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DISSERTATION

METHANE DECREASING PROPERITES, NUTRIENT DIGESTIBILITY, AND ENERGY VALUE OF BREWER'S OR DISTILLER'S GRAINS EXAMINED IN VIVO AND/OR IN VITRO

Submitted by:

Michael J. Jarosz

Department of Animal Sciences

In partial fulfillment of the requirements

for the degree of Doctor of Philosophy

Colorado State University

Fort Collins, CO

Fall 2000

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY MICHAEL J. JAROSZ ENTITLED “METHANE DECREASING PROPERTIES, NUTRIENT DIGESTIBILITY, AND ENERGY VALUE OF BREWER’S OR DISTILLER’S GRAINS EXAMINED IN VIVO AND/OR IN VITRO” BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Colin M. Clay

Jack C. Whittier

Terry L. Stanton

Donald E. Johnson

Advisor

Department Head/Director

ABSTRACT OF DISSERTATION

METHANE DECREASING PROPERITES, NUTRIENT DIGESTIBILITY, AND ENERGY VALUE OF BREWER'S OR DISTILLER'S GRAINS EXAMINED IN VIVO AND/OR IN VITRO

An in vitro and an in vivo experiment were conducted to further evaluate methane losses and nutrient availability of brewer's grains (BG) and distiller's grains (DG) as components of ruminant diets. The objectives of the in vitro experiment were to determine the component(s) and concentration of BG or DG which cause methane production to decrease. Five in vitro trials included: increasing concentrations of BG or DG from 0 to 100% replacing alfalfa, increasing concentrations of corn oil from 0 to 10% replacing corn gluten feed (CGF), BG and DG with and without lipid, corn gluten feed with 3% yeast or 14% beer, and 80% BG or 80% DG with 20% grass hay.

Methane (percent of non-lipid GE) production was linearly decreased ($P < 0.01$) when BG was increased from incubating 20 to 100% of the substrate. Methane production was decreased ($P < 0.01$) for the DG trial only when the substrate contained 100% DG. Adding corn oil to CGF linearly ($P < 0.01$) decreased methane production over concentrations ranging from 0 to 10%. Incubating BG or DG after removing the lipid from these by-products did not increase methane production. Incubating beer with CGF increased ($P < 0.01$) methane production 50%, whereas yeast had no effect. A mixture of 80% BG with grass hay responded similarly to the alfalfa mixture by decreasing methane (percent of non-lipid GE) production below that from forage alone, whereas no change in methane production was measured when incubating 80% DG with grass hay.

The in vivo experiment evaluated methane decreasing effects, nutrient digestibility, and energy value of DG. Six steers were fed 0, 20, or 40% DG as part of a 60% concentrate diet in a 3 x 3 Latin square. Methane production was not significantly decreased by including DG as part of the diet. The only nutrients digested differently with DG addition were NDF and DE. Adding 20 and 40% DG to the diet increased ($P < 0.05$) NDF digestibility 17 and 30%, respectively when compared to the control at 46.7%. Digestible energy was 6.8% greater ($P < 0.05$) for the diet containing 40% DG, than the 0 and 20% DG diets and ME (Mcal/kg DM) content of the DG diets was 7% greater ($P < 0.05$) for each 20 percentage unit addition of DG to the diet. The higher ME content of the DG diets combined with numerically greater partial efficiency of ME use, suggests the NEm and NEg content of the DG diets were found to be at least 10% greater ($P < 0.05$) than the 1.95 and 1.56 Mcal/kg found for the control. Calculated by difference and extrapolated to NRC (1996) equivalents suggest the NEg content of the DG is approximately 1.83 Mcal/kg DM, about 20% above the current estimate.

Michael James Jarosz
Department of Animal Sciences
Colorado State University
Fort Collins, CO 80523
Fall, 2000

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INTRODUCTION

By-product feeds such as brewer's grains (BG) and distiller's grains (DG) are commonly fed to cattle as an economical protein, energy, and mineral source. These feeds are produced during the brewing and distilling process, where most of the starch is extracted concentrating the fiber, protein, lipid, and mineral portion of the grain. When BG and DG are fed to feedlot or dairy cattle, these feeds typically replace a portion of the grain. Not only do these two by-products function as economic nutrient sources, they also have been shown to decrease methane production of ruminants by 25 to 50% when compared to a ruminant consuming common forages (Wainman et al., 1984 and Jarosz, 1998). Methane losses and nutrient availability from substrates or diets containing these by-products were explored by in vitro and in vivo experiments.

The objectives of the in vitro experiment were: 1) to determine the effect of BG and DG concentration (0, 20, 40, 60, 80, or 100%; % of DM) in substrates containing the specified amount of each by-product mixed with alfalfa on methane and carbon dioxide production. 2) to determine the effects by-product components (yeast, ethanol, or lipid) have on methane production. 3) to determine the effect(s) of incubating grass hay (GH) with BG or DG, instead of alfalfa, on gas production and DM digestibility.

The hypotheses are: 1) at least 55% of the substrate (% of DM) must be BG or DG, replacing alfalfa, before a significant decrease in methane production will be measured 2) the high lipid content (10 to 12%) of BG and DG is the unique property causing methane production of ruminants to decrease 3) incubating 80% BG and 80% DG with 20% grass hay will decrease methane production below the 100% grass hay substrate.

The objectives of the in vivo experiment were to evaluate the effects of feeding 0, 20, or 40% DG in a 60% concentrate lactating dairy cow on in vivo methane production, nutrient digestibility, and energy value. Distiller's grains were chosen to be to be evaluated since DG are commonly fed to this group of ruminants and DG have been shown to cause the most dramatic decrease in methane production of ruminants Wainman et al., 1984 and Jarosz, 1998). Dairy cows are commonly fed DG at concentrations of 15 to 20% of diet DM, so 20% was chosen as one concentration of DG to feed. The higher concentration of 40% DG was approximately twice that used in industry, which was chosen to increase the sensitivity of by-product evaluation. Dietary energy partitioning was evaluated with steers as an experimental convenience. The hypotheses are: 1) methane production of steers fed 20 and 40% distiller's grains will not be decreased 2) nutrient digestibility will not differ 3) diet energy value (NEg) will be greater for the DG containing diets.

CHAPTER ONE

LITERATURE REVIEW

The following review focuses on recent experiments evaluating the effect of brewer's grains (BG) and distiller's grains (DG), yeast, ethanol, and lipid on ruminant methane production. For a more extensive review of by-product terminology, processing procedures, feeding value, energy value, and methane production of ruminants fed by-product feeds, consult Jarosz (1998).

By-product feeds described. Brewer's and distiller's grains are fibrous feed products of beer and ethanol or whiskey production. During processing most of the starch is removed from the substrate grain concentrating the other nutrients (lipid, protein, fiber, minerals) two to three fold. Since the fiber content of the by-products is high (40 to 60% NDF), these feeds are best suited for ruminants. Although BG and DG are highly fiberous, recent research has indicated a high energy value of these by-product feeds when fed to ruminants (Larson et al, 1993 and Jarosz, 1998).

Methane and by-products. Partially responsible for the high energy value of BG and DG, is the ability of these feeds to decrease ruminant methane production when fed in combination with forages. Wainman et al. (1984), while determining the ME content of BG and DG, discovered methane production of sheep to be 30 to 50% lower than predicted. (Table 1.1). Jarosz (1998) later evaluated BG and DG when fed to steers and found results similar to Wainman et al. (1984) (Table 1.1). These

Table 1.1. Measured and predicted energy lost as methane from each by-product feed

Source	Brewer's grains	Distiller's grains
Measured methane, % of GE		
Wainman et al. (1984)[a]	4.1	3.5
Wainman et al. (1984)[b]		2.5
Jarosz (1998)	3.4	2.9
Predicted methane, % of GE		
Moe and Tyrrell, (1979)	6.5	5.8

[a] Values averaged for barley and corn distiller's grains.

[b] Values averaged for corn distiller's grains only.

experiments provide evidence that ruminant methane production is decreased when fed BG or DG as opposed to feeding other high fiber feeds.

Global warming. The methane suppressing ability of BG and DG could become important when considering the effects of methane on global warming and ruminant energy balance. Ruminants lose 2 to 12% of the gross energy they consume as methane, with an average loss of approximately 6.0% (Johnson and Johnson, 1995). The energy lost as methane by cattle accounts for an estimated 2% of the total global warming effect (Johnson and Johnson, 1995). If the energy lost as methane could be decreased it could potentially be captured by the animal for maintenance or production, as well as decrease the impact on climatic change.

Although methane production is detrimental to the environment and a loss of energy to the ruminant animal, methane formation is important to ruminal microorganisms to function efficiently. Methane acts in the rumen as a final electron acceptor by removing hydrogen produced during fermentation. Without removal, hydrogen could accumulate in the rumen limiting organic matter digestion (Russell and Jeraci, 1984). Therefore, methane production becomes vital for efficient microbial digestion.

The challenge is to decrease ruminant methane production, while maintaining efficient microbial digestion. Feeding ruminants BG and DG with alfalfa or other forages has the potential to decrease methane production with few detrimental effects. If BG or DG are to be used as a potential methane suppressor, the concentration at which each needs to be included in a ruminant diet becomes important.

Effect of by-product concentration. The minimum concentration at which BG or DG need to be included in a ruminant diet and decrease methane production of ruminants has not been extensively evaluated. However, some evidence of a non-linear or associative suppression with mixtures exists. Wainman et al., (1985) conducted an experiment with barley starch:distiller's grains mixtures and found as long as 55% or more of the diet dry matter was wet barley distiller's grains, methane production of sheep was reduced from 15% of GE to 4% of GE intake. This experiment demonstrated a non-linear suppressing effect of DG concentration on methane production, but was evaluated with barley flour, an uncommon basal feedstuff.

Not only is the concentration of BG or DG important to evaluate, but the unique property causing methane of ruminants to decrease is important. Several unique properties and/or nutrient concentrations of BG and DG were considered as possible methane suppressors. Following review of the literature for possible constituents of BG and DG that cause methane production of ruminants to decrease, the three most likely constituents were: yeast, lipid, and ethanol.

Yeast. Yeast is a common constituent of BG or DG and may cause methane production to decrease by stimulating acetogenesis. Acetogenesis is the process by which a particular species of bacteria use carbohydrate and hydrogen to form acetate. Yeast may stimulate acetogenesis by supplying a specific nutrient or providing a more favorable environment for the acetogens. If stimulated these bacteria may diminish the supply of rumen hydrogen leaving less for methanogens to bond with carbon dioxide and produce methane.

Chaucheyras, et al. (1995) found by in vitro incubation, the yeast strain *Saccharomyces cerevisiae* increased ($P < 0.001$) hydrogen use for acetate production by an acetogenic bacterial strain. *Saccharomyces cerevisiae* also increased ($P < 0.01$) use of hydrogen by the methanogens, but did not significantly increase methane. Their theory was that the yeast had the potential to enhance the acetogenic strain allowing the acetogens to partially compete with the methanogens and obtain more hydrogen. Relevant to BG and DG, yeast present in BG and DG could stimulate ruminal acetogens, increase acetogen hydrogen use, increase acetate production, and leave less hydrogen for methanogens to combine with carbon dioxide and produce methane. In contrast to Chaucheyras, et al. (1995), Mackie and Bryant (1994) found acetogenesis may be difficult to attain in the rumen because of the low ruminal hydrogen concentrations and the high affinity methanogens have for hydrogen.

Lipid. A second possible methane suppressing constituent of BG and DG is the relatively high (10-12%) lipid concentration. Zinn, (1988) added 4% supplemental lipid to a high concentrate feedlot diet and found a decrease in acetate, an increase in propionate, and a reduction ($P < 0.05$) in estimated methane production based on volatile fatty acid analysis. Lipid has the potential to decrease methane by acting as an alternative hydrogen sink, increase propionate, and/or decrease the protozoal population or activity. Lipid and especially unsaturated lipid supplementation can provide an alternate hydrogen sink in the rumen by way of double bond saturation (Johnson and Johnson, 1995). Sauer et al. (1998) found methane production did not change when dairy cows were supplemented 3.5% soybean oil in a total mix ration. However, the soybean oil did increase ($P < 0.01$) the concentration of linoleic acid (18:2) and all the biohydrogenation products in the milk. The increased biohydrogenation measured, suggests an alternative use of hydrogen in the rumen. Potentially the biohydrogenation did not use enough of the hydrogen to produce a significant change in methane production.

A problem with the unsaturated lipid theory, is the large amount of lipid a ruminant would have to consume to completely decrease methane production. Weimer (1998) suggests a cow producing 90 kg of methane yearly would have to consume greater than 4 kg of unsaturated lipid each day, which is unrealistic. Although ruminants would have to consume large amounts of lipid to completely eliminate methane, consumption of lower amounts of lipid may marginally decrease methane.

Aside from the effects of bio-hydrogenation on methane production lipid also has the potential to increase propionate production. By increasing propionate production, hydrogen is used during propionate formation instead of produced instead of produced as when acetate is formed. By increasing propionate less hydrogen is available for methane. Plascencia et al. (1999) fed 5% griddle grease, 5% conventional yellow grease, or 5% of a 50:50 mix of griddle and yellow grease to steers fed a high grain diet. These researchers found propionate production increased ($P < 0.10$) when steers were supplemented griddle or yellow grease, but propionate production decreased ($P < 0.10$) when the combination was fed. Ruminal acetate production did not change when feeding griddle grease, but decreased ($P < 0.10$) when fed yellow grease and increased ($P < 0.10$) when steers were fed the combination lipid. The change in propionate production was most likely a cause of the estimated methane production to decrease ($P < 0.05$) when steers were fed both the griddle grease and yellow grease, but increase ($P < 0.05$) when fed the combination. The data suggest 5% lipid supplementation to a high grain feedlot diet will decrease estimated methane production, deducing that the increase in propionate production is leaving less hydrogen for methanogens to use for methane. By increasing the propionate production or decreasing the acetate:propionate ratio, more hydrogen is used leaving less for methanogens.

Zinn and Plascencia (1996) fed 6% yellow grease in a high grain feedlot diet with either 10 or 30% alfalfa. These researchers found an interaction between forage and lipid concentration on acetate:propionate ratio and estimated methane production. Adding yellow grease to the low forage diet increased ($P < 0.05$) the acetate:propionate ratio with minimal effects on methane. Whereas, adding yellow grease to the high forage diet decreased ($P < 0.05$) the acetate:propionate ratio along with a 15% reduction ($P < 0.05$) in estimated methane production. The higher acetate concentration of the 30% alfalfa diet appeared to allow lipid supplementation to have a greater effect on volatile fatty acid and methane production. Nevertheless, methane production of ruminants was decreased when fed 100% BG or DG, therefore, modest reductions in methane production could result from lower dietary concentrations of unsaturated lipid.

Along with the effects of lipid on the acetate:propionate ratio, lipid has been shown to have detrimental effects on protozoa found in the rumen. During normal conditions, protozoa in the rumen produce high amounts of hydrogen and as a result provide more substrate for the methanogens to use. Methanogens have been found living within the protozoa and latching on to hydrogen the protozoa produce (Krumholtz et al., 1983), suggesting methanogens want hydrogen and will go to great measures to get it. Defaunation (eliminating the protozoa) alters methane production in certain dietary conditions (Ushida et al., 1997).

Defaunation decreased methane production of ruminants fed high concentrate diets, but did not change methane production when ruminants were fed hay diets (Ushida et al., 1997). Ushida et al. (1997) suggested the difference between high concentrate and hay diets may be due to the protozoa existing as a larger population when ruminants are fed a high concentrate diet. Therefore, since the ruminal protozoa population is greater, dietary lipid addition has potential to effect the protozoa population and subsequently decrease methane production of ruminants fed high concentrate diets.

The effects of defaunation on volatile fatty acid (VFA) profiles provides more evidence as to the effects of defaunation on methane production. Acetate production was decreased ($P < 0.05$) by defaunation whether sheep were fed a hay or high concentrate diet. Defaunation increased ($P < 0.01$) propionate production when sheep were fed a high concentrate diet with variable results when sheep were fed hay. Although methane production has only been demonstrated to decrease when ruminants are fed BG or DG with forages, the effects of the lipid on protozoa still may be valid.

Volatile fatty acid. Comparing the effects of lipid supplementation on VFA profiles to the VFA profiles found when BG or DG are fed to ruminants may give more insight as to whether or not lipid of BG or DG is decreasing the methane production of ruminant. Adding DG to high grain ruminant diets suggests VFA changes are just the opposite as commonly seen when lipid is supplemented to ruminant diets. Peter et al. (2000) added 20% dried distiller's grains replacing corn starch in a high grain feedlot diet. These researchers found ruminal acetate production increased 19% ($P < 0.05$) and ruminal propionate production decreased 37% ($P < 0.05$). Based on the findings of Peter et al. (2000), methane production would be expected to increase when DG is fed to ruminants consuming high grain diets. Methane production of ruminants fed BG and DG as a portion of high grain diets has not been evaluated and indeed may increase or remain unchanged when DG is fed.

On the contrary, methane production of ruminants fed BG and DG as a portion of high forage diets has been evaluated by respiration calorimetry and show that methane production is decreased (Jarosz, 1998, and Wainman et al., 1984). One difference between the high grain versus the forage diet is that the acetate production from a high forage diet would be greater. Possibly, the lipid or other fermentation altering factors of the BG and DG has greater potential to decrease the acetate:propionate ratio of the high forage diets and as a result decrease methane production.

Ethanol. Along with yeast and lipid, the residual ethanol remaining in the BG and DG due to inefficient processing may be a third possible factor altering methane production of ruminants. However, the effect of ethanol on methane has not been evaluated, but Pol and Demeyer (1988) found methane production of sheep ruminally infused with methanol to increase. Ethanol is structurally similar to methanol, so the residual ethanol of BG and DG may have effects on methane. While the ethanol content of BG or DG, particularly dried grains, seems an unlikely candidate responsible for decreasing ruminant methane production, ethanol is a common uniqueness of the two by-products. Thus, it is important to discount ethanol as a cause of altered methane production.

Minerals. Although, yeast, lipid, and/or ethanol appear to be the most likely factors causing methane production of ruminants to decrease, other factors such as sulfates and/or nitrates may decrease ruminant methane production by acting as alternative ruminal hydrogen sinks. These factors seem unlikely due to the health problems caused when consumed by ruminants. Ruminants consuming high concentrations of sulfates could potentially develop polioencephalomalacia or high nitrate consumption may result in nitrate toxicity. Thus, these compounds cannot be used as significant alternative hydrogen sinks.

In summary, most of the experiments (Wainman et al., 1984 and Jarosz, 1998) evaluating BG and DG thus far have been evaluated when fed with forages. Since the diets consisted of high concentrations of forage, acetate production would be expected to be greater than when a grain is fed with the by-product feeds. Due to the relatively high concentration of lipid in the by-product feeds, the lipid may cause methane to decrease by increasing propionate, decreasing protozoa, increasing bio-hydrogenation and/or decreasing acetate production of the high forage/by-product combination.

Net energy for gain. Brewer's and distiller's grains have been shown to have a greater NEg value (Larson et al., 1995 and Jarosz, 1998) than suggested by NRC (1996) and greater than expected based on BG and DG digestibility. Larson suggested 9 to 10% of the extra energy may be a result of the relatively high lipid content of the by-products. Another reason for the extra NEg may be a result of less methane produced when ruminants are fed BG or DG. The exact reason for the above expected energy content of BG and DG is not clear and may be a combination of reasons.

In vitro methods for measuring gas production during fermentation. With the expense and time requirements of conducting in vivo experiment to measure nutrient digestibility and/or metabolizability, more interest has been placed on conducting in vitro experiments. In vitro experiments allow researchers to examine a higher number of samples/treatments in less time, with less labor and fewer animals. The primary method used in the past (Tilley and Terry, 1963), has been to incubate feed samples in test tubes with ruminal fluid and buffer for 48 h to determine digestibility. Nutrient disappearance is measured by filtering the residue in the test tubes remaining after incubation.

