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Effects of Soybean Meal Coated with Fat on *in vitro* Organic Matter Fermentation and Gas Production

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Abstract: Researchers utilized *in vitro* rumen gas production technique to evaluate soybean meal coated with different types and levels of hydrogenated fatty acids for total gas production, organic matter digestibility. The aim of this study was also to investigate the digestion kinetic of Soybean Meal (SBM) protected with different types: Hydrogenated Tallow (HT); Hydrogenated Palm oil (HP) and levels (0, 200, 400, 600 and 800 g kg^{-1}) of fatty acids to decrease rumen digestibility of organic matter and Gas Production (GP). Approximately, 200 mg (DM basis) of sample is weighed and inserted in glass syringes then mixed with the inoculum and artificial saliva which the initial volume of the syringes reached to approximately 30 mL and incubated at 39°C in a ventilated oven. GP was recorded after 0, 2, 4, 6, 8, 12, 24, 48, 72 and 96 h. There were differences among fat coated treatments and SBM in total GP at 6, 8, 12, 24 and 48 h of *in vitro* incubation and the treatments differed ($p<0.01$) in rate of and potential, gas production. The result of the present study showed that experimental fats which mixed by soybean meal to protect it from microbial fermentation, reduced *in vitro* digestibility of organic matter and GP during the time of incubation. In compare to HT, coating soybean meal with HP resulted in significant reduced GP ($p<0.01$). Furthermore, the values of b and a+b reduced significantly since, soybean meal coated with these two types of fat ($p<0.01$). It seems that one of the possible strategies to reduce total GP from dairy cows or feedlot cattle is coating some portions of dietary concentrate with supplemental fats in the form of hydrogenated free fatty acids like HT or HP. Accordingly a reduction in the fermentation rate of organic matter and proteins could reduce the total GP as NH_3, CO_2 and CH_4 in the rumen and may provide more protein for absorption in the small intestine.

Key words: Soybean meal, fat coated soybean meal, gas production, hydrogenated tallow, hydrogenated palm oil, Iran

INTRODUCTION

The addition of fat to dairy cows diets is known to enhance the energy density of the diet and milk production. The addition of supplemental fat as well as improve the energy status of cows also, it might be used to coat proteins in dairy cow rations and beside offering more energy could supply supplementary protein and amino acids in the small intestine (Sklan, 1989). Whereas, it has been shown that supplemental fats also could reduce carbohydrates and proteins fermented in the rumen to decrease gas emission from dairy herds (Getachew *et al*., 2001). Likewise, it is known that there is a reduction in the amount of feed fermented with addition of fats (Mathison *et al*., 1997). The addition of medium chain length fatty acids has been reported to lower methane production (Dohme *et al*., 2001). A study concluded that adding unsaturated fats may help to decrease methane production but may also have negative effects on feed intake or animal performance (Giger-Reverdin *et al*., 2003). But some researchers have reported added fat had no adverse effects on fiber digestibility and dry matter intake (Hussein *et al*., 1995; Bateman and Jenkins, 1998; Getachew *et al*., 2001).

Ruminants are a major source of methane emissions with the world's cattle emitting about 100 million tons of methane into the atmosphere annually, about 12.5-20% of the total global CH_4 emissions (France *et al*., 1993; Crutzen, 1995). A suppressing influence of ration fat content on CH_4 production has been reported elsewhere (Sauer *et al*., 1998; Dohme *et al*., 2001; Lee *et al*., 2003) and Dohme *et al*. (2001), Getachew *et al*. (2001) and Fievez *et al*. (2003) have suggested that it is not only the total amount of fat but also, its composition that exerts

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biologically important influences on rumen fermentation. Recently, emission of CH_4 and other volatile organic compounds from ruminants and their effect on air quality has attracted the attention of air regulatory agencies in many parts of the world.

The major greenhouse gases (CH_4 and CO_2) associated with ruminants are produced as a result of natural biological processes of microbial breakdown of feed components. On average, about 4-12% of gross energy intake is converted to CH_4 gas (Holter and Young, 1992; Johnson *et al*., 1996). Methane emissions from dairy cattle represented about 25% of total enteric CH_4 emissions while beef cattle accounted for 71% (18% of global emissions) (Westberg *et al*., 1991). There may be potential to reduce the extent of CH_4 and total gas production by manipulating diet and management practices that influence ruminal microbial fermentation. Considerable efforts have been made to explore the possibility of reducing methane or total GP from animals using an *in vitro* technique (Soliva *et al*., 2003; Mohammed *et al*., 2004).

