UTILIZATION OF BY-PRODUCT FEEDS TO MAINTAIN ANIMAL PERFORMANCE WHILE DECREASING FEED COST IN POST-WEANING BEEF CATTLE DEVELOPMENT PROGRAMS

by

JEFFERSON MENEZES LOURENÇO

(Under the Direction of Robert Lawton Stewart, Jr.)

ABSTRACT

Two experiments were conducted to evaluate the utilization of alternative diets in beef cattle operations. The first experiment evaluated the use of three diets, which were offered to developing bulls. Of these three diets, one was a commercial diet whereas the other two were alternative diets based on by-products from the corn and soybean industries (soybean hulls, corn gluten feed, and dried distillers grains plus solubles). Additionally, the experimental diets were subjected to in vitro digestion for evaluation of fermentation end products. The alternative diets reduced the cost of gain. In the second experiment, three protein supplements were tested in a stocker system based on corn silage. The three supplements were: soybean meal, canola meal, or sunflower meal. Rations containing these supplements were used in feeding trials and also as substrates for in vitro fermentations. Rations containing either canola or sunflower meals decreased the daily feeding cost per animal.

INDEX WORDS: canola meal, corn gluten feed, developing bulls, dried distillers grains plus solubles, stocker animals, sunflower meal.
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May 2014
DEDICATION

I want to dedicate this work to my wife, Daniela, and to all my loved family members, in special, my father – Edinei, and my mother – Neide.
ACKNOWLEDGEMENTS

First of all, I would like to thank God for this achievement. He has given me a supernatural strength throughout this whole process and I am very thankful for this. Secondly, I want to express my gratitude to every person who has been involved in each of the many steps that brought me to this point. I want to say thanks to my wife and all my loved family members. Also, I would like to thank Dr. Lawton Stewart, Dr. Alex Stelzleni, Dr. Keith Bertrand, Dr. Michael Azain, Dr. Mark Froetschel, Dr. Nicolas DiLorenzo, Dr. Ignacy Misztal, Sherie Hulsey, Martin Ruiz Moreno, Tessa Schulmeister, Matthew Studstill, Elyse Ford, Darlene Bloxham, Rogério Moura, Jorge Matos, Erico Mattos, and everybody else who has helped me either with their professional or personal skills. Thank you very much!
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CHAPTER 1

INTRODUCTION

In most beef cattle production systems, profitability is closely related with feed costs. Moreover, feed prices are seasonal and might change quickly due to a myriad of factors influencing their production and commercialization. Data from the USDA illustrate this reality: national average prices of corn grain increased about 20% in a period of only sixty days (between June and August of 2012), moreover, between the years of 2006 and 2013, corn prices have increased 170% (USDA National Agricultural Statistics Service, 2014). It is beyond the scope of this research to investigate the reason for such dramatic changes in feed prices. However, conducting research using alternative products that can replace certain feeds when they become impractical may generate valuable information to the agricultural industry.

Feed costs represent a large proportion of total cost associated with post-weaning development of bulls for breeding stock. Nevertheless, there is a lack of information on feeding by-products to developing bulls and the associated outcomes regarding costs and animal performance. Another significant issue to address is the management of weanling animals. Although most beef cattle operations in the Southeast have been oriented toward cow/calf production, stocker operations represent a relatively simple mean of adding value to calves, and it has great importance for a significant number of producers in the Southeast (Anderson et al., 2004).
The expansion of the ethanol industry over the last years has provided increased availability of dried distillers grains plus solubles. In the process where this by-product is produced, the starch in the corn kernel is fermented to ethanol. The remaining nutrients, including protein, are concentrated about 3-fold (Klopfenstein et al., 2008). Corn gluten feed is another major by-product from the corn industry. It is resultant from the process that produces starch for ethanol or fructose for corn sweeteners (Ham et al., 1995). This by-product typically contains over 18% CP, and, like dried distillers grains plus solubles, is high in digestible fiber (Weigel et al., 1997).

Canola and sunflower meals are resulted from the oil extraction process of their corresponding seeds. These by-products are classified as protein supplements (Conrad et al., 1982). Finding a low-cost, locally available protein supplement is critical in many production systems (Patterson et al., 1999). This is particularly true when dealing with growing animals, such as stocker animals, that require more protein. Even though canola and sunflower meals typically have more fiber and less protein than soybean meal, literature has shown that these protein by-products can efficiently replace cottonseed meal (Richardson et al., 1981; Ahmad et al., 2004), and in some instances, even soybean meal (Stake et al., 1973; Rule et al., 1994).

This research was divided into two main experiments. In the first experiment, two by-product-based diets (containing 73.5% of by-products in their composition) were compared to a commercial diet in yearling bulls. The experiment was carried out for two consecutive years. Animal performance data was evaluated through the use of ultrasound, evaluation of individual dry matter intake, and by weighing animals. In addition, the experimental diets were subjected to in vitro digestion to elucidate possible treatment
effects observed during the feeding trial. Production of gas, methane, hydrogen sulfide, ammonia nitrogen, volatile fatty acids, and in vitro true dry matter digestibility were quantified. Results from this study helped to develop a better understanding of the ruminal digestion process by the treatments.

In the second experiment, three protein supplements were tested in a stocker system based on corn silage. The three supplements were: soybean meal, canola meal, and sunflower meal. Animals were fed a basal diet containing corn silage, ground ear corn, and one of the three supplements. The feeding trial was performed one year and then repeated two more years. A total of 276 animals were used over the three years. Animals were weighed at the beginning and after every 28 days. The cost of feeding was computed and, in addition, samples of the diets were subjected to in vitro fermentations that lasted 0, 6, 12, 24, or 48 hours. This companion in vitro trial helped to understand how production of fermentation end products is affected by fermentation length.

Overall, data from these experiments will be useful from several perspectives: from the producer standpoint, information provided by these studies may help cattle producers to make their decisions based on reliable scientific literature. In addition, scientists will be able to use these data as reference to guide future research and to compare their results. Therefore, this research is relevant to the agricultural industry. It will contribute with useful information to both producers and the scientific community.


CHAPTER 2
THE REVIEW OF THE LITERATURE

Utilization of By-products

One important field of study in Animal Science is the evaluation of alternatives to traditionally used feeds. Major feeds like corn and soybeans have been extensively used in animal feeding programs, and their value as feeds is well known, as well as their ordinary by-products like soybean meal. However, historical data reveals that corn has had its average price increased in United States by over three times in the last decade. The same trend can be observed for soybean. Prices of this oilseed have risen from US$4.43/bushel in 2001 towards US$14.00 in 2012 (USDA National Agricultural Statistics Service, 2014). Given that these main commodities have had their prices sharply increased in the last years, and that feeding animals is generally one of the main costs in animal production systems (Herd et al., 2003), the search for alternative products is a real need if producers want to maintain profitability. As ruminants, beef cattle can potentially utilize a large range of ingredients, including those with high fiber content, which are generally not suitable for non-ruminant species. Therefore, the inclusion of lower-cost by-product feeds in beef cattle diets may be a viable strategy to reduce costs while keeping similar animal performance (Segers et al., 2013).

Variability of the Cost of Gain

Fluctuations in feed prices and cattle performance contribute to volatility of the cost of gain (COG). Beef cattle producers should know the most profitable rations for a
given set of feed ingredients. Simply using cheaper feed ingredients in rations may not be the best strategy to maximize profits. According to Fox et al. (2001), producers who want to market animals at their optimum economic endpoint have to take into account factors such as sale prices and the COG. Using a computer model, these authors calculated the effects on expected profits when two important variables – ADG and feed efficiency – are improved by 10%. Considering the average finished steer marketed in the United States (approximately 531 kg), they found that a 10% improvement in ADG alone would improve profits by 18%, primarily as the result of fewer days on feed. In turn, a 10% improvement in feed efficiency would improve profit by 43%. Regarding the COG, an improvement of 10% in ADG would reduce it in 4%, whereas an improvement of 10% in feed efficiency would reduce COG in 10.2%, demonstrating that feed efficiency has higher impact on COG.

In a two-year study with a stocker system where cattle were fed diets based on corn silage plus different supplements (75% corn silage plus 25% tested supplements, DM basis), Segers et al., (2013) evaluated the replacement of a corn-soybean meal supplement with two corn by-products: dried distillers grains with solubles and corn gluten feed. The authors found that cattle receiving the dried distillers grains with solubles supplementation had the lowest COG, followed by animals fed corn gluten feed, and by the ones receiving corn-soybean meal ($P < 0.01$). The reported values were $1.14$, $1.29$, and $1.35$ per kg of BW gain, respectively. Although numerically inferior, the cost reported for the corn gluten feed treatment was not statistically different from the cost reported for the corn-soybean meal treatment. The main factors influencing the observed differences in COG were animals’ ADG, feed conversion, and cost of the supplements.
In a study utilizing data from 7,292 pens of steers, gathered from Kansas feedyards over 11 years, Albright et al. (1994) used regression analysis to determine the relative impacts of grain prices and animal performance on COG variability. These authors found that corn price alone explained 63 to 66% of COG variability. Feed conversion accounted for 25 to 29%, and average daily gain was responsible for approximately 2% of the volatility in COG. Regression analysis showed that corn price, feed conversion, and ADG explained about 93% of the volatility in COG ($P < 0.01$). They also found that the COG was not constant throughout the year, instead, it followed a seasonal pattern which was influenced by the weather, losses related with health issues or death, animal performance, and feed prices. Because the studied feedyards fed finishing rations based primarily on dry rolled corn, corn price variance was able to explain over 60% of the variability in COG. Although other beef cattle operations will deal with different operating costs, the importance of animal performance and feed costs in the COG are evident. Thus, the same reasoning can be extrapolated to other beef cattle operations.

**The Potential of Corn Gluten Feed and Dried Distillers Grains as Feeds**

Production of by-products has grown over the last years, especially due to the rapid expansion of the ethanol industry (Klopfenstein et al., 2008). Corn is the primary cereal grain used in alcohol production. Manufacturers use two major processes to produce alcohol or other starch-based products. These two processes are the dry milling and the wet milling, which are very different and generate different by-products. The main by-products from the dry milling are distillers grains, whereas from the wet milling are the gluten products (Weigel et al., 1997a).
Most distillers by-products are produced in the manufacturing of ethanol from corn (Weigel et al., 1997b). The leading by-products from this industrial activity are distillers grains plus solubles, which are utilized very efficiently by ruminants. Once the starch in corn is fermented to ethanol, the remaining nutrients are concentrated approximately 3-fold (Klopfenstein et al., 2008). In order to find the optimum level of dried distillers grains with solubles (DDGS) in feedlot diets, Buckner et al. (2007) conducted a study with increasing levels of this by-product. They evaluated inclusion levels of DDGS ranging from 0 to 50% of the total DM and measured the associated effects in feedlot performance. Results from this study showed a quadratic effect of increasing levels of DDGS on final BW, with the highest value observed when DDGS was fed at 20% of the DM ($P = 0.04$). A quadratic trend was observed for ADG as DDGS levels increased ($P = 0.08$). Maximum ADG was achieved when animals were fed 20% DDGS: 1.68 kg/d. Authors reported that the control group (0% DDGS) gained 1.49 kg/d, and, the ones fed other levels of DDGS averaged 1.61 kg/d. Although feed conversion tended to be linear as DDGS levels increased from 0 to 50% ($P = 0.08$), the best efficiency (lowest F:G) was reported when animals were fed 20% DDGS (5.60 vs. 6.04 – average for all other treatments offered). Concerning carcass traits, although statistical analysis did not show significant effects ($P \geq 0.07$), the 20% inclusion level also yielded the highest hot carcass weight, longissimus muscle area, 12th rib fat thickness, marbling score, and calculated yield grade. Similarly, Depenbusch et al. (2009) carried out a trial to identify the optimum level of DDGS in flaked corn finishing diets. They tested six levels of inclusion ranging from 0 to 75% of DDGS in the total DM. Except for feed conversion (linear effect, $P = 0.01$), authors reported a quadratic effect of increasing levels of DDGS
 Moreover, they found maximized ADG, dry matter intake, G:F, hot carcass weight, and final BW for heifers receiving 15% of DDGS. Furthermore, compared to the control diet (0% DDGS), all treatments improved myofibrillar tenderness and overall tenderness. However, 12th rib fat thickness decreased as the inclusion level of DDGS increased ($P = 0.05$). A meta-analysis of five experiments performed by Klopfenstein et al. (2008) revealed maximum ADG when DDGS was included between 20 to 30% of the dietary DM, whereas maximum G:F was achieved when including 10 to 20% of DDGS.

