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Decreasing gas production rate and digestibility of barley grain using fat coating method

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Abstract

With the help of rapid method for measuring gas production (GP) during incubation of feeding stuffs with rumen liquor *in vitro,* the present study was conducted to investigate the GP rate and digestibility of barley grain coated with different types [hydrogenated tallow (HT) and hydrogenated palm oil (HP)] and levels (0, 20, 40, 60 and 80 percent) of fat to decrease gas produced during fermentation process. Approximately 200 mg (DM basis) of sample was weighed and inserted in glass syringes, then mixed with the inoculum and artificial saliva, when the initial volume of the syringes reached to 30 ml and incubated at 39ºC in ventilated oven. GP was recorded after 0, 2, 4, 6, 8, 12, 24, 48, 72 and 96 h. Data were analyzed using SAS programs. The equation of $p= a+b (1-e^{-ct})$, and the values of a, b, and c were computed using F-curve software. The outcome of the present study showed that experimental fat added to barley grain to protect it from fermentation, reduced *in vitro* degradability determined using gas test technique. Addition of experimental fat (HT and HP) to barley grain could significantly decrease GP during incubation times ($P<0.01$). In compare to HP fat, coating barley grain with HT fat resulted in significant reduction of GP ($P<0.01$). Consequently, it seems that if we could use experimental fats as a coating factor to slow down the fermentation of cereal grains (like barley and wheat grains) in the rumen, it could lead to prevention of metabolic diseases like acidosis. Moreover, this processing could alter the values of starch reaching the small intestine to produce more glucose for high producing animals like dairy cows.

Keywords: Barley grain; fat coating method; Gas production; Hydrogenated tallow; hydrogenated palm

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Introduction

The majority of starch in the ration of high-yielding dairy cows originates from cereal grains and maize silage. Supplemental fat has been shown to increase milk yield in high producing dairy cows (Coppock and Wilks, 1991). Fat has more than three times net energy of lactation relative to protein and carbohydrate. Replacing fermentable carbohydrate with fat in the diet of high-producing dairy cows may limit synthesis of microbial protein and will decrease the flow of microbial protein to the small intestine (Jenkins, 1993). Both rate and extent of starch degradation in the rumen can affect the composition of the VFA, ruminal pH and passage of undegraded starch into the small intestine (Mills et al., 1999). Starchy feedstuffs such as barley grain consisted considerable starch values, which mostly consumed by amylolytic bacteria. Also the inclusion of readily digestible carbohydrates in forage based diets for ruminants can restrict microbial digestion of structural polysaccharides (Mertens and Loften, 1980; Mould et al., 1983; Fondevila et al., 1994). This effect is caused by a shift in rumen environmental conditions, making them unfavourable

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for microbial fibrolysis. It has been stated that rumen acidification of pH to below 6.2–6.0 is the main factor causing this effect (Stewart, 1977; Hiltner and Dehority, 1983). However, some authors (Mould et al., 1983; Piwonka and Firkins, 1993) have speculated that readily digestible carbohydrates can slow, or reduce, cell wall degradation even at optimum rumen pH.

Methane production is an unavoidable by-product of ruminal fermentation and represents a significant energy loss to the host animal. Energy losses as methane can range from 2 to 15% of gross energy intake (Holter and Young, 1992; Johnson and Johnson, 1995). Methane contributes to climatic change and global warming (Johnson and Johnson, 1995) by trapping outgoing terrestrial infrared radiation 20 times more effectively than CO_2, which leads to increased surface temperatures, and it indirectly affects atmospheric oxidation reactions that produce CO_2. Thus, there is increased worldwide interest in addressing mitigation of CH_4 in animal agriculture. There may be potential to reduce the extent of CH_4 production by manipulating diet and management practices that influence ruminal microbial fermentation. The type of carbohydrate, the addition of dietary fat, the quantity of feed ingested, and the processing of forages all affect methane output (Johnson and Johnson, 1995).

Our hypothesis was that coating some starchy ingredients of the diary cow diets by the supplemental fat could reduce rumen GP and fermentation rate of starchy cereal grains to reduce destructive effect of quickly fermentable carbohydrates. The objective of this research was to investigate the GP rate and digestibility of fat coated barley grain to reduce GP from feedlots as determined by *in vitro* GP technique.