There are numbers of *in vitro* techniques available to evaluate the nutritive value of feeds at relatively low cost such as *in vitro* GP technique. The gas measuring technique was considered to be a routine method of feed evaluation (Menke *et al*., 1979) where a high correlation between *in vitro* GP and *in vivo* apparent digestibility was reported. Gas production techniques are based on the principle that anaerobic microbial digestion of organic matter releases gas (primarily CO_2 and CH_4) and VFA (Lanzas *et al*., 2007). Rumen protected vegetable proteins such as heat-treated rapeseed meal or soybean meal can be fed as alternatives to FM to increase digestible undegradable protein supply.

Science fat alone sometimes is not very palatable in the diet of high producing dairy cows and because there is a reduction in the amount of feed fermented with additions of fats, it is assumed that feeding a fat coated protein may alter the amount of rumen degradable protein through reducing fermentable organic matter and finally decrees the total GP in the rumen.

The hypothesis was that if there is an intake about 600-700 g of experimental fat per day per cow to reach maximal efficiency (National Research Council, 2001), coating some ingredients of the diet by the experimental fat could lead to a reduction in GP and fermentation of protein source to supply more bypass protein. The objective of this research was to investigate the GP rate and digestibility of fat coated soybean meal to reduce GP.

MATERIALS AND METHODS

All experimental procedure has been carried out in accordance with the EC Directive 86/609/EEC for animal experiments.

Preparing samples and fat coating technique: Before the beginning of the experiment, soybean meal was ground through a sieve with 1 mm pore size in a hammer mill. Fat coating method was done according to pan coating method with some modification (Grass and Unangst, 1972; Sklan, 1989). The procedure was accomplished using two types of experimental fat (HT and HP) to embed soybean meal particles (particle size 1 mm) in very thin layers of fat to make a continuous film of fatty acids on a core of soybean meal. For this purpose, soybean meal was added into Teflon beakers containing melted experimental fat in different ratios. The experimental fats were weighed before heating into the beaker. An automatic mixer (300 watt, Moulinex, ABM641, Brazil) mixed the combination gently until the mixture gets cold slightly. The beaker finally transferred into cold water (5°C) to cool down and the blend continuously mixed by the mixer until small beads of fat coated SBM formed. Therefore, SBM was encapsulated using 0, 200, 400, 600 and 800 (g kg^{-1}) of experimental fats.

The final form of the product was small beads ranging from 1000-1500 μm in diameter depend upon the type of fat used for encapsulation and the optimal size for GP Method. Hydrogenated palm oil was obtained from PALMAC (vegetable derived fatty acids, Pan-Century Oleochemicals, SDN BHD, Malaysia) and hydrogenated tallow supplied by Mirshamsi Co. (Food grade hydrogenated tallow, Kaveh industrial city, Saveh, Iran).

Chemical composition: Dry Matter (DM) was determined by drying at 135°C for 4 h followed by equilibration in a desiccator (AOAC, 1995, ID 930.15) and Organic Matter (OM) was calculated as weight lost upon ignition at 600°C (AOAC, 1995, ID 942.05). Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) were determined as described by Van Soest *et al*. (1991). Both ADF and NDF are reported on an ash-free basis. Fat content was determined by ether extraction (AOAC, 1995, ID 930.39). Crude protein was determined by a standard Kjeldahl Method. Fatty Acid (FA) profiles of two experimental fats were determined using gas chromatography (Agilent Technologies, hp, 6890 N; USA) that was equipped with a capillary column (DB-FFAP, ID:

0.32 mm×0.25 μm×30 m; SGE-incorporated, Texas, SGE, USA) was used in this study for determination of fatty acids profile.

***In vitro* gas production:** GP was determined by the procedure of Menke and Steingass (1988). Samples (200 mg) were weighed into 100 mL calibrated glass syringes with pistons lubricated with vaseline. Buffered mineral solution (Menke and Steingass, 1988) was prepared and placed in a water bath at 39°C under continuous flushing with CO_2. Rumen fluid was collected before the morning feeding from three ruminally fistulated steers that were fed diet containing alfalfa hay (600 g kg^{-1}) plus a concentrate mixture (400 g kg^{-1}) at 9:00 and 18:00 h. Rumen fluid was pumped from the rumen with a manually operated vacuum pump and transferred into two pre-warmed thermos flasks, transported to the laboratory, combined, filtered through eight layers of cheesecloth and flushed with CO_2. Rumen fluid was added to the buffered mineral solution with constant stirring while maintained in a water bath at 39°C. About 30 mL of buffered rumen fluid was dispensed into syringes containing the samples. All handling was under continuous flushing with CO_2. After closing the clips on the silicon tube at the syringe tip, syringes were gently shaken and the clips were opened to remove gas by pushing the piston upwards to achieve complete gas removal. The clip was closed, the initial volume recorded and the syringes were affixed to a rotary shaker platform (lab-line instruments Inc Melors dark, USA) set at (120 rpm) housed in an incubator at 39°C. Incubation was completed in triplicate with readings of GP after incubation for 0, 2, 4, 6, 8, 12, 24, 48, 72 and 96 h for fat coated and uncoated samples. Kinetics of total GP was calculated (Orskov and McDonald, 1979) for fat coated and uncoated soybean meal. Differences in the composition and activity of rumen fluid inoculum were controlled by parallel measurements in with incubation of buffered ruminal fluid without substrate (Blank test). Cumulative GP data were fitted to the exponential equation:

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

Where:

Y = The gas produced at t time
a = The GP from the immediately soluble fraction (mL)
b = The GP from the insoluble fraction (mL)
a+b = Potential of GP (after 96 h) from fermentable fraction (mL/200 g DM)
c = The GP rate constant for b
t = The time of incubation (h)

The Metabolizeable Energy (ME) contents and Organic Matter Digestibility (OMD) were calculated using equations of Menke and Steingass (1988) as:

$$ME\ (MJ\ kg^{-1}\ DM) = 2.20+0.136\times Gp+0.057\times CP+0.0029\times CP^2$$

$$OMD\ (g/100\ g\ DM) = 14.88+0.889\times Gp+0.45\times CP+0.0651\times XA$$

Where:

CP = Crude protein in g/100 g DM
XA = Ash in g/100 g DM
Gp = The net gas production (mL) from 200 mg after 24 h of incubation

Statistical analysis: Data on *in vitro* GP were subjected to analysis of variance in a completely randomized design using the SAS Program General Linear Model (GLM) procedure (SAS, 2005). Significant means were compared using the least square means method. Mean differences were considered significant at $p<0.05$. Standard errors of means were calculated from the residual mean square in the analysis of variance.

RESULTS AND DISCUSSION

Chemical composition: The chemical composition of soybean meal coated with HP and HT is presented (Table 1). The CP content of treatments ranged from 91.6 g kg^{-1} DM in $SBHP_{80}$ to 374.1 g kg^{-1} DM in $SBHP_{20}$ and 468.1 g kg^{-1} DM for soybean meal. The EE content of feeds ranged from 739.2 g kg^{-1} DM in $SBHP_{80}$ to 171.6 g kg^{-1} DM in $SBHT_{20}$. The DM content increased likewise as the inclusion of fats increased.

Table 1: Chemical composition of soybean meal coated with HP and HT (g kg^{-1} DM)

		Hydrogenated Tallow (HT)				Hydrogenated Palm oil (HP)			
Variables	SBM*	$SBHT_{20}$	$SBHT_{40}$	$SBHT_{60}$	$SBHT_{80}$	$SBHP_{20}$	$SBHP_{40}$	$SBHP_{60}$	$SBHP_{80}$
Dry matter	920.9	936.2	952.1	968.3	984.1	932.7	944.5	956.3	968.1
CP[1]	468.1	372.5	282.9	187.1	93.4	374.1	289.9	181.2	91.6
NDF[2]	208.1	162.5	121.9	83.2	41.4	166.2	124.1	84.2	41.4
ADF[3]	63.2	51.1	38.5	25.9	13.1	52.1	38.5	25.2	12.9
EE[4]	16.7	171.6	326.4	480.3	643.1	196.6	379.9	559.9	739.2
OM[5]	924.8	942.0	956.1	970.8	983.4	942.7	936.1	960.7	981.4
ASH	75.2	58.0	43.9	29.2	16.6	57.3	63.9	39.3	18.6

*Soybean Meal (SBM) coated with 200, 400, 600 and 800 (g kg^{-1}) of Hydrogenated Tallow (SBHT) and Hydrogenated Palm oil (SBHP). [1]Crude Protein (CP); [2]Neutral Detergent Fiber (NDF); [3]Acid Detergent Fiber (ADF), [4]Ether Extracts (EE); [5]Organic Matter (OM)

The ash content in contrast decreased by the addition of fats to soybean meal and consequently the OM content substantially enhanced (Table 1).

Fat sources varied in their fatty acids composition as expected (Table 2). The HT was more saturated fat source than HP. The HT has more C18:0 content than HP (42.3 vs. 0.99% of DM) while HP contained large amount of C16:0 fatty acid. Furthermore, the HT contained the odd carbon fatty acids (C15 and C17) in contrast to HP. In the current experiment, the C16:C18 ratio was higher for HP in compare to HT.

