African Journal of Biotechnology Vol. 10(39), pp. 7710-7716, 27 July, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1603
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

In vitro gas production of wheat grain flour coated with different fat types and levels

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Accepted 28 April, 2011

Gas production (GP) is a rapid method for feedstuffs assessment. A study was done to investigate wheat grain coated with hydrogenated tallow (HT) and hydrogenated palm oil (HP) of different fatty acids types and levels to study total gas production. Approximately, 200 mg (DM basis) of sample was weighed and inserted in glass syringes, mixed with the inoculum and artificial saliva, then incubated at 39°C in a ventilated oven. The outcome of this study showed that experimental fat reduced *in vitro* degradability using the gas test technique. The addition of experimental fat (HT or HP) to wheat grains significantly decreased GP during incubation ($P < 0.01$). In comparison to HP fat, coating wheat grains with HT fat resulted in significantly reduced GP ($P < 0.01$). Accordingly, it seems that experimental fats could be used as a coating substance to reduce the speed of cereal grains (like barley and wheat grains) fermentation in the rumen. Consequently, it could prevent dairy cows from metabolic diseases like acidosis. Moreover, this processing could alter the amount of starch reaching the small intestine to produce more glucose for high producing animals like dairy cows.

Key words: Wheat grain, fat coating method, gas production, hydrogenated tallow, hydrogenated palm oil.

INTRODUCTION

The majority of starch in the rations of high-yielding dairy cows originates from cereal grains and maize silage. Excessive grain feeding increases the energy density of the diet but often causes undesirable rumen fermentation and milk fat depression. Supplemental fat has been shown to increase milk yield in high producing dairy cows (Coppock and Wilks, 1991). Replacing fermentable carbohydrate with fat in the diet of high-producing dairy cows may limit the synthesis of microbial protein and will decrease the flow of microbial protein to the small intestine (Palmquist et al., 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 wheat grain contain considerable amounts of starch, which is 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 unfavorable for microbial fibrolysis. It has been stated that rumen acidification of pH to below 6.0 to 6.2 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 contributes to climatic change and global

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Abbreviations: **(HT),** Hydrogenated tallow; **(HP),** hydrogenated palm oil; **(WG),** wheatgrain; ($WGHT_{20}$, $WGHT_{40}$, $WGHT_{60}$, $WGHT_{80}$), wheatgrain coated with 20, 40, 60 and 80% of hydrogenated tallow; ($WGHP_{20}$, $WGHP_{40}$, $WGHP_{60}$, $WGHP_{80}$), wheatgrain coated with 20, 40, 60 and 80% of hydrogenated palm oil; GP, gas production.

warming (Johnson and Johnson, 1995) by trapping outgoing terrestrial infrared radiation 20 times more effectively than CO_2, which leads to increased surface temperature of the earth and it indirectly affects atmospheric oxidation reactions that produce CO_2. Thus, there is increasing worldwide interest in addressing mitigation of biogases in animal agriculture. There may be potential to reduce the extent of CH_4 and CO_2 production by manipulating animal diets and modifying 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 is that coating some starchy ingredients of cow diet with fat supplements could reduce rumen GP and the fermentation rate of starchy cereal grains, thereby reducing the undesirable effects of quickly fermentable carbohydrates. The objective of this study was to investigate the GP rate and digestibility of fat coated wheat 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, wheat grain was ground into flour through a sieve with 1 mm pore size in a hammer mill. Fat coating was done according to the pan coating method with some modifications (Grass and Unangst, 1972; Sklan, 1989). The process was done using two types of experimental fat (HT and HP) to embed wheat grain particles (particle size 1 mm) in very thin layers of fat to make a continuous coat of fatty acids on a core of wheat grain flour. In brief, different ratios of wheat grain flour were added to experimental fats in a Teflon beaker containing melted fat. 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 cooled slightly. The beaker was then transferred to cold-water bath (5°C) to cool and the blend continuously mixed by the mixer until small beads of fat coated sample formed. Wheat grain was encapsulated using zero, 20, 40, 60 and 80% of experimental fats (w/w basis). As the percentage of fat increased for encapsulation, the fermentable substrate decreased inversely.

