www.roavs.com EISSN: 2223-0343
RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Investigation of Digestion kinetics and gas production of fat coated protein sources using *in vitro* gas production technique

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Abstract

An *in vitro* rumen gas production (GP) technique was utilized to evaluate fish and soybean meal coated with different types of fat for total gas production and digestion kinetics. About 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 GP was recorded after zero to 96 h of incubation. There were differences among different fat coated proteins and uncoated control treatment in total GP during incubation, and the treatments differed ($P<0.05$) in rate and potential of gas production. The result of the present study showed that experimental fats which mixed with fish and soybean meal resulted in significant reduction in GP ($P<0.05$). Furthermore, the values of b and a+b reduced significantly since these supplemental proteins coated with both types of fat in comparison to uncoated treatments ($P<0.05$). Consequently, one of the possible approach is to reduce total GP from dairy cattle 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 feeding feedlot cattle or dairy cattle.

Keywords: protein supplements; gas production; hydrogenated tallow (HT); hydrogenated palm oil (HP); fat coating

To cite this article: Palizdar MH, H Sadeghipanah, H Amanlou, HM Tabrizi and AS Amiri, 2012. Investigation of Digestion kinetics and gas production of fat coated protein sources using *In vitro* gas production technique. Res. Opin. Anim. Vet. Sci., 2(5), 372-376.

Introduction

The gas measuring technique was considered to be a routine method of feed evaluation (Menke et al., 1979) where a high correlation between *in vitro* GP and *in vivo* apparent digestibility was reported. Maximizing energy intake by increasing energy density of the diet is a logical feeding strategy for early lactation cows. Moreover, use of partially or completely HT and/or HP also might be a less expensive method of providing fatty acid (FA) to dairy cows (Elliott et al., 1994). The addition of supplemental fat improves the energy status of cows; also it 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). Our hypothesis was that if there is an intake about 600–700g 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 *in vitro* digestibility of fat coated fish meal to reduce GP.

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Materials and Methods

Preparing samples and fat coating technique

Fat coating method was used according to pan coating method with some modifications (Grass and Unangst, 1972; Sklan, 1989). The procedure was accomplished using two types of experimental fats (HT and HP) to embed fish and soybean meal particles (particle size 1 mm) in very thin layers of fat to make a continuous film of fatty acids on a core of protein supplements. Therefore protein supplements were encapsulated using 200 (g/kg) HT and HP. The final form of the product was small beads ranging from 1000 to 1500 µm in diameter depend upon the type of fat used for encapsulation and the optimal size for GP method (Palizdar et al., 2011).

Chemical composition and *in vitro* gas production

The chemical composition was determined according to Palizdar et al. (2011). 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 dioxide carbon (CO_2). Rumen fluid was collected before the morning feeding from three ruminally fistulated steers that were fed diet containing alfalfa hay (600 g/kg) 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 was 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 in with incubation of buffered ruminal fluid without substrate (Blank test). Cumulative GP data were fitted to the exponential equation:

$Y = a+b\ (1-\exp^{-ct})$ where Y 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 96h) from fermentable fraction (ml/200g DM), "c" the GP rate constant for "b", and "t" is the time of incubation (h).

Statistical analysis

Data on *in vitro* GP were subjected to analysis of variance in a completely randomized design using the SAS program General Linear Model (GLM) procedure (SAS 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

Cumulative GP volume (ml 200 mg^{-1} DM) and GP parameters are presented in Table 1 and 2.There was a difference ($P<0.05$) in GP among treatments at the time of incubation (Table 1). The FM, FMHT and FMHP were produced the lowest volume of gas in compare to soybean meal treatments ($P<0.05$). The SBM treatment produced the highest volume of gas at the beginning of the experiment until to the end. Within protein supplements the treatments, which coated with HT had dissimilar gas production significantly compare to HP coated treatments (Table 1).

Table 1: *In vitro* gas production (ml/200mg DM) of protein supplements coated with HT and HP, incubated in buffered rumen fluid at different incubation times.

	Treatments						SEM
Time (h)	FM	FMHT	FMHP	SBM	SMHT	SMHP	
2	*3.1 ^{c}	3.4 ^{c}	3.5^{c}	7.4^{b}	8.9 ^{a}	7.2^{b}	0.43
4	5.5 ^{c}	5.8 ^{c}	6.1 ^{c}	17.3 ^{a}	17.2 ^{a}	15.0^{b}	0.91
6	6.9 ^{c}	6.8 ^{c}	7.6 ^{c}	23.4 ^{a}	22.3^{a}	19.0^{b}	0.85
8	7.6 ^{d}	7.0 ^{d}	7.9^{d}	31.8 ^{a}	25.8 ^{b}	22.1^{c}	0.60
12	10.2 ^{d}	7.8^{e}	9.0 ^{e}	43.3 ^{a}	29.6 ^{b}	24.7^{c}	0.91
24	13.5 ^{d}	8.5 ^{e}	10.5 ^{e}	54.3 ^{a}	36.0^{b}	29.8^{c}	0.23
48	13.9^{d}	9.4^{f}	11.1^{e}	60.4^{a}	42.0^{b}	32.4^{c}	0.96

*Means within a row with different superscripts differ ($P<0.05$). SEM= Standard error of mean. Soya bean meal (SBM), Fish-meal (FM), fishmeal coated with 200 (g/kg) of hydrogenated tallow and hydrogenated palm oil (FMHT and FMHP).

