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In Vitro Rumen Fermentation Characteristics of Intact or Oil Free of Various Protein Sources

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Authors' contributions

This work was carried out in collaboration between all authors and all authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: An in vitro gas production technique was conducted to investigate the effect of oil content of nine ruminant protein feedstuffs including whole soybean, soybean meal, flaked soybean, linseed, fish meal, canola meal, cottonseed meal, sunflower meal and corn gluten, on protein fermentation.
Study Design: Complete randomized block design
Methodology: Nine protein feedstuffs were chosen and 15 mg N of both intact or oil free dried and milled samples were inserted into 125 mL serum bottles. Buffered rumen fluid was added 10 g of rapidly fermentable carbohydrates and incubated for 4 h. Then, 60 mL of the incubated medium was poured in each serum bottle to start the gas production procedure.
Results: Gas production constant rate of the feed samples diminished after oil extraction (0.026 vs. 0.022 respectively). Whole and flaked soybean displayed the greatest magnitude of gas production constant rate among the other feedstuffs (P=.05). Also, the extent of gas production for fish meal, flaked and whole soybean was numerically lower than the other feed samples. A fast gas production was observed for flaxseed among the oil free samples. A decline in gas production constant rate occurred after the oil extraction might be due to the suppression in protein degradability of feedstuffs evaluated.
Conclusion: The oil free samples generally produced, or tended to produce, less in vitro

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gas production constant rate than the intact samples. In addition, results of this study provided evidences that rumen fermentation patterns, as evaluated by gas production technique, is affected when different protein feed sources were incubated.

Keywords: Fermentation; in vitro; oil; protein.

1. INTRODUCTION

In vitro fermentation of feed results in production of gas. The volume of produced gas should reflect the fermentation profile of a feed in the rumen. This led to development of in vitro gas production techniques (IVGPT), which simulate the rumen environment and allows estimation of kinetics of rumen fermentation by measuring cumulative gas production [1]. Microbial fermentation of feeds produces carbon dioxide, methane and short chain volatile fatty acids (VFA) [2]. However, gas production technique can be adapted to obtain gas production profiles that reflect fermentation of protein. To achieve this, incubations must be completed with an excess of rapidly fermenting carbohydrates. Completing the incubations in a nitrogen-free environment makes nitrogen (N) the limiting factor to microbial growth, which then depends on the availability of N from the feed samples.

Many factors influence the rate of fermentation of feeds [3]. Only few studies have been undertaken so far on the interference of oil content of feedstuffs with protein fermentation characteristics. Lipids added to ruminant diets can greatly disrupt fermentation in the rumen, causing reduced digestibility of non-lipid energy sources. Protein metabolism in the rumen is also altered when fat supplements interfere with fermentation. Infusion of linseed oil into the rumen of sheep decreased protein digestion in the rumen and was accompanied by decreased ammonia concentration and increased N flow to the duodenum [4]. Similar changes occurred when sheep were fed additional lipid as either corn oil or lecithin [5]. Increased efficiencies of microbial protein synthesis in the rumen often accompany those changes in protein digestion. This efficiency has been attributed to the reduction of protozoal numbers in the rumen and less bacterial N recycling [4,6] or to increased dilution rate of solids in the rumen because of the added fat [7,8]. The aim of the current research was to investigate the effect of oil content of several protein feedstuffs (containing 11 to 366g oil/ kg DM) on protein fermentation using a proposed gas production technique.

2. MATERIALS AND METHODS

2.1 Feed Samples and Chemical Analysis

Investigations were carried out using 9 ruminant feed protein source samples (Table 1). The feed samples used in this experiment were whole soybean (WS), soybean meal (SM), flaked soybean (FS), linseed (LS), fish meal (FM), canola meal (CM), cottonseed meal (CSM), sunflower meal (SFM) and corn gluten (CG). All feed samples were ground to pass through a 2 mm screen and dry matter was measured after drying for 48 h in an air-forced oven at 65 º C. Crude protein (CP)was determined using Kjeldahl method (Kjeltec 2300 Auto analyzer, Foss Tecator AB, Hoganas, Sweden) [9].