Materials and Methods

Preparing samples and fat coating technique

Before the beginning of the experiment, barley grain 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 process was done using two types of experimental fat (HT and HP) to embed barley grain particles (particle size 1 mm) in very thin layers of fat to make a continues coat of fatty acids on a core of barley grain. In brief barley grain in different ratios to experimental fats was added into Teflon beaker containing melted fat (Palizdar et al., 2012). 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 barley grain formed. Barley grain was encapsulated using zero, 20, 40, 60 and 80 percent of experimental fats. The final shape of the product was like small globules ranging from 1000 to 1500 µm in diameter. 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 hrs followed by equilibration in a 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). Acid detergent fibre (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 (EC, 1993). Fatty acid (FA) profiles of two experimental fats were determined using gas chromatography. The gas chromatograph was (Agilent Technologies, hp, 6890 N; USA) 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 after the morning feeding from three ruminally fistulated steers that were fed 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 barley grain. 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:

$Y = a+b\ (1 - e^{-ct})$ 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/200g 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:

$ME\ (MJ/kg\ DM) = 2.20 + 0.136 \times Gp + 0.057 \times CP + 0.0029 \times CP2$

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

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* GP were subjected to Analysis of Variance (ANOVA) in a completely randomized design using the SAS program General Linear Model (GLM) procedure (SAS, 9.1). 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 barley grain coated with HP and HT are presented in Table 1. The CP content of treatments ranged from 23.1 g/kg DM in $BGHT_{80}$ to 93.2 g/kg DM in $BGHP_{20}$ and 117.1 g/kg DM for barley grain. The EE content of feeds ranged from 723 g/kg DM in $BGHP_{80}$ to 171.9 g/kg DM in $BGHT_{20}$. The DM content increased likewise as the inclusion of experimental fats increased. The NDF and ADF content diminished corresponding to the ratio of fat used for coating procedure. The Ash content in contrast decreased by the addition of fats to barley grain 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 contained the odd carbon fatty acids (C15 and C17) in contrast to HP. The ratio of palmetic to estearic acids was higher for HP in compare to HT. Increasing saturation of fat sources increases ruminal inertness but decreases FA digestibility (Grummer, 1993); extensively hydrogenated triglycerides, such as hydrogenated tallow (HT), are poorly digested (Macleod and Buchanan-Smith, 1972; Eastridge and Firkins, 2000).

In vitro gas production

In vitro cumulative GP (ml 200 mg^{-1} DM) of barley grain coated with HT and HP, GP parameters and calculated amounts of OMD and ME of barley grain are presented in Table 3 and 4.There was a difference ($P<0.01$) in GP among treatments (Table 3). Effect of type and level of experimental fats to protect barley grain were significant, particularly at 6h after incubation to latter times of incubation ($P<0.05$). The GP volume at first time of incubation did not differ among treatments; however fat coated treatments produced less gas compare to BG. The $BGHT_{80}$ and $BGHP_{80}$ were produced the lowest volume of gas after 6h of incubation in compare to other treatments that coated or uncoated by fat ($P<0.01$).

There were significant differences among treatments coated with HT and HP after 6, 8, 12, 24 and 48h of incubation compare to BG ($P<0.01$). The GP reduced, as barley grain coated with HT and HP but the fermentation pattern of uncoated barley grain was not distinctly different compared to BGHT and BGHP treatments at first times of incubation (Table 3 and Fig. 1). However the fermentation pattern of BG was differed at the last time of incubation comparing to other treatments (Fig. 1).

Table 1: Chemical composition of barley grain coated with HP and HT (g/kgDM).

		Hydrogenated Tallow (HT)				Hydrogenated Palm Oil (HP)			
Variable	BG*	$BGHT_{20}$	$BGHT_{40}$	$BGHT_{60}$	$BGHT_{80}$	$BGHP_{20}$	$BGHP_{40}$	$BGHP_{60}$	$BGHP_{80}$
M	912.9	930.3	947.4	965.1	982.8	922.3	931.7	941.6	950.8
CP	117.1	92.9	71.3	45.3	23.1	93.2	72.6	46.1	25.1
NDF	166.1	132.2	99.1	66.1	33.3	142.1	101.9	66.9	36.1
ADF	28.1	22.3	16.7	11.5	5.5	22.9	17.4	11.9	6.3
EE	28.9	171.9	328.1	485.2	693.0	191.1	368.3	595.9	723.0
OM	972	977.9	984	988.3	994.5	977.9	982.7	900	993.8
ASH	28.0	22.1	16.0	11.7	5.5	22.1	17.3	10.0	6.2

*Barley grain (BG) coated with 200,400,600 and 800 (g/kg) of hydrogenated tallow (BGHT) and hydrogenated palm oil (BGHP).