Table 2: Fatty acids (DM%) composition of Hydrogenated Tallow (HT) and Hydrogenated Palm oil (HP)

Components	HT	HP
C8	7.180	4.446
C10:0	2.201	1.457
C10:1	0.943	0.632
C12:0	1.335	0.972
C14:0	2.638	2.024
C14:1	0.893	0.652
C15	0.728	-
C16:0	29.324	74.634
C16:1	0.438	0.402
C17	2.230	-
C18:0	42.320	0.995
C18:1	0.348	6.758
C18:2	0.247	1.550
C18:3	0.414	0.080
Others*	3.770	3.400
Total fatty acids	95.000	98.000
Saturated fatty acids	87.950	84.520
Unsaturated fatty acids	3.280	10.070
Unsaturated to saturated ratio	0.037	0.119
C16:C18	0.692	74.970

*Unknown fatty acids which not detected by GC apparatus

***In vitro* gas production:** Cumulative GP volume (mL/200 mg DM), GP parameters and calculated amounts of OMD and ME of soybean meal coated with HT and HP are shown in Table 3 and 4. There was a difference ($p<0.01$) in GP among treatments (Table 3). Effect of fat type and level to protect soybean meal were significant, particularly at 8 h after incubation to latter times of incubation ($p<0.01$). The GP volume at first time of incubation did not differ among treatments however, fat coated treatments produced less gas compare to SBM.

The $SMHT_{80}$ and $SMHP_{80}$ were produced the lowest volume of gas after 6 h of incubation (12.43 and 13.76) in compare to other treatments ($p<0.05$). Effect of level of HP used to protect SBM was not significant at 6 h of incubation.

There were no significant differences in GP volume among SMHP in level of 200, 400 and 600 g kg^{-1}. There were significant differences among treatments coated with HT and HP after 8, 12, 24 and 48 h of incubation compare to SBM ($p<0.01$). The GP reduced as soybean meal coated with HT and HP but the fermentation pattern of uncoated soybean meal was not distinctly different compared to SMHT and SMHP treatments at first times of incubation (Table 3 and Fig. 1). Nonetheless the fermentation pattern of SBM was principally differed at later times of incubation comparing to other treatments (Fig. 1). Potential GP (a+b), GP from the insoluble fraction (b) and fractional rates of GP (c) differed ($p<0.01$) among treatments (Table 4).

Table 3: *In vitro* gas production (mL/200 mg DM) of soybean meal coated with HT and HP incubated in buffered rumen fluid at different incubation times

	Hydrogenated Tallow (HT)					Hydrogenated Palm oil (HP)				Significance		
Time (h)	SBM	$SMHT_{20}$	$SMHT_{40}$	$SMHT_{60}$	$SMHT_{80}$	$SMHP_{20}$	$SMHP_{40}$	$SMHP_{60}$	$SMHP_{80}$	Fat	Level	SEM
2	7.40	8.95	7.99	7.84	7.86	7.22	8.36	9.04	7.39	NS	NS	0.42
4	17.29	17.27	13.63	12.07	11.00	15.07	14.90	14.27	12.28	NS	NS	0.95
6	23.44^{a}	22.37^{a}	17.87^{b}	15.05^{bc}	12.43^{d}	19.07^{be}	19.10^{be}	17.03^{be}	13.76^{df}	*	NS	1.22
8	31.78^{a}	25.82^{b}	19.91^{c}	16.78^{d}	13.21^{e}	22.14^{f}	19.78^{cg}	18.05^{cg}	14.00^{eh}	**	**	2.63
12	43.30^{a}	29.67^{b}	23.20^{c}	19.13^{e}	14.31^{f}	24.72^{cg}	21.89^{h}	19.95^{eh}	14.44^{if}	**	**	2.95
24	54.37^{a}	36.02^{b}	26.80^{c}	21.01^{d}	14.63^{e}	29.82^{f}	25.91^{cg}	21.57^{dh}	14.99^{ei}	**	**	3.33
48	60.44^{a}	42.06^{b}	30.17^{c}	22.97^{d}	15.02^{e}	32.41^{cf}	28.35^{gf}	22.99^{dh}	17.51^{ei}	**	**	3.96

$^{a-d}$Means within a row with different superscripts differ ($p<0.05$). Fat = Effect of experimental fat source; Level = Effect of experimental fat level; NS = Not Significant; * = $p<0.05$; ** = $p<0.01$ and SEM = Standard Error of Mean

Table 4: The gas production parameters, Metabolizable Energy (ME) and Organic Matter Digestibility (OMD) contents of soybean meal coated with HT and HP