Corn gluten feed is a by-product of the wet corn milling industry that produces starch for ethanol or fructose for corn sweeteners (Ham et al., 1995). In a feedlot trial utilizing English crossbred yearling steers and heifers, these authors replaced dry-rolled corn by dry corn gluten feed (CGF). On a DM basis, diets contained 92.5% concentrate. CGF was present at 0% (control diet based on dry-rolled corn) or 70% (CGF-based diet). Although not statistically different, ADG was improved in animals receiving 70% CGF (1.51 vs. 1.45 kg/d). Dry matter intake was higher in the group of animals fed 70% CGF versus the ones fed 0% (13.37 vs. 11.57 kg/d). In contrast, G:F decreased. It went from 0.126 to 0.113 for animals in the control or 70% CGF groups, respectively. The authors concluded that animals fed 70% CGF were less efficient than the ones fed 0% CGF.

Concerning carcass characteristics, no statistical differences were observed in this trial ($P > 0.10$). A metabolism trial with steers conducted by Ham et al. (1994) included one treatment containing 77% of dry-rolled corn, and another treatment containing 37% of dry-rolled corn plus 40% CGF (DM basis). Although organic matter intake was not different between these treatments ($P > 0.10$), starch intake was lower (2.88 vs. 4.05 kg/d), and NDF intake was higher (1.87 vs. 0.99 kg/d) for the group receiving 40% of
DM as CGF. Ruminal pH and total VFA concentration were similar for both treatments ($P > 0.10$).

Although dried distillers grains and corn gluten feed can efficiently replace traditional feeds when properly used, they may contain high levels of sulfur. Elevated levels of this element may reduce dry matter intake and ADG, reduce liver Cu stores, and cause polioencephalomalacia (Klopfenstein et al., 2008). Therefore, the potential risk of high sulfur in the total diet may limit the level of inclusion of these by-products in ruminant diets. Another issue associated with the use of these feedstuffs is their elevated phosphorus level, which in turn may cause formation of urinary calculi in cattle, however, this problem can easily be minimized by supplementing additional calcium (Klopfenstein et al., 2008; Myer and Hersom, 2011).

**The Potential of Canola Meal and Sunflower Meal as Feeds**

Canola and sunflower meals are resulted from the oil extraction process of their corresponding seeds. These by-products are classified as protein supplements (Conrad et al., 1982). Patterson et al. (1999) stated that finding a low-cost, locally available protein supplement is critical in many production systems. These authors carried out a 2-yr study in which they reported mean CP values for canola and sunflower meal as 39.95% and 33.35%, respectively. They also reported ADF levels for both feedstuffs. Canola meal averaged 20.15% and sunflower meal 29.1% ADF considering the two years.

According to Bell (1993), canola meal typically contains less gross energy, less protein, and more fiber when compared to soybean meal. Nonetheless, canola meal is higher than SBM in most of the B-vitamins and essential minerals. Among other things, fiber, protein, and oil levels are factors that greatly influence canola meal metabolizable
energy content. These factors are influenced by variety and seed quality as well as by processing methods. Bell (1993) reported that the hull represents about 16% of the seed weight and about 30% of meal weight. Hull is largely fiber and all of it remains in the meal after oil extraction. Therefore, the relatively low ME values of canola meal are associated with its high level of fiber.

Considering that canola meal is a protein supplement, it is reasonable to compare its feeding value to soybean meal, which is widely used as source of protein in animal diets. Ponnampalam et al. (2005) compared the effects of supplementing small amounts of different protein sources to lambs fed a hay-based diet (supplementation with 7 to 7.8% of their daily DMI). These authors did not find statistical differences for the groups receiving canola meal or soybean meal when comparing traits like ADG and G:F \((P \geq 0.05)\). In comparison to the control group (only hay, no supplementation), total DMI was increased when animals were supplemented \((P < 0.001)\). The authors rationalized that part of this observed response may be attributed to improved fiber digestion in the rumen, resulting from increased availability of amino acids and ammonia for microbial growth.

Sunflower meal can be a practical choice for protein supplementation. Richardson et al. (1981) carried out three experiments to evaluate the nutritional value of sunflower meal as a protein supplement for growing ruminants. In one trial the authors compared the effects of isonitrogenously substituting cottonseed meal for sunflower meal in growing-finishing feedlot diets. They reported similar values for dry matter digestibility, \(N\) retention, and protein digestibility for both groups and came to the conclusion that sunflower meal can effectively replace cottonseed meal in growing-finishing diets. These findings are in alignment with Ahmad et al. (2004) who conducted a feeding trial with
growing heifers and reported similar daily weight gain and feed efficiency for heifers receiving sunflower meal or cottonseed cake as their major source of protein. Additionally, the authors reported that the sunflower meal-based diet had a lower cost for each kg increased in animals’ BW.

Although protein and energy levels in sunflower meal are lower than in canola and soybean meal (NRC, 2000) it has been shown that sunflower meal can substitute soybean meal without reducing animal performance. Titi (2003) studied the substitution of soybean meal by sunflower meal in young goat rations. No statistical differences were found ($P < 0.05$) regarding ADG, total gain, final weight, and F:G. The author inferred that sunflower meal could effectively substitute soybean meal as a protein source for animal feeding.

**In Vitro Dry Matter Digestibility**

*In vitro* experiments have been successfully used to simulate *in vivo* results. Besides being the more convenient option in many instances, this type of study is more easily replicated for a better understanding of ruminal behavior characteristics. In the ruminant nutrition field, features such as gas production, dry matter digestibility, and VFA concentrations have been frequently studied and measured using *in vitro* procedures.

*In vivo* digestibility is not a constant characteristic. It varies according to the level of feed intake, the manner in which the feed is prepared, and even from one animal to another (Tilley and Terry, 1963). Given these natural variations, prediction of dry matter digestibility as it would occur *in vivo* is challenging. However, since *in vivo* determination of digestibility involves a long metabolism trial and requires large
quantities of feeds, the *in vitro* technique may be a more feasible option. Measurement of *in vitro* dry matter digestibility (IVDMD) has been widely used to assess the nutritional quality of feeds. Furthermore, according to Getachew et al. (2004), there is a high correlation between IVDMD and *in vivo* digestibility.

The IVDMD of a given feed is closely related to its chemical composition. Getachew et al. (2004) working with samples of 12 different feedstuffs collected from commercial dairy farms found highest values of IVDMD for corn grain and soybean meal, and lowest values for corn and wheat silages. They also found that NDF was negatively correlated with IVDMD ($r = -0.77; P < 0.001$). In the same study, they verified that the best prediction equation for IVDMD was obtained with the inclusion of CP, fat, and non-fiber carbohydrate (NFC) in the regression model ($r^2 = 0.87$).

In a study testing different levels of wet distillers grains plus solubles (DG) in crossbred beef steers (15% or 30% of DG inclusion on a DM basis), Quinn et al. (2011) observed higher ADG, G:F, HCW, and calculated yield grade for steers on the 15% DG diet over the ones on the 30% DG diet ($P < 0.05$). However, when testing similar diets for IVDMD, these authors found non-significant differences between the inclusion levels of 15% or 30% DG in substrates. In contrast, Larraín et al. (2009) compared feedlot performance of steers finished with diets based on corn, high-tannin sorghum and a mix of both grains (all diets were mixed with an inclusion of 76.5% of one grain or the mix, DM basis). They observed reduced ADG, G:F, final BW, and HCW for steers fed the sorghum-based diet versus the ones fed the corn-based diet. When analyzing the IVDMD after a 48 hours incubation period, these authors found 88.5% for the sorghum and 90.9%
for the corn diet. The lower digestibility values possibly accounted in part for the observed differences in performance.

*Production of Methane*

Ruminants lose from 5 to 12% of their ingested energy as combustible gases, among which the primary gas is methane (Ferrell, 1988). Many factors influence methane emissions from cattle and include: the level of feed intake, type of carbohydrate in the diet, feed processing, addition of lipids or ionophores to the diet, and alterations in the ruminal microflora (Ferrell, 1988; Johnson and Johnson, 1995). The same authors state that apart from being an environmental problem, methane produced by beef cattle represents a waste of energy and thus, the development of management strategies to mitigate methane emissions from cattle are desirable. Besides the environmental contributions, these strategies may improve animal productivity and feed efficiency through enhanced utilization of dietary carbon.

Hart et al. (2009) carried out a trial utilizing low and high DM digestible forages. They found higher daily methane production ($P = 0.003$) for animals consuming the high DM digestible forage over the ones consuming low DM digestible forage (193 versus 138 g/day). However, they reported that methane produced per unit of DMI was statistically similar ($P = 0.95$) for both groups of animals (25.7 vs 25.6 g/kg of DMI). Furthermore, the predicted growth rate of cattle fed high DM digestible forage was greater than the ones fed low DM digestible forage (1.41 against 0.37 kg/day), resulting in a substantially quicker finish, and therefore, resulting in less overall lifetime methane emissions. Likewise, Monteny et al. (2006) proposed that one feasible strategy for methane
mitigation is to improve feed intake and feed composition, thus promoting greater animal performance.

Smith et al. (2010) conducted two in vitro experiments utilizing different levels of monensin ranging from 0 to 6 mg/L of culture volume. Both experiments revealed the same trend, which was a linear decrease in calculated methane production as monensin concentrations increased. Decreases in calculated methane production agree with the findings of Quinn et al. (2009), which reported a 17% decrease in methane production when comparing 0 vs. 5 mg/L of monensin added to the in vitro cultures.

Also acting as an important influencer in methane production is the available hydrogen level in the rumen, which is dependent on the molar proportions of VFA produced. Wolin and Miller (1988) suggested that if all carbohydrate is fermented to acetic acid and no propionic acid is produced, the energy lost as methane would be 33%. However, if the ratio of acetic:propionic acid is 0.5, the loss of substrate energy as methane would be 0%. In most cases, the ratio acetic:propionic acid varies from 0.9 to 4.0, and consequently, corresponding methane losses vary widely as well. Fermentation of cell wall fiber yields higher acetic:propionic acid ratios and therefore, higher methane losses are expected (Johnson and Johnson, 1995). In agreement with these authors, Ferrell (1988) stated that methane inhibitors usually induce a decrease in the acetate to propionate ratio.