Table 2: Fatty acids (DM percent) 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	42.32	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 not detected by GC apparatus.

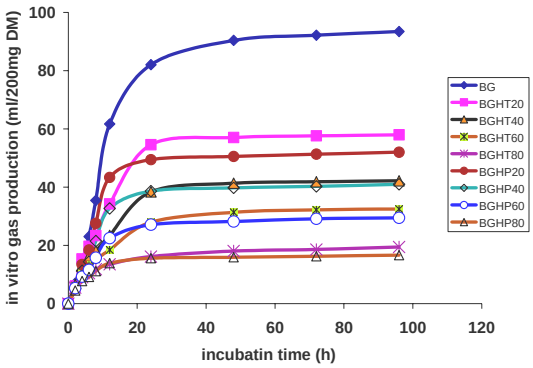

Fig. 1: Pattern of *in vitro* gas production on incubation of BG coated with 200, 400, 600 and 800 g/kg HT (BGHT) and HP (BGHP) in buffered rumen fluid.

Potential GP (a+b), GP from the insoluble fraction (b) and fractional rates of GP(c) differed (P<0.01) among treatments (Table 4). Fat type as well as fat level significantly affected b, a+b and c parameters for coated treatments in compare to BG (P<0.05). The potential GP (a+b) of uncoated BG was greater (94.3 ml) than other fat coated treatments (P<0.05). Fat coating method results in a reduction of GP potential level to 18.28 ml and 16.26 ml in $BGHT_{80}$ and $BGHP_{80}$ in contrast to BG. The GP from the insoluble fraction (b) of barley grain coated with these experimental fats reduced significantly (P<0.05) because fermentable fraction decreased along with the addition of 200 to 800 g/kg of experimental fats to BG (Table 4). In contrast to b and a+b parameters, fractional rates of GP(c) increased significantly for coated treatments in compare to BG (P<0.05) and the value of (c) was greater for $BGHT_{80}$ and $BGHP_{80}$ (Table 4).

Metabolizable energy (ME) and Organic matter digestibility (OMD)

The OMD and ME content could be evaluated by 24 h in vitro GP data and chemical composition of feed samples (Menke and Steingass., 1988; Menke and Steingass., 1979; McDonald et al., 1995). The OMD and ME content results are shown in Table 4. The ME content of fat coated treatments decreased significantly in compare to BG (P<0.01). Moreover the OMD content reduced significantly (P<0.01) as BG coated with HT and HP in different ratios (Table 4).

Discussion

Attempts have been made to manipulate rumen fermentation using ration manipulation strategies to reduce gas production, including addition of ionophores, fatty acids and yeast cultures (Sauer et al., 1998; Dohme et. al., 2001). Using fat to protect cereal grains to decrease ruminal fermentation of starchy feeds may have a positive effect to reduce total GP and may possibly have a carryover outcome on volatile fatty acids production. To our knowledge this technology only used to protect feed proteins (Sklan., 1989; Sklan and Tinsky., 1993) and amino acids (Arambel et al., 1987) but there is still lack of evidence investigating the effect of fat coated starchy feeds like cereal grains.

The CP content of BG (117 g/kg DM) used in the current study was comparable to other researchers (Cone et al, 2009; Cone et al, 2005) that reported 105 and 114 g/kg DM of CP for BG. The NDF content of BG (166 g/kg) was not in consistent with other report that expressed 204 g/kg NDF for BG.

Discrepancy in chemical composition of experimental treatments observed within the present study is related to variation of fats in experimental treatments. Coating BG with HT and HP reduced the CP and increased EE content of experimental treatments. Since the level of fat increased to protect BG, the CP, ADF, NDF and ASH content decreased reasonably.

