ENHANCING BEEF AND DAIRY CONJUGATED LINOLEIC ACID CONTENT THROUGH OIL SUPPLEMENTATION ON FORAGES

by

VANESSA ANN CORRIHER

(Under the direction of Gary M. Hill)

ABSTRACT

Research was conducted to evaluate the effect of increasing dietary linoleic acid through corn oil supplementation fed to grazing steers and feedlot steers on beef conjugated linoleic acid (CLA) cis-9, trans-11 isomer content and on animal performance and carcass traits. Sixteen Angus crossed steers grazing ryegrass were supplemented with ground corn (1%BW) without and with corn oil (0.075% BW). Oil supplementation decreased DMI but neither performance nor carcass traits were affected. Corn oil supplementation increased *cis* 9, *trans* 11 in the s.c. tissue of steers grazing ryegrass. Twenty commercial steers were finished in drylot fed corn silage without and with corn oil supplementation (7% DMI). The longissimis dorsi (LD) of steers finished in drylot had significantly decreased palmitic (C16:0) and myristic (C14:0) acids. Oil supplementation decreased cis 9, trans 11 and had no effect on trans 10, cis 12 in LD samples. A trend was observed in subcutaneous (s.c.) lipids for increased cis 9, trans 11 and trans 10, cis 12 with corn oil supplementation. Performance of finishing steers fed corn silage or low grain forage sorghum silage with corn oil supplementation was determined along with the effect of diet on the concentration of CLA in beef. Steers supplemented with corn oil had higher concentrations of cis-9 trans-11 in LD and s.c. samples. Steers fed corn silage with oil

supplemented diets had higher concentrations of *trans*-10, *cis*-12. The effect of supplemental pigeon peas on the performance of lactating dairy cows fed diets based on corn silage was determined along with the concentration of CLA in milk. The *cis* 9, *trans* 11 and *trans* 10, *cis* 12 isomers in rumen fluid exhibited a time by treatment interaction. The highest peak of fatty acids occurred 2 hours prior to feeding and then again 4-6 hours post feeding. The two main CLA isomers (c9, t11 and t10, c12) were not significantly different among treatments in milk samples (P < 0.77). The isomer c9t11 was numerically higher for cows fed the control diet, and t10c12 was numerically higher for the 10% pigeon pea treatment. Results from the first three studies demonstrate that beef nutraceutical properties could be enhanced by increasing its CLA *cis*-9, *trans*-11 isomer content through supplementation of grazing steers, drylot steers or finishing steers on low grain forage sorghum with corn oil. Results from the fourth study demonstrate that pigeon peas may be used as a protein supplement in dairy diets affecting neither milk production, DM intake nor the rumen environment but milk CLA content was not altered.

INDEX WORDS: Beef, Carcass, CLA, Dairy, Fatty Acids, Forage, Oil, Steers

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DEDICATION

I dedicate this work in memory of my loving grandmother, Ruby Corriher. She believed in me and supported my quest for a higher education. I only wish she could have seen its completion.

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CHAPTER ONE

INTRODUCTION

Enhancing the content of conjugated linoleic acid (CLA), the *cis*-9, *trans*-11 isomer in consumer beef, has acquired attention as a result of its anticarcinogenic and antiatherogenic effects (Scollan et al., 2006; Kritchevsky, 2003; Parodi, 2002). Milk and beef represent the major sources of CLA in the human diet (Ritzenthaler et al., 2001). Research in lactating dairy cows (Griinari et al., 2000; Kay et al., 2004; Mosley et al., 2006) has shown that over 85% of CLA, *cis*-9, *trans*-11 isomer, results from desaturation of *trans*-11 vaccenic acid (TVA) via the stearoyl-CoA desaturase (SCD) enzyme present in mammalian adipose tissues (Ntambi, 1995). In beef, TVA is present in adipose tissue at levels 1.4 times higher than the CLA *cis*-9, *trans*-11 isomer (Gillis et al., 2004). Because the majority of CLA in beef fat originates from TVA, enhancing the proportion of TVA and CLA in beef products is potentially of importance for human health. In grazing cattle, higher levels of CLA *cis*-9, *trans*-11 and TVA proportions in milk or beef fats have been reported (Dhiman et al., 1999; Scollan et al., 2001a; Realini et al., 2004).