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 (hydrogenated tallow- food grade, 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. The gas chromatograph (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 the determination of fatty acids profile.

In vitro gas production technique

GP was determined by the procedure of Menke and Steingass (1988). Samples (200 mg DM basis) 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 morning feeding from three ruminally fistulated steers that were fed a 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. Triplicate incubations were conducted with readings of GP after 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 wheat grain flour. Differences in the composition and activity of rumen fluid inoculum were controlled by parallel measurements by incubating 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 is the GP from the immediately soluble fraction (ml); b is the GP from the insoluble fraction (ml); a+b is the potential of GP (after 96 h) from the fermentable fraction (ml/200 g DM); c is 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 follows:

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

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

Where, CP is the 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 of sample after 24 h of incubation.

Gas production data, OMD and ME values was adjusted for 200 mg of WG (DM basis) sample incubated *in vitro*.

Statistical analysis

In vitro GP data 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

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

Variable	Hydrogenated tallow (HT)					Hydrogenated palm oil (HP)			
	WG	$WGHT_{20}$	$WGHT_{40}$	$WGHT_{60}$	$WGHT_{80}$	$WGHP_{20}$	$WGHP_{40}$	$WGHP_{60}$	$WGHP_{80}$
Dry matter	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

WG, Wheat grain; WGHT. wheat grain coated with 200, 400, 600 and 800 (g/kg) of hydrogenated tallow; WGHP, wheat grain coated with 200, 400, 600 and 800 (g/kg) of hydrogenated palm oil. CP, Crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; EE, ether extracts; OM, organic matter.

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

Component	HT	HP
C8	7.2	4.4
C10:0	2.2	1.5
C10: 1	0.9	0.6
C12:0	1.3	1.0
C14:0	2.6	2.0
C14: 1	0.9	0.7
C15	0.7	-
C16:0	29.3	74.6
C16: 1	0.4	0.4
C17	2.2	-
C18:0	42.3	1.0
C18: 1	0.3	6.8
C18: 2	0.2	1.6
C18: 3	0.4	0.1
Unknown fatty acids	3.8	3.4
Total fatty acids	95	98
Saturated fatty acids	87.9	84.5
Unsaturated fatty acids	3.3	10.0
Unsaturated to saturated ratio	0.04	0.120

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 wheat grain coated with HP and HT is shown in Table 1. The crude protein (CP) content of treatments ranged from 23.1 g/kg DM in $WGHT_{80}$ to 25.1 g/kg DM in $WGHP_{20}$ and 117.1 g/kg DM for wheat grain. The EE content of treatments ranged from 723 g/kg DM in $WGHP_{80}$ to 171.9 g/kg DM in $WGHT_{20}$. 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 wheat grain and consequently the OM content substantially enhanced (Table 1).

Fat sources varied in their fatty acids composition as expected (Table 2). The HT contained more saturated fatty acids than HP. The HT contained some odd numbered carbon fatty acids (C15 and C17) in contrast to HP. The ratio of palmitic to stearic acids was higher for HP in comparison to HT. Increasing the saturation of fat sources increases ruminal inertness but decreases FA digestibility (Grummer, 1993); extensively hydrogenated triglycerides, such as 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 wheat grain coated with HT and HP, GP parameters and calculated amounts of OMD and ME of wheat grain are presented in Tables 3 and 4.There was a significant difference ($P < 0.01$) in GP among treatments (Table 3). The effect of type and amount of fats applied to the wheat grain flour was significant, particularly at 6 h and longer incubation times ($P < 0.05$). The GP volume at the first times of incubation did not differ among treatments; however, fat coated treatments produced less gas compared with WG only. The $WGHT_{80}$ and $WGHP_{80}$ produced the lowest volume of gas after 6 h of incubation compared with other treatments ($P < 0.01$).