It was shown obviously in the present study that HT was more saturated fat than HP, and HT fat has more C18:0 than HP. Hydrogenated fatty acid also has lower digestibility coefficients than corresponding unsaturated ones, indicating that increasing saturation of fat sources increases ruminal inertness but decreases fatty acid digestibility (Grummer, 1993). Commonly hydrogenated triglycerides are inadequately digested (Eastridge and Firkins, 2000).

The HT used in the current study has lower monounsaturated fatty acids (C18: 1 isomers) in

Table 3: *In vitro* GP of barley grain coated with HT and HP, incubated in buffered rumen fluid at different incubation times.

	Hydrogenated Tallow					Hydrogenated Palm Oil				Significance		
	BG	$BGHT_{20}$	$BGHT_{40}$	$BGHT_{60}$	$BGHT_{80}$	$BGHP_{20}$	$BGHP_{40}$	$BGHP_{60}$	$BGHP_{80}$	Fat	Level	SEM
2h	6.99	6.06	6.54	6.23	5.61	5.31	5.16	5.33	4.50	Ns	Ns	0.42
4h	14.31	15.38	13.09	11.06	9.04	13.44	11.27	9.25	7.77	Ns	Ns	0.95
6h	23.03^{a}	19.72^{b}	16.35^{c}	13.24^{d}	10.12^{e}	18.44^{b}	14.40^{d}	11.60^{e}	9.16^{ef}	*	*	1.22
8h	35.45^{a}	23.36^{b}	18.14^{c}	15.18^{d}	11.76^{e}	27.57^{f}	21.51^{bg}	15.75^{d}	11.18^{e}	*	**	2.63
12h	61.72^{a}	34.24^{b}	23.44^{c}	18.46^{d}	13.48^{e}	43.36^{f}	32.71^{bg}	22.50^{c}	13.90^{e}	**	**	2.95
24h	82.08^{a}	54.58^{b}	38.40^{c}	27.97^{d}	15.97^{e}	49.46^{f}	38.66^{c}	27.12^{d}	15.61^{e}	**	**	3.33
48h	90.41^{a}	57.07^{b}	41.36^{c}	31.40^{d}	17.53^{e}	50.55^{f}	39.76^{c}	28.22^{g}	15.93^{e}	**	**	3.96

$^{a, b, c, d}$: 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 GP parameters, Metabolizable Energy (ME) and Organic Matter Digestibility (OMD) contents of barley grain coated with HT and HP.

	Hydrogenated Tallow					Hydrogenated Palm Oil				Significance		
	BG	$BGHT_{20}$	$BGHT_{40}$	$BGHT_{60}$	$BGHT_{80}$	$BGHP_{20}$	$BGHP_{40}$	$BGHP_{60}$	$BGHP_{80}$	Fat	Level	SEM
a+b	94.3^{a}	58.92^{b}	42.54^{c}	32.33^{d}	18.28^{e}	52.33^{f}	41.06^{c}	29.25^{d}	16.26^{e}	*	**	0.005
B	99.9^{a}	60.68^{b}	42.13^{c}	31.14^{d}	17.29^{e}	55.87^{f}	43.15^{c}	29.72^{d}	16.11^{e}	*	**	0.026
C	0.075^{a}	0.077^{a}	0.076^{a}	0.077^{a}	0.127^{b}	0.106^{c}	0.103^{c}	0.103^{c}	0.149^{d}	*	*	0.0002
ME	14.43^{a}	10.55^{b}	8.23^{c}	6.73^{d}	5.08^{e}	9.85^{f}	8.27^{c}	6.62^{d}	5.01^{e}	**	**	0.05
OMD	93.22^{a}	63.14^{b}	52.47^{c}	41.14^{d}	29.83^{e}	67.70^{f}	52.24^{c}	41.89^{d}	30.34^{e}	**	**	0.38

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

compare to tallow (Getachew et al., 2001). It was reported approximately 28.9 (DM, per percent) C18: 1 for tallow (Getachew et al., 2001), whereas the content of C18: 1 in HT and HP in the presenting study was 0.34 and 6.75 (DM, per percent), respectively.

Along with the inclusion of HT and HP to coat BG, the GP decreased during the incubation times and the depression trend was greater for HP than HT treatments. The GP after 24h (422 ml/g OM equivalent to 82.08 ml/ 200 mg DM) was different from those reported by Chai et al., (2004) which reported 315 ml/g OM for BG. Greater GP in this study may due to higher proportion of starch and sugars (data not shown) in compare to that reported by Chai et al. (2004).