	Hydrogenated Tallow (HT)					Hydrogenated Palm oil (HP)				Significance		
	SBM	$SMHT_{20}$	$SMHT_{40}$	$SMHT_{60}$	$SMHT_{80}$	$SMHP_{20}$	$SMHP_{40}$	$SMHP_{60}$	$SMHP_{80}$	Fat	Level	SEM
a+b	61.930^{a}	44.420^{b}	32.150^{c}	24.000^{d}	15.810^{e}	34.340^{f}	29.000^{g}	22.670^{dh}	17.500^{i}	*	**	0.0050
b	63.620^{a}	42.580^{b}	30.770^{c}	23.010^{d}	15.810^{e}	33.590^{f}	27.960^{g}	22.170^{dh}	16.990^{i}	*	**	0.0260
c	0.092^{a}	0.096^{ab}	0.112^{c}	0.149^{d}	0.234^{e}	0.119^{fc}	0.147^{gd}	0.222^{eh}	0.241^{ei}	*	*	0.0002
ME	18.620^{a}	13.830^{b}	10.800^{c}	8.740^{d}	7.110^{e}	12.990^{f}	10.680^{g}	8.820^{dh}	7.160^{ei}	**	**	0.0500
OMD	84.750^{a}	64.140^{b}	51.630^{c}	42.170^{d}	32.190^{e}	58.620^{f}	50.830^{b}	42.670^{d}	32.520^{e}	**	**	0.2100

$^{a-d}$Means within a row with different superscripts differ ($p<0.05$); a+b: Potential GP (mL/200 mg DM); b: The GP from the insoluble fraction (mL); c: Fractional rate of GP (mL*h^{-1}), Fat = Effect of experimental fat source; Level = Effect of experimental fat level; ME = Metabolizable Energy (MJ kg^{-1} DM); OMD = Organic Matter Digestibility (g/100 g DM); NS = Not Significant; * = $p<0.05$; ** = $p<0.01$ and SEM = Standard Error of Mean

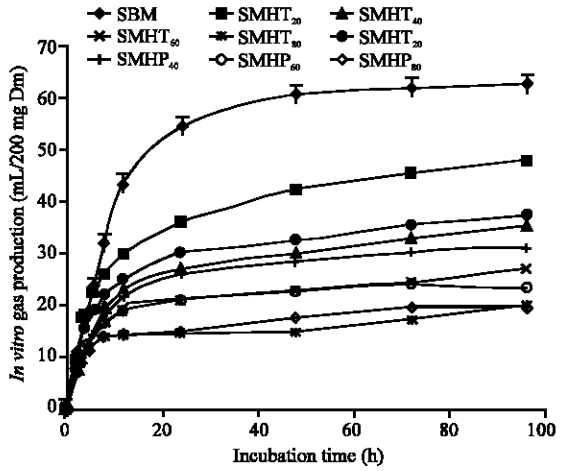

Fig. 1: Pattern of *in vitro* gas production on incubation of SBM coated with 200, 400, 600 and 800 g kg^{-1} HT (SMHT) and HP (SMHP) in buffered rumen fluid

Fat type as well as fat level significantly affected b, a+b and c parameters for coated treatments in compare to SMB ($p<0.05$). The potential GP (a+b) of uncoated soybean meal was greater (61.93 mL) than other treatments which coated with HT or HP ($p<0.01$). Fat coating method results in a reduction of potential GP level to 15.8 and 17.5 mL in $SMHT_{80}$ and $SBHP_{80}$ in contrast to SBM (61.93 mL).

The GP from the insoluble fraction (b) of soybean meal coated with these experimental fats reduced significantly ($p<0.05$) because fermentable fraction decreased along with the addition of 200-800 g kg^{-1} of experimental fats toSBM (Table 4). In contrast to b and a+b parameters, fractional rates of GP (c) increased significantly for coated treatments in compare to SBM ($p<0.05$) and the value of (c) was greater for $SMHT_{80}$ and $SMHP_{80}$ (Table 4).

Metabolizable Energy (ME) and Organic Matter Digestibility (OMD): According to some studies (Menke *et al*., 1979; Menke and Steingass, 1988; McDonald *et al*., 1995) OMD and ME could be evaluated by 24 h *in vitro* GP data and chemical composition of feed samples. The results are shown in Table 4. The ME content of fat coated treatments decreased significantly in compare to SBM ($p<0.01$). Effect of fat type as well as fat level was significant on the content of ME ($p<0.01$). Moreover, the OMD value, reduced significantly ($p<0.01$) as SBM coated with HT and HP in different ratios (Table 4). The type and level of fat used in this trial reduced digestibility of organic matter in all treatments compare to SBM with no experimental fat ($p<0.01$). There were no significant differences in calculated OMD and the ME values for SMHT and SMHP at 600 and 800 g kg^{-1} level (i.e., $SMHT_{60}$ vs. $SMHP_{60}$ and $SMHP_{80}$ vs. $SMHT_{80}$). Recently using fats, to protect amino acids and protein sources is in the case of interest for nutritionist (Dhiman *et al*., 2001; Rulquin and Kowalczyk, 2003; Rulquin *et al*., 2006). Besides protection of protein sources with fat to reduce protein degradability, it may have a side effect on ruminal fermentation to reduce total GP in the rumen. The technology of fat coating nutrients relies on achieving a physical protection of feed to reduce nutrient microbial digestion that could lead to reduced GP. On the other hand, the physicochemical properties of feed and fat as well as the technology used for protection are important factors to reach a fine surface coating.