Ruminant products are saturated fatty acid (SFA)-rich components of the human diet (Demeyer and Doreau, 1999), and consumption of SFA has been linked with coronary heart disease (Kromhout et al., 2002). However, ruminant fat is the main dietary source of CLA (Chin

et al., 1992), which has a number of beneficial health effects anticarcinogenic, antiobesity, and decreasing insulin resistance (Ip et al., 1994; Belury, 1995; Pariza et al., 2001).

Ruminant products can be a significant source of n-3 polyunsaturated fatty acids (PUFA) in the human diet (Scollan et al., 2001a) when the consumption of n-3 PUFA-rich foods such as fish is low. French et al. (2000) demonstrated that pasture finishing of cattle increased the concentration of CLA and n-3 PUFA in muscle compared with cereal-based concentrate finishing. Noci et al. (2005a) reported that the nutritional improvement in fatty acid composition was dependent on the duration of grazing. Conjugated linoleic acid is produced in the rumen by incomplete biohydrogenation of dietary C18:2n-6 but is also synthesized in adipose tissue and in the mammary gland by desaturation of C18:1trans-11 produced during ruminal biohydrogenation of C18:2n-6 and C18:3n-3 (Griinari et al., 2000).

Management strategies exert strong effects on fatty acid composition in animal tissues. The CLA content in tissues of beef cattle raised under a variety of feeding regimens has been widely investigated (Flachowsky 2000; French et al., 2000; Jahreis 2000; Pastushenko et al., 2000; Geay et al., 2001). The CLA content is variable among tissues and is influenced by diet. Pasturing animals has a positive effect on levels of beneficial fatty acids in beef (Laborde et al., 2002; Rule et al., 2002), while maintaining carcass quality. Grass-fed cattle contained 7.4 mg CLA g⁻¹ lipid in the top round, and those supplemented with 8.5 kg of cracked corn contained 5.1 mg CLA g⁻¹ lipid (Shantha et al., 1997).

CHAPTER TWO

REVIEW OF LITERATURE

Conjugated linoleic acid: General overview

Conjugated linoleic acid (CLA) is a collective term for a series of conjugated dienoic positional and geometric isomers of linoleic acid (*cis*-9, *cis*-12-octadecadienoic acid, 18:2). The CLA isomers are found naturally in foods, especially those of ruminant origin (Chin et al., 1992). The CLA isomers can also be synthesized in the laboratory from C18:2 or from sources high in C18:2 such as sunflower, safflower, soybean, or corn oils, by a reaction involving alkaline water isomerization (Christie et al., 1997) and isomerization in propylene glycol (Sehat et al., 1998). The CLA and *trans*-octadecenoic acids are produced in the rumen as intermediates in the biohydrogenation of dietary linoleic acid to stearic acid (Bauman et al., 1999). Conjugated linoleic acid (mixtures of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers) has demonstrated anticancer properties in studies using animal models (Ip et al., 1994, 1995, 1999). It is known that CLA is produced in the mammary gland or adipose tissue by desaturation at the 9th carbon of *trans*-vaccenic acid (*trans*-11 C18:1 TVA), a product of partial biohydrogenation in the rumen (Griinari and Bauman 1999).

Linoleic acid is an 18-carbon unsaturated fatty acid with two double bonds in positions 9 and 12, respectively, and both are in the *cis* configuration (Ip, 1994). Conjugated linoleic acid (CLA) contains *cis* and *trans* isomers at carbons 8 and 10, 9 and 11, 10 and 12, or 11 and 13 (Ip,

1994; Garcia et al., 1998). There are multiple potential isomers, but the *cis-9*, *trans-*11 and *trans-*10, *cis-*12 isomers are thought to be active as potential antioxidant, anticarcinogenic, antiobesity, and immune-modulating agents (Lin et al., 1995; Park et al., 1999). The *cis-9*, *trans-*11 isomer of CLA is an anticarcinogen which reduced tumor proliferation when topically applied to mice with experimentally induced epidermal carcinogenesis (Ha et al., 1987; Ip et al., 1994, 1995, 1996). The *cis-9*, *trans-*11 isomer is the principle dietary form of CLA exhibiting biological activity and accounts for 73 to 94% of total CLA in milk, dairy products, meat, and processed meat products of ruminant origin (Parodi, 1976; Chin et al., 1992; Kramer et al., 1997; Sehat et al., 1998).

