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Full Length Research Paper

In vitro organic matter digestibility and gas production of fish-meal coated with fat

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In this study, an *in vitro* rumen gas production technique was utilized to evaluate fish-meal coated with different types and levels of fats for total gas production, Metabolizable energy (ME) and organic matter digestibility (OMD) contents. Approximately 200 mg of sample was weighed and inserted in glass syringes, then mixed with the inoculum and artificial saliva, incubated at 39 °C in a ventilated oven and gas production (GP) was recorded after zero to 96 h of incubation. There were differences among different fat coated fish-meals and uncoated fish-meal (FM) in total GP at 12, 24, and 48 h of 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 fish-meal, reduced in vitro digestibility of organic matter and GP during the time of incubation. In compare to hydrogenated palm oil (HP), coating fish-meal with hydrogenated tallow (HT) resulted in significant reduced GP (P<0.01). Furthermore the values of b and a+b reduced significantly since fish-meal coated with both types of fat in comparison to uncoated fish-meal (P<0.01). It seems that one of the possible strategies to reduce total GP from dairy cows is coating some portions of dietary concentrate with supplemental fats in the form of hydrogenated fats like HT or HP.

Keywords: fish-meal; gas production; hydrogenated tallow; hydrogenated palm oil, fat coating.

INTRODUCTION

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). 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 were reported. Maximizing energy intake by increasing energy density of the diet is a logical feeding strategy for early lactating dairy cows (Elliott, 1994). The addition of supplemental fat as well as improving the energy status of cows might be used to coat proteins in dairy cow rations and besides offering more energy, could supply supplementary protein and amino acids in the small intestine (Sklan, 1989). 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). Ruminants are a major source of methane emissions, with the world's cattle emitting about 100 million tonnes of methane into the atmosphere annually, about 12.5 to 20% of the total global methane (CH_4) emissions (France et al., 1993). Recently, emission of CH_4 and other volatile organic compounds from ruminants and their effects on air quality has attracted the attention of air regulatory agencies in many parts of the world. An average of about 4 to 1 2% gross energy

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Abbreviations: HT, hydrogenated tallow**; HP,** hydrogenated palm oil**; FM,** fish-meal; **FMHT20, FMHT40, FMHT60, FMHT 80,** fish-meal coated with 200, 400, 600 and 800 (g/kg) of hydrogenated tallow; **FMHP20, FMHP40, FMHP60, FMHP 80,** fish-meal coated with 200, 400, 600 and 800 (g/kg) of hydrogenated palm oil; **GP,** gas production; **DM,** dry matter.

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., 2001). There may be potentials to reduce the extent of CH_4 and total gas production by manipulating diet and management practices that influence ruminal microbial fermentation.

Feeding fish-meal (FM) to dairy cows can be used as an alternative to other plant protein sources to increase digestible undergradable protein supply. Similarly, properly processed FM is highly resistant to rumen degradation and has an excellent amino acids profile in terms of ruminant needs (Rook, 1985). Although, heating is an inherent part of the processing of fish-meal and may lead to decreased ruminal degradation, nevertheless, it is thought that coating fish meal with fatty acids may significantly decrease organic matter and protein digestibility and ruminal GP. It is assumed that, feeding a fat coated fish meal could alter the amount of rumen degradable protein through reducing fermentable organic matter and finally decreases total GP in the rumen.

Our hypothesis was that, if there is an intake of about 600 to 700 g of experimental fat per day, per cow to reach maximal efficiency (NRC, 2001), coating some ingredients of the diet like fish meal with fat, may possibly 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 FM to reduce GP.

MATERIALS AND METHODS

Preparing samples and fat coating technique

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 fish-meal particles (particle size 1 mm) in very thin layers of fat to make a continuous film of fatty acids on a core fish- meal. For this purpose, fish- 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 was finally transferred into cold water (5°C) to cool down and the blend continuously mixed by the mixer until small beads of fat coated FM was formed. Therefore, FM was encapsulated using, 200, 400, 600 and 800 (g/kg) of experimental fats. The final form of the product was small beads ranging from 1000 to 1500 µm in diameter depending on 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 desiccators (AOAC, 1995, ID 930.15) and organic matter (OM) was calculated as weight lost upon ignition at 600°C (AOAC, 1995, ID 942.05). Fat content was determined by ether extraction (AOAC, 1995, ID 930.39). Crude protein was determined by a standard Kjeldahl method (EC, 1993). 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) and was used in this study for determination of fatty acids profiles.