There were significant differences among treatments coated with HT and HP after 6, 8, 12, 24 and 48 h of incubation compare to WG ($P < 0.01$). Potential GP (a+b), GP from the insoluble fraction (b) and fractional rates of GP(c) showed significant difference ($P < 0.01$)

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

Time (h)	Hydrogenated tallow					Hydrogenated palm oil				Significance		SEM
	WG	$WGHT_{20}$	$WGHT_{40}$	$WGHT_{60}$	$WGHT_{80}$	$WGHP_{20}$	$WGHP_{40}$	$WGHP_{60}$	$WGHP_{80}$	Fat	Level	
2	6.02	5.51	5.19	4.99	4.83	5.16	6.01	6.08	6.09	ns	ns	0.42
4	12.37	12.82	12.19	11.08	9.53	9.61	9.75	8.27	7.79	ns	ns	0.95
6	20.26^{a}	17.97^{ab}	15.93^{bc}	13.41^{bc}	11.09^{ce}	13.71^{bc}	11.7^{ce}	10.30^{e}	9.19^{ef}	*	*	1.22
8	36.23^{a}	21.95^{b}	18.20^{c}	15.52^{d}	12.26^{e}	20.97^{bf}	13.75^{e}	13.89^{e}	10.91^{e}	*	**	2.63
12	62.68^{a}	33.05^{b}	24.76^{c}	19.43^{d}	14.46^{e}	41.09^{f}	21.31^{cd}	22.78^{c}	14.64^{e}	**	**	2.95
24	81.38^{a}	56.02^{b}	42.74^{c}	30.19^{d}	18.36^{e}	53.40^{f}	39.39^{c}	30.27^{d}	18.07^{e}	**	**	3.33
48	90.61^{a}	58.52^{b}	46.34^{c}	32.96^{d}	19.93^{e}	54.89^{f}	41.60^{c}	31.29^{d}	18.31^{e}	**	**	3.96

Means within a row with different superscripts differ ($P < 0.05$). WGHT, wheat grain coated with HT; WGHP, wheat grain coated with HP; 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 wheat grain coated with HT and HP.

Parameter	Hydrogenated tallow					Hydrogenated palm oil				Significance		SEM
	WG	$WGHT_{20}$	$WGHT_{40}$	$WGHT_{60}$	$WGHT_{80}$	$WGHP_{20}$	$WGHP_{40}$	$WGHP_{60}$	$WGHP_{80}$	Fat	Level	
a+b	94.74^{a}	57.05^{b}	43.43^{c}	32.26^{d}	18.89^{e}	60.9^{b}	47.99^{c}	33.67^{d}	20.03^{e}	*	**	0.005
b	99.23^{a}	61.41^{b}	44.59^{c}	32.23^{d}	18.51^{e}	63.66^{b}	48.45^{c}	32.91^{d}	18.87^{e}	*	**	0.026
c	0.074^{a}	0.083^{a}	0.065^{a}	0.087^{a}	0.116^{b}	0.072^{ac}	0.069^{ac}	0.079^{c}	0.119^{bd}	*	*	0.0002
ME	14.35^{a}	10.41^{b}	8.39^{c}	7.06^{d}	5.36^{e}	10.77^{b}	8.84^{c}	7.05^{d}	5.40^{e}	**	**	0.05
OMD	92.57^{a}	66.72^{b}	53.18^{c}	43.97^{d}	32.04^{e}	69.05^{b}	56.15^{c}	43.91^{d}	32.30^{e}	**	**	0.38

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); WGHT, wheat grain coated with hydrogenated tallow; WGHP, wheat grain coated with hydrogenated palm oil; 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.

among treatments (Table 4). Fat type as well as fat level significantly affected b, a+b and c parameters for coated treatments in comparison with WG ($P < 0.01$). The potential GP (a+b) of uncoated WG was higher (94.7 ml) than other fat coated treatments ($P < 0.01$). The fat coating method resulted in a reduction of GP potential level to 18.89 and 20.03 ml in $WGHT_{80}$ and WGHP80, respectively. The GP from the insoluble fraction (b) of wheat grain coated with these experimental fats reduced significantly ($P < 0.01$), because fermentable fraction decreased along with the addition of 200 to 800 g/kg of experimental fats to WG (Table 4). Fractional rates of GP(c) increased significantly for the fat-coated treatments compared to WG ($P < 0.05$) and the value of (c) was greater for $WGHT_{80}$ (0.116) and $WGHP_{80}$ (0.119) (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 the feed samples (Menke and Steingass, 1988; McDonald et al., 1995). The OMD and ME content results are shown in Table 4. The OMD and ME content of fat coated treatments decreased significantly ($P < 0.01$) in comparison with WG (Table 4).