Potential GP (a+b), GP from the insoluble fraction (b) and fractional rates of GP (c) differed ($P<0.05$) among treatments (Table 2). The potential GP (a+b) of uncoated fish- meal was greater (14.88 ml) than other FM treatments coated with HT and HP ($P<0.05$) although the SBM treatments had higher potential GP compare to FM treatments and the SBM significantly

had the highest a+b. Fat coating method results in a reduction of potential GP compare to FM and SBM (14.88 and 61.93 ml). The GP from the insoluble fraction (b) of fish- meal and soybean meal coated with these experimental fats reduced significantly (P<0.05) because fermentable fraction decreased along with the addition of 200 g/kg of experimental fats (Table 2). In contrast to b and a+b parameters, fractional rates of GP (c) increased significantly for coated treatments in compare to FM and SBM (P<0.05) and the value of (c) was greater for FMHT (0.171 ml/h) and FMHP (0.151 ml/h) (Table 2).

Table 2: The gas production parameters of protein supplements coated with HT and HP calculated using F-curve.

	Treatments						SEM
	FM	FMHT	FMHP	SBM	SMHT	SMHP	
a+b	*14.88^{d}	9.86^{f}	11.68^{e}	61.93^{a}	44.42^{b}	34.34^{c}	0.003
b	14.50^{d}	9.42^{f}	11.25^{e}	63.62 ^{a}	42.58 ^{b}	33.59^{c}	0.021
c	0.100^{a}	0.171^{d}	0.151^{c}	0.092 ^{a}	0.096 ^{a}	0.119^{b}	0.0002

*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), SEM= Standard error of mean. Fish-meal (FM), fishmeal coated with 200 (g/kg) of hydrogenated tallow and hydrogenated palm oil (FMHT and FMHP).

Discussion

The decrease in GP during the incubation times is along with the inclusion of HT and HP to protect protein supplements. 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). The reduction of GP over the time of incubation by coating with HT and HP may be associated with microbial attachments. It has been suggested that dietary fats may coat fibre 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 other study Stewart (1977) observed a depression in cotton fibre degradation when the cotton yarn had been soaked in either tallow or fatty acids. Other explanation could be stated as; some unsaturated fatty acids may be toxic for rumen methanogen bacteria (Hunter et al., 1976; Kim et al., 2000). The HT and HP in the current study had slightly unsaturated fatty acids (Palizdar et al. 2011), which might interpret the reduced GP in these treatments.

Results from the present study confirm earlier findings that showed free fatty acids and long-chain fatty acids inhibit methane and total GP in the rumen, and free fatty acids may be more potent inhibitors than triacylglycerols (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 (Jenkins, 1993). A free carboxyl group was also proposed to be necessary to inhibit microbial growth (Demeyer and Van Nevel, 1995).

Potential GP (a+b) has the same decreasing trend for FMHT, FMHP, SMHT and SMHP treatments, which indicated the reduced GP, could be achieved successfully, with coating some ingredients of diet with fat. Likewise potential GP reported in this paper for SBM (61.93 ml/200mg DM) is similar to previous report (Getachew et al., 2002), which they stated 49.5ml/200 mg DM for plant protein sources.

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).

Although feeding rumen un-degradable proteins along with fat or fat coated protein provided no further improvement in milk yield compared with fat alone, but partially alleviated the depression in protein content caused by supplemental fat and increased the daily yield of milk protein (Dhiman et al., 2001). It has been suggested that feeding supplemental fat alone in transition period (Afzalzadeh et al., 2010) or oilseeds and bypass fats along with proteins or as fat coated proteins in lactation periods (Sklan and Tinsky, 1993; Dhiman et al., 2001; Petit et al., 2005) could affect some metabolites, blood plasma hormones, feed digestibility and milk composition and fatty acids profile. In a study, Sklan (1989) showed 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. 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.

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. 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.

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 with supplemental fats in the form of long chain free fatty acids, particularly in high concentrate eater feedlot cattle or dairy cattle.

Acknowledgements

Authors would like to express their gratitude to the Deputy for Research of the Azad Islamic University, Chalous Branch for financial support of this research. This research was carried out as a research project at the Islamic Azad University, Chalous Branch, Mazandaran.

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