Conjugated linoleic acid originates from either ruminal biohydrogenation of C18:2 and C18:3 or from endogenous synthesis in tissues. Ruminally, CLA is produced as an intermediate product during the biohydrogenation of dietary C18:2 or C18:3 to stearic acid (C18:0). Biohydrogenation of dietary linoleic acid to stearic acid is sometimes incomplete, yielding several intermediates, including various *trans-* or *cis-*octadecenoic acids and CLA isomers (Bauman et al., 1999). Endogenously, CLA is synthesized from t11, C18:1 vaccenic acid (TVA), another intermediate of ruminal biohydrogenation, via delta-9-desaturase (Bauman et al., 1999). The endogenous synthesis of CLA from TVA has been proposed as being the major pathway of CLA synthesis in lactating cows, accounting for an estimated 78% of the CLA in milk fat (Griinari et al., 2000; Corl et al., 2001).

The dynamic nature of the rumen environment results in the production of a complex pattern of fatty acids from a limited intake of dietary fatty acids. Therefore it is difficult to predict the output of fatty acids from the rumen based on fatty acid intake. When dietary lipid enters the rumen, the initial step in lipid metabolism is the hydrolysis of the ester linkages found in triglycerides, phospholipids, and glycolipids. Rumen bacteria are predominantly responsible

for the hydrolysis of dietary lipids with little contribution by rumen protozoa and fungi or salivary and plant lipases. Hydrolysis of lipids occurs extracellularly, and the glycerol and sugars that are liberated are readily metabolized by ruminal bacteria. Although the extent of hydrolysis is generally high (>85%), a number of factors that affect the rate and extent of hydrolysis have been identified. The extent of hydrolysis is reduced as the dietary level of fat is increased, or when factors such as low rumen pH and ionophores inhibit the activity and growth of bacteria.

Biohydrogenation of unsaturated fatty acids is the second major transformation that dietary lipids can undergo in the rumen. The process of biohydrogenation requires a free fatty acid to proceed; as a consequence rates are always less than those of hydrolysis, and factors that affect hydrolysis also impact biohydrogenation. The initial step in ruminal biohydrogenation typically involves an isomerization of the *cis*-12 double bond to a *trans*-11 configuration resulting in a conjugated di- or trienoic fatty acid (c9, t11 isomer). Linoleate isomerase (EC 5.2.1.5) is the enzyme responsible for forming conjugated double bonds from the *cis*-9, *cis*-12 double bond structure of linoleic as well as alpha and gamma-linolenic acids. Part of the c9, t11 CLA is rapidly reduced to TVA (Kemp et al., 1984; Kellens et al., 1986), becoming available for absorption in the small intestine. The c9, t11 CLA and TVA often escaping complete ruminal biohydrogenation are absorbed from the intestine and incorporated into milk fat (Jiang et al., 1996; Griinari et al., 1999). The next step is a hydrogenation reaction, which results in the conversion of an unsaturated double bond to a saturated single bond. In the case of linoleic and linolenic acid this is a reduction of the cis-9 double bond resulting in a trans-11 fatty acid. In vitro studies using labeled linoleic acid cultured with rumen contents demonstrated that isomerization of the cis-12 double bond was followed by rapid conversion of cis-9, trans-11 CLA to trans-11 octadecenoic acid. Hydrogenation of the trans-11 monoene occurred less

rapidly, and therefore it increased in concentration (Tanaka and Shigeno, 1976; Singh and Hawke, 1979). The final step is a further hydrogenation of the *trans*-11 double bond producing stearic acid (linoleic and linolenic acid pathways) or *trans*-15 18:1 (linolenic acid pathway).