**Production of Hydrogen Sulfide**

Due to higher availability, inclusion of distillers grains and corn gluten feed in ruminant diets has increased. These corn by-products often contain high concentrations of sulfur (Drewnoski et al., 2012). In the process of producing distillers grains plus solubles,
sulfuric acid is used for pH control and cleaning, resulting in sulfur levels of 0.6 to 1.0% or greater (Klopfenstein et al., 2008). Corn gluten feed may also have high sulfur levels since sulfur dioxide is added during the milling process to aid in the extraction of starch. Many geographic regions have ground water that is high in sulfate, and thus, even water consumption can contribute to excessive sulfur intake. Feedstuffs with high concentration of sulfur-containing amino acids can also provide significant amounts of sulfur. The NRC (2000) establishes 0.4% as the maximum tolerable concentration of sulfur in beef cattle diets.

Excessive intake of sulfur may cause overproduction of free sulfide in the rumen, which combined with hydrogen forms hydrogen sulfide (H$_2$S), a poisonous gas. While non-reduced forms of sulfur such as sulfate and elemental sulfur are relatively nontoxic, H$_2$S and its various ionic forms are highly toxic substances that interfere with cellular energy metabolism (Merck, 2010). Eructated H$_2$S might be inhaled and absorbed into the blood, potentially resulting in poor animal performance, metabolic anoxia, and sulfur-induced polioencephalomalacia (Smith et al., 2010; Kessler et al., 2012). Given that the central nervous system requires a high and continuous level of energy production, it might be significantly affected by energy deprivation. Polioencephalomalacia may cause blindness, recumbency, seizures, and coma. Animals that survive often manifest significant neurologic impairment that necessitates culling (Merck, 2010). These harmful symptoms have drawn attention and there is an undeniable demand for further research on H$_2$S metabolism.

Kung et al. (2000) suggested that interactions between methanogens and sulfate-reducing bacteria might lead to an increase in H$_2$S production when monensin was added
at 5 mg/L. However, Smith et al. (2010) reported no effects of monensin on in vitro hydrogen sulfide production when it was included at 0, 2, 4, and 6 mg/L of culture volume. In another in vitro study, Quinn et al. (2009) did not find any effect of ionophores (monensin sodium, lasalocid sodium, and laidlomycin propionate potassium) or antibiotics (chlortetracycline hydrochloride and tylosin tartrate) on the production of H₂S gas. Moreover, both Smith et al. (2010) and Quinn et al. (2009) reported very strong evidence that increasing the sulfur amount in the substrate leads to a linear increase in H₂S production, implying that dietary sulfur plays a major role. May et al. (2010) also found increased in vitro H₂S production per g of fermentable DM when the substrates contained higher concentrations of wet distillers grains with solubles, and consequently, higher sulfur concentrations. They suggested that the sulfur concentration of the substrate is likely the most important factor contributing to in vitro H₂S production.

Drewnoski et al. (2012) conducted an in vivo experiment and related greater ruminal H₂S concentrations in high sulfur-fed steers versus low sulfur-fed steers during the transition and finishing phases. However, in a previous period when steers were on a forage-based diet, the high sulfur and the low sulfur-fed groups had similar ruminal H₂S concentrations. These findings indicate that other factors beyond sulfur intake may contribute to concentrations of ruminal H₂S. Also, the level of roughage in the diet seems to have an important role. Kessler et al. (2012) studied if molybdenum supplementation could bind excess sulfur in the rumen of steers receiving high-S water, and thus, improve health and performance of these animals. However, they found that Mo did not effectively bind excess sulfur, resulting in even greater H₂S production, along with Mo toxicity signs.
Volatile Fatty Acids

Volatile fatty acids (VFA) are produced in the gastrointestinal tract mainly by microbial fermentation of carbohydrates. The amount of fiber in the diet seems to affect the amount of VFA produced, and thus being, the contribution of VFA to animal’s energy requirements are variable. However, estimates are that VFA contribute approximately 70% to the caloric requirements of ruminants (Bergman, 1990). Several studies have been conducted to determine other factors influencing VFA production. Drewnoski et al. (2012) found no significant differences on molar proportions and total VFA concentration ($P \geq 0.21$) when comparing different levels of sulfur in steer diets. May et al. (2010) tested different levels of inclusion of corn or sorghum wet distillers grains with solubles and did not report significant differences in VFA concentration and molar proportions. Smith et al. (2010) reported no differences on in vitro proportions of VFA, total VFA concentration, and acetate:propionate ratio when comparing substrates ranging from 0.2 to 0.8% of sulfur on a DM basis. However, when comparing inclusion levels of monensin ranging from 0 to 6 mg/L, these authors found decreasing concentrations of acetate, increasing rates of propionate, and decreasing acetate:propionate ratio, suggesting that, unlike sulfur, ionophores may influence VFA proportions. It has been reported that ruminal VFA concentrations are raised with increased forage digestibility (Park et al., 1994; Rinne et al., 1997). However, Hart et al. (2009) reported no differences in ruminal VFA concentration when comparing cannulated steers receiving either low or high dry matter digestible forages. In addition, these authors did not find any differences in VFA molar proportions.
CONCLUSIONS

Literature has shown that by-product feeds have potential to maintain animal performance and decrease feed cost. Animal response to dried distillers grains plus solubles and corn gluten feed in steers and heifers is well documented, however little is available for developing bulls. Additionally, data is limited on describing stocker cattle response to the replacement of soybean meal with canola and sunflower meals in silage-based stocker systems. The research that follows includes two experiments to address these issues.
LITERATURE CITED


http://quickstats.nass.usda.gov/results/054D529A-BB78-34C3-9572-225405DBC297


CHAPTER 3

UTILIZATION OF BY-PRODUCT FEEDS TO DECREASE FEED COST WHILE MAINTAINING PERFORMANCE OF YEARLING BEEF BULLS

ABSTRACT

In a two-year study, 58 yearling bulls (initial BW = 280 ± 31 kg) were individually fed for 90 d (YR 1) and 99 d (YR 2) to evaluate two by-product-based diets and a commercial diet. The diets were: 1) Commercial ration (COM); 2) Ration composed of 49% soybean hulls, 24.5% corn, 24.5% corn gluten feed and 2% minerals (CGF); or 3) Ration composed of 49% soybean hulls, 24.5% corn, 24.5% dried distillers grains plus solubles and 2% minerals (DDGS). Bulls were individually fed ad libitum using Calan gates system. On d 0, 49, and 90 (YR 1) or 99 (YR 2), BW were recorded, and, longissimus muscle area, 12th rib fat thickness, intramuscular fat, and rump fat thickness were assessed via ultrasound. Additionally, the diets were subjected to in vitro digestion to evaluate production of CH₄, H₂S, in vitro true dry matter digestibility (IVTDMD), NH₃-N, and VFA concentrations. Animal performance data showed that ADG was greater (P < 0.05) for bulls fed COM compared with CGF and DDGS (2.04, 1.83, and 1.82 kg/d, respectively). Feed conversion and DMI expressed as a function of the BW were similar for all treatments (P > 0.06). Predicted intramuscular fat was higher for COM and DDGS (P < 0.05) compared to CGF, whereas longissimus muscle area was similar across all treatments (P = 0.57). Twelfth rib fat thickness and rump fat thickness were both higher in the COM group (P < 0.01). Data from the in vitro tests revealed greater IVTDMD and H₂S production for CGF (P < 0.001). Total CH₄ production, CH₄ production per g of incubated DM and per g of fermented DM were all greater for the CGF and DDGS treatments (P < 0.01). Molar proportions of acetate, acetate to propionate ratio, and total VFA concentrations were higher for CGF and DDGS (P < 0.001). Molar proportion of propionate was significantly higher for COM (P < 0.01).
Data from this study also suggest that there is a linear relationship between dietary S levels and H$_2$S production. Moreover, production of CH$_4$ was greater for the CGF and DDGS treatments, which in turn represents higher energy lost as gas. However, considering that animal performance was minimally affected when using CGF and DDGS, and that the cost of gain was significantly reduced, utilization of these diets may be preferable over COM for developing bulls.

**Key words:** by-products, developing bulls, distillers grains, corn gluten feed, cost of gain, soybean hulls.
INTRODUCTION

Increasing prices of traditional feedstuffs such as corn and soybean meal have motivated many cattle producers to explore new strategies to minimize feed costs. Feed costs represent a large proportion of total cost associated with post-weaning development of cattle (Herd et al., 2003); especially for developing bulls for breeding stock. Concurrently, the use of by-product feeds such as dried distillers grains plus solubles and corn gluten feed are becoming more popular in all segments of cattle production (Ham et al., 1995; Depenbusch et al., 2009; Segers et al., 2013). However, data comparing these ingredients as replacements for traditional feeds in developing bull diets is limited.

The milling of corn to produce both corn gluten feed and dried distillers grains plus solubles results in reduced proportions of starch and increased proportions of fiber and protein. The fiber portion is highly digestible in both corn gluten feed and dried distillers grains (Weigel et al., 1997; Segers et al., 2013). Additionally, dried distillers grains plus solubles has a high level of RUP, even when compared with traditional protein supplements such as soybean meal (NRC, 2000). Although these by-products have potential as feed ingredients, they may contain high levels of S. Excessive intake of S may cause overproduction of free sulfide in the rumen, which combined with H forms hydrogen sulfide (H₂S), a poisonous gas. Eructated H₂S may be inhaled and absorbed, potentially resulting in poor animal performance, metabolic anoxia, and S-induced polioencephalomalacia (Merck, 2010; Smith et al., 2010; Kessler et al., 2012).

The objective of this study was to compare the use of two by-product-based rations to a commercial ration for developing bulls. Additionally, in order to better understand the digestion and metabolism of these rations, *in vitro* production of CH₄,
H₂S, ammonia nitrogen (NH₃-N), and VFA were quantified, along with the in vitro true dry matter digestibility (IVTDMD).

**MATERIALS AND METHODS**

All procedures involving animals were verified and approved by The University of Georgia’s Animal Care and Use Committee (OACU).

The research was divided into two experiments. Experiment 1 was a 2-yr feeding trial conducted during the fall and early winter of 2011-2012 (YR 1) and 2012-2013 (YR 2). The experiment was conducted at the University of Georgia Wilkins Beef Unit, located in Rayle, GA. Experiment 2 was an in vitro trial in which the same diets fed to bulls were used as substrates, and production of CH₄, NH₃-N, H₂S, VFA, and IVTDMD were quantified.