2.2 Oil Extraction Procedure

Oil content of the intact feedstuffs was extracted using automatic Soxhlet extraction (Soxtec system 1043, Foss Tecator, Seweden) [9]. A specific amount of each feedstuff which contained 15 mg nitrogen was applied to separate the oil content. Oil free samples were stored at 4º C for the subsequent gas production incubations.

2.3 Gas Production Incubations

The gas production technique was conducted as described by Cone et al. [10]. Two rams (42±4 kg, body weight) of 9 months were used as rumen fluid donors. The animals were fed on a diet containing wheat straw, dried alfalfa and concentrate (CP; 150 g/kg DM) offered once a day in the morning. Animals had free access to water. Rumen fluid samples were withdrawn 2 h after the morning feeding into pre-warmed insulated flasks, previously filled with CO_2. Rumen fluid was strained through two layers of cheesecloth and kept at 39 ˚C under CO_2. The buffer/mineral solution was N-free and contained (/l) 10.03 g $NaHCO_3$, 1.43gNa_2HPO_4, 1.55g KH_2PO_4, 0.15 g $MgSO_4 \cdot 7H_2O$, 0.52 g Na_2S, 0.017 g $CaCl_2 \cdot 2H_2O$, 0.015 g $MnCl_2 \cdot 4H_2O$, 0.002 g $CoCl_3 \cdot 6H_2O$, 0.012 g $FeCl_3 \cdot 6H_2O$ and 0.125mg resazurin. To avoid a too high input of N from the rumen fluid, the rumen fluid was diluted 1:19 with the buffer/mineral solution.

To be certain that nitrogen was the limiting factor to fermentation, 10 g/l rapidly fermentable carbohydrates (glucose, 3.33 g/l; xylose, 3.33 g/l and wheat starch, 3.33 g/l) were added to the buffered rumen fluid and incubated at 39 ˚C. This pre-incubation was performed in a 4 l bottle and also in four 125 ml bottle with 60 ml buffered rumen fluid to follow the gas production. During this pre-incubation, all available nitrogen from the rumen fluid was incorporated into bacterial nitrogen components, mainly protein, in order to make nitrogen limiting to microbial growth. After only 4 h of the incubation, gas production had ceased and 60 ml of the buffered rumen fluid, with the rapidly fermentable carbohydrates, was added with a dispenser to bottles, already containing the feed samples, with exactly 15 mg nitrogen. All gas production procedures were run for intact and oil-free feed protein samples.

Table 1. Content of dry matter, crude protein and crude fat in the investigated samples

Feed [1]	DM (g/Kg)	Crude protein (g/Kg)	Crude fat (g/Kg)
Whole Soybean	928	302	205
Soybean meal	922	435	34
Flaked soybean	941	452	123
Flaxseed	958	175	366
Cottonseed meal	979	305	127
Canola meal	935	303	11
Fish meal	920	524	151
Corn gluten	926	612	35
Sunflower meal	950	305	23
S.E.M.[2]	5.8	4.4	2.9

[1]*All samples were analyzed in duplicate in each sample*
[2]*Standard error of means*

Gas production parameters were estimated using a non-linear equation of $P=b\ (1-e^{-c(t-L)})$ [11], where P is the gas production at time t, b is the gas production from fermentable fraction (ml), c is the gas production rate constant (ml/h), t is the incubation time (h) and L is the lag time (h).