In contrast to our results, Getachew et al. (2001) reported that tallow did not affect GP, although we used HT that differed extensively from tallow. Moreover the coating procedure of BG generally varied from the procedure of adding fat to a total mixed rations, as supplemental fat. The levels of fat used to protect BG (up to 800g/kg) was noticeably different from those levels reported in the study of Getachew et al. (2001) which utilize 50 to 250 g/kg added fatty acids as tallow or yellow grease to deists. The reduction of GP over the time of incubation by coating BG with HT and HP may associate with microbial attachments, whereas these unsaturated fatty acids act as toxins for rumen bacteria (Henderson., 1973, Kim et al., 2000 Hunter et al., 1976). It has been suggested that dietary fats may coat some nutrients and interfere with microbial attachment and depressed digestibility (Devendra and Lewis, 1974). Depression in cotton fiber degradation has been reported (Stewart, 1977), when the cotton yarn had been soaked in either tallow or fatty acids.

It has been shown some compounds that directly inhibit activity of methanogen bacteria are likely to reduce, or eliminate, CH4 production (Baker, 1999). Although it is very difficult to explain the biological basis why HT and HP coated BG would be considered to produce lower gas.

In vitro GP at 2 h from BG (35ml/g OM or 6.99 ml/ 200mg DM) and BG coated with HT and HP was comparable to values of GP after 3h incubation reported by Cone et al. (2006), which reported 34.9 ml/g OM from degradable fraction for BG. Although it has been shown that GP from the washout fraction of BG is very high (90.9 ml/g OM) after 3h incubation, but this fraction behaves like the degradable fraction (Cone et al., 2006).

It is well documented that soluble components of feeds are very fast fermented (Madsen and Hvelplund, 1994; Lopez et al., 1994; Hvelplund and Weisbjerg, 2000).

In this study we did not measure different fractions of BG separately but it is appear that coating processes could not affect initial gas produced from BGHT and BGHP compare to BG. It may reflect that the coating processes did not influence the soluble fraction of treated BG. Starchy feedstuffs do not only consist of starch, but also contain other components such as neutral detergent fiber (NDF), sugars and protein. This

GP from these starchy feedstuffs does not originate solely from fermentation of starch, but also from the other components. Cone et al. (1997) and Cone and Van Gelder (1999) showed that initially gas is produced from fermentation of the water-soluble components, such as sugars and protein.

In the present study BG produced approximately 118.5 ml gas per g OM (23.04 ml/200 mg DM, Table 3) at 6h after incubation in which it was 107 ml gas per g DM reported by previous work (Getachew et al., 2004). This difference may be related to differences in chemical composition of BG used in two studies.

Results from the current study confirm earlier findings that showed free fatty acids and long-chain fatty acids inhibit methane and total GP in the rumen, although the mechanism by which this occurs is still not completely known (Van Nevel and Demeyer, 1996). HT and HP in the form of long chain and free fatty acids reduced GP as the time of incubation approached (Table 4). Some explanation may be due to the reduced availability of calcium needed for appropriate microbial function (Jenkins, 1993; Galbraith et al., 1971), and the negative effect of unsaturated fats (Jenkins, 1984), and free fatty acids content of these fat sources that caused a larger negative effect than the corresponding triglycerids (Bateman and Jenkins, 1988). In the present study fat sources used to protect BG consisted of hydrogenated free fatty acid with the fatty acids profile which expose 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 Chai et al. (2004). These authors reported a gas volume of 61 ml/mg 200 DM for BG after 24h incubation time. In the current study it was approximately 82 ml/200mg DM after 24h incubation time. The greater gas produced after 24h for BG may relate to higher content of starch and NDF in BG. Higher starch and NDF contents of BG used in the present study (208 g/kg DM and 68 g/kg DM), also may describe in part the difference between our results from those reported by Getachew et al. (2002).

The $BGTH_{20}$ achieved 82.4 ml/g DM (15.9 ml/200mg DM) GP after 24 h, which was comparable to $SMPH_{20}$ that produced 76 ml/g DM (15.6 ml/200mg DM). Generally the BGHP treatments produced lower gas than BGHT, which means the HT had more inhibitory effect on rumen microbial ecosystem.