In vitro gas production

GP was determined by the procedure of Menke and Steingass, (1988). Samples (200 mg) weighed into 100 ml calibrated glass syringes with pistons lubricated and Vaseline. Buffered mineral solution (Menke and Steingass, 1988) was prepared and placed in a water bath at 39°C under continuous flushing with carbon dioxide (CO_2). Rumen fluid was collected before the morning feeding from three ruminally fistulated steers that were fed with diet containing alfalfa hay (600 g/kg) plus a concentrate mixture (400 g/kg) 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 (Ørskov and McDonald, 1979) for fat coated and uncoated fish- meal. Differences in the composition and activity of rumen fluid inoculum were controlled by parallel measurements 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 is 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" and "t" is the time of incubation (h).

The metabolizable energy (ME) contents and organic matter digestibility (OMD) were calculated using equations of Menke and Steingass (1988) as:

$$ME\ (MJ/kg\ DM) = 2.20 + 0.136 \times Gp + 0.057 \times CP + 0.0029 \times CP^2$$

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

Where, CP is crude protein in g/100 g DM, XA ash in g/100 g DM and Gp is the net gas production (ml) from 200 mg after 24 h of incubation.

Statistical analysis

Data on *in vitro* gas production were subjected to analysis of

Table 1. Chemical composition of fish- meal coated with HP and HT (g/kgDM).

Variable	FM*	Hydrogenated tallow (HT)				Hydrogenated palm oil (HP)			
		$FMHT_{20}$	$FMHT_{40}$	$FMHT_{60}$	$FMHT_{80}$	$FMHP_{20}$	$FMHP_{40}$	$FMHP_{60}$	$FMHP_{80}$
Dry matter	924.6	926.2	927.9	928.5	930.1	925.3	925.9	927.2	929.6
OM♣	796	836.8	877.6	918.4	959.2	836.8	871.4	906.1	960.2
CP‡	578.6	462.8	347.1	231.4	115.7	451.3	352.9	237.2	127.2
EE†	114.1	251.3	411.3	571.3	731.3	262.7	422.7	582.7	742.7
ASH	204	163.2	122.4	81.6	40.8	163.2	128.5	93.8	39.7

*Fish-meal (FM) coated with 200, 400, 600 and 800 (g/kg) of hydrogenated tallow (FMHT) and hydrogenated palm oil (FMHP) ‡ Crude protein (CP), † Ether extracts (EE) and ♣ Organic matter (OM).

Table 2. Fatty acids (DM percent) composition of hydrogenated tallow (HT) and hydrogenated palm oil (HP).

Component	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	42.32	0.995
C18: 1	0.348	6.758
C18: 2	0.247	1.550
C18: 3	0.414	0.080
Others*	3.77	3.4
Total fatty acids	95	98
Saturated fatty acids	87.95	84.52
Unsaturated fatty acids	3.28	10.07
Unsaturated to saturated ratio	0.037	0.119
C16: C18	0.692	74.97

*Unknown fatty acids which were not detected by GC apparatus.

variance in a completely randomized design using the SAS program General Linear Model (GLM) procedure (SAS, 9.1, 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

Chemical composition

The chemical composition of fish-meal coated with HP and HT is presented (Table 1). The CP content of treatments ranged from 115.7 g/kg DM in $FMHP_{80}$ to 462.8 g/kg DM in $FMHT_{20}$ and 578.6 g/kg DM for fish-meal. The ether extract (EE) content of treatments ranged from 742.7 g/kg DM in $FMHP_{80}$ to 251.3 g/kg DM in $FMHT_{20}$. The DM content increased likewise as the inclusion of fats increased. The ash content in contrast decreased by the addition of fats to fish- 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 versus 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

Table 3. *In vitro* gas production (ml/200 mg DM) of fish-meal coated with HT and HP, incubated in buffered rumen fluid at different incubation times.