2.4 Statistical Analyses

Statistical analyses were performed using PROC GLM of SAS. The model used for the analysis was $Y_{ij} = \mu + T_i + B_j + e_{ij}$, where Y_{ij} was an observation of the dependent variable ij; μ was the population mean for the variable; T_i was the main effect of individual feedstuff i; B_j was the effect of oil content; e_{ij} was the random error associated with the observation ij.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition of Feed Samples

Crude protein ranged from 175 g/kg DM in flaxseed to 612 g/kg DM in corn gluten. All samples except flaxseed contained more than 300 g CP/kg DM. Crude fat ranged from 11 g/kg DM in canola meal to 366 g/kg DM in flaxseed (Table 1). The crude protein content of soybean meal and canola meal were lower than those reported by Cone et al. [10] and Getachew et al. [12]. All the experimental feed samples except for flaxseed used in the current study were by-product feeds. This inconsistency could be arising from the composition of original plant material and method of processing [13].

3.2 Gas Production Incubations

Gas production parameters and lag time for intact and oil free feed samples with exactly 15 mg N are shown in Table 2. Oil content of the feedstuffs did not alter extent of gas production (b) significantly (P>.05). However, gas production constant rate (c) of the feed samples declined after oil extraction (0.026 vs. 0.022 respectively). In contrast, Lag time of the feed samples was increased after oil extraction. Flaxseed did not show any lag time (0 h) and fish meal had a lag time of 0.9 h. Whole and flaked soybean showed the greatest magnitude of parameter (c) among the other feedstuffs (P=.05) (Table 2). Also, the extent of gas production for fish meal, flaked and whole soybean was numerically lower than the other feed samples (Table 2). A moderate amount and rate of produced gas resulted from exactly incubated 15 mg nitrogen was found for sunflower meal, cottonseed meal and soybean meal. Canola meal posed higher rate of gas production before oil extraction in comparison with other feed samples (Fig. 1). A fast gas production was observed for flaxseed among oil free samples (Fig. 2). Moreover, both intact and oil free corn gluten were fermented slower than the other feedstuffs (Fig. 1 and 2). In the present study, corn gluten showed the lowest gas production constant rate, whereas it showed a high gas production. The low gas production constant rate might be due to the slow releasing of nitrogen content of corn gluten in the incubation bottles. Furthermore, the high nitrogen content of the feedstuff caused an elevated amount of gas. The positive correlation between crude protein content and gas production of corn gluten is in agreement with the study of Labri et al. [14] who reported a positive correlation between crude protein content and potential gas production.

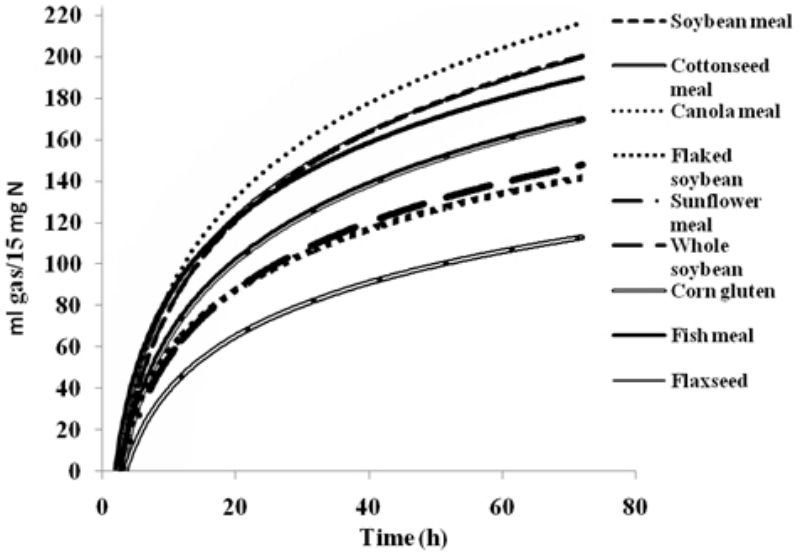

Fig. 1. Gas production profile of different intact feed samples with 15 mg N after a 4 h pre-incubation with rapidly fermentable carbohydrates to bind all free existing N of medium. As the standard errors were the same for all of the feed samples, they are not shown in the figure.