DISCUSSION

Efforts have been made to control rumen fermentation using ration manipulation approaches 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 in reducing the total GP and may possibly have a carryover effect on volatile fatty acids production. To our knowledge, this technology is 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 for the effect of fat coated starchy feeds like cereal grains.

The CP content of WG (117 g/kg DM) used in this study was comparable to other researchers (Cone et al, 2009, 2005) who reported 132 and 114 g/kg DM of CP for WG. The NDF content of WG (166 g/kg) was not inconsistent with other report that expressed 120 g/kg NDF for WG (Cone et al., 2009).

Discrepancy in chemical composition within the treatments is related to variation in the fats used in the experimental treatments. Coating WG with HT and HP reduced the CP and increased EE content of experimental treatments. Since the level of fat increased to protect WG, the CP, ADF, NDF and ASH content decreased reasonably. It is shown in this study that HT was more saturated fat than HP, and HT fat has more C18:0 than HP. Hydrogenated fatty acids of a particular chain length have lower digestibility coefficients than the 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 this study has lower monounsaturated fatty acids (C18: 1 isomers) in comparison to tallow (Getachew et al., 2001). It is reported that C18:1 for tallow is approximately 28.9% (DM) (Getachew et al., 2001), whereas the content of C18:1 in HT and HP in this study was 0.34 and 6.75% (DM), respectively.

Along with the inclusion of wheat grain coated with HT and HP, the GP decreased during incubation and the depression trend was greater for HP than HT treatments. The GP after 24 h for WG (421 ml/g OM equivalent to 81.38 ml/ 200 mg DM) was different from that reported by Chai et al. (2004), which reported 585 ml/g OM for WG. Lower GP from WG in this study may be due to a lower proportion of starch and sugars (data not shown) compared with those reported by Chai et al. (2004).

Although, we used HT, which differs extensively from tallow, Getachew et al. (2001) reported that tallow do not affect GP. Moreover, the coating procedure of WG used in this study varied from the procedure of Getachew et al. (2001) who add fat to total mixed rations, as supplemental fat. The levels of fat used to protect WG (up to 800 g/kg) was significantly different of those reported by Getachew et al. (2001) which added 50 to 250 g/kg fatty acids as tallow or yellow grease to diets. The reduction of GP over incubation time after coating WG with HT and HP may be associated with microbial attachments, whereas these unsaturated fatty acids act as toxins for rumen bacteria (Henderson, 1973, Hunter et al., 1976; Kim et al., 2000). 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 cotton yarn had been soaked in either tallow or fatty acids. It has been shown that some compounds directly inhibit the activity of methanogen bacteria are likely to reduce, or eliminate, CH4 production (Baker, 1999). Although, it is very difficult to explain the biological basis of lower gas production of wheat grain coated by HT and HP.

In vitro GP from WG after 2 h (29.49 ml/g OM or 6.02 ml/ 200 mg DM) and WG coated with HT and HP were not comparable to the values of Chai et al. (2004), who reported 14.0 ml/g OM from the degradable fraction for WG. Although, it has been shown that after 3 h of incubation, GP from the washout fraction of WG is very high (227 ml/g OM); however, this fraction behave like the degradable fraction (Cone et al., 2006). It is well documented that soluble components of feeds are fermented very quickly (Madsen and Hvelplund, 1994; Lopez et al., 1994; Hvelplund and Weisbjerg, 2000).

In this study, we did not measure different fractions of WG separately, but it is clear that coating processes could not affect initial gas produced from WGHT and WGHP compared to WG. It may reflect that the coating process did not influence the soluble fraction of treated WG. Cone et al. (1997) and Cone and Van Gelder (1999) showed that gas is initially produced from fermentation of water-soluble components, such as sugars and protein.