	Hydrogenated Tallow (HT)					Hydrogenated Palm Oil (HP)				Significance		
	FM	FMHT20	FMHT40	FMHT60	FMHT80	FMHP20	FMHP40	FMHP60	FMHP80	Fat	Level	SEM
2h	3.1	3.4	4.2	4.4	4.6	3.5	3.4	5.0	5.3	Ns	Ns	0.42
4h	5.5	5.8	6.1	6.3	6.6	6.1	5.6	7.2	7.5	Ns	Ns	0.95
6h	6.9	6.8	6.7	6.9	7.4	7.6	6.6	8.0	8.3	Ns	Ns	0.85
8h	7.6	7.0	7.1	7.1	7.9	7.9	7.0	8.3	8.6	Ns	Ns	0.63
12h	10.2^{a}	7.8^{d}	7.7^{d}	7.8^{cd}	8.1^{c}	9.0^{b}	8.1^{c}	9.2^{b}	9.2^{b}	**	**	0.95
24h	13.5^{a}	8.5^{c}	7.9^{cd}	7.7^{de}	7.6^{de}	10.5^{b}	8.8^{c}	9.56^{b}	8.7^{c}	**	**	0.29
48h	13.9^{a}	9.4^{c}	8.6^{cd}	7.4^{e}	7.2^{e}	11.1^{b}	9.5^{c}	9.4^{c}	8.7^{cd}	**	**	0.96

a, b, c, 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; * = $P < 0.05$; ** = $P < 0.01$; SEM = standard error of mean.

Table 4. The gas production parameters, metabolizable energy (ME) and organic matter digestibility (OMD) contents of fish-meal coated with HT and HP

	Hydrogenated Tallow (HT)					Hydrogenated Palm Oil (HP)				Significance		
	FM	$FMHT_{20}$	$FMHT_{40}$	$FMHT_{60}$	$FMHT_{80}$	$FMHP_{20}$	$FMHP_{40}$	$FMHP_{60}$	$FMHP_{80}$	Fat	Level	SEM
a+b	14.88^{a}	9.86^{c}	9.64^{c}	8.82^{c}	8.66^{c}	11.68^{b}	10.47^{b}	10.34^{b}	9.68^{ce}	*	NS	0.004
b	14.50^{a}	9.42^{c}	8.98^{c}	8.50^{c}	8.56^{c}	11.25^{b}	9.88^{c}	9.96^{c}	9.54^{c}	*	NS	0.022
c	0.10^{a}	0.17^{d}	0.18^{d}	0.27^{c}	0.34^{b}	0.15^{d}	0.14^{d}	0.26^{c}	0.36^{b}	**	**	0.0002
ME	17.04^{a}	12.87^{b}	10.07^{c}	8.10^{d}	6.93^{e}	13.14^{b}	10.20^{c}	8.35^{d}	7.08^{e}	**	**	0.05
OMD	54.26^{a}	44.39^{b}	38.36^{c}	32.70^{cd}	27.19^{e}	46.13^{b}	39.20^{cd}	34.33^{cd}	28.16^{e}	**	**	0.28

$^{a, b, c, d}$ Means within a row with different superscripts differ ($P < 0.05$); a + b: potential GP (ml/200 mgDM); 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 DM); OMD = organic matter digestibility (g/100 gDM); NS = not significant; * = $P < 0.05$. ** = $P < 0.01$; SEM = standard error of mean.

ratio was higher for HP in comparison to HT.

In vitro gas production

Cumulative gas production (GP) volume (ml 200 mg^{-1} DM), GP parameters and calculated amounts of OMD and metabolizable energy (ME) of fish- meal coated with HT and HP are presented in Tables 3 and 4. There was a difference ($P < 0.01$) in GP among treatments at 12, 24 and 48 h after incubation (Table 3). Effect of fat type and level to protect fish- meal were significant, particularly at latter times of incubation ($P < 0.01$). The GP volume at first time of incubation (2, 4, 6 and 8 h) did not differ among treatments. The $FMHT_{20}$, $FMHP_{40}$ and $FMHP_{60}$ produced the lowest volume of gas after 12 h of incubation in comparison to other treatments ($P < 0.01$). Generally, the FMHT treatments produced lower gas compared to FMHP treatments. At 24 and 48 h after incubation, fat coated treatments produced lower gas compared to fish-meal ($P < 0.05$). Potential GP (a + b), GP from the insoluble fraction (b) and fractional rates of GP (c) differs ($P < 0.01$) among treatments (Table 4). Fat type as well as fat level significantly affected 'c' parameter for coated treatments in comparison to FM ($P < 0.05$). However, level of fat did not affected the Potential GP (a + b) and GP from the insoluble fraction (b) actually ($P > 0.05$).