**Experiment 1**

**Animal and Diet Management.** A total of 58 yearling bulls were used (26 in the first and 32 in the second year). Animals were fed for 90 d in YR 1, and 99 d in YR 2. In both years, the feeding period began in October and ended in January. Because there were three different genetic groups (Limousin x Angus, Simmental x Angus, and pure Angus) animals were first stratified by breed, so that each treatment could be offered to similar groups of bulls. Following, animals were randomly assigned to one of three experimental diets: 1) a commercial ration composed of cottonseed hulls, corn, rolled oats, and soybean meal (COM); 2) a ration composed of soybean hulls, corn and corn gluten feed (CGF); 3) a ration composed of soybean hulls, corn and dried distillers grains plus solubles (DDGS). All rations had limestone and trace mineral mix added to their composition. They can be visualized in Table 3.1.
Animals were managed similarly across years. Within treatment, steers were randomly assigned to one of four pens for feeding. Concrete pens were 75 m², with automatic waterers and equipped with a system for control of individual feed intake – Calan Broadbent Feeding System (American Calan, Northwood, New Hampshire). The trough area was covered so rations were protected against precipitation. Animals were fed once a day and had free access to their individual ration throughout the day. Each morning at 0800 h, troughs were inspected to evaluate preceding day’s consumption. After, feed was offered to provide approximately 10% of excess based on the intake from previous day. Once a week, orts were individually weighed. The equipment utilized to weigh and distribute the diets was the Calan Super Data Ranger (American Calan, Northwood, New Hampshire). The same machine was used to remove and weigh the orts. Animals were allowed to adapt to this feeding system for 10 days prior to the experimental period. Manure was removed from all pens on a weekly basis, providing a clean area for the animals.

At the beginning of the trial, bulls were dewormed using injectable 1% ivermectin (Ivomec – Merial Limited, Duluth, GA), weighed (Toledo Scale – Toledo Scale Corporation, Toledo, Ohio) and assessed with ultrasound (Aloka SSD-500V – Hitachi Aloka Medical Ltd., Tokyo, Japan) for estimation of longissimus muscle area (LM), 12th rib fat thickness (FT), intramuscular fat (IMF), and rump fat thickness (RF). The weighing and ultrasound assessments were repeated on the 49th day and at the end of the experiment (90th day on the first year and 99th day on the second year). Measurements were taken in the morning, prior to feeding. Ultrasound assessments were made by a trained technician from the University of Georgia Meat Science Technology Center. The
left side of each animal was clipped to remove hair on the areas between the 12th and 13th ribs, as well as between the *tuba coxae* of the *ilium* and *tuba ischiadicum* of the *ischium* for image collection. Vegetable oil was spread on the clipped areas to assure quality on ultrasound wave transmission. For collection of LM and FT data, a wave guide was also used to adjust the probe to animals’ dorsum (Designer Genes Technologies Inc., Harrison, AR). Captured ultrasound images were analyzed using Beef Image Analysis Feedlot software (Designer Genes Technologies Inc., Harrison, AR).

**Statistical Analysis.** Analysis of variance for animal performance and ultrasound data were done using R (The R Foundation for Statistical Computing, Vienna, Austria) in a completely randomized design with three treatments. Each animal was considered as an experimental unit. Treatment, year, breed and lot were considered as fixed effects. Models included treatment x year interaction. For the ultrasound data, treatment, year, breed, lot and day were considered fixed effects. Interactions between day, year, and treatment also were analyzed. Orthogonal contrasts were tested using Tukey's honest significant difference test. A significance level of 0.05 was used.

**Experiment 2**

Representative samples of the experimental diets were separated by yr and subjected to *in vitro* digestion to evaluate production of CH$_4$, NH$_3$-N, H$_2$S, VFA, and the IVTDMD. *In vitro* procedures were performed at the University of Florida (North Florida Research and Education Center, Marianna, FL). Two ruminally cannulated steers were used as ruminal fluid donors for the incubations. They were fed an 85% concentrate diet for an average of 10 days prior to collection of ruminal fluid. After collection, ruminal fluid was transported to laboratory in thermic equipment. Approximately 15 min after
collection, 500 mL of ruminal fluid from each donor were strained through cheesecloth and composited. Following, 2 L of McDougall’s artificial saliva were added to the ruminal fluid blend, resulting in a 2:1 ratio of saliva:rumen fluid (the inoculum). McDougall’s artificial saliva was prepared replacing magnesium sulfate with magnesium chloride to produce a solution without sulfur. Substrate diets were ground to pass a 2 mm screen using a Model 4 Wiley Mill (Thomas Scientific, Swedesboro, NJ). Approximately 1.4 g was placed into 250 mL glass bottles and 100 mL of inoculum was added. Carbon dioxide was flushed over the blend during the mixing and inoculation processes to maintain an anaerobic environment.

In Vitro Incubations

Incubations were conducted at 39°C for 24 h in an oscillating incubator (Sheldon Manufacturing, Inc., Cornelius, OR). Duplicate bottles were used per each treatment. One set of bottles contained the inoculum but no substrate (blanks). Incubations were performed on three separate days (replicates). Fourteen 250 mL bottles were used on each replicate day. Tedlar bags (1 L capacity, Sigma-Aldrich, St. Louis, MO) were attached to the bottles for collection of fermentation gases. After 24 h of incubation, they were detached from the bottles and submitted to analysis of their content. Bottles had their pH measured and fermentation was stopped by addition of 1 mL of 20% (v/v) H2SO4. Two samples were collected from each bottle and immediately frozen at -20°C for subsequent VFA and NH3-N analyses.

Ammonia Nitrogen and VFA Analyses

Frozen subsamples were allowed to thaw prior to analysis for NH3-N and VFA. Ammonia nitrogen analysis was made by adding 20 μL of sample into 12 x 75 mm
disposable culture tubes, along with 1 mL of phenol reagent and 0.8 mL of hypochlorite reagent (Broderick and Kang, 1980). Tubes were covered and placed in a water bath for 5 min at 95°C. After cooling, duplicated samples of 200 μL were taken from each tube and transferred into a flat-bottom 96-well plate, and absorbance was measured. Measurements were done at 620 nm using a model AD 340 spectrophotometer (Beckman Coulter, Inc. Indianapolis, IN). For VFA analyses, samples were strained and centrifuged at 10,000 x g for 10 min. Then, 5 mL of supernatant were mixed with 1 mL of meta-phosphoric acid-2 ethyl butyrate solution. Tubes were placed in an ice bath for 30 min and centrifuged for 10 min at 10,000 x g. Supernatant was removed and analyzed by gas chromatography on Agilent 7820A GC System (Agilent Technologies, Inc., Santa Clara, CA).

**Methane Analyses**

Analysis of CH₄ concentration in the tedlar bags was performed using gas chromatography (Agilent 7820A GC; Agilent Technologies, Inc., Santa Clara, CA). Total gas produced in 24 h, bottle headspace, and bag volume corrected by lab temperature were used in calculation of CH₄ concentration. Gas production was obtained using cumulative pressure data over the 24-h incubation period (Ankom Technology, Macedon, NY).

**Hydrogen Sulfide Analyses**

Hydrogen sulfide concentrations were quantified by sampling 5 mL of the gas present in the tedlar bags and bubbling it into a vacutainer containing a solution of 5 mL of alkaline water (prepared by adding 0.1 N NaOH to deionized water to reach a pH of 8.5 – 9.0), 0.5 mL of N,N-dimethyl-p-phenylenediamine sulfate, and 0.5 mL of ferric chloride reagent. A reaction time of 30 min at 25°C was allowed, then, the absorbance
was read in a spectrophotometer at 665 nm (DU 530 Spectrophotometer - Beckman Coulter, Inc. Indianapolis, IN). Hydrogen sulfide concentration was calculated comparing the absorbance values from the samples to standards of known concentration, applying linear regression and correcting by the blank values. Final calculations adjusted the H$_2$S produced by the total gas volume and by the amount of fermentable DM incubated.

**In Vitro True Dry Matter Digestibility**

In order to assess IVTDMD, a separate incubation was carried out. Twelve 125-mL plastic scintillation flasks were prepared with 0.7 g of substrate and 50 mL of inoculum. Two flasks were incubated with only the inoculum (blanks) and two other flasks were included for determination of ruminal fluid DM. Carbon dioxide was flushed while the inoculum was being added to maintain anaerobic conditions. Flasks were capped with rubber stoppers with a 16-gauge needle for gas release, and incubated at 39°C for 24 h under agitation at 60 rpm (Sheldon Manufacturing, Inc., Cornelius, OR). After 24 h, flasks were removed from the incubator and frozen at -20°C. Seven days later, they were freeze dried at -50°C for 36 h (FreeZone 6 Liter Console Freeze Dry System – Labconco Corporation, Kansas City, MO). Following, they were placed in a drying oven for 16 h at 100°C (Thermo Fisher Scientific Inc., Waltham, MA). The IVTDMD was obtained by difference between initial and final incubated DM mass, corrected by blanks and ruminal fluid DM contribution.

**Statistical Analysis.** Analyses of *in vitro* data were conducted using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Bottles or flasks were considered the experimental unit. Replicate day was considered as random effect. Both treatment and
year were considered as fixed effects. Interaction between treatment and year were also included in the model. Differences were considered significant at $\alpha < 0.05$.

**RESULTS AND DISCUSSION**

*Experiment 1*

Animal performance data revealed highest ADG for bulls fed COM ($P = 0.03$) compared to those fed CGF or DDGS treatments (2.04, 1.83, and 1.82 kg/d, respectively). However, the additional gain in the COM group was associated with more fat deposition instead of lean tissue. This is supported by the fact that LM was similar across treatments ($P = 0.57$), and that bulls eating COM had greater FT and RF ($P < 0.01$). Coulter and Kozub (1984) found that excessive gains may reduce reproductive potential of bulls. Therefore, assuming that a leaner growth is preferable for developing bulls, higher gains based on increased fat deposition might not be desirable. Ultrasound assessment also revealed that IMF was higher in COM and DDGS groups ($P = 0.01$; Table 3.3).

Although differences were observed between treatments in the current study, the ADG across treatments was higher than those previously reported on corn by-products. Klopfenstein et al. (2008) conducted a meta-analysis of 5 trials and reported gains ranging from 1.69 to 1.70 kg/d when feeding diets containing 20% and 30% of dried distillers grains plus solubles, respectively. In the present study, the percentage of inclusion of this by-product was 24.5% in the DDGS treatment (Table 3.1). Similarly, Ham et al. (1995) reported that animals receiving a treatment containing corn gluten feed gained 1.51 kg/d, whereas, findings from the present study showed that animals in the CGF group gained 1.83 kg/d. However, the percentage of inclusion of corn gluten feed in that mentioned study was 70%, whereas, in our study it was 24.5%. Additionally, the fact
that our experiment was conducted used yearling bulls may explain part of these mentioned differences, since the other studies used heifers and steers.

Total DMI was highest for COM and CGF ($P = 0.02$); however, no differences were observed when DMI was expressed as a function of the BW ($P = 0.06$). Feed efficiency, expressed as G:F, was similar across treatments ($P = 0.62$). This finding is in contrast with the ones reported by Segers et al. (2013). These authors fed either corn gluten feed, dried distillers grains plus solubles, or a mix of corn-soybean meal to stocker animals at 25% of their DMI. They found that G:F was lowest in animals supplemented with corn gluten feed ($P = 0.01$).

The costs of the diets were calculated considering local market prices for each ingredient. Two-yr average cost per metric ton were $345.32, $304.08 and $298.68 for COM, DDGS and CGF, respectively. These prices, along with DMI and G:F, were used to calculate the cost to gain 1 kg of BW (cost of gain). Table 3.2 shows that the cost of gain was 13% less when animals were fed DDGS, compared to COM. Furthermore, it shows that the cost of gain was significantly lower for CGF and DDGS ($P = 0.003$).