Due to filtering complications and to better characterize carbohydrate and protein pools (NRC, 1996), researchers (Wilkins, 1974, Menke et al, 1979, and Pell and Schofield, 1993) have begun to use gas production as an indirect measure of nutrient disappearance and/or metabolizability. To determine if gas production could be used as a measure of nutrient digestibility, researchers (Menke et al, 1979) have compared in vitro gas production to in vivo dry matter digestibility and found a strong correlation between the two.

During microbial digestion, some of the carbohydrates are hydrolyzed to glucose. To metabolize the glucose, the carbon, hydrogen, and oxygen is restructured to form volatile fatty acids, carbon dioxide, water, and methane. Wolin (1960) suggested the following equation relating the degradation of glucose into VFA's, carbon dioxide, methane, and water.

57.5 ($C_6H_{12}O_6$) $\longrightarrow$ 65 acetate + 20 propionate + 15 butyrate + 60 carbon dioxide + 35 methane + 25 water

The equation suggests a relationship between carbohydrate degradation and gas production, the more carbohydrate digested, the more carbon dioxide and methane produced.

To measure gas production, various methods have been implemented. One method used (Menke et al., 1979, Blummel and Orskov, 1993) is to incubate substrates in syringes and read the syringe plunger as a means of measuring gas volume produced. The procedure consisted of adding 0.2 to 1.0 g of dry matter to syringes along with ruminal fluid and a buffer solution. The syringes are then placed in a water-bath maintained at 39 °C. The gas produced by the microorganisms digesting the substrate cause the syringe plunger to move. The volume of gas is periodically measured every few hours by reading and recording the volume using the syringe plunger.

Researchers have begun to use pressure transducers to measure gas volume due to difficulty with the syringe method (Wilkins, 1974 and Pell and Shoefield, 1993. The pressure transducer method incorporates the use of tightly sealed vaccine vials to use as incubation vessels. Sealed vaccine vials are used to allow the gas to accumulate in the head-space and increase pressure, which can then be measured using a pressure transducer equipped with a needle. The pressure measurements can be made faster and possibly with less error than reading gas volume with a syringe. Pressure readings from the transducer are then converted to volume using Boyle's gas law or other related equations. To validate if using a pressure transducer will yield results similar to the syringe method, Varadyova et al. (1998) compared measuring volume using the pressure transducer technique to the syringe technique and found similar results.

Using gas volume for in vitro experiments allows the user to sample the head-space gas collected in the vials to measure not only volume but gas composition. The gas sample can be injected into a gas chromatograph to determine the carbon dioxide, methane, and hydrogen composition. Gas composition becomes important when researchers are examining the effects of various treatments on factors such as methane production. In summary, the pressure transducer method is an effective and efficient method to measure gas volume and composition.

Summary. Global warming is an important issue to animal and all of agriculture, finding ways to reduce the contribution of animals to the warming effect must be considered. One means is by reducing the methane production of ruminants through rumen manipulation. By-products such as BG or DG have reduced the methane production of ruminants to one-third to one-half of predicted. The concentration required to decrease methane or the unique factor causing the methane suppression of ruminants has not been extensively examined.

Wainman et al. (1985) suggested 55% of a sheep diet must be DG before methane production of ruminants is decreased, but this was the only experiment examining concentration effect of DG or BG. The unique constituents of BG or DG with potential to alter methane production of ruminants is the yeast, beer/ethanol, and lipid content. Lipid is the primary constituent that has demonstrated the potential to decrease methane production of ruminants, primarily by decreasing the hydrogen supply in the rumen available to the methanogens.

The in vitro pressure transducer fermentation system developed by Mauricio et al. (1999) allows users to measure quickly measure gas pressure on several incubating vials. The pressure can then be used to estimate gas volume produced and a sample of the gas can be collected and analyzed for composition. Other in vitro systems such as Rusitec are more labor intensive and require more complicated equipment to collect gas produced. One disadvantage of the transducer system is the difficulty calibrating the transducer and finding a way to validate the transducer is measuring the proper pressure. The in vitro pressure transducer system appears to be a functional way to evaluate the effects of BG and DG concentration and to better establish the unique constituent(s) of BG and/or DG causing methane production to decrease with minimal labor and animals.

CHAPTER 2

MATERIALS AND METHODS

A 40-vial in vitro fermentation system was used to measure by-product and by-product constituent effects on gas volume and composition, associative effects, and dry matter digestibility during five separate trials. During each trial, six different substrates/treatments were evaluated, resulting in a total of 36 different substrates. The first two in vitro trials were structured with the primarily objective to examine the effect of by-product concentration on gas production. Incremental concentrations of brewer grains (BG) or distiller's grains (DG) replaced every 20 percentage units alfalfa from 0 to 100% (Table 2.1).

Substrates. After careful consideration of possible common constituents of BG and DG causing ruminant methane production to decrease, it was decided that yeast, lipid, and ethanol should be investigated. Additional trials (three through five) were structured to examine the effects of three by-product constituents (yeast, lipid, or ethanol) and BG or DG mixed with grass hay on gas production, associative effects, and DM digestibility. Trial three examined the effect of lipid by incubating corn gluten feed (CGF) with increasing increments of corn oil (CO) every two percentage units from 0 to 10% replacing CGF (Table 2.2). Food grade CO was selected as the fat source to act as a similar lipid source as found in BG or DG. The CO was added to CGF, since CGF is a by-product produced and with similar nutrient content as BG and

Table 2.1. Ratios of brewer's grains (BG) or distiller's grains (DG) to alfalfa (ALF) incubated as substrate during in vitro trial one and two

	Ingredient, DM basis		
Treatment	Brewer's grains	Distiller's grains	Alfalfa
Trial one:			
100% BG	100		0
80% BG	79.5		20.5
60% BG	59.8		40.2
40% BG	39.7		60.3
20% BG	20.1		79.9
0% BG	0		100
Trial two:			
100% DG		100	0
80% DG		79.8	20.2
60% DG		60.2	39.8
40% DG		40.4	59.6
20% DG		20.1	79.9
0% DG		0	100

Table 2.2. **Ratios of corn gluten feed (CGF) and corn oil (CO) incubated as substrate during in vitro trial three.**

	Ingredient, DM basis	
Treatment	**Corn gluten feed**	**Corn oil**
0% CO	100	0
2% CO	96.7	3.3
4% CO	94.9	5.1
6% CO	93.4	6.6
8% CO	91.5	8.5
10% CO	89.2	10.8

DG, but methane production is not decreased when CGF is fed to ruminants (Deaville et al., 1994 and Jarosz, 1998).

Trial four further evaluated the effect of lipid by removing the lipid portion of BG or DG. The lipid was removed by ether extraction (AOAC, 1990), the residual BG or DG was dried to eliminate any residual ether, and a specified amount of the dried residual placed in vials to use as in vitro substrate (Table 2.3).

Trial five evaluated the effects of yeast, beer, and a second forage (grass hay) on methane production and DM digestibility. The yeast *Saccharomyces cerevisiae* along with other fermentation products (Conklin Inc., Shakopee, MN) were added to CGF to examine the effect of yeast. The concentration of yeast added with CGF was 2.9% (% of DM), which is the concentration suggested on the yeast product label (Table 2.4).

Also during trial five, approximately 14% (% of DM) light beer was added to CGF (Table 2.4) to evaluate the effects of ethanol on methane production and DM digestibility. The addition of beer to CGF simulates residual beer or ethanol remaining in the BG or DG, respectively, due to inefficient processing. The concentration of beer added to CGF was 14%, which brought the concentration of ethanol in the substrate incubated to approximately 9%. Nine-percent ethanol was the concentration remaining in wet DG, as suggested by Larson et al. (1993) due to inefficient distillation.

The final substrates examined during trial five, were BG or DG each incubated with 20% grass hay (GH). Grass hay was chosen to determine if methane is decreased when BG or DG are incubated with another forage in the same manner as when these

Table 2.3. Ratios of brewer's grains (BG) and distiller's grains (DG) with and without lipid and alfalfa incubated as substrate during in vitro trial four.

	Ingredient, DM basis				
Treatment	BG	BG without lipid[a]	DG	DG without lipid[a]	Alfalfa
100% BG	100				
100% BG w/o lipid		100			
80% BG w/o lipid		80.0			20.0
100% DG			100		
100% DG w/o lipid				100	
80% DG w/o lipid		79.6			20.4

[a] Residual remaining following lipid removal using ether extract.

Table 2.4. Ratios of corn gluten feed (CGF), brewer's grains (BG), distiller's grains (DG), beer, yeast, and grass hay (GH) incubated as substrate during in vitro trial five.

	Ingredient, DM basis					
Treatment	Corn gluten feed	Yeast	Beer	Brewer's grains	Distiller's grains	Grass hay
100% CGF	100					
CGF + yeast	97.1	2.9				
CGF + beer	86.3		13.7			
100% GH						100.0
80% BG 20% GH				79.1		20.9
80% DG 20% GH					79.5	20.5

by-products are incubated with alfalfa (Table 2.4).

Inoculum source. Ruminal fluid was supplied by two ruminally cannulated heifers fed a diet of chopped alfalfa hay with similar nutrient content as early bloom alfalfa hay reported in NRC (1996) (Table 2.5). The heifers were adapted to the alfalfa diets at least 14 d prior to ruminal fluid collection and fed the hay diet once daily to appetite. The heifers had free choice access to water and a trace mineralized salt block (96.5% NaCl, not less than 0.350% Zn, 0.280% Mn, 0.175 Fe, 0.035% Cu, 0.007% I, or 0.007% Co, Morton Salt, Morton International, Inc. Chicago, IL).

Ruminal fluid from the heifers was collected at approximately 0730, which was just prior to feeding at 0800, as recommended by Mauricio et al. (1999). Mauricio et al. (1999) found less variability during in vitro incubations when collecting ruminal fluid prior to feeding rather than 2 h post-feeding. Approximately 1 L of ruminal fluid was collected from each of the two cannulated heifers. The fluid was collected by suction from approximately four locations, two anterior and two posterior in the rumen into a pre-warmed thermos. Following collection: ruminal fluid was strained through four layers of cheesecloth, equal amounts of fluid from each heifer were mixed, placed in a pre-warmed beaker, and held at 39 °C in a water-bath, while continuously flushing with carbon dioxide to maintain an anaerobic environment.

In vitro system. An in vitro system modeled after Mauricio et al. (1999) and Pell and Schofield (1993) was developed to calculate gas volume by measuring head-space gas pressure in vaccine vials serving as containers for in vitro digestibility. Previous researchers (Pell et al., 1993 and Mauricio et al. 1999) primarily used in

Table 2.5. Nutrient composition of the chopped alfalfa fed to the cannulated heifers

Nutrient, Percent of DM	Measured	NRC, 1996
Dry matter	89.5	91.0
Crude protein	18.9	19.9
Lipid	4.3	2.9
Ash	9.8	9.2
NDF	36.4	42
ADF	26.4	
Lignin	5.3	7.1
NSC[a]	30.6	26
GE, Mcal/kg	4.42	

[a] Non-structural carbohydrate, 100 - (CP + EE +ash + NDF).

vitro systems to measure nutrient disappearance. The focus of the current trial was to use a similar in vitro system to measure gas volume production and analyze the gas produced for composition (carbon dioxide, methane, and hydrogen gas).

Incubation vessels consisted of 125 mL vaccine vials (Wheaton Science Products; Millville, NJ), capped with rubber septum, and kept tightly closed with a crimped aluminum seal. Prior to incubation, 1 g of substrate DM, ground through a Wiley mill equipped with a 2-mm screen, was measured into each vial. The day prior (Mauricio et al., 1999) to incubation a buffer (Tilley and Terry, 1963) was mixed by adding the following chemicals to a small amount of distilled water in a volumetric flask and then the volume diluted to 1 L with distilled water:

1) 9.8 g $NaHCO_3$
2) 3.71 g Na_2HPO_4
3) 0.75 g KCl
4) 0.47 g NaCL
5) 0.12 g $MgSO_7 * 7H_2 0$
6) 0.5 g urea
7) 0.04 g $CaCl_2$, added approximately 1 hour prior to initiating incubation

Following addition of $CaCl_2$ on the day of incubation, the pH of the buffer was adjusted to 6.8 to 7.0 by bubbling carbon dioxide through the buffer.

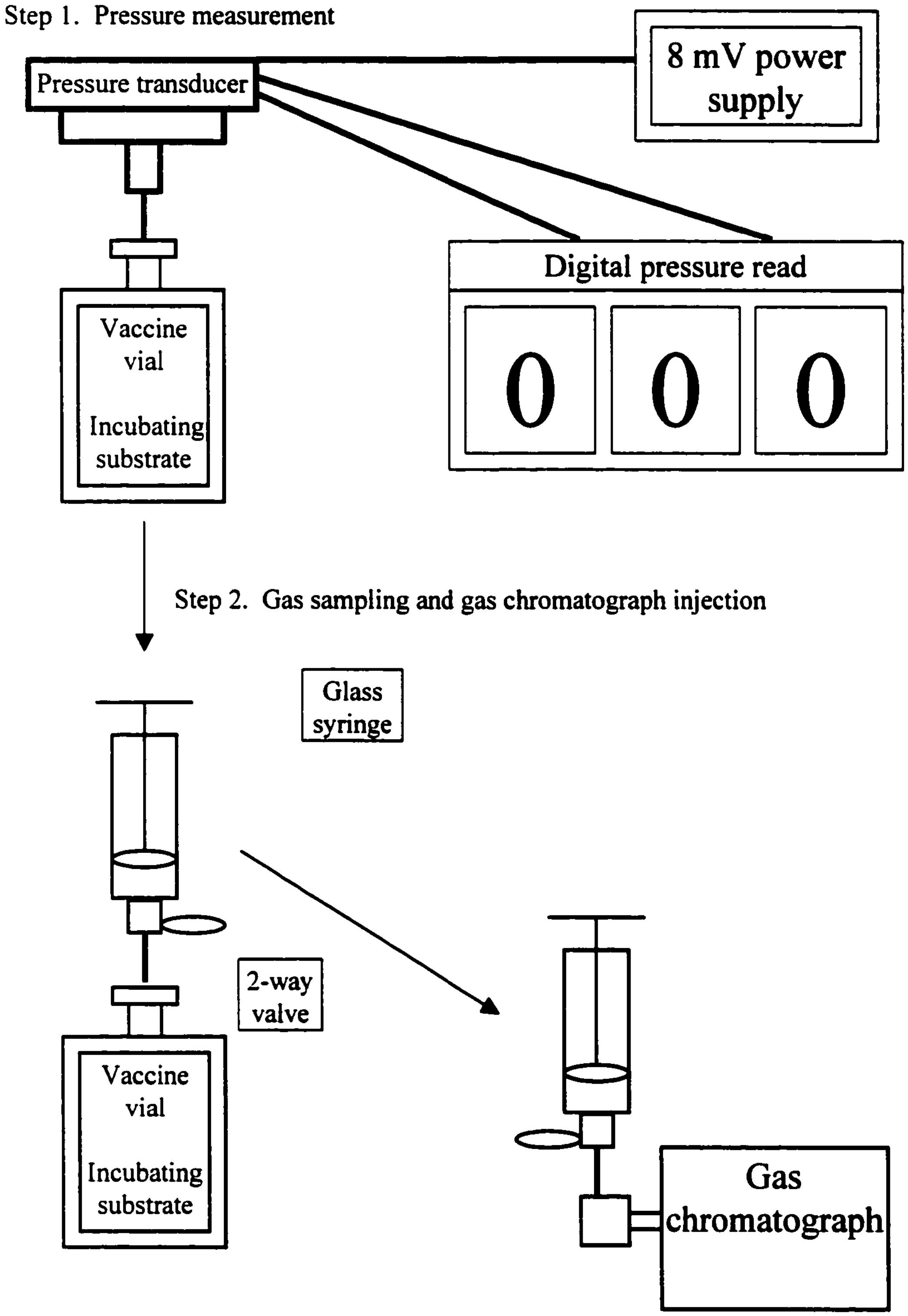
Due to time limitations when analyzing each gas sample for composition with the gas chromatograph, a total of only 40 vials could be incubated during each in vitro run. Four of the 40 vials received buffer and ruminal fluid alone. Gas volume and composition of the four vials were measured to adjust the other 36 vials containing substrate for gas produced by the microorganisms incubated with buffer alone. Of the remaining 36 vials, six vials each were equally divided to evaluate one of six different treatments. At 90 min prior to incubation initiation the buffer and vials with substrate were heated to 39 °C. Thirty min prior to incubation initiation ruminal fluid was collected and handled as previously described. To initiate fermentation, 90 mL of buffer and 10 mL of ruminal fluid were sequentially added with syringes, vials were flushed with carbon dioxide, sealed with septa and aluminum caps, and placed in a water-bath maintained at 39 °C. One vial was inoculated every 3 min to coordinate the time required to analyze each gas sample for composition in the gas chromatograph. Gas pressure and gas composition were measured at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72, and 96 hr after initiating incubation.

Gas pressure. Gas pressure was measured with a P-272 Omega (Stamford, CT) pressure transducer fitted with a 22 gauge, 1.9 cm long hypodermic needle (Figure 2.1). The pressure transducer was excited with an eight mV external power source and the signal read using a DP25-E Omega (Stamford, CT) digital reader. At each designated time, one vial was removed from the water-bath and the needle connected to the transducer was inserted through the septum and the head-space gas pressure measured and recorded (Figure 2.1).

To calculate gas volume from the pressure readings, the following equation was implemented: gas volume $= 0.18 + 3.697P + 0.0824P^2$ (Mauricio et al., 1999). Where P is the gas pressure measured with the pressure transducer. The Mauricio et al. (1999) equation was used instead of Boyle's Gas Law, because the author's found the equation they developed accounted for head-space gases diffusing into the liquid phase, which Boyle's Gas Law did not.

To check the pressure transducer and equation for accuracy, a tightly capped

Figure 2.1. Procedure followed to measure gas pressure and composition



vial was released of its gas contents by inserting a needle through the septum and allowing the gas to equilibrate with atmospheric pressure. The vial was then tared on a balance and filled with a designated amount of room temperature water with a syringe equipped with a hypodermic needle. Once the water was injected, the needle was quickly removed to minimize gas loss and the vial with water weighed (known volume). Weighing 10 mL of water into a vial (known volume) resulted in a mean weight/volume of 10.1 mL and a standard deviation of 0.07. Measuring the same vial using the pressure transducer to determine the volume of water resulted in a mean volume of 10.1 with a standard deviation of 0.8. Linearity of the pressure transducer was checked by injecting increasing known weighed amounts (i.e. 2, 4, 6, 8, 10, and 12 mL) of water and measuring the gas volume using the transducer method as described. Caution should be taken when calibrating the transducer reader, make sure the same amount of liquid is in the vials during calibration as contained in the vials during incubations (~100 mL).

One problem encountered during incubation was the way in which the pressure transducer was calibrated. During calibration head space volume was not the same as in the incubating vials during the experiment, resulting in biased estimates of pressure and subsequent volume. Approximately 40 mL of water was placed in the vials to calibrate the transducer reader, whereas the vial should have contained 100 mL. The researchers estimate the actual gas volumes may be as much as 50% lower than reported, but this is an estimate and should not be taken as absolute.

The pressure transducer implemented measured gas pressure as referenced to the local atmospheric pressure around the transducer, so daily changes in atmospheric pressure can have an effect on the pressure measured. The atmospheric pressure was not recorded but, Mauricio et al. (1999) suggested a minor effect of daily changes in atmospheric pressure on gas pressure. Due to the problems encounter while measuring pressure, the gas volumes should not be considered as absolute, but each vial was consistently handled in the same manner so relative substrate/treatment comparisons within a trial can be made.