Potential GP (a+b) has the same decreasing trend for BGHT and BGHP treatments for 200, 400, 600 and 800 g/kg DM levels, which indicated that the reduced GP could be achieved successfully, with coating some ingredient of diets with fat. In the same way potential GP reported in this paper for BG (94.3 ml/ 200mg DM) is extensively different from previous report (Chai et al., 2004), which stated 49.7 ml/ 200 mg DM for BG. The extent of potential GP reported here is even much more than those reported for citrus pulp and corn grain (74.3 and 75.6 ml/ 200mg DM) reported previously (Getachew et al., 2002). The GP from the insoluble fraction (b) also decreased similar to potential GP noticeably, due to a reduction of insoluble part of treatment by adding fat to BG.

When supplemental fat is included at levels normally fed in commercial dairy rations (50 –60 g/kg DM), possibly it is difficult to find such evidence that support adverse effects of HT and HP on microbial activity and GP. In the present study the presence of negative effects on rumen fermentation kinetic parameters was seen with inclusion of fat as a free fatty acid form, for coating BG.

The results showed that the fractional rate of GP (c: ml/h^{-1}) ranged from 0.149/h for $BGHP_{80}$, to 0.075/h for BG. the fractional rate of GP for BG in this study is in close agreements with Getachew et al., (2004) which reported a 0.074/h of fractional rate of GP for distillers' dried grains. The greatest fractional rate of GP was seen for $BGHT_{80}$ and $BGHP_{80}$ treatments (0.127 and 0.149). Methane production is associated with fiber fermentation, however in highly digestible feeds such as BG; a higher quantity of gas is produced in early hours of fermentation due to high digestibility of the nutrients in this feed. There is a reduction in NDF content by coating BG with HT and HP (from 208 in BG to 41.4 g/kg in BGHT and BGHP), which reflects the postponed fibre digestion of fat coated treatments. Consequently it was predictable that the organic matter digestibility of treatments will be diminishing by adding fat to BG and by reduction in GP. Likewise fat could influence the final GP (Fig. 1) and the GP after 3h incubation can be regarded as fast initial fermentation of the water-soluble components and the GP between 3 and 20h as moderate fast fermentation of the non-soluble components (Van Gelder et al., 2005). Consequently the pattern of fermentation of BG was noticeably different from BGHT and BGHP between 3 to 20h incubation because they might have more insoluble fractions because of high content of fat in these treatments (Fig. 1).

The OMD values were highest for BG (93.2) and lowest for $BGHT_{80}$ (29.8) and $BGHP_{80}$ (30.3) as we expected. High OM digestibility for BG can be predicted due to high concentration of starch polysaccharides and other carbohydrates that are highly digestible by rumen microbes. Low digestibility of OM in fat coated treatments compared to BG was due to high concentration of fat in these treatments. These findings agree with Getachew et al. (2001) who found a decline in GP 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 BGHT and BGHP treatments can be related to differences in GP after 24h of incubation and the differences in chemical composition of these treatments.

It has been shown that 84-90 % of whey powder and soybean meal coated with calcium salts of fatty acids remained *in sacco* after 20h incubation in the rumen of sheep (Sklan., 1989). This author 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 calculated OMD measured *in vitro* in this study may lead to supply more nutrient to small intestine for milk synthesis and affect the animal performance.

The ME values of the fat coated treatments and the calculated ME for BG 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 BG was greater than previous work (Getachew et al., 2002). It could be due to high production of gas after 24 h of incubation and the different chemical composition of BG from those reported previously (Getachew et al., 2002) that resulted to larger ME value.

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). BG has the greatest OMD in this study compare to other fat coated treatments, consequently the greatest ME value for BG was expected.

Conclusions

Starchy feedstuffs such as cereal grains could provide a major part of energy in practical rations of ruminants. Reducing the rate and speed of fermentation of these energy sources may help nutritionist to use higher quantities in dairy cows and feedlot cattle dietaries. As a result, the technology used for protection of starch to reduce its fermentation rate in the rumen may be an important aspect to reduce high risk of metabolic disorders and total GP in the rumen. According to our findings the *in vitro* rumen gas technique can be used successfully to study the nutritional quality of feed ingredients that covered physically with fat as well as mixed rations and individual feed ingredient.

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