Variation in chemical composition of experimental treatments observed in the current study is associated with inclusion of fats to coat SBM in different ratios. Coating SBM with HT and HP reduced the CP and increased EE content of experimental treatments. As the level of fat inclusion increased to protect SBM, the CP, ADF, NDF and ASH content decreased reasonably. High content of C18:0 fatty acid in HT is expected because of the hydrogenation process that occurs in industries could lead to exchange polyunsaturated fatty acids to subsequent saturated ones.

One of the challenges associated with feeding HT is that the digestibility of hydrogenated fatty acid is lower than unsaturated fatty acids which are extensively digestible. Increasing saturation of fat sources increases ruminal inertness but decreases fatty acid digestibility, commonly hydrogenated triglycerides such as tallow is inadequately digested (Macleod and Buchanan-Smith, 1972; Eastridge and Firkins, 2004).

The difference between fatty acids profile of tallow (Getachew *et al*., 2001) and HT used in the current study indicate that tallow has more monounsaturated fatty acids (C18:1 isomers) than HT. Getachew *et al*. (2001) reported approximately 28.9 (DM, per percent) C18:1 for tallow whereas the content of C18:1 in HT and HP in the presenting study was 0.34 and 6.75 (DM, per percent), respectively. The decrease in GP during the incubation times is along with the inclusion of HT and HP to protect SBM. In contrast to the findings, Getachew *et al*. (2001) reported that tallow did not affect GP. There is a great difference between tallow and HT for fatty acids profile and also variations between fat coating procedure and adding fat in a total mixed rations as supplemental fat (Getachew *et al*., 2001). In addition, the levels of fat used to protect SBM (up to 800 g kg^{-1}) varies noticeably from those levels reported in the study of Getachew *et al*. (2001) which utilize 50-250 g kg^{-1} added fatty acids *in vitro* as tallow or yellow grease to deists. The reduction

of GP over the time of incubation by coating SBM with HT and HP may associate with microbial attachments. It has been suggested that dietary fats may coat fiber and interfere with microbial attachment (Devendra and Lewis, 1974). Perhaps this could explain in part the lower GP in HT and HP coated SBM. In other study (Stewart, 1977) observed a depression in cotton fiber degradation when the cotton yarn had been soaked in either tallow or fatty acids. Other explanation could be stated as some unsaturated fatty acids may act as toxin for rumen methanogen bacteria (Hunter *et al*., 1976; Kim *et al*., 2000). The HT and HP in the current study at high levels of handling (>400 g kg^{-1} DM) have large amounts of unsaturated fatty acids which might slightly interpret the reduced GP in these treatments. Methanogen bacteria are a separate group of organisms which are an ordinary component of the rumen microbial ecosystem. Hydrogen (H_2) and CO_2 are the principal substrates used by rumen methanogens to produce CH_4. Consequently, compounds that directly inhibit activity of methanogens are likely to reduce or eliminate, CH_4 and total gas production (Baker, 1999). It is actually difficult to explain the biological basis why HT and HP coated SBM would be produce lower total gas volume *in vitro* compare to SBM. It is assumed that coating SBM with thin layers of fat could reduce the accessibility of microbial enzymes to the feed.

In vitro GP at 6 h from SBM was not comparable to that reported by Getachew *et al*. (2004). In the present study, SBM produced approximately 117.2 mL gas per g DM (23.44 mL/200 mg DM, Table 3) at 6 h after incubation in which it was 101.4 mL gas per g DM reported by previous research (Getachew *et al*., 2004). Results from the present study confirm earlier findings that showed free fatty acids and long-chain fatty acids inhibit methane and total GP in the rumen and free fatty acids may be more potent inhibitors than triacylglycerols (Nevel and Demeyer, 1996). Although, the mechanism by which this happen is still not known. Similarly, HT and HP in the form of long chain and free fatty acids, reduced gas production in this study as the time of incubation approached. One explanation may be the reduced availability of calcium needed for appropriate microbial function. This could be a mechanism by which dietary fat inhibit microbial fermentation (Jenkins, 1993). Some researchers employing *in vivo* experiment (El-Hag and Miller, 1972) and using pure culture techniques (Galbraith *et al*., 1997) demonstrated that individual fatty acids inhibited microbial growths but inclusion of calcium reversed the inhibitory effect. It is clearly elucidated that unsaturated oils had greater negative effect than saturated fats (Macleod and Buchanan-Smith, 1972; Jenkins and Palmquist, 1984) and free fatty acids caused a larger negative effect than the corresponding triglycerids (Macleod and Buchanan-Smith, 1972; Bateman and Jenkins, 1998). A free carboxyl group was also proposed to be necessary to inhibit microbial growth (Demeyer and van Nevel, 1995).

