 fluid was obtained from two fistulated cattle fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). The samples were incubated in the rumen fluid in calibrated glass syringes following the procedures of Menke and Steingass [6] 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. 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 [10].

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

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, [11] 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 , [11] 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 [6] 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 including dry matter (DM), crude protein (CP), crude Ash (Ash), ether extract (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) compounds, 93.20, 11.65, 6.05, 1.5, 33 and 18.02 percent, respectively measured. The chemical composition of mixed 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 as table1.

Table1. Chemical composition of experimental diet (%)

Compound	Dry matter	Crude protein	Ether extract	Ash	Acid detergent fiber	Neutral detergent fiber
total mixed ration	93.20	11.65	1.5	6.05	18.02	33

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

	Incubation times								
Treatment	2	4	6	8	12	24	48	72	96
0 % SFO	9.65	23.21[b]	34.65	44.31[ab]	52.25[a]	63.08[a]	71.26[a]	72.04	72.19
2.5% SFO	9.93	24.37[a]	35.70	44.61[a]	51.79[a]	62.03[a]	70.23[a]	72.58	73.83
5% SFO	10.41	24.42[a]	34.60	42.84[b]	49.53[b]	59.26[b]	67.58[b]	70.53	71.62
P value	0.33	0.048	0.18	0.045	0.045	0.04	0.04	0.21	0.16
SEM	0.34	0.30	0.40	0.42	0.57	0.65	0.68	0.70	0.69

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

Treatment	Estimated Parameters							
	a	b	|a|+b	c	OMD	ME	SCFA	NEL
0 % SFO	-6.28[a]	77.28[a]	83.56[a]	0.122	74.33[a]	10.84[a]	1.39[a]	6.64[a]
2.5% SFO	-4.68[a]	75.85[a]	80.53[a]	0.119	73.38[a]	10.70[a]	1.37[a]	6.53[a]
5% SFO	-1.88[b]	70.80[b]	72.68[b]	0.112	70.88[b]	10.32[b]	1.31[b]	6.27[b]
P value	0.01	0.002	0.002	0.125	0.005	0.001	0.005	0.005
SEM	0.34	0.40	0.42	0.57	0.34	0.090	0.014	0.062

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)
S.E.M: standard error of the mean

The gas production of Treatments shown in Table 2. 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. 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 blank or without SFO supplementation than 2.5 and 5% of SFO supplemented Treatments. Higher level of SFO caused lower energy for feed.

DISCUSSION

Various factors can affect volume of gas production; experimental samples, cell wall percentage, metabolites, preventive compounds and ruminal fluid content and ability for fermentation process [12]. Also, *in vitro* gas production has negative correlation with structural carbohydrates (cell wall) and has positive correlation with non-fiber carbohydrates [4] that this subject was observed in present study. Menke and Steingass [6] had reported when we applied gas production test for digestive characterizes of feedstuffs it is proposed that only effective agents on gas production are physical and chemical traits of feed, but in fact any change in microbial activity of ruminal fluid can be efficient for fermentation process. With attention to P values in tables 2-4, in most of hours of fermentation, significant changed between gas production and energy indices can be observed; so that with inclusion of SFO to 2.5%, there was not any significant difference between SFO included treatments and control one. But with inclusion of 5% SFO, significant decreases in gas production volume for both of soluble (a) or insoluble (b) fractions were observed at most of fermentation hours [table3]. Decreases in gas production potential or decreases in gas production from insoluble or soluble fractions may be because of prevention of substrate availability for bacteria in SFO included feed or possible toxicity for microorganism because of SFO fatty acids. With attention to methanogenic bacteria is sensitive for high level of oil [13], the SFO may be act as a toxin for proliferation and activity of this group of ruminal bacteria. Kumar et al., [14] had stated lower gas production parameters with addition of some oil seed cakes in ration. It seems that thin layer of SFO on feed (substrate for rumens bacteria) may be destroyable for fermentation process. As conclusion, dietary inclusion of 5% sunflower oil may cause considerable decreases in in vitro gas production parameters and energy indices of mixed ration (40%: forage and 60% concentrated feed) for ruminant. Obtained results also shows efficiency of *in vitro* gas production bio-technique for evaluation of ruminal fermentation process and relative factors.

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