It is possible to differentially affect steps in the biohydrogenation process. This can be caused by dietary factors including the addition of oils high in PUFA or a reduction in rumen pH. The major biohydrogenation substrates are linoleic and linolenic acids. The predominant C18:3 fatty acid in feedstuffs is alpha-linolenic acid (*cis-9 cis-12 cis-15* octadecatrienoic acid). Rumen biohydrogenation of alpha-linolenic acid produces *cis-9*, *trans-11*, *cis-15* conjugated octadecatrienoic acid as the predominant initial isomerization product, and is followed by reduction of the *cis*-double bonds. The rate of rumen biohydrogenation is typically more rapid with increasing unsaturation. For most diets linoleic acid and linolenic acid are hydrogenated to the extent of 70-95% and 85-100% respectively.

It has been suggested that the biohydrogenation pathways are affected by several factors related to the composition of the diet consumed by the animal including the rumen environment and the bacterial population (Griinari et al., 1999; Leat et al., 1977; Van Soest, 1994; Griinari et al., 1998). Diet and changes in the rumen environment can shift the pathways of biohydrogenation resulting in dramatic changes in the fatty acid intermediates produced. Isomerization and biohydrogenation are strongly affected by the rumen pH (Bessa et al., 2000), because decreased rumen pH can result in a shift of the bacterial population (Van Soest, 1994), which then influences the pattern of the fermentation end products (Bauman et al., 1999). Diets that cause a low ruminal pH and the feeding of ionophores inhibit the final step in biohydrogenation resulting in an accumulation of trans-18:1 fatty acids. The extent of the inhibition is much lower than their inhibition of hydrolysis (Van Nevel and Demeyer, 1995;

1996). The extent of hydrolysis (of triglycerides, phospholipids and glycolipids; initial step of lipid metabolism in rumen) is reduced as the dietary level of fat is increased (Beam et al., 2000), or when factors such as low rumen pH and ionophores inhibit the activity and growth of bacteria (Van Nevel and Demeyer, 1995; 1996; Demeyer and Doreau, 1999). Based on the role of ruminal bacteria in the biohydrogenation of unsaturated fatty acids, there has been much interest over the past few years in maximizing CLA formation in the rumen with the goal of increasing CLA levels in milk and meat products.

Lipid entering the small intestine is virtually identical to that leaving the rumen. There is no significant absorption or modification of the long and medium chained fatty acids in the omasum or abomasum (Noble, 1981). The lipid entering the small intestine consists of fatty acids that are highly saturated, mainly palmitic and stearic acids. The total amount of lipid entering the duodenum may exceed lipid intake because of the contribution of microbial lipid synthesis. The greatest increase typically occurs with high forage diets (Bauman et al., 2003). Dietary lipid supplements can result in higher, similar or lower post-ruminal flow of fatty acids relative to intake, resulting from the range of effects they can have on microbial lipid synthesis (Demeyer and Doreau, 1999; Lock and Shingfield, 2003). Approximately 80-90% of the lipid entering the small intestine is in the form of free fatty acids attached to feed particles (Davis, 1990; Doreau and Chilliard, 1997).

Two key biohydrogenation intermediates are *trans*-11 18:1 (vaccenic acid) formed from linoleic and linolenic acids and *cis*-9, *trans*-11 conjugated linoleic acid formed in the biohydrogenation of linoleic acid. These intermediates are present in appreciable quantities in ruminant fat at a ratio of about 3:1, but in the rumen *cis*-9, *trans*-11 CLA is only transitory intermediate and instead it is vaccenic acid (VA) that accumulates. Most of the *cis*-9, *trans*-11

CLA found in ruminant fat originates in the mammary gland and adipose tissue from endogenous synthesis involving the enzyme delta-9 desaturase with rumen-derived VA as the substrate (Bauman et al., 2003). In lactating dairy cows, mammary epithelial cells have a high activity of delta-9-desaturase (Bickerstaffe and Annison, 1969; Kinsella, 1972; McDonald and Kinsella, 1973; Ward et al., 1998), which is consistent with the mammary gland being a major site for endogenous synthesis of *cis*-9, *trans*-11 CLA. However, delta-9-desaturase also occurs in the small intestine and adipose tissue of ruminants (Bickerstaffe and Annison, 1969; St. John et al., 1991; Ward et al., 1998), suggesting some endogenous synthesis may occur in these tissues as well. There are reported species differences in the tissue distribution of delta-9-desaturase. Enzyme activity and mRNA abundance of delta-9-desaturase are highest in the adipose tissue of growing sheep and cattle (Chang et al., 1992; Cameron et al., 1994; Page et al., 1997). In lactating ruminants, the highest activity of delta-9-desaturase is found in the mammary tissue (Kinsella, 1972).