In the present study, fat sources used to protect SBM were hydrogenated free fatty acid products and their fatty acids profile confirmed that they have more long chain fatty acids than short chain ones (Table 2). The amount of gas produced after 24 h (Table 3) is not similar to those found by Getachew *et al*. (2004). These researchers reported a gas volume of 213 mL g^{-1} DM of soybean meal after 24 h incubation time. In the current study, it was approximately 271 mL g^{-1} DM (54.37 mL/200 mg DM) for soybean meal after 24 h incubation time.

The greater GP after 24 h for soybean meal may relate to higher content of NDF in SBM from those reported by Getachew *et al*. (2004). Higher NDF contents of SBM used in the present study (208 g kg^{-1} DM) also may describe in part the difference between the results (300 mL g^{-1} DM after 48 h or 60.4 mL/200 mg DM, Table 3) from those reported by Getachew *et al*. (2004) that reported SBM reached 237 mL gas/g DM after 48 h. The $SMTH_{20}$ achieved 180 mL g^{-1} DM (36.02 mL/200 mg DM) GP after 24 h which was greater than $SMPH_{20}$ that produced 130 mL g^{-1} DM (25.91 mL/200 mg DM). Generally, the SMHP treatments produced lower gas than SMHT which indicate that the HT had more inhibitory effect on rumen microbial ecosystem. In the other hand it could be explicated that HP has more potent to protect SBM. It showed that unsaturated fatty acids act as toxins for rumen bacteria (Hunter *et al*., 1976; Kim *et al*., 2000), therefore because the HP is more unsaturated fat than HT, covering SBM with this fat source may interfere with methanogenes bacteria and lead to a reduction in GP over the time of incubation.

Potential GP (a+b) has the same decreasing trend for SMHT and SMHP treatments for 200, 400, 600 and 800 g kg^{-1} DM levels which indicated the reduced GP could be achieved successfully with coating some ingredient of diets with fat. Likewise potential GP reported in this paper for SBM (61.93 mL/200 mg DM) is different from previous report (Getachew *et al*., 2002) which stated 49.5 mL/200 mg DM for SBM. The extent of potential GP reported here is similar to soybean hull pellets potential GP (62.6 mL/200 mg DM) reported previously (Getachew *et al*., 2002). Therefore, the discrepancies may be due to difference in protein and fiber content of SBM (468 g kg^{-1} CP and 208 g kg^{-1} NDF) used in this research and SBM (535 g kg^{-1} CP and 102 g kg^{-1} NDF) used in the

companion research made by Getachew *et al*. (2002). The GP from the insoluble fraction (b) also decreased similar to potential gas production noticeably due to a reduction of insoluble part of treatment by adding fat to SBM.

The results showed that the fractional rate of GP (c:mL h^{-1}) ranged from 0.241 h for $SMHP_{80}$ to 0.092 h for SBM. The fractional rate of GP reported in the present study for SBM is in close agreement with other report (Getachew *et al*., 2004). The greater fractional rate of GP for SMHT and SMHP treatments is comparable to that reported for canola meal (0.169) and alfalfa silage (0.134) (Getachew *et al*., 2004). One explanation for this could be due to protein and fat content of SBM coated with HT and HP whereas it showed that protein fermentation influenced GP mainly in the initial hours of incubation because the major part of protein is part of the soluble fraction and may determine the rate of GP and the shape of the GP curve (Cone and Van Gelder, 1999). Similarly, fat could influence the final GP (Fig. 1). The GP caused by fermentation of protein is not the same as that of carbohydrates and lipids. Relative to potato starch, casein was fermented in an earlier stage of incubation and fatty acids would be fermented relatively at the latter time of incubation (Fig. 1).

Moreover, the GP profiles could be separated in three sub-curves, representing GP caused respectively by fermentation of the water-soluble components by fermentation of the non-soluble components and by microbial turn over (Cone *et al*., 1997). Consequently, the pattern of fermentation of SBM was noticeably different from SMHT and SMHP treatments that had more insoluble fractions because of high content of fat in these treatments. Although, higher methane production is associated with fiber fermentation in highly digestible feeds such as SBM, a higher quantity of gas is produced in early hours of fermentation due to high digestibility of this feed. There is a reduction in NDF content by coating SBM with HT and HP which reflects the reduced fiber digestion and may lead to delayed organic matter digestion of fat coated treatments. As a result, we expected the overall digestibility of fat coated treatments should be declined by addition of fat to SBM.