Gas composition. Following each pressure measurement, a 2 to 10-mL gas sample was taken from the vial. The gas sample was taken using a 10-mL glass syringe fitted with a two-way valve and equipped with a 22-gauge, 1.9-cm long hypodermic needle (Figure 2.1). The needle was inserted through the rubber septum of the vial, the two-way valve opened and the syringe filled by allowing pressure in the vial to push the syringe plunger out. The two way valve was then closed once adequate gas was collected. During the initial time periods less than 10 mL of gas was produced and as a result less gas was injected into the gas chromatograph, but at least 2 mL of gas was required to obtain a chromatogram. After the gas was sampled, the syringe was removed and the needle was left in the vial septum to allow the remaining gas in the vial to escape. Researchers (Mauricio et al., 1999) deemed it necessary to exhaust the gas after each collection to minimize the chance of accumulated gases causing decreased microbial growth by feedback inhibition or pressure. Once the gas was released, the vial was carefully swirled to mix the contents and placed back in the water-bath. On the days with only one gas measurement, the vials were mixed three times each day.

Following collection of the gas aliquot, the gas was injected into a GC-14A Shimadzu (Kyoto, Japan) gas chromatograph equipped with a Poropak (Framingham, PA) column designated to separate hydrogen, methane, and carbon dioxide (Figure 2.1). The column was originally purchased to analyze hydrogen gas, but following injection of higher concentrated hydrogen, methane, carbon dioxide, and nitrogen to confirm the peaks observed on the chromatograph for the unknown samples, the gas appeared to most similar to nitrogen. The nitrogen was prevalent primarily during the first 20 h of incubation and did not increase after that.

Two vials of each substrate/treatment were removed after 48 hr of incubation and filtered to determine DM digestibility. Residual contents of the vials incubated for 48 hr were filtered with #4 Whatman filter paper with suction and then washed with approximately 30 mL of distilled water. The previously tared filter paper plus residual was then dried at 105 °C, cooled in a dessicator, and weighed to determine DM digestibility. Gas pressure and composition on the 48 hr DM digestibility vials were measured only to 48 h.

Statistics. To analyze gas production over the 96 h of incubation, two parameters were first determined by fitting the data for each vial with the equation: volume = $V_F(1 - \exp(-kt))$ (Schofield et al, 1994). Where V_F is the estimated final volume (similar to extent of digestion) and k is the estimated rate constant. Curve fitting was done using procedure NLIN or non-linear regression of SAS (SAS Inst., Inc., Cary, NC). The curve fitting was performed to determine the final volume (V_F) and rate (k) parameters. Once each of the parameters were determined for each vial, the parameters were then analyzed for treatment differences using procedure GLM of SAS (see Appendix: statistical programs).

Linear, quadratic, and cubic effects of BG, DG, and CO on various dependent variables for the in vitro experiment were sequentially analyzed using procedure GLM of SAS. To sequentially analyze for polynomials, the quadratic effect was considered significant after the linear, whereas the cubic effect was significant after the linear and quadratic.

To analyze in vitro results at discrete times (48 or 96 h), statistics were performed using procedure MIXED of SAS. The model included the independent variable substrate/treatment with vial as the experimental unit. Substrate/Treatment means were considered different when the whole model was significant at the ($P < 0.05$) level. Least square means were used to estimate substrate/treatment means due to missing observations and pdiff was used to determine which substrates/treatments differed.

In vivo experiment. A second experiment was conducted using six steers in a 3 x 3 Latin square experiment to evaluate the effects of 0, 20, and 40% DG on methane production, nutrient digestibility, metabolizability, and energy value. Diets consisted of one of three treatments, the control diet was formulated to represent a 60% concentrate dairy cow diet and the other two treatments consisted of 20% and 40% dried DG (Table 2.6). Distiller's grains replaced part of the corn, alfalfa, whole Cotton, and soybean meal, holding the ratios of each of the basal diet ingredients constant (Table 2.6).

Diets were balanced to meet or exceed NRC (1989) requirements for lactating

Table 2.6. Diet ingredient and chemical composition fed to steers in experiment two, DM basis

Item	0% distiller's grains	20% distiller's grains	40% distiller's grains
Diet composition, %			
Chopped alfalfa	40.1	32.0	23.9
Cracked corn	41.2	33.1	25.0
Distiller's grains	0	19.7	39.3
Whole cotton	9.0	7.1	5.3
Soybean meal	7.9	6.2	4.6
Monosodium Phosphate	.22	.22	.22
Vitamin/mineral supp[a]	1.6	1.6	1.6
Chemical composition, %			
DM	88.0	87.4	86.8
CP	17.5	20.4	23.3
Lipid	5.4	6.3	7.2
NDF	23.5	24.6	25.7
ADF	15.1	15.0	15.0
Lignin	3.2	3.5	3.9
NSC[b]	47.2	42.5	37.8
Ash	6.4	6.2	6.0
GE, Mcal/kg	4.53	4.70	4.88

[a] Supplement supplied: 200 KIU vitamin A, 50 KIU vitamin D, 500 IU vitamin E, 6 g niacin, 1 g Cu SO4, 1 g ZnO, 0.75 g MnO, 12.5 mg EDDI, 4.5 mg Se, 2 g Zinpro 100, 2 oz MgO, and 2 oz yeast•cow^{-1}•d^{-1}.

[b] Non-structural carbohydrate, 100 - (CP + lipid + NDF + Ash).

dairy cows during peak lactation. While diets were not of optimum nutrient balance, holding basal ingredients ratios constant provided less confounding results to evaluate DG effects. The nutrient content of the diets differed primarily in their crude protein and lipid content. As the amount of DG increased in the diet from 0 to 40%, the concentration of crude protein and ether extract increased 2.9 and 0.9 percentage units with each 20 percentage unit increase in DG.

An adequate supply of DG and all other feeds were obtained from local sources prior to trial initiation to help ensure consistency. Distiller's grains were obtained in the dried and ground form to ease handling facilities and to ensure storage life.

Animals. To evaluate the effects of DG in the diets described, seven Red Angus and Red Angus crossbred steers (mean experiment body weight = 282 kg ± 2.6) were purchased from the Colorado State University (CSU) Eastern Research Center located in Akron, CO. Prior to arrival at the metabolic laboratory, steers were vaccinated against IBR, BVD, PI_3, and BRSV, branded, and ear tagged for identification. In addition, the Colorado State University Animal Care and Use Committee approved (protocol #00-007A-01) steer handling for the experiment. Steers were purchased during the month of February, 2000 and delivered to the CSU metabolic laboratory where the experiment was conducted. The steers were vaccinated against clostridium and treated for internal and external parasites (Ivermectin, Merk and Co, Rahway, NJ). Steers were fed their treatment diets twice daily at 0730 and 1400.

During the first 21 days after arriving at the metabolic lab, steers were accustomed to halters, digestion stalls, urine harnesses, and respiration calorimetry chambers. After completion of the animal training period, six of the seven steers were selected based on disposition to be included in the experiment. One steer died during the first period due to bloat and was replaced. His observations during the first period were considered missing.

The experimental design was a replicated Latin square, where two steers were initially randomly assigned to each treatment and treatments switched following each collection period. Each period of the Latin square consisted of a 14-d diet adaptation period followed by a 7-d collection period. Steers were housed in an open roof barn in 4 x 4 m dirt floor pens during diet adaptation. The steers spent 5 d in 1.5 x 2.5 m rubber-matted metabolism stalls and 2 d in calorimetry chambers. Feces and urine were collected during the 5 d the steers spent in the metabolism stalls and the 2 d in the calorimetry chambers.

Prior to each collection period two meals per head per day were weighed into plastic sacks to supply 3 d prior and during the 7-d collection period. Individual feed ingredients were periodically sampled during sacking and frozen at -10 °C in airtight bags until further analysis could be performed.

Digestion. Collection periods consisted of a 7-d total collection of orts, feces and urine. Orts was collected daily prior to the 0730 feeding by removing any uneaten feed from the bunk and on the surrounding floor. Feces accumulated in stainless steel pans placed under grates behind the steers and was collected daily following the morning feeding. Urine was vacuumed directly from nylon pouches suspended beneath the steers, accumulated in glass collection vessels, and was collected once daily before the morning feeding. The urine vessels were pre-acidified daily with 250 mL of 18.5% hydrochloric acid and adjusted to pH 4 or lower before freezing. Following collection, weight of orts, feces, and urine was measured and 10% was removed after homogenization and placed in a -10 °C freezer in air tight bags or the urine in tightly capped jars until further analysis could be performed.

Calorimetry. During the 7-d collection periods, steers were rotated through a two chamber, open-circuit indirect, respiration calorimetry system. Steers spent two consecutive 24 hr periods in the calorimetry chambers to measure carbon dioxide and methane production, and oxygen consumption. Heat production was calculated by the Brouwer (1963) equation based on the gas measurements. The calorimetry system was calibrated during the trial by burning absolute ethanol in the chambers with a mean carbon dioxide recovery of 98 ± 0.9% and a mean oxygen recovery of 96 ± 2.4%.

Chemical analyses. Following each collection period, orts, fecal, and urine samples for each animal were thawed, combined, homogenized, and sub-sampled for analysis. Feces was dried at 60 °C for 48 hr and ground through a Wiley mill equipped with a 2-mm screen. Feed, orts, and feces were analyzed for DM, ash (AOAC, 1990), ether extract (AOAC, 1990) and sequentially analyzed for neutral and acid detergent fiber using the filter bag technique (Komarek, 1993; Ankom Co., Fairport, NY). Feed was sequentially analyzed for lignin following NDF and ADF analysis (Ankom Co., Fairport, NY). Feed, orts, feces, and urine samples were analyzed for nitrogen by the Kjeldahl procedure (AOAC, 1990) and gross energy using an isoperibol bomb calorimeter (Parr Instrument Co., Moline, IL). Before analyzing the urine for gross energy, 15 mL of urine was dried in small plastic bags in a 55 °C oven. The plastic bag containing the dried urine sample was then placed in the calorimeter to determine the GE content of the bag plus the urine. To determine the GE content of the urine alone, the GE content of the bag was subtracted from the total by measuring the GE content of several empty bags.

Many different terms and abbreviations are used to describe various energy terms and as a result, terms used in the text must be described (Table 2.7). Metabolizable energy was determined based on the average urinary energy (UE) measured during the 7-d collection period and a 2-d average methane energy loss measured in respiration calorimetry chambers. Retained energy (RE) was then calculated by subtracting heat energy (HE) from ME. Heat energy was determined indirectly from the average steer gas production during the 2 d respiration calorimetry measures.

Metabolizable energy required for maintenance (MEm) was determined by iteratively solving for the point where HP = ME from regression of the log of heat production versus ME intake (MEI; kcal/kg BW$^{.75}$) using all observations for each treatment (Lofgreen and Garrett, 1968). Efficiency of ME use for maintenance (km) was calculated as the fasting heat production (FHP) divided by MEm. Fasting heat production was assumed to be 77 kcal/kg BW$^{.75-1}$ as suggested by NRC (1996). Net energy for maintenance (NEm, Mcal/kg diet DM) was calculated as the ME of the diet multiplied by the km. Efficiency of ME use for gain (kr) was calculated as

Table 2.7. List of energy terms.

GE	Gross energy, gross energy of a feed/diet determined by bomb calorimetry
GEI	Gross energy intake, total gross energy intake of an animal
$BW^{.75}$	Body weight of the animal raised to the ¾ power,
MBS	Metabolic body size, $BW^{.75}$
HE	Heat energy, energy given off by an animal as heat, determined by the Brouwer equation (1963) based on measurement of O_2 consumption, and CO_2 and CH_4 production in the respiration calorimetry chambers
FE	Fecal energy, energy lost in the feces
UE	Urinary energy, energy lost in the energy
CH_4E	Methane energy, energy lost as methane measured in the respiration calorimetry chambers
ME	Metabolizable energy, GE - (FE + UE + CH_4E)
RE	Retained energy, ME - HE = RE
MEm	Metabolizable energy required for maintenance, calculated using the semilog solution of Lofgreen and Garrett (1968)
FHP	Fasting heat production, assumed to be 77 Kcal/kg $BW^{.75}$ as suggested by NRC, (1996)
km	Partial efficiency of ME use for maintenance, calculated as FHP/MEm
kr	Partial efficiency of ME use for gain, calculated as RE/ME - MEm
NEm	Net energy for maintenance, calculated as ME value of the diet multiplied by the km of the diet
NEg	Net energy for gain, calculated as the ME value of the diet multiplied by the kr of the diet

RE/(MEI - MEm). Net energy for gain (NEg, Mcal/kg DM) was calculated for each diet by multiplying the diet ME by kr.

Statistics. Statistical design and analysis was performed using a replicated 3 x 3 Latin square design experiment. Procedure MIXED of SAS was used to perform the analysis (see Appendix: statistical programs) with animal as the experimental unit. To account for potential level of intake effects, data were analyzed using intake level (kcal gross energy intake/kg $BW^{.75}$ or g nitrogen intake) as a continuous covariate. Treatment means were determined by lsmeans to account for missing observations. Differences among treatment means were considered different when a significant F test ($P < .05$) was detected for the whole model. The complete model contained period, animal, treatment, level of intake, and the interaction of treatment x period. The interaction of treatment and level of intake was also checked, but was not significant.

CHAPTER 3

RESULTS AND DISCUSSION

Substrates. The nutrient content of the three by-product feeds evaluated during the in vitro experiment was found to be similar to NRC (1996) and findings of British researchers, Wainman et al. (1984) and Deaville et al. (1994) (Table 3.1). The lipid and NDF content of the BG in the current experiment were most similar to other samples of American by-products such as reported in NRC (1996), whereas the CP content was most like British by-products (Wainman et al., 1984). The NDF content of BG in the current experiment and other American samples of BG (NRC, 1996) contained nine to ten percentage units less NDF than British BG (Wainman et al., 1984). The CP and lipid content of all three sources of CGF were similar, however, the NDF content was at least 17 percentage units lower for the American by-products than the British. The concentration of CP of the DG in the current experiment was most similar to NRC (1996), whereas DG evaluated by Wainman et al. (1984) contained five to six percentage units more CP. The lipid for all three sources of DG was similar, but the NDF content of the current trial and reported for DG by Wainman et al. (1984) was 12 and 22 percentage units lower, respectively, than reported by NRC, 1996. Differences in nutrient content between the by-product sources can likely be attributed to differences in grain sources and processing procedures.

Although, differences in nutrient content exist between by-product feed

Table 3.1. Comparison of by-product nutrient composition of the current in vitro experiment to British by-products and NRC (1996), DM basis

Feedstuff/nutrient	n	British reports range	British reports mean	NRC, (1996)	Current trial
Brewers[a]	6				
CP, %		19.6 - 27.6	24.5	29.2	22.8
Lipid, %		6.6 - 9.3	7.7	10.8	9.6
NDF, %		49.9 - 64.3	57.2	48.7	47.1
ADF, %		21.6 - 26.6	24.4		17.1
GE, Mcal/kg		5.05 - 5.22	5.144		5.00
Corn gluten feed[b]	1				
CP, %			21.7	23.8	21.4
Lipid, %			4.6	3.91	3.9
NDF, %			56.3	36.2	37.5
ADF, %			11.8		31.0
GE, Mcal/kg			4.51		4.53
Distillers grains[a]	5				
CP, %		30.5 - 43.7	36.0	30.4	31.4
Lipid, %		10.8 - 13.0	11.9	10.7	12.5
NDF, %		23.2 - 44.4	33.8	46	27.8
ADF, %		15.1 - 24.7	19.0		10.5
GE, Mcal/kg		5.33 - 5.65	5.49		5.39

[a] Wainman et al. (1984).

[b] Deaville et al. (1994).

sources, BG and DG whether sourced from the U. S. or Britain, have been shown to decrease methane production of ruminants. Overall, the primary difference between BG and DG by-products and normal methane producing feeds such as alfalfa or CGF was the lipid content (Table 3.2). Brewer's and distiller's grains contained at least 146% more lipid than CGF or alfalfa, similar to previous research by Jarosz (1998), where the relatively high lipid content of BG and DG was the primary constituent with the greatest potential to cause methane production of ruminants to decrease.

In vitro system. Incubation of substrates during the in vitro trials resulted in accumulative gas productions over time (Figures 3.1 and 3.2) similar to those for other feedstuffs as analyzed and reported by Mauricio et al. (1999) and Pell and Schofield (1992). Accumulative gas production from substrates incubated over time formed a sigmoidal to curvilinear shape. Initially, gas production increases at an increasing rate, then increases at a decreasing rate, until beginning to reach a plateau at 72 to 96 hours (Figures 3.1 and 3.2). An attempt was made to describe the relationship between gas production across time using non-linear procedures in SAS by estimating the asymptote (F) and slope (k) according to: $V = F(1-e^{-kt})$. Many of the values determined by non-linear analysis were unrealistic and may be a result of the time period (0 to 96 h) the data was collected. Data were collected at specific time points beginning at time zero and ending at 96 h of incubation. Gas production appeared to keep increasing for many of the treatments past 96 h suggesting gas production did not yet plateau (Figures 3.1 and 3.2). It was not valid to estimate the asymptote occurring past 96 h using data collected from 0 to 96. Thus, the curve analysis estimates (Appendix Tables 6.1 to 6.5) will be discussed only tangentially.

Table 3.2. Chemical composition of substrates incubated during experiment one

	Nutrient, percent DM basis								
Ingredient	DM	CP	Lipid	Ash	NSC[g]	NDF	ADF	Lignin	GE, Mcal/kg
BG	93.7	22.8	9.6	4.4	16.1	47.1	17.1	2.6	5.00
CGF	90.1	21.4	3.9	6.2	37.5	31.0	7.6	0	4.53
DG	87.2	31.4	12.5	6.0	22.3	27.8	10.5	0.57	5.39
Alfalfa	89.3	17.6	1.5	9.1	29.6	42.2	31.1	5.9	4.49
Grass hay	91.7	10.3	4.3	6.9	12.0	66.5	35.4	1.8	4.54
Yeast[d]	90.4	13.5	4.2	15.9	37.2	29.2	11.1	1.2	3.99
$BG_{w/olipid}$[e]	98.0	25.2	0	4.9	17.8	52.1	18.9		4.65
$DG_{w/olipid}$[e]	97.9	35.9	0	6.9	25.5	31.8	12.0		4.69
Light beer[f]	1.4	4.2	0	2.1	27.0	0	0		5.83

[a] Brewer's grains.

[b] Corn gluten feed.

[c] Distiller's grains.

[d] Contained yeast culture (Saccharomyces cerevisiae), rice hulls, calcium carbonate, dried Enterococcus faecium fermentation product, dried Lactobacillus acidophilus fermentation product, dried Aspergillus oryzae fermentation product, dried Bacillus subtillis fermentation product (Conklin Company Inc., Shadopee, MN 55379).

[e] Brewer's or distiller's grains without lipid. All values except dry matter and gross energy determined by calculating by difference from values of the normal by-products assuming 100% of the lipid was removed.

[f] All values except dry matter were reported from USDA (1999).

[g] Non-structural carbohydrate = 100 - (%CP + %EE + %NDF + %Ash).