**Experiment 2**

*In vitro* true dry matter digestibility was highest for CGF and lowest for COM ($P < 0.001$; Table 3.4). The IVTDMD procedure differs from the traditional *in vitro* digestion (Tilley and Terry, 1963) because it does not include the pepsin digestion stage. Therefore, the IVTDMD procedure resembles the ruminal digestion process. Thus, the highest IVTDMD for CGF is indicative that this diet was more fermented in the rumen than the other diets. Moreover, the lowest IVTDMD for COM may partially explain the higher ADG observed for this group. This would suggest that more compounds from this
diet might have escaped ruminal fermentation and reached the small intestine in these animals. Total VFA production was inferior for the COM treatment ($P < 0.0001$). Since VFA are the primary end products of fermentation in the rumen (Sharp et al., 1982), this finding is in harmony with the IVTDMD values, implying that less ruminal fermentation occurred for this treatment.

Molar proportions of VFA and A:P ratio showed distinct fermentation patterns for the diets (Table 3.4). Fermentation from the COM diet yielded the highest molar proportion of propionate ($P = 0.002$), whereas DDGS and CGF yielded the highest proportion of acetate ($P < 0.0001$) and the highest A:P ratio ($P < 0.001$). Johnson and Johnson (1995) found that fermentation of cell wall fiber yields higher A:P ratio. So, the findings from the present study are indicative that both DDGS and CGF were diets with higher fiber content than COM. And this was confirmed by NDF and ADF analyses, (Table 3.1).

In vitro data also showed that production of CH$_4$ (mM/g of Fermented DM) was lowest when COM was used as substrate ($P = 0.007$; Table 3.5). According to Ferrell (1988) ruminants lose from 5 to 12% of their ingested energy as combustible gases, among which the main one is CH$_4$. In agreement with this author, McGinn et al. (2004) conducted an experiment where steers were placed in chambers for measurements of gas emissions. They reported that approximately 6.5% of the GE consumed was lost in the form of CH$_4$ emissions. Therefore, a reduced loss of energy due to inferior CH$_4$ production further explains the higher ADG observed in bulls on the COM treatment.

Ruminal H$_2$S production is dependent on the sulfur content of the diet. Quinn et al. (2009) and Smith et al. (2010) reported very strong evidence that an increase in the
amount of sulfur in the substrate leads to increased H$_2$S productions. In the current study, laboratory analysis showed that sulfur content increased for the by-product-based diets (Table 3.1). This resulted in highest production of H$_2$S from the CGF diet, and lowest from the COM diet ($P < 0.0001$). Therefore, data from the present study is in agreement with the findings of Quinn et al. (2009), May et al. (2010), and Smith et al. (2010). Despite the fact that CGF generated greater H$_2$S production than the other treatments, no clinical signs of intoxication by this gas were observed in bulls receiving this treatment. The NRC (2000) establishes 0.4% as the maximum tolerable concentration of sulfur in beef cattle diets. So, the absence of intoxication symptoms is in alignment with NRC guidelines, since the maximum concentration of S found was 0.29% of the DM.

**CONCLUSIONS AND IMPLICATIONS**

In conclusion, results from this study showed an overall harmony between *in vitro* and *in vivo* findings. *In vitro* data provided support and helped to explain some of the animal performance results. Furthermore, our findings give further support to the perception that dietary levels of sulfur are strictly correlated with ruminal production of H$_2$S.

Even though the COM treatment yielded slightly higher ADG, estimation of carcass traits revealed that animals consuming either DDGS or CGF had a leaner growth, more desirable for developing bulls. Moreover, important traits like DMI as a percentage of BW, feed conversion, and longissimus muscle area were not affected by the treatments. In addition, the by-product-based diets were able to maintain adequate level of performance while significantly reducing the cost of gain. Therefore, results from this
study have demonstrated that the replacement of COM with DDGS or CGF is a viable alternative for developing bulls.


Table 3.1. Composition and chemical analysis of COM, DDG, and CGF diets fed to yearling bulls\(^1\)

<table>
<thead>
<tr>
<th>Ingredient, % of inclusion (DM basis)</th>
<th>Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COM</td>
</tr>
<tr>
<td>Cottonseed hulls</td>
<td>25.0</td>
</tr>
<tr>
<td>Soybean hulls</td>
<td>-</td>
</tr>
<tr>
<td>Ground corn</td>
<td>43.9</td>
</tr>
<tr>
<td>Rolled oats</td>
<td>16.8</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>12.1</td>
</tr>
<tr>
<td>Corn gluten feed</td>
<td>-</td>
</tr>
<tr>
<td>Dried distillers grains plus solubles</td>
<td>-</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.7</td>
</tr>
<tr>
<td>Trace mineral mix</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Analyzed nutrient content(^2)</strong></td>
<td></td>
</tr>
<tr>
<td>Dry matter percentage</td>
<td>88.7</td>
</tr>
<tr>
<td>Crude protein, % of DM</td>
<td>13.8</td>
</tr>
<tr>
<td>Neutral detergent fiber, % of DM</td>
<td>31.6</td>
</tr>
<tr>
<td>Acid detergent fiber, % of DM</td>
<td>19.5</td>
</tr>
<tr>
<td>Calcium, % of DM</td>
<td>0.95</td>
</tr>
<tr>
<td>Phosphorus, % of DM</td>
<td>0.38</td>
</tr>
<tr>
<td>Sulfur, % of DM</td>
<td>0.19</td>
</tr>
<tr>
<td>Cost, $/metric ton(^3)</td>
<td>345.32</td>
</tr>
</tbody>
</table>

\(^1\)COM = commercial diet; DDGS = by-product-based diet containing dried distillers grains plus solubles; CGF = by-product-based diet containing corn gluten feed.

\(^2\)Cumberland Valley Analytical Services, Hagerstown, MD. Average of two years.

\(^3\)Average of the two years.
### Table 3.2. Performance of yearling bulls receiving the experimental diets

<table>
<thead>
<tr>
<th>Item</th>
<th>COM</th>
<th>DDGS</th>
<th>CGF</th>
<th>Pooled SE²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>20</td>
<td>18</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial BW, kg</td>
<td>275.6</td>
<td>275.6</td>
<td>288.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final BW, kg</td>
<td>469.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>449.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>461.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.1</td>
<td>0.03</td>
</tr>
<tr>
<td>Average daily gain, kg</td>
<td>2.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.06</td>
<td>0.03</td>
</tr>
<tr>
<td>Dry matter intake, kg/d</td>
<td>9.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.41&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.23</td>
<td>0.02</td>
</tr>
<tr>
<td>Dry matter intake, g/kg BW</td>
<td>26.4</td>
<td>24.7</td>
<td>25.1</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>Gain:feed, kg</td>
<td>0.21</td>
<td>0.20</td>
<td>0.20</td>
<td>0.01</td>
<td>0.62</td>
</tr>
<tr>
<td>Cost of BW gain, $/kg of BW gain</td>
<td>1.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.05</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

<sup>a</sup>Means within a row with different superscripts differ (P < 0.05).

<sup>1</sup>COM = commercial diet; DDGS = by-product-based diet containing dried distillers grains plus solubles; CGF = by-product-based diet containing corn gluten feed.

<sup>2</sup>Standard Errors are based on unequal numbers of bulls per treatment (18 for DDGS, and 20 for COM and CGF).
Table 3.3. Ultrasound data for yearling bulls receiving the experimental diets

<table>
<thead>
<tr>
<th>Item</th>
<th>COM</th>
<th>DDGS</th>
<th>CGF</th>
<th>Pooled SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longissimus muscle area, cm²</td>
<td>65.82</td>
<td>65.39</td>
<td>66.20</td>
<td>0.89</td>
<td>0.57</td>
</tr>
<tr>
<td>12th rib fat thickness, cm</td>
<td>0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Rump fat thickness, cm</td>
<td>0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Intramuscular fat, %</td>
<td>3.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.97&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.05</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means within a row with different superscripts differ ($P < 0.05$).

<sup>1</sup>COM = commercial diet; DDGS = by-product-based diet containing dried distillers grains plus solubles; CGF = by-product-based diet containing corn gluten feed.

<sup>2</sup>Standard Errors are based on unequal numbers of bulls per treatment (18 for DDGS, and 20 for COM and CGF).
Table 3.4. Molar proportions of VFA (mol/100 mol), acetate:propionate ratio (A:P), total VFA concentration (mM), and IVTDMD after 24 hours of *in vitro* fermentation

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment¹</th>
<th>COM</th>
<th>DDGS</th>
<th>CGF</th>
<th>SE²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td></td>
<td>55.8b</td>
<td>60.7a</td>
<td>60.6a</td>
<td>0.50</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Propionate</td>
<td></td>
<td>25.5a</td>
<td>24.0b</td>
<td>23.6b</td>
<td>0.30</td>
<td>0.002</td>
</tr>
<tr>
<td>Butyrate</td>
<td></td>
<td>14.8a</td>
<td>11.4b</td>
<td>12.0b</td>
<td>0.23</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Valerate</td>
<td></td>
<td>2.9</td>
<td>2.8</td>
<td>2.8</td>
<td>0.08</td>
<td>0.52</td>
</tr>
<tr>
<td>Isovalerate</td>
<td></td>
<td>0.05</td>
<td>0.14</td>
<td>0.11</td>
<td>0.06</td>
<td>0.56</td>
</tr>
<tr>
<td>Isovalerate + 2-Methyl butyrate</td>
<td></td>
<td>0.96</td>
<td>0.94</td>
<td>0.85</td>
<td>0.05</td>
<td>0.33</td>
</tr>
<tr>
<td>A:P</td>
<td></td>
<td>2.20b</td>
<td>2.53a</td>
<td>2.58a</td>
<td>0.05</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total VFA</td>
<td></td>
<td>108.7b</td>
<td>132.0a</td>
<td>130.2a</td>
<td>1.83</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>IVTDMD, %</td>
<td></td>
<td>69.9c</td>
<td>72.6b</td>
<td>74.5a</td>
<td>0.58</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

¹Means within a row with different superscripts differ (*P* < 0.05).

¹COM = commercial diet; DDGS = by-product-based diet containing dried distillers grains plus solubles; CGF = by-product-based diet containing corn gluten feed.

²Standard error of the main-effect means.
Table 3.5. Percentage of S, H$_2$S production, CH$_4$ production, final pH, and NH$_3$-N concentration of the experimental diets after 24 hours of \textit{in vitro} fermentation

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment$^1$</th>
<th>COM</th>
<th>DDGS</th>
<th>CGF</th>
<th>SE$^2$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$S, $\mu$mol/g of fermented DM</td>
<td></td>
<td>10.10$^c$</td>
<td>14.75$^b$</td>
<td>18.63$^a$</td>
<td>0.79</td>
<td>$&lt; 0.0001$</td>
</tr>
<tr>
<td>Total CH$_4$, mM</td>
<td></td>
<td>2.09$^b$</td>
<td>2.53$^a$</td>
<td>2.73$^a$</td>
<td>0.12</td>
<td>0.009</td>
</tr>
<tr>
<td>CH$_4$, mM/g of incubated DM</td>
<td></td>
<td>0.97$^b$</td>
<td>1.24$^a$</td>
<td>1.34$^a$</td>
<td>0.05</td>
<td>0.001</td>
</tr>
<tr>
<td>CH$_4$, mM/g of fermented DM</td>
<td></td>
<td>1.39$^b$</td>
<td>1.71$^a$</td>
<td>1.80$^a$</td>
<td>0.07</td>
<td>0.007</td>
</tr>
<tr>
<td>pH after 24 h of incubation</td>
<td></td>
<td>6.3$^a$</td>
<td>6.0$^b$</td>
<td>6.0$^b$</td>
<td>0.01</td>
<td>$&lt; 0.0001$</td>
</tr>
<tr>
<td>NH$_3$-N, mM</td>
<td></td>
<td>0.80$^a$</td>
<td>0.55$^c$</td>
<td>0.60$^b$</td>
<td>0.01</td>
<td>$&lt; 0.0001$</td>
</tr>
</tbody>
</table>

$^{a-c}$Means within a row with different superscripts differ ($P < 0.05$).