The potential GP (a + b) of uncoated fish-meal was greater (14.88 ml) than other treatments, which were coated with HT or HP ($P < 0.01$). Fat coating method results in a reduction of potential GP level to 8.62 and 9.68 ml in $FMHT_{80}$ and $FMHP_{80}$ in contrast to FM (14.88 ml). The GP from the insoluble fraction (b) of fish-meal coated with these experimental fats reduced significantly ($P < 0.05$) because fermentable fraction decreased

alongside with the addition of 200 to 800 g/kg of experimental fats to FM (Table 4). In contrast to b and a + b parameters, fractional rates of GP (c) increased significantly for coated treatments in comparison to FM ($P < 0.05$) and the value of (c) was greater for $FMHT_{80}$ (0.34 ml/h^{-1}) and $FMHP_{80}$ (0.36 ml/h^{-1}) (Table 4).

Metabolizable energy and organic matter digestibility

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 comparison to FM ($P < 0.01$). 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 FM 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 compared to FM with no fat coating ($P < 0.01$). There were no significant differences in calculated OMD and the ME values for FbMHT and FMHP at 600 and 800 g/kg level (That is, $FMHT_{60}$ versus $FMHP_{60}$ and $FMHP_{80}$ versus $FMHT_{80}$).

DISCUSSION

Protection of protein sources with fat to reduce protein degradability 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 FM in different ratios. Coating FM with HT and HP reduced CP and ash content reasonably, but increased EE content of experimental treatments, as the level of fat inclusion increased to protect FM. High content of C18:0 fatty acid in HT is expected because of the hydrogenation process that occurs in industries, leading to exchange of polyunsaturated fatty acids to subsequent saturated ones.

One of the challenges associated with feeding HT is that, the digestibility of hydrogenated fatty acids is lower than unsaturated fatty acids, which the unsaturated fatty acids are extensively digestible. Increasing saturation of fat sources increases ruminal inertness but decreases fatty acid digestibility, generally hydrogenated triglycerides such as tallow, is inadequately digested (Macleod and Buchanan-Smith, 1972; Eastridge and Firkins, 2000).

The difference between fatty acids profile of tallow (Getachew et al., 2001) and HT used in the current study indicated that, tallow has more monounsaturated fatty acids (C18: 1 isomers) than HT. In the study of Getachew et al. (2001), they reported approximately 28.9 (DM, per percent) C18: 1 for tallow, whereas the content of C18; 1 in HT and HP in the current 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 FM. In contrast to our 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 ration, as supplemental fat (Getachew et al., 2001). In addition, the levels of fat used to protect FM (up to 800 g/kg) varies noticeably from those levels reported in the study of Getachew et al. (2001), which utilized 50 to 250 g/kg added fatty acids *in vitro* as tallow or yellow grease to diets. The reduction of GP over the latter time of incubation by coating FM 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 FM. In another study (Stewart, 1977), a depression in cotton fiber degradation when the cotton yarn had been soaked in either tallow or fatty acids was observed. Other explanation could be stated as, some unsaturated fatty acids may be toxic 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 (more than 400 g/kg 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 dioxide carbon (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 CH_4 and total gas production (Baker, 1999). It is actually difficult to explain the biological basis why HT and HP coated FM, would produce lower total gas volume *in vitro* when compared to FM. It is assumed that, coating FM with thin layers of fat could reduce the accessibility of microbial enzymes to the feed.

In vitro GP at 12 h from FM was not comparable to that reported by Cone et al., (2005), which used some protein sources to evaluate protein fermentation in the large intestine of pigs using gas test technique. In the present study, FM produced approximately 10.2 ml gas/200 mg DM (Table 3) at 12 h after incubation. Results from the present study confirm earlier findings, 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 (Van 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., 1971) 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 (Macleaod 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 FM 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 for FM (Table 3) is not similar to those feeds investigated by Getachew et al. (2004). These authors reported a gas volume of 213 ml/g DM for soybean meal after 24 h incubation time. In the current study, it was approximately 51 ml/g DM (10.2 ml/200 mg DM) for fish- meal after 24 h incubation time. The lower GP after 24 h for fish-meal may relate to higher content of crude protein and ash from those reported by Getachew et al. (2004) for soybean meal. Higher protein and ash contents of FM used in the present study, also may describe part of the difference between our results from those reported for other feeds (Getachew et al., 2004).