Figure 3.1. Effect of brewer's grains (BG) concentration when replacing alfalfa on accumulative gas production

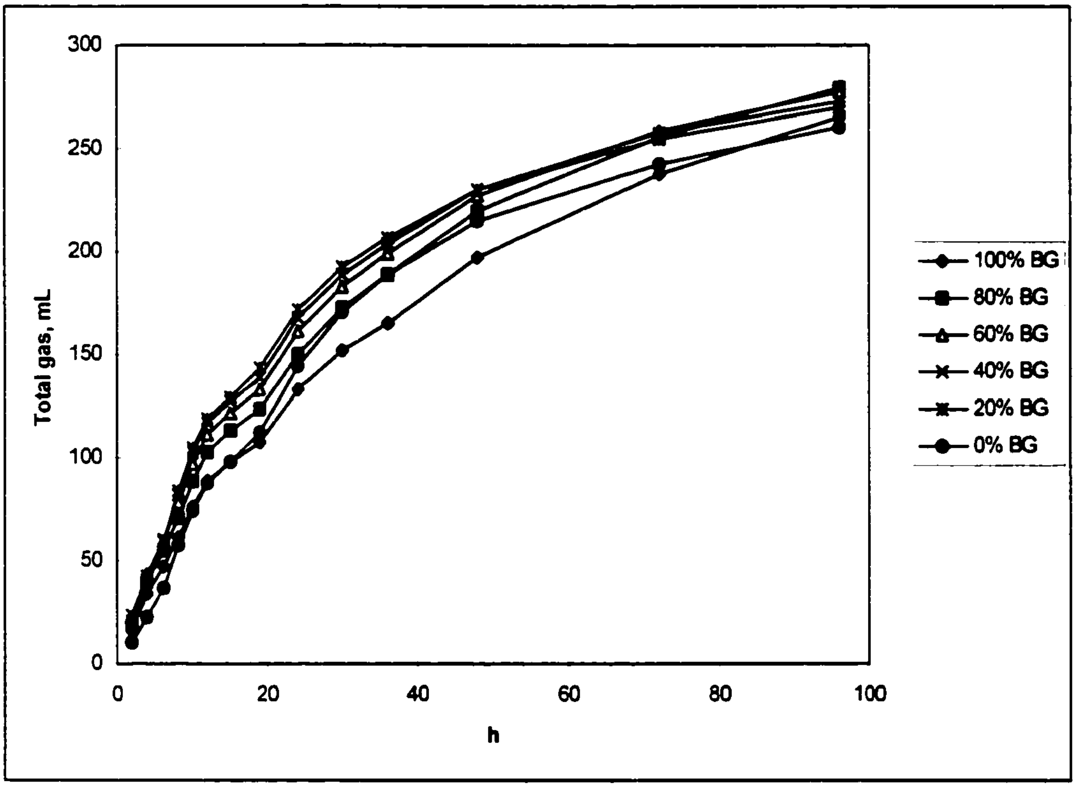
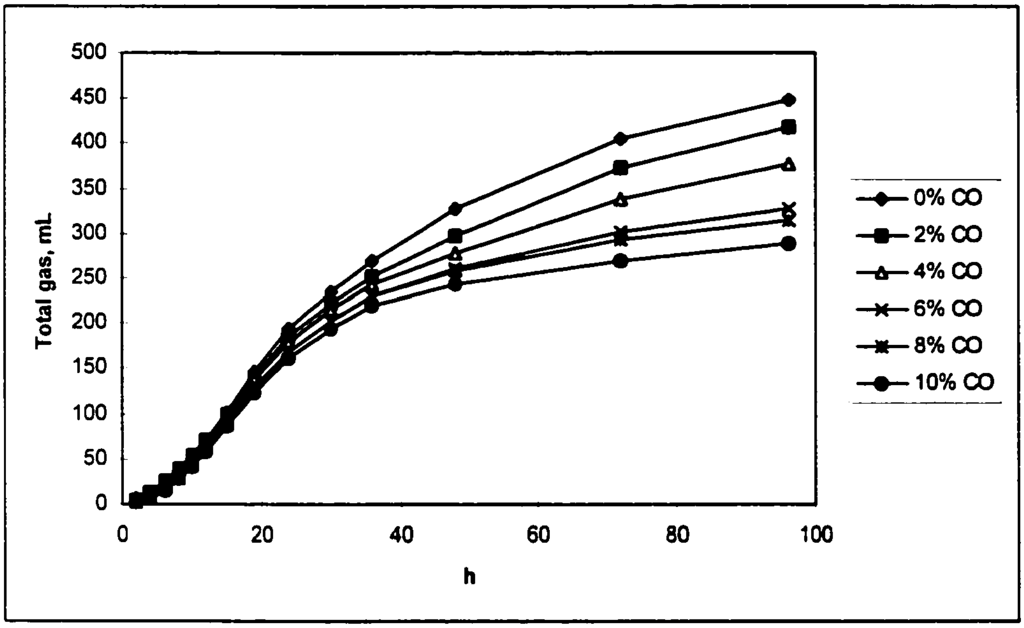


Figure 3.2. Effect of corn oil (CO) concentration when replacing corn gluten feed on accumulative gas production



At 48 h, gas composition for all five in vitro trials averaged 196 mL of carbon dioxide, 29 mL of methane, and 42 mL of a unique and unexpected peak, which was assumed to be nitrogen. During the first two trials approximately 60 mL of nitrogen (Figure 3.3) were released, while during the last three trials gas patterns were similar, but nitrogen release decreased to approximately 30 mL (Figure 3.4). Although uncertain, authors of the current experiment assumed the unique gas generated during incubation was nitrogen, since the peak generated during analysis of a sample of unknown gas most closely matched a sample with known nitrogen. The origin of the nitrogen is puzzling and was most likely residual atmospheric nitrogen gas remaining in solution of the vials that was not removed during flushing with carbon dioxide prior to incubation. Nitrogen release during the first hours of incubation may result in biased estimates of DM digestibility, depending on how well the vials are flushed with carbon dioxide and if the nitrogen source was in fact residual atmospheric nitrogen. Unfortunately, the duration and/or vigor of flushing with carbon dioxide was not recorded.

The following discussion of in vitro results will focus on gas production, gas composition, and digestibility of the substrates incubated during in vitro trials one through five measured at 48 h of incubation. Forty-eight hour digestibilities were measured (Tilley and Terry, 1963) and to coordinate gas analysis with digestibility, gas production and composition were analyzed at 48 h. Forty-eight hours was also similar to the rumen mean retention time of 32 h as reported by Torrent et al. (1994), for one of the by-products, BG, and is an incubation time commonly used during in vitro analysis (Tilley and Terry, 1963).

Figure 3.3. Average nitrogen (N_2), carbon dioxide (CO_2), and methane (CH_4) composition of head-space gas, while incubating brewer's grains substrates during trial one

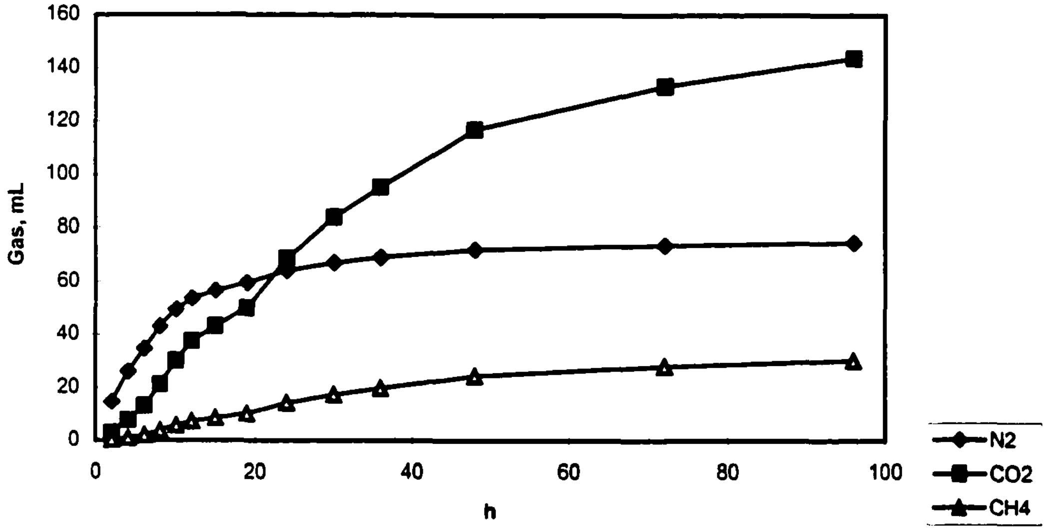
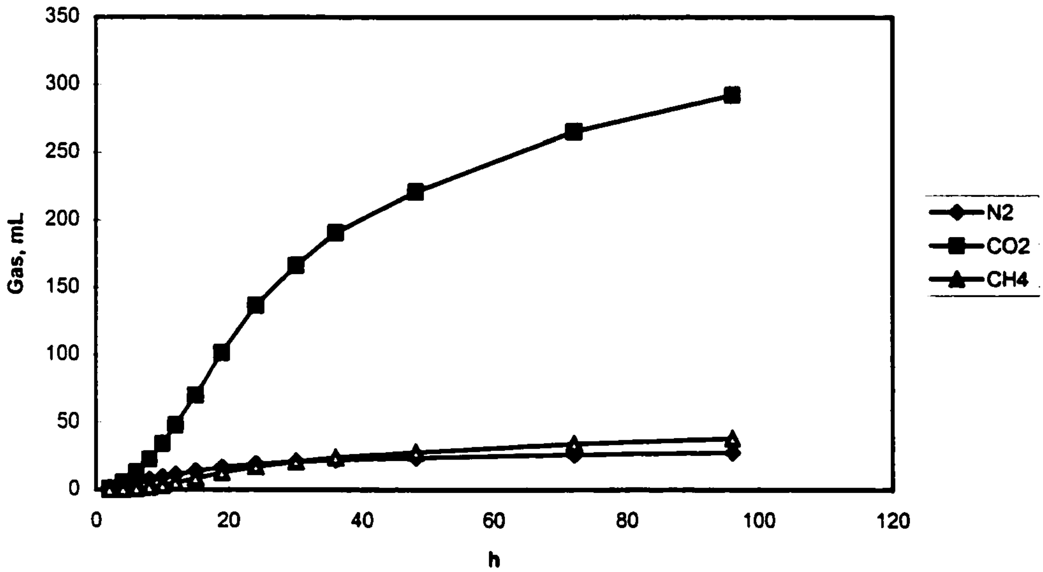


Figure 3.4. Average nitrogen (N_2), carbon dioxide (CO_2), and methane (CH_4) composition of head-space gas, while incubating corn oil substrates during trial three



Along with 48 h measures, gas production and composition were analyzed at 96 h of incubation (Appendix tables 6.6 to 6.10), but were not discussed due to reasons previously mentioned. Polynomial analysis and individual treatment comparisons at 96 h did not always mimic results found at 48 h, possibly a result of changes in fermentation patterns from 48 to 96 h of incubation.

In vitro trial one. At the beginning of this trial researchers inoculated one extra 0% BG vial and one less vial containing 20% BG than planned. To account for the unbalanced number of observations, lsmeans were used for the statistical analysis.

The pH was checked during trial one and was found to measure 6.8 at 0 h and 6.4 at 96 h. Accumulative gas production over 48 h of incubation finds a quadratic effect due to BG concentration ($P < 0.01$; Table 3.3), where gas production was greater for mixtures of BG and alfalfa than when the two feeds were incubated alone. Dry matter digestibility followed a similar curvilinear (quadratic; $P < 0.01$) pattern with increasing concentration of BG. The quadratic increases in DM digestibility and related gas production by incubating BG with alfalfa indicates positive associative effects between the two feeds. The reason for the associative effects are difficult to explain. The curvilinear increase and then decrease in gas production due to BG concentration was not a result of one particular gas, but due to a combined quadratic ($P < 0.01$) effect of all three gases (carbon dioxide, methane, and nitrogen) (Table 3.3). These data provide additional evidence for associative effects between the BG and alfalfa and appears to be consistent for each gas.

Total accumulative gas production is becoming more widely accepted as a measure of DM or other nutrient digestibility and was previously found to correlate

Table 3.3. Effect of brewer's grains concentration on total gas production, gas composition, and digestibility at 48 h of incubation

	Percent brewer's grains replacing alfalfa							P ≤		
Item	0	20	40	60	80	100	SE	L[a]	Q[b]	C[c]
n	7	5	6	6	6	6				
Gas, mL										
Total	201	227	229	226	213	196	5.4	0.05	0.01	0.49
N[d]	66.2	73.9	76.5	77.5	72.6	68.1	0.92	0.46	0.01	0.40
CO_2[e]	107	125	126	124	119	110	3.7	0.73	0.01	0.46
CH_4[f]	28.1	29.1	26.2	24.5	21.2	17.5	1.1	0.01	0.01	0.93
DMD, %[g]	38.2	40.4	37.6	37.6	34.8	25.2	1.8	0.01	0.01	0.40

[a] Linear effect.

[b] Quadratic effect.

[c] Cubic effect.

[d] Nitrogen

[e] Carbon dioxide

[f] Methane

[g] Dry matter digestibility. Two observations for each treatment.

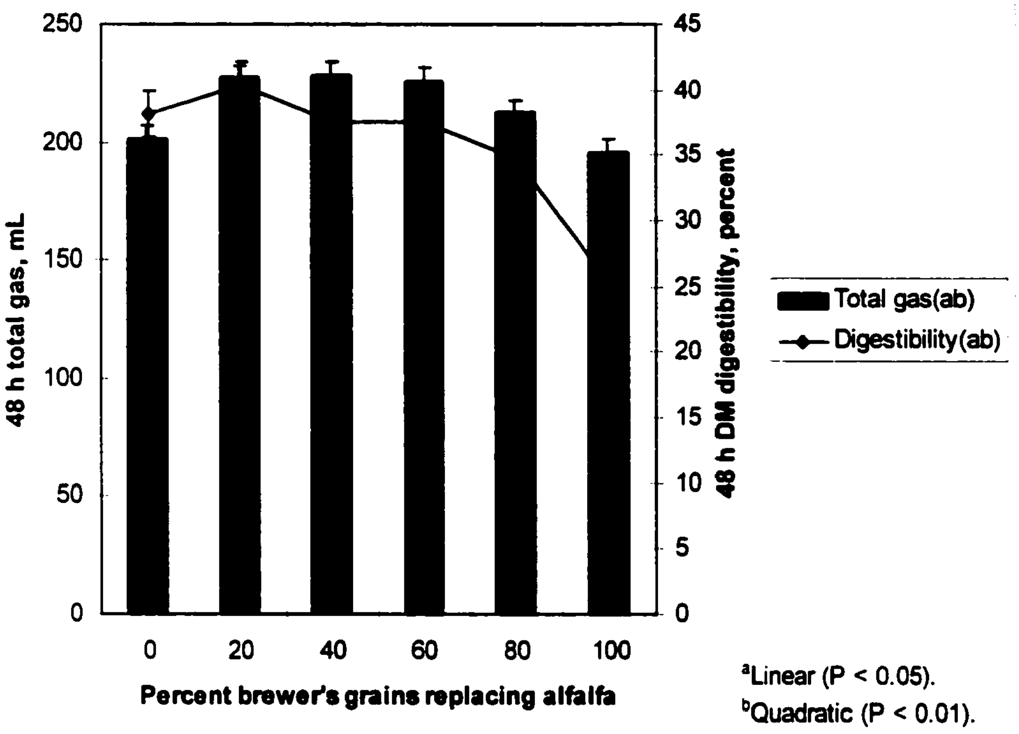
($r^2 = 0.99$) with DM digestibility (Pell and Schofield, 1992). During the current trial BG concentration had a similar curvilinear effect on total gas production and DM digestibility, suggesting gas production changed in a similar manner as digestibility (Table 3.3; Figure 3.5)

The 48-h in vitro DM digestibility (Table 3.3) of the 100% BG substrate alone was 25.2%, which was at least 27% lower (linear; $P < 0.01$) than all the other substrates incubated. Dry matter digestibility of the 80% BG substrate was 34.8% during the in vitro measurements. These in vitro digestibilities compare to 62 and 59.3% measured in vivo for diets containing 100 and 80% BG, respectively (Jarosz, 1998).

Potential explanations for the difference between the in vivo and in vitro digestibilities may be due to methods used to measure digestibility in vitro versus in vivo. Digestibility at 48 h was lower when measured in vitro than in vivo and may have been lower due to the in vitro only simulating ruminal digestibility. Whereas in vivo measures would take into account digestibility occurring in the lower gastrointestinal tract along with ruminal digestibility resulting in higher total digestibility values.

A second possible reason for differences in vivo versus in vitro may be due to lipid digestibility. The relatively high lipid concentrations incubated as substrate had the potential to bias the digestibility values due in part to low lipid digestibility during in vitro incubation and methods implemented to filter the residual DM. Dry matter digestibility was adjusted for lipid, and the resulting values measured in vitro become 31.3 and 20.5 for the 80 and 100% BG substrates, which were lower still than in vivo.

Figure 3.5. Effect of brewer's grains concentration on total gas production as compared to DM digestibility at 48 h of incubation



^{a}Linear ($P < 0.05$).
^{b}Quadratic ($P < 0.01$).

The third and final reason for differences in vitro versus in vivo may be due to microbial population differences in the ruminal fluid between in vitro and in vivo measures. The cannulated heifers providing ruminal fluid for the in vitro measures were fed alfalfa, whereas the steers used for in vivo measures were fed the actual diets containing BG. Due to differences in diets, the microbial population may have been different between the in vivo and in vitro experiments and caused DM digestibility to differ.

When examining methane production at 48 h, a quadratic effect ($P < 0.01$) was found on methane production with increasing BG concentration (Table 3.4). Appearance of a linear decrease (Figure 3.6) in methane production with increasing BG concentration from 20 to 100% BG lead researchers to omit the 0% BG from the analysis. Once the 0% BG substrate was omitted, a linear ($P < 0.01$) decrease in methane production with increasing BG concentration was found. Methane production (percent of non-lipid GE) decreased 0.4% with every one percentage unit addition of BG to the substrate replacing alfalfa when substrates contained 20 to 100% BG (Table 3.4; Figure 3.6).

In vitro trial two. The significance of the cubic effect suggests the polynomial does not adequately provide information describing the effect DG concentration has on total gas production or DM digestibility. Since the polynomial did not informatively describe the data, individual treatment comparisons were conducted by mean separation to obtain more relevant descriptions. Total gas production was greater ($P < 0.01$) for the substrates containing 40 to 80% DG than the 0, 20, or 100% DG substrates with the 100% DG substrate lower ($P < 0.01$) than all other substrates

Table 3.4. Effect of brewer's grains concentration on methane production at 48 h of incubation

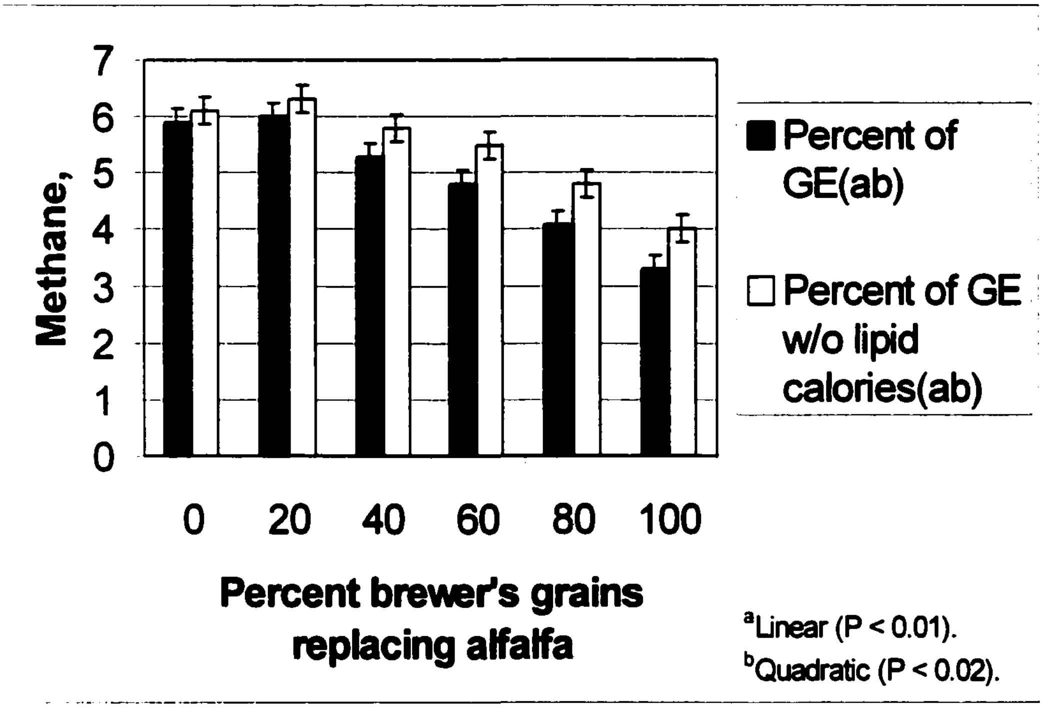
	% brewer's grains replacing alfalfa							P ≤		
Methane	0	20	40	60	80	100	SE	L[a]	Q[b]	C[c]
n	7	5	6	6	6	6				
Calories	266	275	247	232	201	165	10.3	0.01	0.02	NS
% of GE	5.9	6.0	5.3	4.8	4.1	3.3	0.23	0.01	0.05	NS
% of GE w/o lipid	6.1	6.3	5.8	5.5	4.8	4.0	0.24	0.01	0.01	NS

[a] Linear effect.