The CLA found in milk and inter- and intramuscular fat of ruminants originates from two sources (Griinari and Bauman, 1999). One source is CLA formed during ruminal biohydrogenation of linoleic acid. The second source is CLA synthesized by the animal's tissues of the animal from *trans*-11 C18:1, another intermediate in the biohydrogenation of unsaturated fatty acids. The uniqueness of CLA in food products derived from ruminants relates to the incomplete biohydrogenation of dietary unsaturated fatty acids in the rumen. Rumen biohydrogenation of dietary lipids is responsible for the high levels of saturated fatty acids in fat of ruminants, a feature considered undesirable for some aspects of human health. Biohydrogenation is responsible for ruminant fat containing CLA, fatty acids with many putative beneficial effects on human health.

Conjugated linoleic acids and fatty acids in food products

Conjugated linoleic acids are components of ruminant fat that are of interest in redesigning foods. Food products derived from ruminant animals are the major source of CLA in human diets (Chin et al., 1992; Fritsche and Steinhart, 1998; McGuire and McGuire, 2000). Recently, the range of positive health effects associated with CLA in experimental models has been extended to include reduction in body fat accretion and altered nutrient partitioning, antidiabetic effects, reduction in the development of atherosclerosis, enhanced bone mineralization, and modulation of the immune system (Belury, 1995; Banni and Martin, 1998; Houseknecht et al., 1998; Whigham et al., 2000). The major isomer of CLA in milk fat is *cis*-9, *trans*-11 and it represents 80 to 90% of the total CLA (Parodi, 1977; Chin et al., 1992; Sehat et al., 1998). Studies have demonstrated that the *cis*-9, *trans*-11 isomer reduces mammary tumor incidence in rats when added to the diet or consumed as a natural component of butter (Ip et al., 1999).

Ritzenthaler et al. (2001) has reported that about 30% of the CLA intake by US consumers is derived from beef products. Beef has always been considered a functional food, providing nutrients such as good-quality protein, vitamins B6 and B12, niacin, and highly available iron and zinc. Beef contains high levels of saturated fatty acids (SFA), especially, palmitic (C16:0) and some stearic (C18:0), which are hyper-cholesterolemic fatty acids. Decreases in SFA and increases in both mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA), such as oleic (C18:1) and linoleic (C18:2) acids, respectively, have been achieved by dietary manipulation (Ashes et al., 1993; Gulati et al., 1996). A substantial increase in oleic acid content of beef is highly desirable, because this fatty acid is known to have hypocholesterolemic properties (Bonanome and Grundy 1988). Beef contains

PUFA in an acceptable n-6 to n-3 ratio ranging between 1.0 and 2.5, which approximates the values recommended for humans [n-6:n-3=2 (Gaey et al., 2001)]. Research studies indicated that CLA (cis-9, trans-11 C18:2 rumenic acid) in beef had an anticarcinogenic effect (Pariza et al. 1979). It was also reported that some trans MUFA such as vaccenic acid (trans-11 C18:1) could be converted to CLA by desaturase enzymes in the human body (Salminen et al. 1998). Therefore, n-3 PUFA, CLA, and trans-11 C18:1 would be desirable fatty acids to increase in beef to provide health benefits to consumers. The C18: 1 content of beef is 45%, which can be increased to 52% with the use of appropriate genetics and feeding systems.

Fogerty et al. (1988) reported that Australian beef muscle contained 2.3 to 12.5 mg of CLA/ g of fatty acids, and Fritsche and Steinhart (1998) reported that German retail beef had an average CLA content of 6.5 mg/g of fat. Surveys from the US found that the CLA content in beef products ranged from 2.9 to 8.5 mg/g of fat (Chin et al., 1992; Shantha et al., 1994), whereas Canadian beef products ranged from 1.2 to 6.2 mg/g of fat (Ma et al., 1999). Takenoyama et al. (2001) reported the CLA concentrations of Japanese beef products ranged from 1.5 to 3.9 mg of CLA/g of lipid. In general, countries with higher levels of CLA concentrations are ones where pasture is a major dietary component throughout the growth and finishing phase, and this is known to increase the CLA content in body fat and milk fat (Bauman et al., 2000b; French et al., 2000). The levels of CLA in ruminant meat can be enhanced by dietary manipulation.