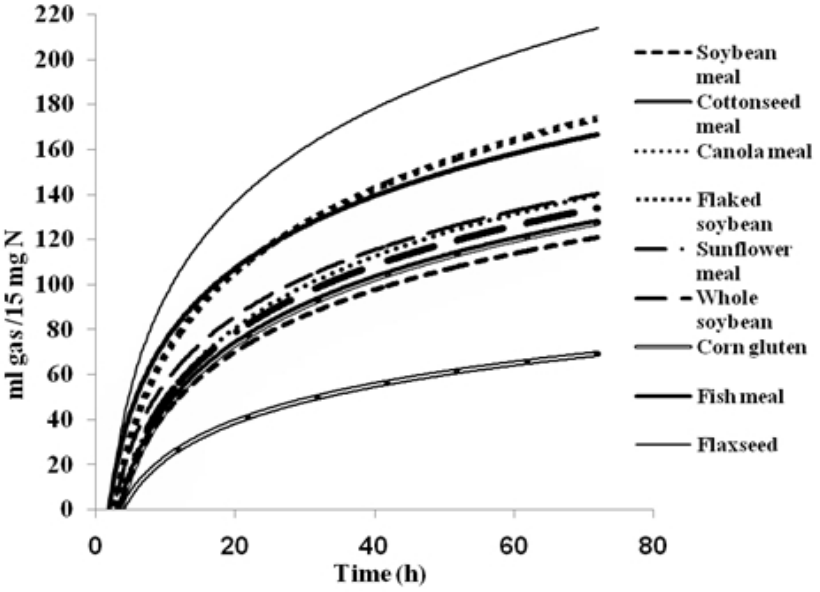

Fig. 2. Gas production profile of different oil free feed samples with 15 mg N after a 4 h pre-incubation with rapidly fermentable carbohydrates to bind all free existing N of medium. As the standard errors were the same for all of the feed samples, they are not shown in the figure.

Table 2. *In vitro* gas production parameters for intact and oil free of ruminant protein feed source samples

Items	Feed Source		Feedstuffs[3]									Oil free		Feedstuffs	
	Intact	Oil free	SBM	CSM	CM	FS	SFM	WS	CG	FM	FLX	SEM	P value[2]	SEM	P value
b	223.13	219.09	229.9^{cd}	218.6^{bc}	255.3^{e}	192.3^{a}	209.1^{abc}	198.3^{ab}	252.3^{de}	190.7^{a}	243.1^{de}	5.52	0.60	11.48	<0.001
c	0.026	0.022	0.025^{c}	0.021^{b}	0.021^{b}	0.036^{e}	0.019^{b}	0.035^{e}	0.008^{a}	0.032^{d}	0.027^{c}	0.0007	<0.001	0.001	<0.001
L	1.69	1.96	2.27^{ab}	2.54^{ab}	2.04^{bc}	2.02^{bc}	2.30^{ab}	2.37^{ab}	2.70^{a}	0.90^{e}	0.00†	0.093	0.003	0.28	0.041

[1]b is the asymptotic gas production (ml); c is the rate of gas production (h^{-1}); L is the lag time (h).

[2] Different superscripts following means within a row indicate differences at P<0.05.

[3] SBM=Soybean meal, CSM= Cottonseed meal, CM= Canola meal, FS= Flaked soybean, SFM= Sunflower meal, WS= Whole soybean, CG= Corn gluten, FM= Fish meal,FLX= Flaxseed.

In contrast with corn gluten, fish meal showed a relatively high gas production constant rate and low accumulated amount of gas in spite of its high nitrogen content. It can be interpreted that the little rumen degradable nitrogen of fish meal could not meet the nitrogen requirements of the rumen micro-biota, as the nitrogen liberating from the feedstuffs is the sole source of nitrogen in the incubation bottles, therefore, microbial fermentation had decreased.