[b] Quadratic effect.

[c] Cubic effect.

Figure 3.6. Effect of brewer's grains concentration on methane production at 48 h of incubation



(Table 3.5). Although gas production differed, DM digestibility did not when analyzed using mean separation, most likely a result of a low number of observations for digestibility (Figure 3.7). Similar to BG, substrates containing combinations of DG and alfalfa produced greater amounts of gas than when the two feeds were incubated alone suggesting associative effects.

Dry matter digestibility values measured in vitro for the substrates containing 80% and 100% DG were 41.4 and 35.9%, respectively (Table 3.5). Results from a prior experiment suggest digestibilities for diets with similar ingredient composition measured when fed to steers were 70.4 and 73.1% (Jarosz, 1998). Again, digestibility measured in vitro was lower than in vivo. The same reasons (in vitro versus in vivo, lipid digestibility, and ruminal fluid conditions) for the discrepancy between the two methods discussed for BG apply to the DG substrates.

Similar to gas production and DM digestibility, the significance of the cubic effect of DG concentration on methane production suggests the polynomial is not an informative means to describe the data. To better characterize the effects of DG on methane production, individual treatment comparisons were conducted. Methane production (percent of non-lipid GE) was greater ($P < 0.01$) for substrates containing 60 and 80% DG than 0% DG. On the contrary, methane production was lower ($P < 0.01$) for the 100% DG substrate than all other substrates incubated during trial two. These data provide evidence that methane production is not decreased in vitro until 100% of the substrate is DG (Table 3.6; Figure 3.8).

Wainman et al. (1985) found when feeding sheep increasing concentrations of wet barley distiller's grains replacing barley flour, methane (percent of GE)

Table 3.5. Effect of distiller's grains on total gas production, gas composition, and digestibility at 48 h of incubation

	Percent distiller's grains replacing alfalfa							P ≤		
Item	0	20	40	60	80	100	SE	L^{a}	Q^{b}	C^{c}
N	6	6	6	6	6	6				
Gas, mL										
Total	271^{h}	280^{hi}	284^{i}	288^{i}	281^{i}	251^{j}	3.3	0.01	0.01	0.01
N^{d}	72.7	69.9	75.1	74.6	74.1	69.1	1.7	0.68	0.04	0.05
CO_2^{e}	163^{h}	163^{h}	173^{hij}	178^{ij}	176^{j}	145^{k}	3.7	0.15	0.01	0.01
CH_4^{f}	30.7^{h}	30.6^{h}	31.8^{h}	32.2^{h}	31.2^{h}	25.0^{i}	0.59	0.01	0.01	0.01
DMD, %^{g}	39.1	38.7	41.2	38.4	41.4	35.9	3.3	0.01	0.01	0.01

[a] Linear effect.

[b] Quadratic effect.

[c] Cubic effect.

[d] Nitrogen

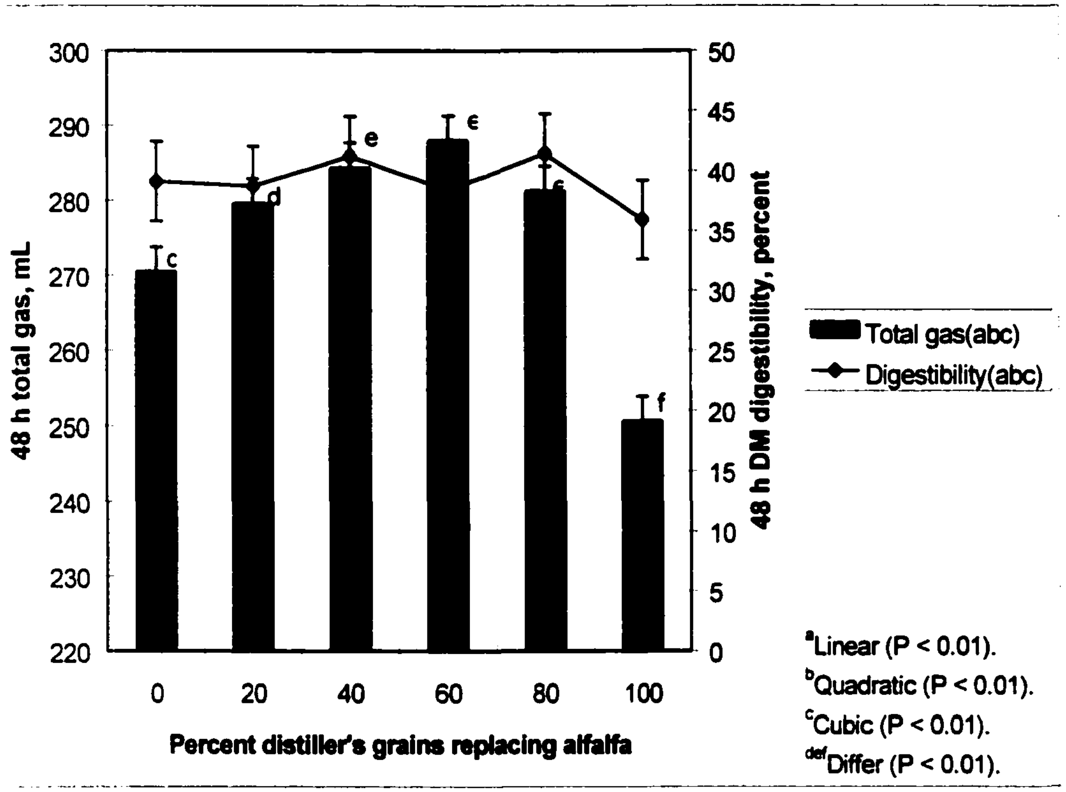
[e] Carbon dioxide

[f] Methane

[g] Dry matter digestibility. Two observations for each treatment.

[hijk] Means with unlike superscripts differ $P < 0.01$.

Figure 3.7. Effect of distiller's grains on total gas production as compared to DM digestibility at 48 h of incubation



^{a}Linear ($P < 0.01$).
^{b}Quadratic ($P < 0.01$).
^{c}Cubic ($P < 0.01$).
^{def}Differ ($P < 0.01$).

Table 3.6. Effect of distiller's grains concentration on methane production at 48 h of incubation

	Percent distiller's grains replacing alfalfa							P ≤		
Methane	0	20	40	60	80	100	SE	L[a]	Q[b]	C[c]
n	6	6	6	6	6	6				
Calories	291^{d}	290^{d}	301^{d}	305^{d}	295^{d}	236^{e}	5.6	0.01	0.01	0.01
% of GE	6.5^{d}	6.2^{d}	6.2^{de}	6.1^{ef}	5.7^{df}	4.4^{g}	0.11	0.01	0.01	0.01
% of GE w/o lipid	6.7^{d}	6.7^{d}	7.0^{de}	7.1^{ef}	7.0^{df}	5.6^{g}	0.13	0.01	0.01	0.01

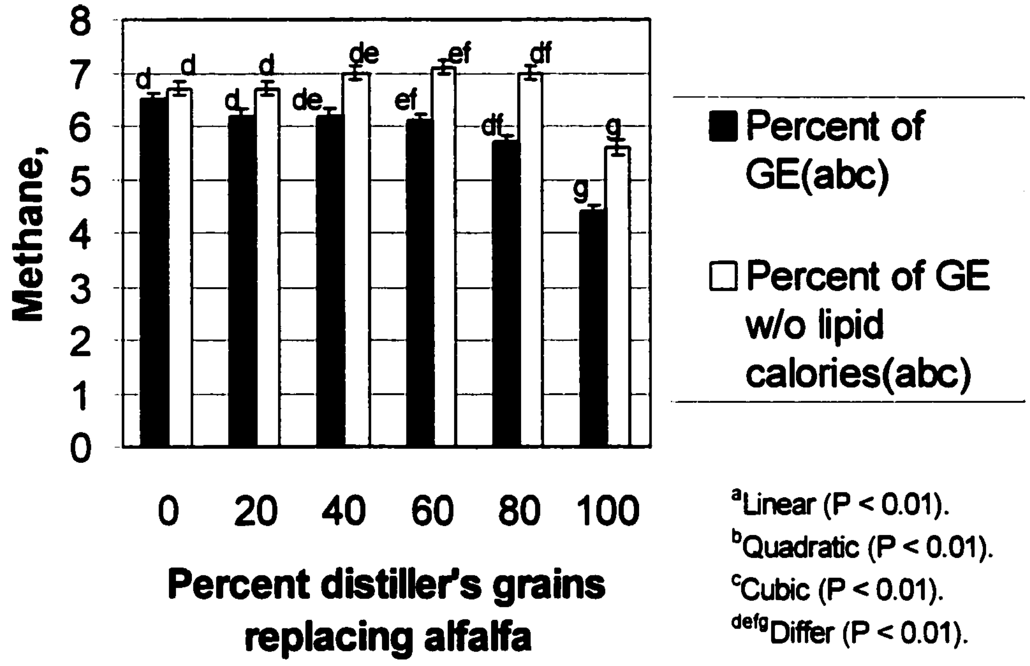
[a] Linear effect.

[b] Quadratic effect.

[c] Cubic effect.

[defg] Means with unlike superscripts differ $P < 0.01$.

Figure 3.8. Effect of distiller's grains concentration on methane production at 48 h of incubation



[a]Linear ($P < 0.01$).
[b]Quadratic ($P < 0.01$).
[c]Cubic ($P < 0.01$).
[defg]Differ ($P < 0.01$).

production was decreased from approximately 15 to 4.5% when 55% or more of the diet was wet distiller's grains. The discrepancy between the in vitro and in vivo (Wainman et al., 1985) experiments may be due to differences in microbial populations or activity in the ruminal fluid. Since the ruminal fluid donor heifers were fed alfalfa and not DG, the microbial population fed alfalfa may have metabolized the DG in a different manner than if the heifers were fed DG. A second possible reason for the discrepancy was differences between the dry DG evaluated in vitro versus the wet DG in vivo. Wet versus dry by-products may not always be digested or metabolized in the same manner as suggested by Ham et al. (1995) who found greater NEg values for wet than dry DG. Another possible cause for the discrepancy is that Wainman et al. (1995) fed barley flour instead of alfalfa with the DG and may have different effects on aspects of fermentation.

In vitro trial three. Total gas production over 48 h of incubation linearly decreased ($P < 0.01$) with increasing addition of corn oil (CO) replacing CGF (Table 3.7). The decrease in total gas production reflects a decrease in both carbon dioxide and methane (mL) with increasing CO and is most likely a result of detrimental effects of CO on digestibility (Table 3.7; Figure 3.9). Further evaluation of the results indicate increasing concentration of CO added to CGF, linearly decreased ($P < 0.01$) DM digestibility (Table 3.7), in a similar manner as gas production (Figure 3.9). The decrease in DM digestibility is most likely caused by the effect of lipid on digestibility and specifically fiber digestibility.

Jenkins (1993) suggested ruminal structural carbohydrate digestion can be decreased 50% when less than 10% lipid is added to a ruminant diet. Other

Table 3.7. Effect of corn oil concentration on gas production, gas composition, and digestibility at 48 h of incubation

	Percent corn oil replacing corn gluten feed							P ≤		
Item	0	2	4	6	8	10	SE	L[a]	Q[b]	C[c]
n	6	6	6	6	6	6				
Gas, mL										
Total	324^{h}	294^{i}	279^{j}	263^{kl}	257^{l}	242^{m}	3.7	0.01	0.01	0.07
N[d]	28.1	28.6	26.5	19.0	22.1	23.4	7.2	0.40	0.72	0.59
CO_2[e]	249	236	223	217	209	188	9.7	0.01	0.85	0.48
CH_4[f]	31.3	29.6	28.2	26.8	26.3	24.0	0.84	0.01	0.99	0.50
DMD, %[g]	65.9^{h}	57.5^{ijk}	57.4^{jk}	55.2^{k}	48.9^{lm}	50.1^{m}	1.0	0.01	0.01	0.01

[a] Linear effect.

[b] Quadratic effect.

[c] Cubic effect.

[d] Nitrogen

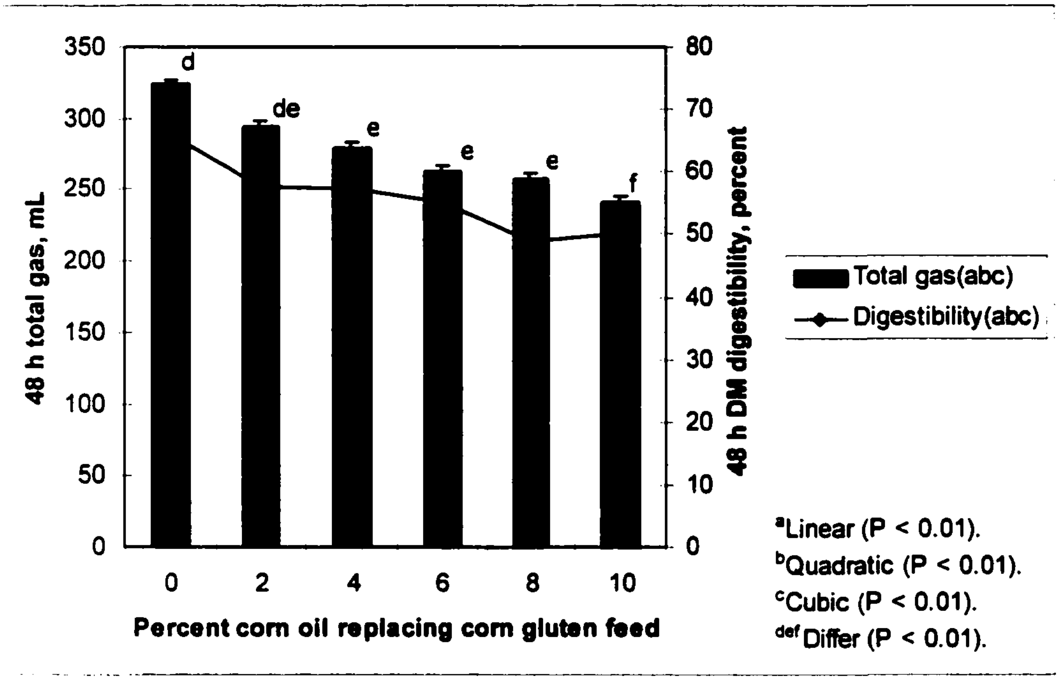
[e] Carbon dioxide

[f] Methane

[g] Dry matter digestibility. Two observations for each treatment.

[hijklm] Means with unlike superscripts differ $P < 0.01$.

Figure 3.9. Effect of corn oil concentration on gas production as compared to DM digestibility at 48 h of incubation



researchers such as Lewis et al. (1998) also reported lipid depressed ruminal fiber digestion and Zinn (1988) reported total tract OM digestion decreased when steers were fed 4% yellow grease as part of a high grain feedlot diet. Jenkins (1993) proposed the two most likely reasons for the decrease in fiber digestion are: the lipid may coat the fiber particles decreasing cellulase contact with fiber or disruption of the cellular membranes of fiber digesting microorganisms may result in microbial death.

Not only did treatment comparisons for total gas production and DM digestibility differ, but DM digestibility of the CGF was lower than expected. Jarosz, 1998 estimated by difference in vivo DM digestibility of CGF to be 72%, which is approximately 9% greater than measured in vitro in the current trial. The steers, in vivo, were fed the CGF diets at least 14 d allowing time for the ruminal microbes to adapt, whereas the microbes from the heifers fed alfalfa were not adapted to the CGF substrate. Since the microbes were possibly not adequately adapted, DM digestibility may be lower than expected for the 100% CGF substrate.

Methane production at 48 h was linearly decreased ($P < 0.01$) with increasing concentration of CO for each method of expressing methane (Table 3.8). Methane production expressed as a percent of GE decreased at a faster rate with increasing CO concentration than when methane production is expressed as a percent of GE without lipid calories (Table 3.8; Figure 3.10). The discrepancy between the two ways of expressing methane production as a percent of GE was most likely due to a dilution effect when dividing the calories of

Table 3.8. Effect of corn oil concentration on methane production at 48 h of incubation

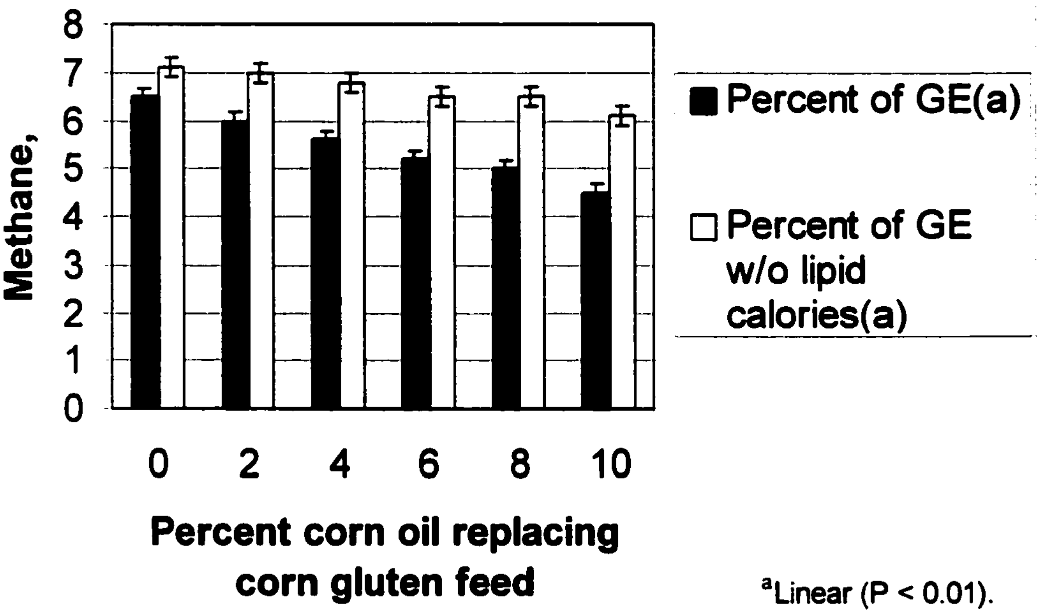
	% corn oil replacing corn gluten feed							P ≤		
Methane	0	2	4	6	8	10	SE	L[a]	Q[b]	C[c]
n	6	6	6	6	6	6				
Calories	295	280	267	254	249	227	7.9	0.01	0.99	NS
% of GE	6.5	6.0	5.6	5.2	5.0	4.5	0.17	0.01	0.63	NS
% of GE w/o lipid	7.1	7.0	6.8	6.5	6.5	6.1	0.20	0.01	0.74	NS

[a] Linear effect.