$^1$COM = commercial diet; DDGS = by-product-based diet containing dried distillers grains plus solubles; CGF = by-product-based diet containing corn gluten feed.

$^2$Standard error of the main-effect means.
CHAPTER 4

UTILIZATION OF CANOLA AND SUNFLOWER MEALS AS REPLACEMENTS FOR SOYBEAN MEAL IN A CORN SILAGE-BASED STOCKER OPERATION

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ABSTRACT

Two experiments were conducted to evaluate 3 stocker diets composed mainly of corn silage. In the first experiment, diets were fed to a total of 276 animals in a period of 3 yr. In the second experiment, these diets were subjected to *in vitro* digestion for 5 distinct lengths: 0, 6, 12, 24, and 48 h, to evaluate IVDMD, production of fermentation end products, and efficiency of transformation of energy. In a DM basis, the experimental diets were composed of: 1) 74% corn silage, 15.2% ground ear corn, and 10.8% soybean meal (SBM); 2) 74.4% corn silage, 9.8% ground ear corn, and 15.8% canola meal (CAN); 3) 74.5% corn silage, 9.8% ground ear corn, and 15.7% sunflower meal (SUN).

Results from the first experiment showed that DMI was similar across all treatments (*P* = 0.167), whereas, ADG was greatest (*P* = 0.007) for animals fed either SBM or CAN, followed by the ones fed SUN (1.29, 1.28, and 1.20 kg/d, respectively). Both CAN and SUN significantly reduced daily feeding cost per animal in comparison to SBM (*P* < 0.001). Data from the second experiment revealed that total VFA production was similar for all treatments (*P* = 0.185), and greatest molar proportions of propionate were observed for SBM and CAN (*P* = 0.02). Additionally, IVDMD was highest for SBM (*P* < 0.001). Regression analysis showed that most of the evaluated traits followed a quadratic trend for incubation times (*P* ≤ 0.02). On average, the *in vitro* technique used was able to account for 97.03% of the caloric transformations suffered by DE throughout the different incubation times. Overall, our findings revealed that although animals receiving SUN had the cheapest daily feeding cost, important traits like final BW, ADG and feed conversion were compromised in this group of animals. In contrast, data showed that CAN was an effective replacer for SBM. This alternative diet was able to maintain
similar animal performance while decreasing daily feeding cost per animal. Therefore, from a producer standpoint, CAN is a viable alternative to replace the more costly diet SBM in stocker operations.

**Key words:** canola meal, corn silage, cost of gain, efficiency of conversion of DE, stockers, sunflower meal.
INTRODUCTION

Recent changes in market have created a new scenario for beef cattle producers. Feeds traditionally used in beef cattle operations have reached unprecedented prices in the last recent years. The national average price of corn grain has increased 170% between 2006 and 2013. The same trend was observed for prices of soybeans, since they have risen 150% in the same period (USDA National Agricultural Statistics Service, 2014). Given these circumstances, to assure profitability, research on alternative feeds is a real need. Ideally, alternative feeds must be able to reduce costs while yielding similar or even improved animal performance (Segers et al., 2013).

Canola and sunflower meals are by-product feeds classified as protein supplements (Conrad et al., 1982). They are both resulted from the oil extraction process of their respective seeds. Canola meal typically contains less gross energy, less protein, and more fiber when compared to soybean meal. Nonetheless, it is higher than soybean meal in several essential vitamins and minerals (Bell, 1993).

The nutritional value of sunflower meal is greatly influenced by the amount of hull in its composition. The proportion of the hull removed before processing differs among processing facilities. In some cases, a portion of the hulls may be added back to the meal after crushing. This variation in processing may result in meals ranging from 25% to over 40% of CP for products containing all the hulls or completely dehulled, respectively (Anderson and Lardy, 2012).

The purpose of this study was to evaluate the replacement of soybean meal with canola or sunflower meals as protein supplements in a corn silage-based stocker operation. Additionally, in order to evaluate the digestibility and formation of end
products related with feed efficiency, \textit{in vitro} assays were performed on the experimental diets.

**MATERIALS AND METHODS**

All practices and procedures used in this study were examined and approved by the University of Georgia Animal Care and Use Committee.

The research was divided in two experiments. The first experiment was a feeding trial conducted for 3 yr: 2010 to 2011 (\textbf{YR1}); 2011 to 2012 (\textbf{YR2}); and 2012 to 2013 (\textbf{YR3}). A total of 276 stocker animals were fed the experimental treatments for periods of 84 d. The second experiment was an \textit{in vitro} assay. The same treatments offered to animals in the feeding trial were incubated for up to 48 h for assessment of digestion, formation of gases, and transformation of dietary energy. Both experiments are further described hereafter.

**Experiment 1**

A 3-yr feeding trial using weanling stocker animals was conducted at the Georgia Mountain Research and Education Center, located in Blairsville, GA. Over the 3 years, a total of 276 animals were used: 93 in YR1, 93 in YR2, and 90 in YR3. On each year, animals were fed for a period of 84 d, which lasted from December through March.

**Animal and Diet Management.** Prior to the experimental period, animals were preconditioned for 55 d. They were delivered at the research station on the third week of October, when they were treated for parasites using transdermal ivermectin (Durvet, Inc., Blue Springs, MO). Animals were then backgrounded approximately 55 d with supplementation of ground ear corn at 4.5 g/kg of BW, and grazed stockpiled tall fescue \textit{(Festuca arundinacea} cv Kentucky 31) and orchard grass \textit{(Dactylis glomerata)}. 
After the backgrounding phase, animals were weighed (initial BW = 285 ± 9.7 kg), stratified by weight, and assigned to an experimental pen. A total of 9 pens were used. Each pen was approximately 4 ha and composed of dormant grasses, which had been previously mowed to ground level to ensure animals would not graze during the experimental period. Each pen was randomly assigned to 1 of 3 treatments, thus, each treatment was offered in 3 different pens. On a DM basis, the experimental treatments were formulated to contain: 1) 75% corn silage, 15% ground ear corn, and 10% soybean meal (SBM); 2) 75% corn silage, 10% ground ear corn, and 15% canola meal (CAN); 3) 75% corn silage, 10% ground ear corn, and 15% sunflower meal (SUN). Due to variation in the DM contents, the actual formulation that animals received was slightly different (Table 4.1). Each day, troughs were inspected at 0800 h to evaluate feed consumption from the previous day. Following, diets were weighed, mixed, and offered to provide approximately 110% the previous day’s estimated DMI. Feed was placed in fence-line concrete troughs that were covered to protect from precipitation and allow at least 41 cm of linear bunk space per animal. Animals had free access to water and a commercial mineral throughout the experiment (12% Ca, 9% P, 24% NaCl, 0.20% K, 0.30% Mg, 0.20% S, 50 mg/kg Co, 150 mg/kg Cu, 70 mg/kg I, 1000 mg/kg Mn, 1750 mg/kg Zn, 1000 mg/kg Fe). Samples from the experimental diets were submitted for chemical analysis at The University of Georgia Feed and Environmental Water Laboratory, located in Athens, GA (Table 4.1).

**Animal Performance.** In order to evaluate animal performance, animals were weighed on d 0 (initial BW) and after every 28 d, until the end of the experiment on d 84 (final BW). Both initial and final BW were assessed on 2 consecutive days and the value
for these measurements was the average of the 2 days. Animals were weighed in the morning, before being fed. The cost to gain 1 kg of BW (or cost of gain) was calculated assuming local prices for feedstuffs in each yr, along with DMI and ADG data.

**Statistical Analysis.** Analysis of variance for animal performance was done with the software R (The R Foundation for Statistical Computing, Vienna, Austria) in a completely randomized design with three treatments (SBM, CAN, and SUN). Pens were considered as the experimental units. Treatment, year, and pen were considered as fixed effects. Initial BW was used as a covariate for analyses of final BW and DMI. The model also included a treatment x year interaction. Orthogonal contrasts were tested using Tukey's honest significant difference test. A significance level of 0.05 was considered.

**Experiment 2**

The diets used in experiment 1 were subjected to *in vitro* digestion to elucidate potential mechanisms caused by the dietary treatments affecting animal performance. Samples from the experimental diets were collected on YR1, YR2, and YR3. These samples were composited across year within diet and used as substrate for the *in vitro* fermentations.

**Substrate Preparation.** Substrates were dried to constant weight (50°C for 72 h) for determination of DM. Afterwards, they were allowed to equilibrate their moisture content by attaining ambient humidity for 24 h. Then, substrates were re-weighed to determine their air-equilibrated DM content. Next, they were ground to pass a 2 mm screen using a Model 4 Wiley Mill (Thomas Scientific, Swedesboro, NJ). Once ground, 1.5 g of the substrate-diets were placed into septum bottles along with 67 ml of McDougall’s buffer. Thirty 160-ml septum bottles were used for the incubations of
substrate, plus, 10 bottles were used as blanks. After these procedures, all bottles were placed in a water bath incubator (Blue M Electric Company, Blue Island, IL) at 39°C overnight, prior to inoculation with rumen fluid in the following morning.

**Inoculation Process.** Ruminal fluid was collected from three lactating dairy cows prior to their morning feeding. Approximately 750 ml was collected from each animal, placed in a sealed thermos and transported to the laboratory. Ruminal fluid from each cow was strained through a 500-micron nylon mesh to remove feed particles and then combined into one mixture. The mixture was placed in a water bath at 39°C and CO₂ was bubbled into it. Following, each of the 160-ml septum bottles received 33 ml of the processed ruminal fluid. Since each bottle had received 67 ml of McDougall’s buffer in the previous day, the achieved ratio of buffer to rumen fluid was 2:1. Bottles were gassed with CO₂, sealed with rubber stoppers, and placed in a water bath incubator at 39°C. Incubations times included: 0, 6, 12, 24, and 48 h post inoculation.

**Collection and Analysis of Gases.** Collection of fermentation gases were conducted at 3-h intervals using a water displacement method. This method consisted of inserting 22-gauge needles into the incubation bottle and into a water-filled bottle connected to a three-way valve and a 60-ml syringe. During gas measurement, the valve was directed to allow gas to flow from the incubation bottle to the 60-ml syringe. Gas pressure moved the syringe plunger until pressure was equilibrated. The incubation bottle was swirled to allow gas bubbles to escape. Once the plunger stopped moving during swirling of the incubation septum bottle, gas pressure was equilibrated and the syringe reading was recorded. The valve was then turned to direct the collected gas into the water-filled bottle. During this procedure, an extra 22-gauge needle was inserted into the
water-filled bottle to allow displacement of water and capture of gas inside the bottle. The apparatus was then disconnected and the water displacement bottle was stored upside down until analysis of its gas content. Compositional analyses were performed for CO₂ and CH₄ using the SRI 310C gas chromatograph (SRI Instruments, Torrance, CA).