Results declared that oil free flaxseed had a significant higher constant rate compared with the intact form (P=.05). However, the amount of produced gas was not altered (Fig. 1 and 2). Gilbery et al. [15] reported that steers fed 40 and 80 g/kg dietary DM had a lower nitrogen concentration in the rumen fluid when replaced linseed meal and the portion of corn. It seems that oil free flaxseed can liberate more nitrogen in the bottles so gas is produced faster than bottles containing intact flaxseed. Moreover, a trial in anaerobic closed fermenter of the batch type showed a decrease in degradability of nitrogenous compounds with diets supplied with flaxseed oil [16]. Oil extraction had no effect on gas production parameters of sunflower meal (Fig. 1 and 2). Based on the previous study conducted by Lund et al. [21] illustrating no effect of heat processing on rumen degradability of sunflower meal, it can be addressed that oil extraction did not interrupt the degradability of nitrogen compounds of sunflower meal so that, the profile of gas production was not switched.

Results exhibited in Table 2 elucidate that the cumulative volume of gas (b) was not affected by oil extraction among the experimental samples (P>.05). Prolonged incubation period provided adequate time for potentially degradable fraction of protein to be degraded in the medium. Therefore, final extent of accumulated gas was not changed. This speculation confirmed the concept proposed by Lund et al. [22], who suggested that a large proportion of protein is degraded in the last part of the degradation period. The results of the present work show that the adapted gas production technique, being depleted from nitrogen, and using an excess of rapidly fermentable carbohydrates, is suitable to recognize differences in nitrogen availability between feed samples.

3.3 Maillard reaction

In the present study, oil extraction was carried out using an automatic soxhlet extraction. This method applies heat treatment (140 ºC) and moisture to extract the oil content of the feedstuffs. It has been proved that heat treatment of protein feedstuffs such as expanding, extruding and etc. inhibits the nitrogen degradation in the rumen, thus the concentration of free amino acid and ammonia nitrogen concentration decrease in the rumen fluid [17,18]. The mechanism of decreasing degradation in the rumen by the combination of heat treatment and moisture involves Maillard reaction in which the free amino acid group of especially lysine reacts with carbonyl compounds from reducing sugars like glucose and fructose [19,20]. This phenomenon would result in a decline in gas production parameters especially the fractional constant rate and a concomitant increase in lag time. The increase in lag time after oil extraction is logical due to the Maillard reaction and extended time needed to cleavage the undesired bonds. Lund et al. [21] concluded in an in situ experiment to investigate the impact of heat processing (expanding) on degradation parameters of some feed sources. They resulted that expander treatment decreases the fractional rate of degradation and water soluble fraction and also increases the potentially degradable fraction of protein. Therefore, as heat processing and moisture interfere with the nitrogenous compound fractions of feed sources, it is of considerable value to employ cold extraction

approaches in order to minimize the effect of the undesirable reactions on estimation of protein degradability.