In this study, WG gas production was approximately 99.27 ml g^{-1} OM (20.26 ml/200 mg DM, Table 3) after 6 h incubation, which was reported 75 ml g^{-1} OM by previous work (Chai et al., 2004). This difference may be related to differences in chemical composition of WG used in the two different studies.

Results from this 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 increased (Table 3). 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 and Palmquist, 1984) and free fatty acids content of these fat sources that caused a larger negative effect than the corresponding triglycerides (Bateman and Jenkins, 1998). In this study, fat sources used to protect WG consisted of hydrogenated free fatty acids with more long chain fatty acids than short chains (Table 2).

The amount of gas produced after 24 h (Table 3) is not similar to those reported by Chai et al. (2004). These authors reported a gas volume of 119 ml/mg 200 DM (585 ml/g OM) for WG after 24 h incubation time. In this study, it was approximately 81.38 ml/200 mg DM after 24 h incubation time. The lower gas produced after 24 h for WG in this study may relate to lower content of starch and NDF in WG used by Chai et al. (2004).

The $WGTH_{20}$ achieved 56.02 ml/200 mg DM GP after

24 h, which was comparable to $WGPH_{20}$ that produced 53.40 ml/200 mg DM. Generally, the WGHP treatments produced less gas than WGHT, indicates that the HT had more inhibitory effect on rumen microbial ecosystem.

Potential GP (a+b) has the same decreasing trend for WGHT and WGHP treatments for 200, 400, 600 and 800 g/kg DM levels, which indicated that the reduced GP could be achieved successfully by coating some ingredient of diets with fat. In the same way potential GP reported in this study for WG (94.74 ml/ 200 mg DM) is comparable to a previous report (Chai et al., 2004). The extent of potential GP reported here is even higher than those reported for citrus pulp and corn grain (74.3 and 75.6 ml/ 200 mg DM, respectively) (Getachew et al., 2002). The GP from the insoluble fraction (b) also decreased similar to the potential GP, due to a reduction of insoluble part of treatment by adding fat to WG.

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

The results showed that the fractional rate of GP (c: ml/h) ranged from 0.119 ml/h in $WGHP_{80}$, to 0.074 ml/h in WG (Table 4). The fractional rate of GP for WG in this study is close to 0.074/h of fractional rate of GP for distillers' dried grains reported by Getachew et al. (2004). The greatest fractional rate of GP was seen for $WGHT_{80}$ and $WGHP_{80}$ treatments (0.116 and 0.119 ml/h, respectively). Methane production is associated with fiber fermentation, however, in highly digestible feeds such as WG; 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 WG with HT and HP (from 166.1 in WG to 33.3 and 36.1 g/kg in $WGHT_{80}$ and $WGHP_{80}$, respectively) (Table 1), which reflects fiber digestion postponed of fat coated treatments. Consequently, it was predictable that the organic matter digestibility of treatments will be diminishing by adding fat to WG and by reduction in GP.

The highest and lowest OMD values were for WG (92.57 g/100 g DM) and $WGHT_{80}$ (32.04 g/100 g DM) as expected. High OM digestibility for WG can be predicted due to high concentrations of starch polysaccharides and other carbohydrates that are highly digestible by rumen microbes. Low digestibility of OM in fat coated treatments compared with WG was due to a high concentration of fat in these treatments. These findings are similar to Getachew et al. (2001) who reported a decline in GP and *in vitro* true digestibility with the addition of fatty acids of yellow grease and tallow to total mixed rations. The variation in OMD values for WGHT and WGHP treatments can be related to differences in GP after 24 h of incubation and the differences in chemical composition of these treatments.

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

The ME values of the fat coated treatments and the calculated ME for WG 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. Because of different production of gas after 24 h of incubation and the different chemical composition of WG from those reported previously (Getachew et al., 2002) for cereal grains, it could result into different ME values for fat coated treatments.

The application of the technique 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). In comparison with other fat coated treatments, WG had the greatest OMD in this study; so the greatest ME value for WG 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.

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