**VFA, Ammonia Nitrogen and Digestibility Analyses.** After the final gas collection at the end of the incubation periods, incubation bottles were opened. The content from each bottle was transferred to Nalgene bottles and frozen at -20°C to stop fermentation and be stored until further analysis. Several days later, the frozen bottles were thawed and their content was separated into liquid and particulate fractions by centrifuging at 1,400 x g for 10 min using a C-6000 centrifuge (International Equipment Company, Needham Heights, MA). The liquid fraction was further prepared for analysis of VFA and NH₃-N. Preparation included freezing the liquid at -20°C for 3 d, thawing, and centrifuging it at 1,400 x g for 15 min. Then, 5 ml of the supernatant was pipetted into 15-ml tubes. A 25% metaphosphoric acid solution was prepared and 1 ml was added to the tubes. Tubes were sealed, mixed with a vortexer, and frozen at -20°C for 3 d. Once thawed, they were centrifuged at 1,400 x g for 20 min, and 1.5 ml of the supernatant was transferred into vials for analysis of VFA on a model 3400 gas chromatograph (Varian, Inc., Santa Clara, CA). Analysis of NH₃-N was done according to the procedure described by Broderick and Kang (1980). Samples were analyzed on a Beckman DU-600 spectrophotometer at 620 nm (Beckman Coulter, Inc., Brea, CA).

The particulate fraction, composed mainly of undigested substrate and microbial organic matter, was dried in a forced-air oven at 55°C until constant weight for determination of DM (Blue M Electric Company, Blue Island, IL). Digestibility was
calculated by difference between the original and residual weights. Following, the dried residues were subjected to GE analysis in a Parr 1261 bomb calorimeter (Parr Instrument Company, Moline, IL). Similarly, samples of the substrate-diets were dried in a forced-air oven at 55°C until constant weight and subjected to GE analysis.

Through the use of standard caloric coefficients, the quantities of VFA and CH$_4$ produced *in vitro* were converted to their equivalents in calories. This information allowed estimation of the extent that both VFA and CH$_4$ contributed to apparent and *in vitro* DE.

**Statistical Analysis.** Analysis of variance was performed to verify the effects of treatment, incubation length, and their interactions on the fermentation traits. Contrasts were compared using Tukey's honest significant difference test. Differences were considered significant at $\alpha < 0.05$. Additionally, regression analysis was performed to evaluate the trends that fermentation traits followed over the incubation times. Traits were tested for both linear and quadratic effects. Analyses were performed using the software R (The R Foundation for Statistical Computing, Vienna, Austria).

**RESULTS AND DISCUSSION**

**Performance Data**

Animal performance data showed that there was no effect of the experimental treatments on DMI, either when expressed in kg/d or when expressed as a percentage of the BW ($P \geq 0.087$; Table 4.2). Regarding development and growth, data revealed that initial BW was similar for all treatments ($P = 0.56$), whereas, final BW was greatest in the SBM and CAN groups ($P = 0.009$). Average daily gain was also highest ($P = 0.007$) for both SBM and CAN treatments, and it was lowest for SUN (1.29, 1.28, and 1.20 kg/d,
respectively). Similar ADG in ruminants fed soybean meal or canola meal as protein supplements have been reported by Rule et al. (1994) and Ponnampalam et al. (2004). However, findings from the present study differ from the ones reported by Stake et al. (1973). These authors reported similar ADG for weaned calves supplemented with either soybean meal or sunflower meal (0.74 and 0.71 kg/d, respectively). Nevertheless, one important observation is that the mentioned authors have used a sunflower meal containing 37% CP, whereas the sunflower meal used in the present study had 32.5% CP, indicating a higher presence of hulls. In the present study, G:F was greatest in SBM, lowest in SUN, with CAN being similar to both ($P = 0.02$). In two feeding trials using corn silage as the roughage source, Rule et al. (1994) found similar G:F in cattle fed either soybean meal or canola meal, supporting our findings. In contrast, Stake et al. (1973) reported that weaned calves receiving soybean or sunflower meals had the same feed efficiency.

The cost to gain 1 kg of BW was similar across all treatments ($P = 0.31$). Nevertheless, the alternative diets CAN and SUN yielded significantly lower cost per head per day when compared to SBM ($P < 0.001$). Considering SBM as reference, the daily feeding cost per animal was reduced up to 9.72% with the use of the alternative diets (Table 4.2).

**In Vitro Data**

Regression analysis revealed that total production of gas increased quadratically over the incubation period ($P < 0.001$; Figure 4.1 and Table 4.4). Moreover, gas production was highest for SBM and CAN, and lowest for SUN ($P = 0.002$). Although no differences were observed across treatments for concentrations of CH$_4$ and CO$_2$ per ml of
gas \((P \geq 0.31)\), across incubation times, a linear effect was observed for concentration of 
\(\text{CH}_4\) \((P = 0.01)\) and a quadratic effect was observed for concentration of \(\text{CO}_2\) \((P = 0.004)\). Production of \(\text{NH}_3\)-N was not influenced by treatments \((P = 0.308)\), but there was a quadratic effect for incubation time \((P < 0.001; \text{Table 4.4 and Figure 4.2})\). Moreover, ANOVA demonstrated that concentration of \(\text{NH}_3\)-N was highest when the incubations lasted 48 h, followed by the 24 h period, and it was lowest for either the 12, 6, or 0 h periods \((P < 0.001; \text{data not shown})\).

No differences were found across treatments \((P = 0.185)\), however, total VFA production over the 48-h incubation increased \((P < 0.001)\) quadratically \((\text{Figure 4.4})\). For the 24-h of incubation length, total concentration of VFA was 102.7 mM. This value is lower than the ones reported by Quinn et al. (2009), May et al. (2010), and Smith et al. (2010) for \textit{in vitro} incubations lasting 24 h. These authors reported concentrations ranging from 124.6 to 166.8 mM, however, the substrate-diets used in their studies were substantially different from the ones used in this experiment. On a DM basis, our diets used as substrates contained approximately 75% roughage as corn silage, whereas, in those mentioned studies, the inclusion of roughage in the substrates varied from 0 to 9%, closely resembling diets offered in feedlots and not in stocker operations.

Regarding the molar proportions of VFA, considering all incubation lengths, a treatment effect was observed on production of acetate, propionate, isovalerate, and valerate \((P \leq 0.03; \text{Table 4.3})\) with SUN yielding the highest proportion of acetate, and the lowest proportion of all the other mentioned VFA. The SBM treatment yielded the lowest proportion of acetate and the highest molar proportion of propionate. Furthermore, SBM yielded the lowest acetate to propionate ratio, and both CAN and SUN yielded the
highest ratio across all the incubation times ($P = 0.006$). Results from the regression analysis are presented in Table 4.4. These data show that the proportions of all measured VFA and the acetate:propionate ratio, followed a quadratic trend for the incubation time ($P \leq 0.02$). As observed for several other traits, IVDMD increased in a quadratic manner as incubation time was extended ($P < 0.001$; Table 4.4 and Figure 4.3). A treatment effect was also observed, with SBM averaging the highest IVDMD, followed by CAN and SUN ($P < 0.001$; Table 4.3).

Assessment of caloric values from the diets, digested residues and blank samples allowed estimation of DE. Through the use of standard coefficients, the produced amounts of VFA and CH$_4$ were also expressed as energy (calories) and converted to a percentage of the DE. A balance showing the transformations of DE along the different incubation lengths is shown in Table 4.5. No effect of treatment was observed on the efficiencies of transformation of energy ($P \geq 0.17$; data not shown), but the effect of changing the length of incubation was very evident in all the evaluated traits ($P < 0.001$; Table 4.5). Considering only the incubation times of 24 and 48 h, more consistent with in vivo digestion lengths, the present study found that the transformation of DE into CH$_4$ ranged from 11.14 to 11.94%. These findings are in alignment with the results reported by McGinn et al. (2004). These authors conducted two experiments where steers were fed a high-forage diet (75% barley silage, DM basis) and placed in chambers to measure gas emissions. In the first experiment, 10.51% of the DE consumed was lost in the form of CH$_4$, whereas in the second experiment, this value was 11.36%.
CONCLUSIONS AND IMPLICATIONS

Overall, although \textit{in vitro} systems have some limitations in simulating \textit{in vivo} conditions, the technique used in the present study allowed production of results that are consistent with the ones observed in live animals. Furthermore, according to the physical law of conservation of energy, energy can neither be created nor destroyed. Accordingly, the \textit{in vitro} technique used in this study was able to account for approximately 100\% of the conversion of DE in other compounds.

Regarding the experimental diets, although SUN cost less per metric ton, animal performance was reduced when animals were fed this diet. However, important traits like ADG, DMI, G:F, and total VFA production were similar for animals receiving either CAN or SBM. In addition, compared to SBM, CAN decreased the average daily feeding cost per animal. Therefore, results from this research have demonstrated that CAN is a viable alternative to the SBM diet in stocker operations, since it was able to keep animal performance at the same level while reducing the daily cost per head of cattle.


with a steam-flaked corn-based substrate with or without added sulfur. J. Anim. Sci. 87:1705-1713.


http://quickstats.nass.usda.gov/results/054D529A-BB78-34C3-9572-225405DBC297
Table 4.1. Composition and chemical analysis of SBM, CAN, and SUN diets fed to stocker animals\(^1\) (DM basis)

<table>
<thead>
<tr>
<th>Item</th>
<th>SBM</th>
<th>CAN</th>
<th>SUN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient, % of DM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn silage</td>
<td>74.0</td>
<td>74.4</td>
<td>74.5</td>
</tr>
<tr>
<td>Ear corn</td>
<td>15.2</td>
<td>9.8</td>
<td>9.8</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>10.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Canola meal</td>
<td>-</td>
<td>15.8</td>
<td>-</td>
</tr>
<tr>
<td>Sunflower meal</td>
<td>-</td>
<td>-</td>
<td>15.7</td>
</tr>
<tr>
<td>Analyzed nutrient content(^2), % of DM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry matter</td>
<td>46.4</td>
<td>46.9</td>
<td>46.9</td>
</tr>
<tr>
<td>Crude protein</td>
<td>13.5</td>
<td>12.9</td>
<td>12.3</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.19</td>
<td>0.23</td>
<td>0.21</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.27</td>
<td>0.36</td>
<td>0.34</td>
</tr>
<tr>
<td>Cost, $/metric ton</td>
<td>80.63</td>
<td>75.34</td>
<td>71.76</td>
</tr>
</tbody>
</table>

\(^1\) SBM: protein supplement was soybean meal; CAN: protein supplement was canola meal; SUN: protein supplement was sunflower meal.