[b] Quadratic effect.

[c] Cubic effect.

Figure 3.10. Effect of corn oil concentration on methane production at 48 h of incubation



^{a}Linear ($P < 0.01$).

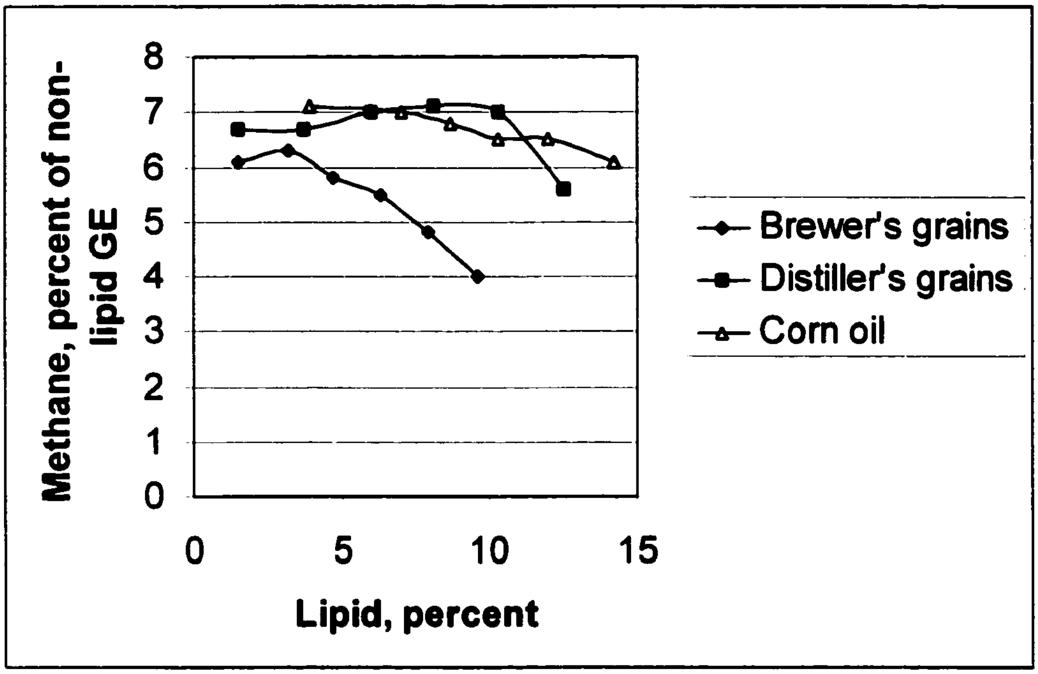
methane by the higher calories of GE when the lipid was included.

Methane production (percent of non-lipid GE) was decreased 0.07% with each percentage unit addition of CO replacing CGF. Although methane production when expressed as a percent of GE without lipid calories did not decrease as rapidly with CO addition as when BG were incubated, CO still caused a linear decrease in methane (Figure 3.11). Jarosz (1998) speculated the relatively high lipid content of BG and DG was one of the factors potentially decreasing methane production of ruminants fed BG or DG. The similar trends for BG and CO substrates provide some evidence that the lipid may play a role with decreasing methane, but is not substantial. A linear decrease in methane with increasing DG relationship was not measured for DG substrates as with BG and CO substrates. Lack of methane suppression when incubating DG in vitro may be

a result of using in vitro techniques instead of in vivo where a dramatic decrease in methane production was measured when sheep or steers were fed DG.

Although, it is still not clear whether or not the lipid of the BG or DG is causing methane production of ruminants fed BG or DG to decrease, the lipid seems to be the most logical constituent. Several potential mechanisms by which lipid can decrease methane have been noted. Johnson and Johnson (1995) suggest lipid may decrease methane production by way of its effects on ruminal protozoa and ruminal hydrogen availability. Protozoa produce high levels of hydrogen, the hydrogen is taken up by the methanogen producing methane. If hydrogen production was decreased by lowering protozoal activity or their numbers, the methanogens have less substrate to produce methane. Additionally, unsaturated lipid can act as an alternative

Figure 3.11. Effect of ether extract concentration of brewer's, distiller's, and corn oil substrates incubated on methane production



hydrogen sink in the rumen, so instead of the hydrogen going to methane production it is used to saturate double bonds.

Examining the effects of lipid on volatile fatty acid concentrations and acetate:propionate ratio may help explain why lipid, BG, and/or DG have methane decreasing properties. Supplemental lipid included in ruminant diets has varied effects on the ruminal acetate:propionate ratios, especially when the concentration of forage in the diet varied. Zinn and Plascencia (1996) fed 6% yellow grease to cattle fed a 90% concentrate feedlot diet with 10% alfalfa and found the acetate:propionate ratio increased, but when 6% lipid was added to a 70% concentrate feedlot diet with 30% alfalfa, the acetate:propionate ratio decreased. Even with the increased acetate:propionate ratio with lipid supplementation and low forage (10%), no effect on estimated methane production was measured. On the contrary, estimated methane production was decreased ($P < 0.05$) 15% when cattle were fed the added fat with the 70% concentrate diet. The data suggest lipid added to ruminant diets, has the largest effect on methane production when lipid is added to diets with 30% forage or more.

Lipid appears to decrease methane production of ruminants fed high forage diets and BG and DG when fed as a major portion of the diet would be considered a high forage or fiber diet with relatively high lipid content. The change in acetate:propionate ratio of ruminants fed BG or DG as a portion of a high fiber diet has not been evaluated, but it has been evaluated when DG were added to a high grain diet. Researchers (Peter et al., 2000 and Ham et al., 1995) found by replacing 20 to 40% of the grain in a high grain feedlot diet with DG, in most cases the concentration of acetate increased and propionate decreased. These results would suggest ruminants fed DG as a portion of a high grain diet would produce greater amounts of methane due to the greater acetate concentration. Ruminants fed BG or DG replacing a portion of another high fiber feed such as alfalfa may have a different effect on methane, since ruminal acetate concentration would already be expected to be relatively high. By adding the BG or DG to the diet, a relatively high lipid source is also added, potentially causing the concentration of acetate to decrease resulting in less methane production.

In vitro trial four. Following 48 h of incubation, gas production did not differ between the three BG substrates, but total gas production was greater ($P < 0.01$) for the 100% DG substrate than the two DG without lipid substrates (Table 3.9). Similar to gas production, DM digestibility did not significantly differ between the BG substrates, although digestibility was numerically lower for the 100% BG than the two BG without lipid substrates (Table 3.9; Figure 3.12). Gas production was also low for the 100% BG substrate, but did not decrease as drastically as DM digestibility when compared to the BG without lipid (Figure 3.12). Dry matter digestibility did not differ due to DG substrate (Table 3.9), but numeric differences were observed and the relationships between the DG substrates were different than those for gas production (Figure 3.12).

Expressing methane release as a percent of GE, suggests methane release was lower ($P < 0.01$) for substrates containing BG with lipid than the BG without lipid substrates incubated with or without alfalfa (Table 3.10; Figure 3.13). Methane (Percent of GE) release was lower ($P < 0.01$) for the 100% DG and 80% DG without lipid than the 100% DG without lipid substrate (Table 3.10; Figure 3.13). When

Table 3.9. Effect of brewer's grains (BG) or distiller's grains (DG) with and without lipid on total gas production, gas composition, and digestibility at 48 h of incubation

Item	100% BG	100% BG w/o lipid	80% BG w/o lipid	100% DG	100% DG w/o lipid	80% DG w/o lipid	SE
n	6	6	6	6	6	6	
Gas, mL							
Total	243^{e}	241^{e}	250^{ef}	301^{g}	257^{ef}	269^{f}	8.5
N^{a}	15.2^{ehij}	18.2^{fgklm}	19.2^{g}	16.9^{gkm}	16.4^{ikl}	17.5^{jm}	0.86
CO_2^{b}	204^{e}	197^{e}	203^{e}	252^{f}	210^{e}	220^{e}	8.1
CH_4^{c}	23.6^{e}	26.2^{fg}	27.6^{g}	32.8^{hj}	29.8^{i}	32.5^{j}	0.59
DMD, %^{d}	23.5	33.7	35.4	32.2	40.9	37.5	3.6

[a] Nitrogen

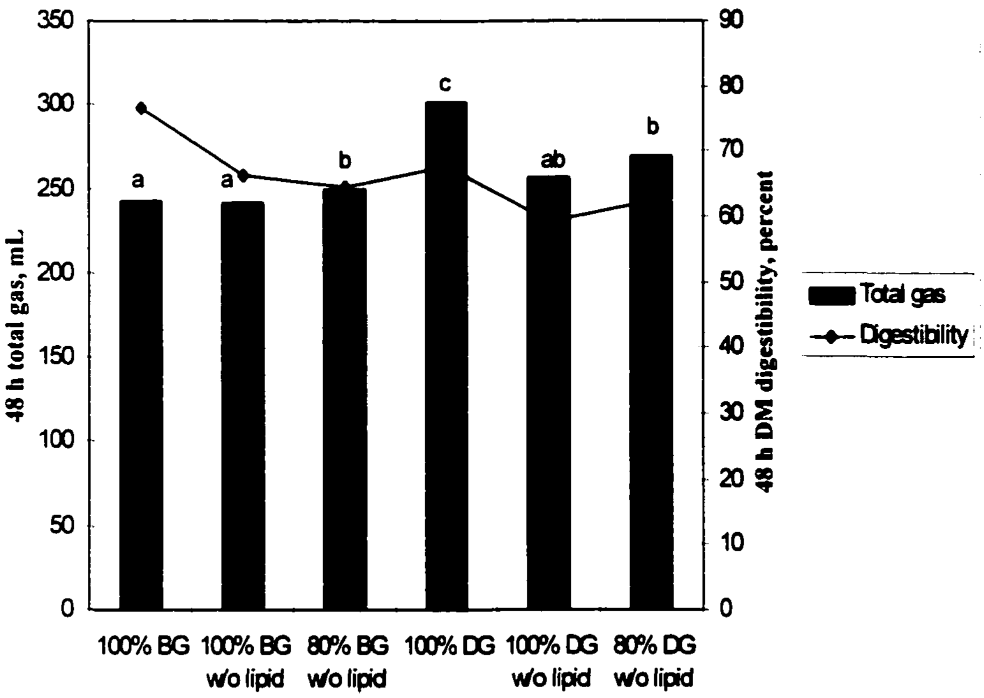
[b] Carbon dioxide

[c] Methane

[d] Dry matter digestibility. Two observations for each treatment.

[efghijklm] Means with unlike superscripts differ ($P < 0.01$).

Figure 3.12. Effect of brewer's grains (BG) or distiller's grains (DG) with and without lipid on total gas production, gas composition, and digestibility at 48 h of incubation



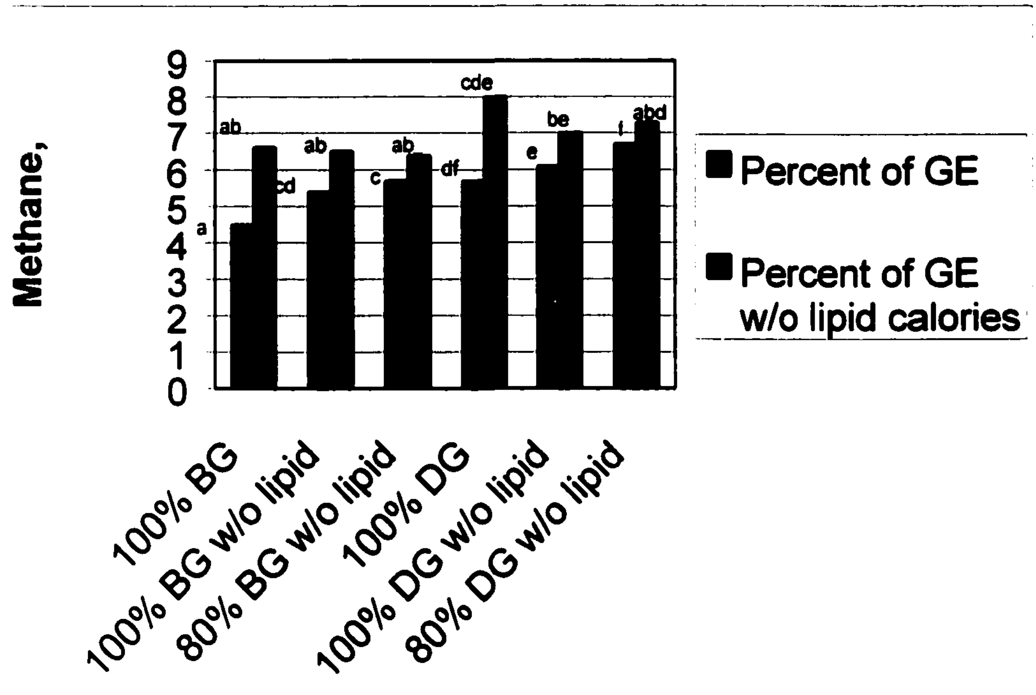
^{abc}Means with unlike superscripts differ P < 0.01.

Table 3.10. Effect of brewer's grains (BG) or distiller's grains (DG) with and without lipid on methane production at 48 h of incubation

Methane	100% BG	100% BG w/o lipid	80% BG w/o lipid	100% DG	100% DG w/o lipid	80% DG w/o lipid	SE
n	6	6	6	6	6	6	
Calories	270^{a}	298^{ab}	289^{ac}	344^{b}	322^{bc}	336^{b}	16.6
% of GE	4.5^{a}	5.4^{bcd}	5.7^{c}	5.7^{df}	6.1^{e}	6.7^{f}	0.12
% of GE w/o lipid	6.6^{ab}	6.5^{ab}	6.4^{ab}	8.0^{cde}	7.0^{bc}	7.3^{abd}	0.37

^{abcdef}Means with unlike superscripts differ ($P < 0.04$).

Figure 3.13. Effect of brewer's grains (BG) or distiller's grains (DG) with and without lipid on methane production at 48 h of incubation



^{abcdef}Means with unlike superscripts differ $P < 0.01$.

comparing the methane release as a percent of GE without lipid, the three BG substrates did not differ, whereas methane release was lower for the 100% DG without lipid substrate than the other two DG substrates (Table 3.10; Figure 3.13).

The results expressed as a percent of GE without lipid calories suggest lipid was not the unique factor of BG or DG causing methane production to decrease (Table 3.10; Figure 3.13). Although unlikely, the ether extraction and subsequent drying may have caused the BG or DG without lipid to act in a different manner resulting in no change in methane production when an increase was anticipated.

In vitro trial five. Analysis of results at 48 h of incubation, finds gas production was greater ($P < 0.01$) for substrate containing beer than the 100% CGF, due to a 41% increase ($P < 0.01$) in both carbon dioxide and methane (mL) (Table 3.11). One possible explanation for the high gas production may be a higher non-structural carbohydrate (NSC) for the beer than CGF providing more substrate for microbial activity and/or growth to increase. However, this is not supported by the analyses which show CGF contains more NSC than beer (37.5 versus 27.0%) (Table 3.2). Further examination of the nutrient analysis of beer, indicates the unique constituent different than CGF was ethanol, which was 66.7% (% of DM) of the nutrient analysis of beer. The ethanol is possibly readily metabolized to acetate and hydrogen during microbial fermentation resulting in greater volumes of gas produced (Berkely website).

Similar to gas production (Figure 3.14), DM digestibility was numerically greater for substrates containing CGF plus beer than the other two CGF substrates (Table 3.11). Digestibility of the substrate containing CGF plus beer was possibly

Table 3.11. Effect of corn gluten feed (CGF), CGF plus yeast, CGF plus beer, and substrates containing brewer's grains (BG) or distiller's grains (DG) mixed with grass hay (GH) on total gas production, gas composition, and digestibility at 48 h of incubation

Item	100% CGF	CGF plus yeast	CGF plus beer	100% GH	80% BG 20% GH	80% DG 20% GH	SE
n	6	6	6	6	6	6	
Gas, mL							
Total	345^{ef}	351^{f}	492^{g}	285^{hj}	247^{i}	284^{j}	11.0
N[a]	35.4	37.3	31.2	35.7	33.4	35.3	9.7
CO_2[b]	278^{e}	282^{e}	399^{f}	215^{ghi}	188^{hi}	220^{i}	17.0
CH_4[c]	32.0^{e}	32.5^{e}	62.2^{f}	31.3^{e}	23.6^{g}	29.5^{e}	1.3
DMD, %[d]	54.8	49.6	63.8	43.7	37.9	38.4	6.9

[a] Nitrogen

[b] Carbon dioxide

[c] Methane

[d] Dry matter digestibility. Two observations for each treatment.

[efghi] Means with unlike superscripts differ ($P < 0.01$).

higher due to higher digestibility of the beer alone resulting in a greater total digestibility when the beer is combined with CGF as substrate.

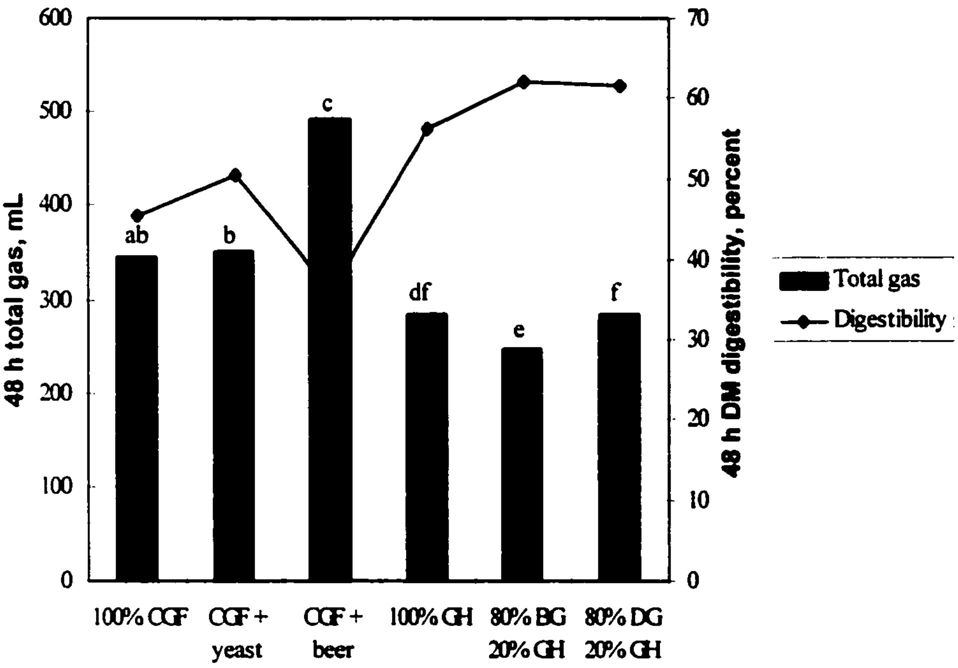
A second objective of in vitro trial five was to compare the 100% grass hay (GH) substrate to those containing 80% BG or DG each with 20% GH. Total 48 h gas production for the substrates containing 80% BG and 20% GH was decreased ($P < 0.01$) 12% below the substrates containing 100% GH or 80% DG with 20% GH (Table 3.11). Total gas production was decreased ($P < 0.01$) for the substrates containing 80% BG and 20% GH due to a decrease ($P < 0.01$) in methane and a numerical decrease in carbon dioxide production (Table 3.11).

Although differences in total gas production were measured between the by-product and GH substrates, no significant differences were measured for DM digestibility (Table 3.11; Figure 3.14). Numeric differences were measured with DM digestibility greatest for the 100% GH, which was unexpected since digestibility of the BG and DG would be expected to be greater than the GH.