Additionally, there is a lack of evidence to support a general theory that added fat exert adverse effects on rumen microbial activity, particularly when supplemental fat is included at levels typically fed in commercial dairy rations. The presence of negative effects on rumen fermentation parameters with inclusion of fat as a free fatty acid form, for coating SBM at high levels used in this study suggest that free fatty acids have more effect on rumen fermentation parameters compared to the corresponding triglycerides reported previously (Getachew *et al*., 2004). In contrast to the triglyceride form of fats, Getachew *et al*. (2004) reported that the K-soap of fatty acid could dissociate in the rumen buffer and consequently fatty acids in their free form depressed Volatile Fatty Acids (VFA) as well as GP. The OMD values were highest for SBM and lowest for $SMHT_{80}$ and $SMHP_{80}$ as we expected. High OM digestibility for SBM can be expected due to high concentration of carbohydrates, protein and fiber that supply available energy as ATP for microbial growth. Low digestibility of OM in fat coated treatments compared to SBM was due to high concentration of fat in these treatments. These finding is in agreement with results obtained later whereas Getachew *et al*. (2001) found a decline in gas production and *in vitro* true digestibility with addition of fatty acids of yellow grease and tallow to total mixed rations.

The variation in OMD values for SMHT and SMHP treatments can be related to differences in GP after 24 h of incubation and the differences in chemical composition of these treatments. Although, feeding rumen un-degradable proteins along with fat or fat coated protein provided no further improvement in milk yield compared with fat alone but partially alleviated the depression in protein content caused by supplemental fat and increased the daily yield of milk protein (Dhiman *et al*., 2001). It has been suggested that feeding supplemental fat alone in transition period (Afzalzadeh *et al*., 2010) or oilseeds and bypass fats along with proteins or as fat coated proteins in lactation periods (Sklan and Tinsky, 1993; Dhiman *et al*., 2001; Petit *et al*., 2005) could affect some metabolites, blood plasma hormones, feed digestibility and milk composition and fatty acids profile. In a study by Sklan (1989), he showed 84-90% of whey powder and SBM coated with calcium salts of fatty acids remained *in sacco* after 20 h incubation in the rumen of sheep. He concluded that proteins coated with calcium soaps are not degraded in the rumen and thus energy and non-degradable protein can be supplied to ruminants by this route (Sklan, 1989). The reduced OMD values calculated in this study may lead to supply more organic matter and proteins to small intestine for milk protein synthesis and influence animal performances.

The ME values of fat coated treatments were within the ranges reported by Menke and Steingass (1988) where the ME values of various European feeds ranged from 4.5-15 MJ kg^{-1} DM. Although, the ME value for SBM was greater than previous research (Getachew *et al*., 2004). The discrepancy is due to high production of gas during the time of incubation and the dissimilar chemical composition of SBM from those reported previously (Getachew *et al*., 2004) which resulted to larger ME values. The gas produced after 24 h of incubation

accompanied by chemical analysis of the feeds are used to estimate the ME contents of feeds for ruminants. The application of the method for ME estimation has been used to evaluate large numbers of feeds and is documented in several studies (Menke and Steingass, 1988; Krishnamoorthy *et al*., 1995; Getachew *et al*., 2002, 2004). According to the findings, the *in vitro* rumen gas technique can be used to study the nutritional quality of feed ingredients that covered physically with fat as well as mixed rations and individual feed ingredient.

CONCLUSION

The fat coating technique was successfully used to assess the impact of HT and HP on total GP and fermentation kinetics. Also, it can be used to identify the influence of fats on GP during the time of incubation, to evaluate empirical equations to estimate the ME and OMD content of fat coated treatments. The *in vitro* rumen gas technique offers a unique tool for researchers to verify greater levels of supplemental fats in ruminant diets to investigate ruminal GP kinetics. In addition, it could be concluded that HP had higher degree of rumen protection for SBM because the HP treatments have the lowest GP over the time of incubation.

The differences identified in the lipid study suggest that the degree of rumen protection required to prevent ruminal protein degradation being depressed varies with lipid type and level. The *in vitro* GP methodology used in this study will allow such treatments to be developed and examined under various rumen conditions prior to animal studies. Likewise it should be demonstrated that GP techniques have good potential to predict rumen OM degradation. Researchers did not measure CH_4 GP in the current study but as the total gas emission and production reduced by coating SBM with fat, it could be concluded that these treatments probably reduced the CH_4 production along with total gas production. Therefore supplemental fats have a good potential to reduce gas emission from dairy and feedlot cattle.

As a general result, one of the possible strategies to reduce total GP from dairy cows is coating some portions of dairy cow dietary concentrate (non-fibrous and high in protein or starch content) with supplemental fats in the form of long chain free fatty acids, particularly in high concentrate eater feedlot cattle or dairy cattle.

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