\(^2\) The University of Georgia Feed and Environmental Water Laboratory, Athens, GA.
Table 4.2. Performance of stocker animals receiving the experimental diets

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>SBM</th>
<th>CAN</th>
<th>SUN</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals$^2$</td>
<td></td>
<td>89</td>
<td>89</td>
<td>98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial BW, kg</td>
<td></td>
<td>285.8</td>
<td>286.1</td>
<td>283.8</td>
<td>1.60</td>
<td>0.56</td>
</tr>
<tr>
<td>Final BW, kg</td>
<td></td>
<td>394.3$^a$</td>
<td>393.6$^a$</td>
<td>384.3$^b$</td>
<td>1.58</td>
<td>0.009</td>
</tr>
<tr>
<td>Average daily gain, kg</td>
<td></td>
<td>1.29$^a$</td>
<td>1.28$^a$</td>
<td>1.20$^b$</td>
<td>0.018</td>
<td>0.007</td>
</tr>
<tr>
<td>Dry matter intake, kg/d</td>
<td></td>
<td>6.67</td>
<td>6.82</td>
<td>6.73</td>
<td>0.05</td>
<td>0.167</td>
</tr>
<tr>
<td>Dry matter intake, g/kg BW</td>
<td></td>
<td>1.96</td>
<td>2.01</td>
<td>2.01</td>
<td>0.02</td>
<td>0.087</td>
</tr>
<tr>
<td>Gain:feed, kg</td>
<td></td>
<td>0.195$^a$</td>
<td>0.188$^{ab}$</td>
<td>0.178$^b$</td>
<td>0.003</td>
<td>0.02</td>
</tr>
<tr>
<td>Cost of gain, $/kg of BW gain</td>
<td></td>
<td>1.13</td>
<td>1.09</td>
<td>1.10</td>
<td>0.02</td>
<td>0.31</td>
</tr>
<tr>
<td>Daily feed cost, $^3$</td>
<td></td>
<td>1.44$^a$</td>
<td>1.38$^b$</td>
<td>1.30$^c$</td>
<td>0.01</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

$^{a-c}$ Means within a row lacking a common superscript differ (P < 0.05).

1 SBM: protein supplement was soybean meal; CAN: protein supplement was canola meal; SUN: protein supplement was sunflower meal.

2 Number of animals used in the 3 years of the experiment.

3 Daily Feed Cost: Average daily cost per animal considering prices across the 3 yr.
Table 4.3. Proportions of VFA (mol/100 mol), total VFA concentration (mM), A:P ratio, IVDMD, NH₃-N, total gas production, and production of CH₄ and CO₂ for different substrates incubated for up to 48 h

<table>
<thead>
<tr>
<th>Item</th>
<th>Incubated Substrate¹</th>
<th>SBM</th>
<th>CAN</th>
<th>SUN</th>
<th>SE²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>57.5ᵇ</td>
<td>58.1ᵃ</td>
<td>58.3ᵃ</td>
<td>0.14</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Propionate</td>
<td>27.72ᵃ</td>
<td>27.24ᵇ</td>
<td>27.16ᵇ</td>
<td>0.13</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>0.75</td>
<td>0.73</td>
<td>0.74</td>
<td>0.006</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Butyrate</td>
<td>10.96</td>
<td>11.01</td>
<td>10.87</td>
<td>0.066</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Isovalerate</td>
<td>1.66ᵃ</td>
<td>1.53ᵇ</td>
<td>1.53ᵇ</td>
<td>0.01</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Valerate</td>
<td>1.42ᵃ</td>
<td>1.39ᵇ</td>
<td>1.37ᵇ</td>
<td>0.01</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Total VFA, mM</td>
<td>81.4</td>
<td>80.2</td>
<td>79.4</td>
<td>0.73</td>
<td>0.185</td>
<td></td>
</tr>
<tr>
<td>A:P ratio³</td>
<td>2.09ᵇ</td>
<td>2.14ᵃ</td>
<td>2.16ᵃ</td>
<td>0.014</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>IVDMD, %</td>
<td>49.91ᵃ</td>
<td>47.47ᵇ</td>
<td>45.95ᵇ</td>
<td>0.6</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>NH₃-N, mM</td>
<td>4.03</td>
<td>4.10</td>
<td>3.67</td>
<td>0.20</td>
<td>0.308</td>
<td></td>
</tr>
<tr>
<td>Total gas production, ml</td>
<td>181.7ᵃ</td>
<td>179.8ᵃ</td>
<td>171.6ᵇ</td>
<td>1.7</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>CH₄, μmoles/ml of gas</td>
<td>6.2</td>
<td>6.7</td>
<td>6.2</td>
<td>0.2</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>CO₂, μmoles/ml of gas</td>
<td>21.5</td>
<td>23.3</td>
<td>21.7</td>
<td>0.9</td>
<td>0.43</td>
<td></td>
</tr>
</tbody>
</table>

ᵃᵇᶜ Means within a row lacking a common superscript differ (P < 0.05).

¹ SBM: protein supplement was soybean meal; CAN: protein supplement was canola meal; SUN: protein supplement was sunflower meal. Substrates were incubated for 0, 6, 12, 24, and 48 h. Values are the means for all incubation times.

² Standard error of the main-effect means.

³ A:P ratio = acetate:propionate ratio.
Table 4.4. Proportions of VFA (mol/100 mol), total VFA concentration (mM), A:P ratio, IVDMD, NH₃-N, total gas production, and production of CH₄ and CO₂ for substrates incubated for up to 48 h

<table>
<thead>
<tr>
<th>Item</th>
<th>Incubation Period, hours¹</th>
<th>P-value²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Acetate</td>
<td>61.7</td>
<td>56.9</td>
</tr>
<tr>
<td>Propionate</td>
<td>24.7</td>
<td>29.0</td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>0.66</td>
<td>0.61</td>
</tr>
<tr>
<td>Butyrate</td>
<td>10.5</td>
<td>10.8</td>
</tr>
<tr>
<td>Isovalerate</td>
<td>1.26</td>
<td>1.27</td>
</tr>
<tr>
<td>Valerate</td>
<td>1.12</td>
<td>1.40</td>
</tr>
<tr>
<td>Total VFA, mM</td>
<td>28.3</td>
<td>65.1</td>
</tr>
<tr>
<td>A:P ratio⁴</td>
<td>2.49</td>
<td>1.97</td>
</tr>
<tr>
<td>IVDMD, %</td>
<td>23.2</td>
<td>39.0</td>
</tr>
<tr>
<td>NH₃-N, mM</td>
<td>1.95</td>
<td>0.96</td>
</tr>
<tr>
<td>Total gas production, ml</td>
<td>-</td>
<td>105.5</td>
</tr>
<tr>
<td>CH₄, µmoles/ml of gas</td>
<td>-</td>
<td>6.1</td>
</tr>
<tr>
<td>CO₂, µmoles/ml of gas</td>
<td>-</td>
<td>19.8</td>
</tr>
</tbody>
</table>

¹ Experimental diets composed of 75% corn silage plus supplements were incubated for 5 different lengths ranging from 0 to 48 h. Values are the means for all treatments.

² Significance of regression coefficients for traits on incubation time. Linear (LIN) and quadratic (Q) effects.

³ Standard error of the main-effect means.

⁴ A:P ratio = acetate:propionate ratio.
Table 4.5. Percentage of the digestible energy (DE) converted into VFA, CH₄ and microorganism cells, and percentage of DE accounted for

<table>
<thead>
<tr>
<th>Item</th>
<th>Incubation Period, hours¹</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>SE²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of DE converted into VFA</td>
<td></td>
<td>0</td>
<td>6</td>
<td>12</td>
<td>24</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.94c</td>
<td>49.37b</td>
<td>56.68ab</td>
<td>59.91a</td>
<td>65.61a</td>
<td>2.07</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>% of DE converted into CH₄</td>
<td></td>
<td>-</td>
<td>5.57b</td>
<td>7.41b</td>
<td>11.14a</td>
<td>11.94a</td>
<td>0.47</td>
</tr>
<tr>
<td>% of DE retained as microorganisms</td>
<td></td>
<td>74.95a</td>
<td>46.47b</td>
<td>34.70c</td>
<td>29.13cd</td>
<td>23.34d</td>
<td>1.48</td>
</tr>
<tr>
<td>Percentage of DE accounted²</td>
<td></td>
<td>83.89</td>
<td>101.41</td>
<td>98.79</td>
<td>100.18</td>
<td>100.89</td>
<td></td>
</tr>
</tbody>
</table>

¹ Means within a row lacking a common superscript differ (P < 0.05).
² Experimental diets composed of 75% corn silage plus supplements were incubated for 5 different lengths ranging from 0 to 48 h. Values are the means for all treatments.
³ Standard error of the main-effect means.
⁴ Percentage of DE accounted = percentage converted into VFA + percentage converted into CH₄ + percentage retained as microorganism cells.
Figure 4.1. Total gas produced (ml) for the substrate-diets SBM, CAN, and SUN on 5 different incubation lengths: 0, 6, 12, 24, and 48 h. Gas production was greatest for SBM and CAN ($P = 0.002$). Regression analysis showed a quadratic effect as incubation time was increased from 0 to 48 h ($P < 0.001$).
Figure 4.2. Production of NH$_3$-N (mM) for the substrate-diets SBM, CAN, and SUN on 5 different incubation lengths: 0, 6, 12, 24, and 48 h. No differences were observed across treatments ($P = 0.308$). Regression analysis showed a quadratic effect ($P < 0.001$). Analysis of variance showed greatest NH$_3$-N concentration for the incubation time of 48 h, followed by 24 h, and it was lowest for either the 12, 6, or 0 h periods ($P < 0.001$).
Figure 4.3. *In vitro* dry matter digestibility (%) for the substrate-diets SBM, CAN, and SUN on 5 different incubation lengths: 0, 6, 12, 24, and 48 h. SBM had the highest digestibility, followed by CAN and SUN ($P < 0.001$). Regression analysis showed a quadratic effect with increasing IVDMD as incubation length increased ($P < 0.001$).
Figure 4.4. Total VFA concentration (mM) for the substrate-diets SBM, CAN, and SUN on 5 different incubation lengths: 0, 6, 12, 24, and 48 h. No effect of treatment was observed ($P = 0.185$). Regression analysis showed that total VFA production increased in a quadratic manner as the incubation length was increased ($P < 0.001$).
CHAPTER 5
CONCLUSIONS AND IMPLICATIONS

In conclusion, results from this study showed an overall harmony between \textit{in vitro} and \textit{in vivo} findings. \textit{In vitro} data provided support and helped to explain the animal performance results.

Results from the first experiment showed that although the COM treatment yielded slightly higher ADG, the estimation of carcass traits revealed that animals eating DDGS or CGF had a leaner growth, more desirable for developing bulls. Furthermore, the use of by-product-based diets to reduce feed costs of developing bulls has proven to be a viable option. Important traits like feed conversion, DMI as a percentage of BW, and longissimus muscle area were not affected by the treatments. Sulfur levels remained below the toxic threshold, and, in addition, the cost of gain was significantly reduced when the two by-product-based rations were used instead of the commercial ration.

Results from the second experiment showed that although SUN cost less per metric ton, animal performance was slightly reduced when animals were fed this diet. On the other hand, important traits like ADG, DMI, feed efficiency, and total VFA production were similar for animals receiving either CAN or SBM. In addition, compared to SBM, CAN decreased the average daily feeding cost per animal. Therefore, results from this research have demonstrated that CAN is a viable alternative to the SBM diet in stocker operations, since it was able to keep animal performance at the same level while reducing the daily cost per head of cattle.
The expansion of some specific industrial sectors may generate further availability of by-products. Compared to non-ruminant animals, ruminants are able to handle and digest higher amounts of fiber. Taking advantage of this unique capacity – to convert rough materials – may be very beneficial to the beef cattle industry. Ultimately, it can reduce the need for traditional feeds such as corn, decrease competition with other animal production segments such as swine and poultry, and reduce cost of feeding. Therefore, further research is needed on utilization of by-products and alternative feeds in beef cattle.