4. CONCLUSION

It was concluded that, under the condition of the present study conducted to compare the in vitro ruminal protein fermentation of intact or oil free of various protein feed samples, the oil free samples generally produced, or tended to produce, less in vitro gas production constant rate than the intact samples. In addition, results of this study provided evidences that rumen fermentation patterns, as evaluated by gas production technique, is affected when different protein feed sources were incubated. However, studies identifying the changes in microbial activities through the incubation period in the gas production system are needed to understand the observed differences.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Menke KH, Steingass H. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim Res Dev. 1988;28:7–25.
2. Palmer MJA, Jessop NS, Fawcett R, Illius AW. Interference of indirect gas produced by grass silage fermentation acids in an in vitro gas production technique. Anim Feed Sci Technol. 2005;123:185–96.
3. Nagadi S, Herrero M, Jessop NS. The influence of diet of the donor animal on the initial bacterial concentration of ruminal fluid and in vitro gas production degradability parameters. Anim Feed Sci Technol. 2000;87:231–39.
4. Ikwuegbu OA, Sutton JD. The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep. Bri J Nutr. 1982;48:365-75.
5. Jenkins TC, Fotouhi N. Effects of lecithin and corn *oil on* site of digestion, ruminal fermentation and microbial protein synthesis in sheep. J Anim Sci.1990;68:460-66.
6. Jenkins TC, Palmquist DL. Effect of fatty acids or calcium soaps on rumen and total nutrient digestibility of dairy ration. J Dairy Sci. 1984;67:978-86.
7. Boggs DL, Bergen WG, Hawkins DR. Effect of tallow supplementation and protein withdrawal on ruminal fermentation, microbial synthesis and site of digestion. J Anim Sci. 1987;64:907-14.
8. Czerkawski JW, Christie WW, Breckenridge G, Hunter ML. Changes in the rumen metabolism of sheep given increasing amounts of linseed oil in their diet. Bri J Nutr. 1975;34:25-44.
9. AOAC. Associations of Official Agriculture Chemists. Official Methods of Analysis, 15th edition. 1990;1298.
10. Cone JW, Rodriguez MAM, Guedes CM, Blok MC. Comparison of protein fermentation characteristics in rumen fluid determined with the gas production technique and the nylon bag technique. Anim Feed Sci Technol. 2009;153:28-38.
11. Orskov ER, McDonald I. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J Agri Sci Camb. 1979;92:499–503.

12. Getachew G, Robinson PH, DePeters EJ, Taylor SJ. Relationships between chemical composition, dry matter degradation and in vitro gas production of several ruminant feeds. Anim Feed Sci Technol. 2004;11:57-71.
13. DePeters EJ, Fadel JG, Arosemena A. Digestion kinetics of neutral detergent fiber and chemical composition within some selected by-product feedstuffs. Anim Feed Sci Technol. 1997;67:127-40.
14. Labri A, Smith JW, Kurdy IO, Adeknle IO, Raji AM, Ladipo DO. Chemical composition, rumen degradation, and gas production characteristics of some multipurpose fodder trees and shrubs during wet and dry season in the humid tropics. Anim Feed Sci Technol. 1998;72:81-96.
15. Gilbery TC, Lardy GP, Hagberg DS, Bauer ML. Effect of flax grain inclusion on rumen fermentation, digestion, and microbial protein synthesis in growing and finishing diets for beef cattle. Anim Feed Sci Technol. 2010;161:1-8.
16. Broudiscou L, Lassalas B. Linseed oil supplementation of the diet of sheep: Effect on the in vitro fermentation of amino acids and proteins by rumen microorganisms. Anim Feed Sci and Technol. 1991;33:161-71.
17. Ljokjel K, Harstad OM, Skrede A. Effect of heat treatment of soybean meal and fish meal on amino acid digestibility in mink and dairy cows. Anim Feed Sci and Technol. 2000;84:83-95.
18. Doiron K, Yu P, McKinnon JJ, Christensen DA. Heat-induced protein structure and subfractions in relation to protein degradation kinetics and intestinal availability in dairy cattle. J Dairy Sci. 2009;92:3319-30.
19. Broderick GA, Wallace RJ, Ørskov ER. Control of rate and extent of protein degradation. In: Tsuda T, Sasaki Y, Kawashima R. (Eds.), Physiological Aspects of Digestion and Metabolism in Ruminants. Academic Press, USA. 1991;541–92.
20. Martins SIFS, Jongen WMF, Van Boekel MAJS. A review of Maillard reaction in food and implications to kinetic modeling trends. Food Sci and Technol. 2000;11:364–73.
21. Lund P, Weisbjerg MR, Hvelplund T. Profile of digested feed amino acids from untreated and expander treated feeds estimated using in situ methods in dairy cows. Livestock Sci. 2008;114:62-74.
22. Lund P, Weisbjerg MR, Hvelplund T. Heat treatment of concentrates ten lkvæg. Grøn Viden Husdyrbrug. No. 6.DanmarksJordbrugsforskning. 1998;8 pp.

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