The CLA content of ruminant-derived food products is related to the initial CLA content in milk and meat fat, and to the total fat content of the food product. According to a review by Parodi (2002) the average dietary intake of CLA is in the range of 100 to 300 mg/d; however, this does not include the contribution of endogenous synthesis of CLA from dietary vaccenic acid. The estimated current average total CLA intakes range between 95 and 440 mg, differing

from country to country because of different cultural diets and variable CLA values in food (Schmid et al., 2006). It was hypothesized that 95 mg CLA per day is enough to show positive effects in the reduction of breast cancer in women; calculations were based on epidemiological data linking increased milk consumption with reduced breast cancer (Enser et al., 1999; Knekt et al., 1996). On the other hand, Ha et al. (1989) extrapolated from rat studies that 3.5g/day were needed to promote human health benefits and Ip et al. (1994) reported 3g/day on the same basis. Ritzenthaler et al. (2001) calculated the CLA intake with a cancer protective effect to be 620 mg/day for men and 441 mg/day for women. However, all these values represent rough estimates and are mainly based on extrapolations from animal data.

The content of CLA in fat from ruminant-derived food products will be dependent on the ruminal production of both CLA and trans-11 C18:1 and the tissue activity of delta-9 desaturase. Using forages to increase CLA

Forages and concentrates are the primary sources of lipid in the ruminant diet, and over the last decade fat supplementation has become a common practice to increase dietary energy density for high producing cattle. Forages typically contain 2 to 3 % of the dry matter of the leaf as lipid, of which the major lipid class is glycolipids. The lipid content of concentrates is usually higher than that of forages, and the majority is present in the form of triglycerides. In vitro studies with rumen cultures suggest that glycolipids are hydrolyzed and hydrogenated similarly to triglycerides (Dawson et al., 1974, 1977; Singh and Hawke, 1979).

Triacylglycerol (90% fatty acid) is the major lipid class in rendered fats, in most cereals, and in oil seeds (>95, 2 to 8, and 18 to 45% ether extract, respectively), whereas fatty acid in forages is often less than 50% of the ether extract (Palmquist and Jenkins, 1980; Van Soest, 1994). A large part of ether extract in forages is comprised of nonsaponifiable substances

(waxes, chlorophyll, cutin, etc.). The majority of lipid in forages is found in the chloroplasts and its proportion of the plant dry weight decreases as the plant matures (Hawke, 1973). Triacylglycerol is potentially completely metabolizable by animals, whereas the nonsaponifiable fraction has no energy value, although it may offer other desirable nutritional characteristics. Glycerol (10 to 11% of the glyceride in weight) has an energy value comparable to other carbohydrates, whereas the fatty acids contribute the highly dense energy value of fats. The ether extract fraction of plants, because it contains numerous nonnutritive substances, is not a nutritionally uniform fraction, whereas fatty acids constitute a uniform fraction (Weiss, 1993, NRC 2001). The FDA defines total fat in foodstuffs as the sum of all fatty acids obtained from a total lipid extract, expressed as triglycerides (Eller, 1999). The lipid composition of forages consists largely of glycolipids and phospholipids, and the major fatty acids are the unsaturated fatty acids linolenic C18:3 and linoleic C18:2 acids.

About 48 to 56% of the total FA in fresh forages consists of C18:3 (Bauchart et al., 1984). Fresh grass supplies C18:3 FA as a substrate for ruminal biohydrogenation. However, the abundant supply of C18:3 from fresh grass only partly explains the large increases in CLA and TVA contents of milk fat from pasture-fed cows. Additionally, the high concentrations of soluble fiber and fermentable sugars present in fresh grass may create an environment in the rumen without lowering the ruminal pH that is favorable to the growth of the microbes responsible for CLA and TVA production. Ruminal pH is generally relatively high in cows grazing pasture compared with cows fed a combination of conserved forage and grain. The addition of grain to dairy diets decreases ruminal pH. A decrease in pH will change the microbial population and affect ruminal fermentation (Van Soest, 1994). It has been suggested that the main ruminal biohydrogenating bacteria are cellulolytic (Harfoot et al., 1997; Martin and Jenkins, 2002).