When expressing methane production as a percent of GE or GE without lipid calories at 48 h of incubation, methane production was at least 50% greater ($P < 0.01$) for substrates containing CGF plus beer than the 100% CGF or CGF plus yeast substrates (Table 3.12; Figure 3.15). Based on the increase in methane production when adding beer, it appears any residual beer or ethanol of the BG or DG will stimulate rather than suppress methane.

Methane production increased when beer was added to CGF, which is similar to findings of Pol and Demeyer (1988) who ruminally infused a similar compound (methanol) into sheep and found methane production increased. The increase in

Figure 3.14. Effect of corn gluten feed (CGF), CGF plus yeast, CGF plus beer, and substrates containing brewer's grains (BG) or distiller's grains (DG) mixed with grass hay (GH) on volume of gas produced as compared to DM digestibility at 48 h of incubation



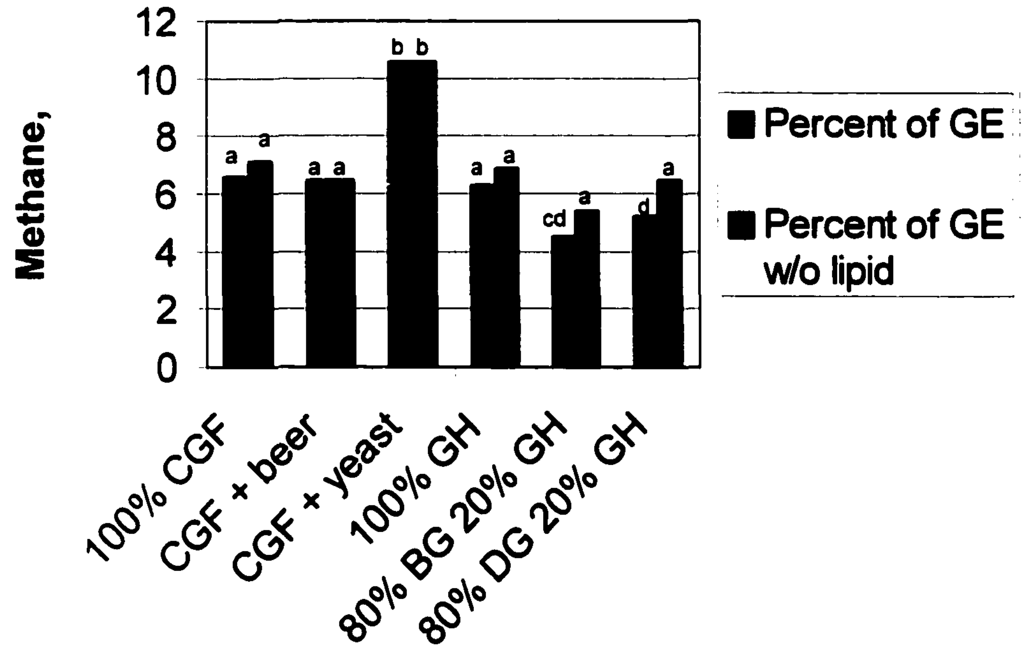
^{abcdef}Means with unlike superscripts differ $P < 0.01$.

Table 3.12. Effect of corn gluten feed (CGF), CGF plus yeast, CGF plus beer, and substrates containing brewer's grains (BG) or distiller's grains (DG) mixed with grass hay (GH) on methane production at 48 h of incubation

Methane	100% CGF	CGF plus yeast	CGF plus beer	100% GH	80% BG 20% GH	80% DG 20% GH	SE
n	6	6	6	6	6	6	
Calories	302^{a}	307^{a}	588^{b}	296^{a}	223^{c}	278^{a}	12.6
% of GE	6.6^{a}	6.5^{a}	10.6^{b}	6.3^{a}	4.5^{cd}	5.2^{d}	0.26
% of GE w/o lipid	7.1^{a}	6.5^{a}	10.6^{b}	6.9^{a}	5.4^{c}	6.5^{a}	0.27

^{abcde}Means with unlike superscripts differ ($P < 0.01$).

Figure 3.15. Effect of corn gluten feed (CGF), CGF plus yeast, CGF plus beer, and substrates containing brewer's grains (BG) or distiller's grains (DG) mixed with grass hay (GH) on methane production at 48 h of incubation



^{abcd}Means with unlike superscripts differ $P < 0.01$.

methane is possibly caused by the conversion of ethanol to acetate and hydrogen by the microorganisms (Berkeley website), thus supplying more hydrogen substrate for the methanogens. Since ethanol was not the only constituent added to CGF, other constituents of beer may be causing the dramatic increase in methane production, although difficult to distinguish with the substrates incubated.

Yeast did not significantly change methane production (Table 3.12; Figure 3.15) when added to CGF. Chaucheyras et al. (1995) found similar results where yeast, incubated in vitro, (*Saccharomyces cerevisiae*) stimulated hydrogen utilization by acetogens and methanogens, but did not change methane production. It appears yeast are unable to significantly stimulate acetogens causing them to use up hydrogen before the methanogens use it, resulting in a reduction in methane production. Adding 80% BG or DG to grass hay decreased ($P < 0.01$) methane (percent of GE) production 28 and 17% below the 100% GH substrate (Table 3.12; Figure 3.15).

These results demonstrate, 80% BG or DG and grass hay mixtures will decrease methane production similar to when the two by-products were mixed with alfalfa. However, when expressing methane production as a percent of GE without lipid, methane production was lower for the substrate containing 80% BG and 20% GH than the 100% GH substrate (Figure 3.15), but did not differ for the DG substrates.

In vivo measure of nutrient digestibility, metabolizability, and energy value of distiller's grains. The analyzed nutrient composition of the ingredients (Table 3.13) used to formulate experimental diets for the in vivo experiment was largely similar to values reported in NRC (1996). The CP content of whole cottonseed and soybean

Table 3.13. In vivo experiment ingredient nutrient composition, DM basis

Item, percent	Whole cotton	Alfalfa	Soybean meal	Distiller's grains	Corn	Vit/min supp[a]	Mono Phos
Analyzed:							
DM	92.2	88.5	89.9	84.9	86.0	92.8	99.8
Ash	3.4	9.6	6.8	4.5	1.4	58.3	91.6
Lipid	17.1	2.8	2.9	9.9	6.0	3.4	0
CP	19.1	20.4	48.4	32.6	8.7	10.7	0
NDF	53.2	33.7	10.5	29.3	10.2	11.9	0
ADF	40.5	24.2	5.9	15.1	2.9	4.2	0
Lignin	9.2	5.8	0	5.0	0	1.3	0
NSC[b]	7.2	33.5	31.4	23.7	73.7	15.8	0
GE, Mcal/kg	5.40	4.49	4.65	5.47	4.46	2.24	0
NRC, 1996:							
Lipid	17.5	2.9	2.3	10.7	4.3		0
CP	24.4	19.9	52.9	30.4	9.8		0
NDF	51.6	42.0	14.9	46.0	9.0		0

[a] Contained (% as-fed) 1465 IU/kg vitamin A, 366.3 IU/kg vitamin D, 3663 IU/kg vitamin E, 4.4% niacin, 0.73% $CuSO_4$, 0.73% ZnO, 0.55% MnO, 1.47% Zinpro 100, 1.63% MgO, 1.63% yeast pack, and the difference made up with wheat midds as a carrier.

[b] Non-structural carbohydrate, 100 - (CP + lipid + NDF + ash).

meal (Table 3.13) was marginally lower than reported in NRC (1996). Likewise, the NDF content of early bloom alfalfa hay and DG was also lower. The DG used during the in vivo experiment were similar to the DG used in vitro, although they came from different sources. The largest difference in nutrient content between the two sources of DG was that the in vivo experiment DG batch contained less ether extract (9.9 versus 12.5; Tables 3.1 and 3.13).

The body weight of the steers used to evaluate the DG diets did not significantly differ across treatment, but did differ by animal and period, which would be expected. Steers fed 20 and 40% DG diets consumed less ($P < 0.05$) dry matter, (kg/d or % of BW) than when fed the control diet (Table 3.14). Steers were observed sorting and leaving the DG during the experiment and nutrient analyses of the orts were most similar to DG (Appendix Table 6.11), suggesting the orts were primarily composed of DG for the DG diets. The intake change in the current trial may have been due to the DG containing an offensive odor or taste. However, energy intake is equal and thus steers may be reaching their energy limit. Peter et al. (2000) did not measure a change and Ham et al. (1994) measured an increase ($P < 0.10$) in DM intake when DG were fed to cattle at 20% or 40% (% of DM) of a high grain diet. The DG fed in the current trial came from a different source than those fed by Peter et al. (2000), so differences in processing may have caused the DMI differences.

Digestibility. Dry matter or OM digestibility did not differ due to treatment and averaged 74.7 and 76.5%, respectively (Table 3.14). Calculated total digestible nutrient content for the 0, 20, and 40% DG diets based on NRC (1996) values, predicts a digestibility of 75.7, 77.8, and 80.4, respectively. Determining by

Table 3.14. Effect of distiller's grains on apparent nutrient digestibility

Item	0% distiller's grains	20% distiller's grains	40% distiller's grains	SE
n	5	5	5	
BW, kg[ab]	278.0	281.2	285.8	2.6
DMI, kg/d[ab]	5.9[c]	5.7[d]	5.4[d]	0.05
DMI, % of BW[b]	2.1[c]	2.0[d]	1.9[e]	0.02
Digestibility, percent:				
DM[a]	73.6	74.7	75.7	0.51
OM[a]	75.4	76.5	77.5	0.53
Lipid	81.4	84.3	81.5	2.2
CP	57.5	61.7	66.1	
NDF[b]	46.7[c]	54.6[d]	61.1[d]	2.1
ADF[ab]	43.0[c]	53.4[d]	61.0[e]	2.2
NSC[f]	100	100	100	

[a] Animal significant at $P < 0.05$.

[b] Period significant at $P < 0.05$.

[cde] Means with unlike superscript differ $P < 0.05$.

[f] Non-structural carbohydrate, calculated by difference.

regression analysis the digestibility of the DG, suggests an OM digestibility of 75.7%, which is similar to 73.4 determined by difference by Jarosz (1998) when 80% DG was fed with 20% alfalfa.

Although DM digestibility did not differ, cell wall constituents were more extensively digested when DG was added. Steers fed diets containing 20 and 40% DG digested NDF 17 and 30.8% better ($P < 0.05$) than steers fed the control diet (Table 3.14). Neutral detergent fiber digestibility determined by regression suggests a digestibility of 68.3 for the DG alone, which is similar to 73.3 determined by Jarosz (1998). Acid detergent fiber digestion was also increased ($P < 0.05$) 24.2 and 41.9% for the diets containing 20 and 40% DG when compared to diets containing 0% DG, respectively.

The increase in fiber digestion may be caused by an increase in ruminal pH due to a lower starch content of the diet. With every 20 percentage unit increase of DG in the diet, non-structural carbohydrate (NSC) content decreased 4.7 percentage units (Table 2.7). Fiber digesting microorganisms are progressively depressed in a ruminal environment when pH drops below 6.5 (NRC, 1996). By removing some of the starch (NSC) in the steer diet and replacing it with fiber, the pH should increase allowing more fiber digestion. Peter et al. (2000) found ruminal pH increased (not significant) from 5.96 to 6.24 and total tract fiber digestibility increased 72% when including 20% DG in a high grain diet replacing corn starch. In the current trial when steers were fed a dairy diet with a moderate grain content, NDF digestibility increased 17% and 12% with the inclusion of 20 and 40% DG, respectively. The increase in fiber digestibility measured in the current trial was not as great as measured by Peter et al. (2000), but the pH of the control diet was likely higher than with the high grain diet.

Although daily DMI was lower ($P < 0.05$) for steers fed the DG treatment diets (Table 3.14), GE intake did not differ between treatments (Table 3.15). The DG contained more GE than the ingredients replaced, so even though DMI decreased, GE intake did not differ. Additionally, the energy digestibility was at least 6% greater ($P < 0.05$) for steers fed the 40% DG diets than the other two (Table 3.15).

Methane. The primary objective of the in vivo experiment was to determine if feeding steers increasing concentrations of DG decreased methane production from moderate concentrate diets. Methane production averaged slightly lower (5.9 versus 6.2% of GE), but was not significantly decreased by DG addition (Table 3.15). These findings agree with in vitro results where a high concentration (100%) of the substrate needed to be DG before methane (percent of GE) production decreased.

Energy partitioning. Although, methane production of steers did not significantly decrease due to feeding 20 or 40% DG, it seems important to consider the lipid concentration of the three diets to further determine if methane changes with increasing lipid. The lipid concentration increased 1.8 percentage units when comparing the 0% DG treatment to the 40% diet and methane (percent of GE) decreased ($P = 0.19$) 0.3 percentage unit. During the current experiment minimal changes in dietary lipid appeared to cause a small change in methane production. The small mean decline in methane combined with similar urine energy and lower fecal energy losses resulted in greater ($P < 0.05$) diet ME for the 40% DG treatments

Table 3.15. Effect of distiller's grains on energy partitioning

Item	0% distiller's grains	20% distiller's grains	40% distiller's grains	SE
Gross energy intake				
Mcal/d[ab]	26.7	26.9	27.2	0.22
kcal/kg $BW^{.75}$	380.0	411.9	407.4	25.0
DE, % of GE	72.7[e]	74.3[e]	78.5[f]	1.3
Urine, % of GE[b]	4.4	4.2	4.6	0.17
Methane, % of GE[ad]	6.2	5.9	5.9	0.17
ME				
% of GE[ab]	62.7[e]	64.8[f]	66.4[f]	0.55
kcal/kg $BW^{.75}$[d]	252.4	262.9	268.2	3.9
ME/DE	0.86	0.87	0.85	0.13
Heat				
%[d]	41.9	41.4	41.3	0.93
kcal/kg $BW^{.75}$[d]	174.4	174.7	175.4	2.5
Retained energy				
%[d]	20.6	21.8	23.3	1.3
kcal/kg $BW^{.75}$[d]	77.9	88.3	92.8	4.8
As fat, %	43.8	55.1	54.9	8.6
As protein, %	56.2	44.9	45.1	8.6

[a] Animal significant at $P < 0.05$.

[b] Period significant at $P < 0.05$.

[c] Period x treatment interaction significant $P < 0.05$.

[d] GE intake/kg $BW^{.75}$ significant at $P < 0.05$.

[ef] Means with unlike superscript differ $P < 0.05$.

than the 0 and 20% DG treatments (Table 3.15). Unlike ME, energy lost as heat did not differ, nor did retained energy or type of retained energy (protein versus fat) differ due to treatment (Table 3.15).

Steers fed 20 and 40% DG could be expected to retain more energy as protein than steers fed 0% DG, since they consumed 17 and 33 g more ($P < 0.05$) nitrogen than steers fed the control diet (Table 3.16). Nitrogen lost as feces and urine was numerically increased when steers were fed DG. Although steers fed the DG treatments consumed more nitrogen ($P < 0.05$) and numerically more nitrogen was lost in feces and urine, resulting in similar nitrogen retention (g/d) across all treatments. Nitrogen retention did not differ since urine nitrogen excretion by steers fed the DG diets was also numerically greater (Table 3.16).

Metabolizable energy required for maintenance (MEm) for the steers fed DG did not significantly differ, but was at least 3% lower than the control fed steers (Table 3.17). Partial efficiency of ME use for maintenance (km) and partial efficiency of ME use for gain (kr) were both approximately 4% greater for the DG diets than the control. Jarosz (1998) measured the kr of steers fed diets consisting of 80% BG or 80% DG along with 20% alfalfa to be 0.55 and 0.54, respectively, which was 12 and 10% greater (not significant) than measured for steers fed 80% CGF, a feed of similar nutrient content acting as the control.

The higher kr values for steers fed BG and DG were possibly a result of the relatively high (10 to 12%) lipid content of each. Lipid typically has a low heat increment when used for lipogenesis resulting in a more efficient gain. Tyrrell et al. (1991) found similar results, where dairy cows fed a high lipid diet had a higher

Table 3.16. Effect of distiller's grains on nitrogen balance

Item	0% distiller's grains	20% distiller's grains	40% distiller's grains	SE
Nitrogen intake, g/d[d]	171.1[e]	188.2[f]	203.9[g]	10.8
Nitrogen loss, g/d				
Fecal[abd]	48.2	52.2	54.4	1.8
Urine	48.1	65.0	74.8	14.5
Nitrogen digestibility, %	72.1	72.3	73.4	0.95
Retained nitrogen, g/d	76.4	70.1	76.6	14.7
% of intake	43.0	38.1	34.9	6.3
% of digested	60.1	52.8	47.0	9.7

[a] Animal significant at $P < 0.05$.

[b] Period significant at $P < 0.05$.

[c] Period x treatment interaction significant $P < 0.05$.

[d] Gross energy intake covariate significant $P < 0.05$.

[efg] Means with unlike superscript differ $P < 0.05$.

partial efficiency (0.72 versus 0.66) of milk synthesis than a low lipid diet.

Net energy for maintenance and gain. With the greater ME content of the DG diets and numerically greater partial efficiencies of ME use, the NEm and NEg content of the DG diets were found to be at least 10% greater ($P < 0.05$) than the 1.95 and 1.56 Mcal/kg found for the control (Table 3.17). To establish a NEg value for the DG alone, by difference calculations were implemented, assuming no associative effects and assuming the additional NEg above the control diet was a result of DG addition. The by difference calculations estimate NEg values value of 2.51 and 2.31 for the DG alone contained in the 20 and 40% diets, respectively.

The NEg values determined in the current trial using respiration calorimetry were greater for all diets (Figure 3.16) than suggested by NRC (1996). Respiration calorimetry typically estimates NEg values to be greater than determined using slaughter balance, (Larson, 1996) upon which NRC (1996) is based. Slaughter balance NEg values are typically 76% of those found for respiration calorimetry (Larson et al., 1996). Similarly, the NRC (1996) predicted NEg of the control diet (Table 3.17) was 76% of the respiration calorimetry value. This further adjustment to the NEg by difference predicts DG to contain 1.83 Mcal/kg, approximately 20% greater than NRC (1996) reports. A very similar value of 1.87 Mcal/kg for DG was estimated from animal performance experiments by Ham et al. (1994).

Table 3.17. Steer maintenance requirements and NEm and NEg values of diets containing distiller's grains

Item	0% distiller's grains	20% distiller's grains	40% distiller's grains	SE
FHP, kcal/kg $BW^{.75}$[a]	77	77	77	
MEm, kcal/kg $BW^{.75}$[bi]	113.0	109.3	108.6	2.1
ME, Mcal/kg[h]	2.84[j]	3.05[k]	3.24[l]	0.03
km[ci]	0.68	0.71	0.71	0.01
NEm, Mcal/kg[di]	1.95[j]	2.15[k]	2.31[k]	0.05
kr[d]	0.55	0.57	0.57	0.02
NEg, Mcal/kg[f]	1.56[j]	1.75[k]	1.86[k]	0.05
NEg, Mcal/kg, NRC, 1996[g]	1.19	1.26	1.31	

[a] Fasting heat production, NRC, 1996.

[b] Metabolizable energy required for maintenance, determined by semilog solution (Lofgreen and Garrett, 1968)

[c] km = partial efficiency of ME use for maintenance (77/MEm).

[d] NEm = ME * km.

[e] kr = partial efficiency of ME use for gain (RE/(ME - MEm)), where RE = retained energy.

[f] NEg = ME * kr.