The $FMHT_{20}$ achieved 8.5 ml/200 mg DM, GP after 24 h, which was lower than $FMHP_{20}$ that produced 10.5 ml/ 200 mg DM. Generally, the FMHP treatments produced higher gas than FMHT, which indicate that the HT had more inhibitory effect on rumen microbial ecosystem. On the other hand, it could be explicated that, HT has more potency to protect FM. 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 a more unsaturated fat than HT, it was expected that, covering FM with this fat source may interfere with methanogenes bacteria and lead to a reduction in GP over the time of incubation. Although it may relate to more potential of HT to coating FM, but still there is no excellent explanation for this event.

Potential GP (a + b) has the same decreasing trend for FMHT and FMHP treatments for 200, 400, 600 and 800 g/kg DM levels, which indicated that, reduced GP could be achieved successfully, when some ingredients of diet is coated with fat. Likewise, potential gas production reported in this paper for FM (14.88 ml/ 200 mg DM) is different from previous report (Getachew et al., 2002), which they stated as 49.5 ml/ 200 mg DM for plant protein sources. The extent of potential GP reported here is not comparable to plant protein feeds reported previously (Getachew et al., 2002). Therefore, the discrepancies may be due to differences in crude protein, ash and fiber content of those feeds used by Getachew et al. (2002) and the FM apply in this research. The GP from the insoluble fraction (b) also decreased similar to potential gas production noticeably because of a reduction in insoluble part of treatment.

The fractional rate of GP (c: ml/h^{-1}) reported in the present study for FM is in the range of other feed reported previously (Getachew et al., 2004). The greater fractional rate of GP for FMHT and FMHP 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 FM 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 (Cone and van Gelder, 1999). In the same way, fat could influence the final GP. The GP caused by fermentation of protein is not the same as that of carbohydrates (Steingass, 1983) and lipids (Data not shown). Relative to potato starch, casein was fermented in an earlier stage of incubation (Steingass, 1983) and fatty acids would be fermented relatively at the latter time of incubation.

Moreover, the GP profiles could be separated in three sub-curves, representing GP caused respectively by fermentation of the water-soluble components (a), by fermentation of the non-soluble components (b) and by microbial turn over (Cone et al., 1997). Consequently, the pattern of fermentation of FM would be noticeably different from FMHT and FMHP 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 some concentrate feeds such as FM, a higher quantity of gas is produced in early hours of fermentation due to high digestibility of soluble protein. 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 all 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 FM 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 (VFAs) as well as GP. As a result, we expected that overall digestibility of fat coated treatments should be declined by addition of fat to FM.

The OMD values were highest for FM and lowest for $FMHT_{80}$ and $FMHP_{80}$ as we expected. High organic matter (OM) digestibility for FM can be expected due to high concentration of protein and carbohydrates that supply available energy for microbial growth. Low digestibility of OM in fat coated treatments when compared with FM was due to high concentration of fat in these treatments. These finding is in agreement with results obtained earlier, 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 FMHT and FMHP 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 undegradable 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, milk composition and fatty acids profile. In a study by Sklan (1989), he showed 84 to 90% of whey powder and soybean meal 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 the supply of more organic matter and proteins to small intestine for milk protein synthesis and influence animal performances.

The metabolizable energy (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 to 15 MJ/kg DM. Although, the ME value for FM was greater than previous work done with different feeds (Getachew et al., 2004). The discrepancy is due to the chemical composition of FM, which contained more crude protein when compared with other protein supplements.

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 our findings, the *in vitro* rumen gas technique can be used to study the nutritional quality of feed ingredients that is covered physically with fat as well as mixed rations and individual feed ingredient.

Conclusions

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 HT had higher degree of rumen protection for FM, because the HT 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. We did not measure CH_4 production in the current study but as the total gas emission and production reduced by coating FM with fat, it could be concluded that, these treatments probably reduced the CH_4 production along with total gas production. Hence, 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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