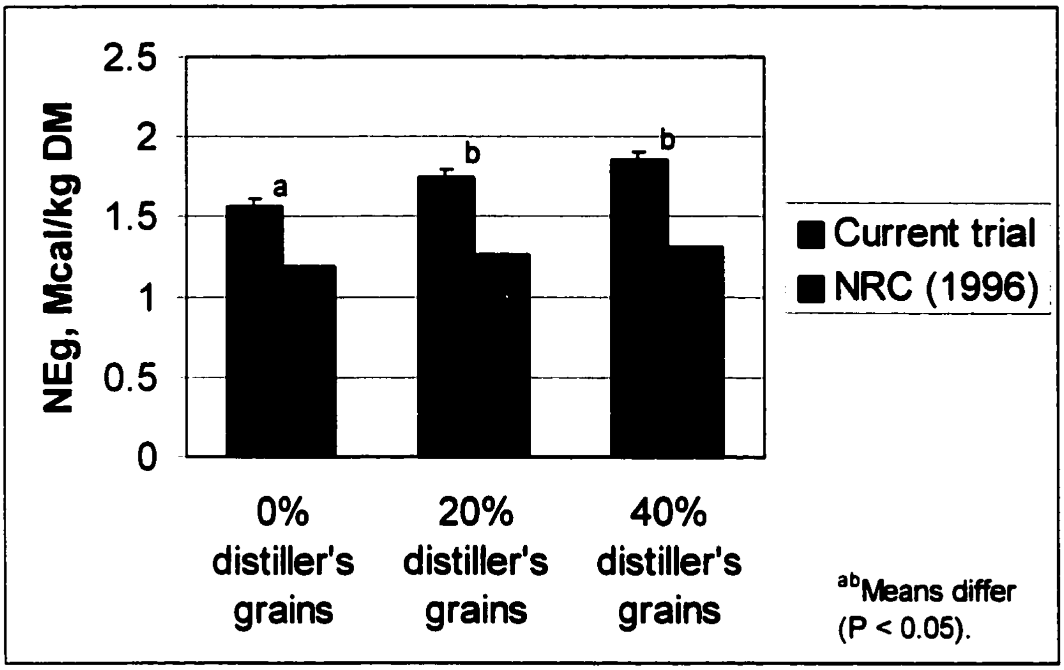
[g] Determined from NRC, 1996 reported values.

[h] Animal significant $P < 0.05$.

[i] Gross energy intake covariate significant $P < 0.05$.

[jkl] Means with unlike superscripts differ $P < 0.05$.

Figure 3.16. NEg content measured in the current trial as compared to NRC (1996)



CHAPTER 4

CONCLUSIONS

Dry matter digestibility of substrates incubated in vitro were typically lower than measured in vivo and may have been different due to differences in rumen fluid sources. Gas production appeared to be related DM digestibility, since DM digestibility and gas production changed in a similar manner when incubating different substrates/treatments. Methane production (percent of non-lipid GE) linearly decreased with increasing brewer's grains (BG) concentration from 20 to 100%. Whereas methane production did not decrease with increasing distiller's grains concentration until the substrate contained 100% DG. Similar to BG, increasing concentration of corn oil replacing corn gluten feed linearly decreased methane production, suggesting for BG the relatively high lipid content may cause methane production to decrease. In contradiction to lipid's role in decreasing methane, when the lipid was removed from both BG and DG before fermentation, methane production did not change in a manner supporting lipid effects on methane. Of the other factors investigated, yeast had no effect on methane production, whereas beer increased methane production 50%.

The in vivo data obtained by indirect respiration calorimetry suggest methane production was not decreased by feeding steers 20 or 40% DG in a 60% concentrate diet. Neutral detergent fiber digestibility was increased by including 20 and 40% DG and digestible energy was greater for the 40% DG diet than the 0 or 20% DG diets.

Metabolizable energy content of the DG diets was greater than the control, which was a result of a higher digestible energy content of the DG diets since no differences in energy lost as methane or urine were measured. The greater metabolizable energy content along with a 4% increase (not significant) in the partial efficiency of metabolizable energy use for maintenance and gain for the DG diets resulted in NEm and NEg values at least 10% greater than the control. Net energy for gain values for the current trial were 35, 39, and 42% greater for the 0, 20, and 40% DG diets, respectively than NRC (1996) predicts. These improvements due to DG additions indicate that the NRC (1996) content/kg DM for DG should be increased from 1.50 to 1.83, following adjustment for respiration calorimetry versus slaughter balance differences.

CHAPTER 5

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CHAPTER 6

APPENDIX: STATISTICAL PROGRAMS

To make treatment comparisons for the 48 h measures such as total gas production, DM digestibility, or methane as a percent of GE, procedure mixed of SAS was used due to missing observations. The following SAS code was used to model the dependent variable methane as a percent of GE, which was repeated for each dependent variable of interest:

```
PROC SORT;
        BY TREATMENT;

PROC MIXED;
        CLASS TREATMENT;
        MODEL METHANE=TREATMENT;
        LSMEANS TREATMENT/PDIFF;
RUN;
```

To analyze for linear, quadratic, and cubic effects of treatments such as 0, 20, 40, 60, 80, and 100% brewer's grains on methane production as a percent of GE, etc., procedure GLM of SAS was used. The following SAS code was used to test for linear, quadratic, and cubic effects due to brewer's grains concentration:

```
DATA INVITRO;
        INPUT METHANE PERCENTBG;
X=PERCENTBG;

CARDS;
raw data

;
PROC SORT;
        BY TREATMENT;

PROC GLM;
        CLASS PERCENTBG;
        MODEL METHANE=PERCENTBG   X   X*X   X*X*X;
RUN:
```

Where X in the model statement tests for the linear effect, X*X tests for the quadratic, and X*X*X test for the cubic effect. To check for the significance of each of the terms, type I sums of squares must be used. The PERCENTBG variable is used in the model statement to test for lack of fit. If the PERCENTBG variable is significant, then no linear, quadratic, or cubic effect explains the data. The previous code was used to test for linear, quadratic, or cubic effects. To get the estimates for each of the parameters, the following SAS code was used:

```
PROC GLM;
        MODEL METHANE=X   X*X    X*X*X/SOLUTION;
QUIT;
RUN;
```

To characterize and statistically analyze the relationship between gas production and time between treatments, the data was first arranged in a manner relating accumulative gas production over time for each individual vial. The accumulative gas data was then analyzed in two steps. The first step was to fit each of the gas curves with an equation developed by Schofield et al. (1994) and use non-linear statistical procedures to estimate two parameters (V_F and *kt*). The data for each vial over 96 h was fit with the equation volume = $V_F(1-\exp(-kt))$ (Schofield et al., 1994). Where V_F is the estimated final volume of gas produced and *k* is the estimated rate constant. The following SAS code was then used to determine the two parameters:

```
PROC SORT;
        BY VIAL;

PRCO NLIN;
        BY VIAL;

PARMS VF=300 k=0 to .01 by .005;
        MODEL mLgas=VF*(1-exp(-k*time));

RUN;
```

Where vial = incubation vessel number, VF = estimated final gas volume parameter, k = estimated rate parameter, mLgas = accumulative gas production at each time point measured, and time = hour at which each measurement is taken. The PARMS statement contains user number guesses or a number range at which the user thinks the estimated parameter will be or fall within. This program was repeated for each vial.

To analyze for treatment differences the estimated parameters VF and k4 estimated from the previous SAS program were then used. Each parameter for each vial was entered into the SAS program as part of the data set and then analyzed. The following SAS code was used to determine treatment differences:

```
PROC SORT;
        BY TREATMENT;

PROC MIXED;
        CLASS TREATMENT;
        MODEL FINALVOLUME=TREATMENT;
        LSMEANS TREATMENT/PDIFF;
RUN;
```

The same code can then be used to compare the rate constants by entering RATE in place of FINALVOLUME.

To analyze the in vivo data for DM digestibility, methane production, etc., procedure MIXED of SAS was used due to missing observations. Gross energy intake per metabolic body size was also used as a covariate to adjust for potential level of intake effects. The following SAS code was used to measure treatment differences for methane production as an example:

```
PROC SORT;
        BY TREATMENT;

PROC MIXED;
        CLASS ANIMAL PERIOD TREATMENT;
        MODEL METHANE=TREATMENT  GEI  ANIMAL*TREATMENT
                TREATMENT*GEI;
        LSMEANS TREATMENT/PDIFF;
RUN;
```

Table 6.1. Effect of brewer's grains concentration on estimated final volume and rate of gas production

	Percent brewer's grains replacing alfalfa							P ≤		
Item	0	20	40	60	80	100	SE	L[a]	Q[b]	C[c]
Total gas										
Volume, mL	280	267	271	277	284	277	3.5	0.21	0.16	0.01
Rate, h^{-1}	0.03	0.04	0.04	0.04	0.03	0.03	0.01	0.01	0.01	0.01
Methane										
Volume, mL	23.0	19.7	16.8	15.7	16.4	10.0	5.7	0.11	0.96	NS
Rate, h^{-1}	0.18	0.21	0.19	0.17	0.09	0.22	0.07	0.91	0.61	NS

[a] Linear effect.

[b] Quadratic effect.

[c] Cubic effect.

Table 6.2. Effect of distiller's grains concentration on estimated final volume and rate of gas production

	Percent distiller's grains replacing alfalfa							P ≤		
Item	0	20	40	60	80	100	SE	L[a]	Q[b]	C[c]
Total gas										
Volume, mL	302	318	335	355	374	389	2.8	0.01	0.97	NS
Rate, h^{-1}	0.04	0.04	0.04	0.03	0.03	0.02	0.00	0.01	0.01	NS
Methane										
Volume, mL	41.4	41.0	44.7	48.2	55.0	81.9	4.1	0.01	0.01	NS
Rate, h^{-1}	0.03	0.03	0.03	0.02	0.02	0.01	0.00	0.01	0.01	NS

[a] Linear effect.

[b] Quadratic effect.

[c] Cubic effect.

Table 6.3. Effect of corn oil concentration on estimated final volume and rate of gas production

	% corn oil replacing corn gluten feed							P ≤		
Item	0	2	4	6	8	10	SE	L[a]	Q[b]	C[c]
Total gas										
Volume, mL	637	566	477	401	375	341	9.2	0.01	0.01	NS
Rate, h^{-1}	0.01	0.02	0.02	0.02	0.02	0.02	0.00	0.01	0.57	NS
Methane										
Volume, mL	99.4	78.4	59.0	45.0	41.9	36.0	6.5	0.01	0.03	NS
Rate, h^{-1}	0.01	0.01	0.01	0.02	0.02	0.2	0.00	0.01	0.78	NS

[a] Linear effect.

[b] Quadratic effect.

[c] Cubic effect.

Table 6.4. Effect of brewer's grains (BG) or distiller's grains (DG) with and without lipid on estimated final volume and rate of gas production

Item	100% BG	100% BG w/o lipid	80% BG w/o lipid	100% DG	100% DG w/o lipid	80% DG w/o lipid	SE
Total gas							
Volume, mL	315^{ac}	340^{ad}	305^{c}	375^{ef}	392^{be}	351^{df}	9.5
Rate, h^{-1}	0.03^{ae}	0.03^{ac}	0.04^{e}	0.03^{ae}	0.02^{bc}	0.03^{ae}	0.00
Methane							
Volume, mL	38.8^{a}	44.8^{bc}	36.9^{a}	46.1^{dc}	48.7^{b}	44.1^{bc}	1.6
Rate, h^{-1}	0.02^{ac}	0.02^{a}	0.03^{bd}	0.02^{cd}	0.02^{a}	0.03^{d}	0.00

^{abcdef}Means with unlike superscripts differ $P < 0.01$).

Table 6.5. Effect of corn gluten feed (CGF), CGF plus yeast, CGF plus beer, and substrates containing brewer's grains (BG) or distiller's grains (DG) mixed with grass hay (GH) on estimated final volume and rate of gas production

Item	100% CGF	CGF plus yeast	CGF plus beer	100% GH	80% BG 20% GH	80% DG 20% GH	SE
Total gas							
Volume, mL	203^{a}	214^{a}	282^{b}	146^{c}	146^{c}	159^{c}	9.1
Rate, h^{-1}	0.24^{ae}	0.25^{ac}	0.23^{e}	0.20^{ae}	0.30^{bc}	0.70^{ae}	0.23
Methane							
Volume, mL	17.9^{ad}	19.0^{a}	34.3^{c}	15.0^{be}	12.5^{b}	15.9^{de}	0.90
Rate, h^{-1}	0.19	0.21	0.19	0.15	0.18	0.12	0.12

^{abcde}Means with unlike superscripts differ $P < 0.01$.

Table 6.6. Effect of brewer's grains concentration on total gas production and composition at 96 h of incubation, DM basis

	Percent brewer's grains replacing alfalfa							P ≤		
Item	0	20	40	60	80	100	SE	L[a]	Q[b]	C[c]
n	4	4	4	4	4	4				
Total gas, mL	263	270	273	278	273	268	4.1	0.30	0.03	0.75
Nitrogen, mL	71.9	78.1	80.6	82.0	76.5	73.3	0.70	0.56	0.01	0.21
Carbon dioxide, mL	150	156	159	163	165	166	3.0	0.04	0.33	0.95
Methane:										
mL	41.2^{a}	35.5^{bc}	33.5^{cd}	33.0^{d}	30.8^{ef}	28.7^{f}	0.73	0.01	0.02	0.01
calories	390^{a}	336^{bc}	317^{cd}	312^{d}	291^{ef}	272^{f}	6.9	0.01	0.02	0.01
% of GE	8.7^{a}	7.3^{b}	6.7^{cd}	6.5^{d}	5.9^{e}	5.4^{f}	0.15	0.01	0.01	0.01
% of non-lipid GE	9.0^{a}	7.7^{bcd}	7.5^{cde}	7.4^{de}	7.0^{ef}	6.6^{f}	0.17	0.01	0.03	0.01

[a] Linear effect.

[b] Quadratic effect.

[c] Cubic effect.

[d] Two observations for each treatment.

Table 6.7. Effect of distiller's grains on total gas production and composition at 96 h of incubation, DM basis

	Percent distiller's grains replacing alfalfa							$P \leq$		
Item	0	20	40	60	80	100	SE	L^{a}	Q^{b}	C^{c}
n	4	4	4	4	4	4				
Total gas, mL	300^{e}	308^{e}	328^{fgh}	333^{gh}	338^{h}	304^{e}	5.8	0.03	0.01	0.01
Nitrogen, mL	76.0	77.4	78.4	77.3	77.1	74.8	1.0	0.37	0.02	0.95
Carbon dioxide, mL	188^{e}	194^{e}	210^{fgh}	216^{gh}	220^{h}	195^{e}	5.1	0.01	0.01	0.02
Methane:										
mL	36.1^{e}	36.7^{e}	39.1^{fgh}	40.0^{gh}	40.5^{h}	34.1^{e}	0.79	0.82	0.01	0.01
calories	341^{e}	347^{e}	370^{fgh}	376^{gh}	383^{h}	322^{e}	7.5	0.82	0.01	0.01
% of GE	7.6^{a}	7.4^{a}	7.6^{a}	7.5^{a}	7.3^{a}	6.0^{b}	0.15	0.01	0.01	0.01
% of non-lipid GE	7.9^{a}	8.0^{a}	8.6^{bcd}	8.8^{cd}	9.0^{d}	7.6^{a}	0.17	0.17	0.01	0.01

[a] Linear effect.

[b] Quadratic effect.

[c] Cubic effect.

[d] Two observations for each treatment.

[efgh] Means with unlike superscripts differ $P < 0.01$.

Table 6.8. Effect of corn oil concentration on gas production and composition at 96 h of incubation, DM basis

	Percent corn oil replacing corn gluten feed							P <		
Item	0	2	4	6	8	10	SE	L[a]	Q[b]	C[c]
n	4	4	4	4	4	4				
Total gas, mL	433	418	376	327	314	279	11.1	0.01	0.89	0.29
Nitrogen, mL	31.3	32.0	29.3	20.9	23.8	24.8	7.3	0.39	0.45	0.40
Carbon dioxide, mL	317	303	278	255	240	213	19.3	0.01	0.55	0.82
Methane:										
mL	41.6	39.8	36.2	32.1	30.6	27.3	2.6	0.01	0.62	0.24
calories	393	377	342	304	289	258	24.4	0.01	0.62	0.23
% of GE	8.7	8.0	7.2	6.2	5.8	5.1	0.52	0.01	0.28	0.41
% of non-lipid GE	10.7	10.6	9.6	8.3	8.1	7.4	0.29	0.01	0.98	0.14

[a] Linear effect.

[b] Quadratic effect.

[c] Cubic effect.

[d] Two observations for each treatment.

[cfghij] Means with unlike superscripts differ $P < 0.01$.

Table 6.9. Effect of brewer's grains (BG) or distiller's grains (DG) with and without lipid on total gas production and composition at 96 h of incubation, DM basis

Item	100% BG	100% BG w/o lipid	80% BG w/o lipid	100% DG	100% DG w/o lipid	80% DG w/o lipid	SE
n	4	4	4	4	4	4	
Total gas, mL	292	298	281	336	304	316	12.6
Nitrogen, mL	17.4	20.4	20.8	18.7	19.4	19.3	1.1
Carbon dioxide, mL	243	242	228	279	248	259	11.4
Methane:							
mL	30.9^{a}	35.0^{bc}	32.3^{ab}	38.0^{c}	36.8^{c}	37.8^{c}	1.2
calories	292^{a}	331^{bc}	305^{ab}	359^{c}	347^{c}	357^{c}	11.5
% of GE	5.9^{a}	7.2^{bc}	6.7^{bc}	6.6^{c}	7.5^{de}	7.7^{e}	0.24
% of non-lipid GE	7.2^{a}	7.2^{a}	6.8^{b}	8.4^{c}	7.5^{a}	7.8^{ac}	0.26

^{abcdefghi}Means with unlike superscripts differ ($P < 0.01$).

Table 6.10. Effect of corn gluten feed (CGF), CGF plus yeast, CGF plus beer, and substrates containing brewer's grains (BG) or distiller's grains (DG) mixed with grass hay (GH) on total gas production and composition at 96 h of incubation, DM basis

Item	100% CGF	CGF plus yeast	CGF plus beer	100% GH	80% BG 20% GH	80% DG 20% GH	SE
n	4	4	4	4	4	4	
Total gas, mL	427^{a}	454^{a}	585^{b}	328^{bc}	295^{c}	336^{c}	17.2
Nitrogen, mL	47.4	27.4	26.6	41.9	41.1	45.6	19.1
Carbon dioxide, mL	338^{ace}	383^{ab}	480^{b}	249^{cd}	224^{d}	255^{e}	31.7
Methane:							
mL	41.4^{a}	44.2^{a}	78.4^{b}	37.0^{ac}	29.6^{c}	35.5^{a}	2.9
calories	392^{ad}	418^{ad}	741^{b}	350^{acd}	280^{c}	336^{d}	27.0
% of GE	8.4^{a}	8.9^{a}	13.4^{b}	7.5^{ad}	5.7^{c}	6.3^{cd}	0.49
% of non-lipid GE	9.3^{a}	8.7^{ad}	13.5^{b}	8.5^{ad}	6.7^{c}	7.9^{d}	0.31

^{abcdef}Means with unlike superscripts differ ($P < 0.01$).

Table 6.11. Nutrient composition of steer orts during in vivo experiment two

Period	Animal	Treatment	Orts, kg/d	Orts, % of intake	CP, %	Lipid, %	NDF, %	Ash, %
1	197	0% DG						
1	198	0% DG						
1	227	20% DG	0.115	2.0	28.6	5.0	18.6	16.8
1	72	20% DG						
1	159	40% DG	0.619	12.4	29.9	6.5	21.5	11.8
1	223	40% DG						
2	197	40% DG						
2	198	20% DG						
2	227	0% DG	0.064	0.92	21.4	5.1	12.5	20.5
2	72	40% DG						
2	159	20% DG						
2	223	0% DG	1.391	20.7	18.8	11.4	28.7	6.2
3	197	20% DG						
3	198	40% DG						
3	227	40% DG	0.047	0.88	31.6	10.1	20.8	13.6
3	72	0% DG						
3	159	0% DG	0.223	4.2	24.7	4.3	12.2	15.8
3	223	20% DG						