Reduction in ruminal pH decreases the population of cellulolytic bacteria and other microbes responsible for lipid biohydrogenation and the production of CLA and TVA (Jiang et al., 1996).

Pasture feeding has been shown to increase milk fat content of CLA compared with feeding either a total mixed ration (TMR) with similar lipid content or conserved forages. Dhiman et al. (1999) reported that cows grazing pasture had 500% higher CLA content in milk fat (2.21% of total FA) compared with cows fed a diet containing 50% conserved forage (hay and silages) and 50% grain (0.38% of total FA). Other researchers have also demonstrated that the CLA content of milk increased linearly as the proportion of fresh grass from pasture in the diet was increased (Ward et al., 2003; Jahreis et al., 1997; Kelly et al., 1998b; White et al., 2001).

Supplementing grain to cows grazing pasture decreases the CLA content of milk fat. Cows supplemented with 0, 6, or 12 kg/d of grain on pasture had 2.21, 1.43, and 0.89% CLA in milk fat respectively (Dhiman et al., 1999). Similarly, supplementing grain to cows receiving grass silage or replacing conserved grass in dairy cow diets with corn silage lowered the CLA content of milk (Jahreis et al., 1997; Chouinard et al., 1998).

Forage maturity seems to be an important factor affecting CLA content in milk fat. Cows fed immature forages had higher levels of CLA in milk than cows fed mature forages. Cows fed grass silage cut at early heading, flowering, and second cutting had 1.14, 0.48, and 0.81% CLA in milk fat, respectively (Chouinard et al., 1998). The high C18:3 and low fiber content of immature grass compared with mature grass probably interacted to increase the production of CLA and TVA. Harvesting forage as hay decreases the proportion of C18:3 and total FA in grass, whereas harvesting forage as silage, when carried out properly, does not (Doreau and Poncet, 2000). The content of C18:3 FA decreased when forage was wilted before ensiling, or if

there was undesirable fermentation during ensiling (Lough et al., 1973; Dewhurst et al., 1998). The amount of C18:3 FA available to the animal as a substrate for CLA and TVA synthesis from fresh grass is much higher than that from hay or silage. Forage lipid content and composition seems to only partly explain observed differences in milk fat content of CLA. Synergistic effects between lipid substrate and other pasture components may also alter rumen biohydrogenation.

Pasture diets have several differences that relate to milk fat CLA when compared with TMR diets. Concentrations of cis-9, trans-11 CLA in milk fat from cows fed pasture diets are typically higher (Bauman et al., 2001b; Stanton et al., 2003). In addition, the lipids in pasture are high in linolenic acid, and rumen biohydrogenation of linolenic acid does not produce cis-9, trans-11 CLA as an intermediate (Harfoot and Hazlewood, 1988; Griinari and Bauman, 1999). Thus, the importance of endogenous synthesis of CLA in milk fat of pasture-fed cows may differ.

Pasturing animals also has a positive effect on levels of beneficial fatty acids in beef (Laborde et al., 2002; Rule et al., 2002), while maintaining carcass quality. Generally, forage sources contain higher concentration of linolenic acid (18:3) whereas linoleic acid (18:2) is the predominant fatty acid in cereal grains and seeds. Grass-fed cattle contained 7.4 mg CLA g^{-1} lipid in the top round, and those supplemented with 8.5 kg of cracked corn contained 5.1 mg CLA g^{-1} lipid (Shantha et al., 1997). Grazing animals on pasture, feeding fresh forages, or increasing the amount of forage in the diet will elevate the percentage of CLA as a proportion of total FA in meat from ruminants.

Grazing beef steers on pasture or increasing the amount of silage in the diet increased the c9, t11 CLA content in fat by 29 to 45% compared with controls (Shantha et al., 1997; McGuire et al., 1998). The increase in beef CLA content varies with the quality and quantity of dietary

forage. Beef from steers raised on green pasture had 200 to 500% more c9, t11 CLA as a proportion of fat compared with steers fed an 87% corn grain-based feedlot diet (French et al., 2000; Poulson et al., 2001). Rule et al. (2002) observed that the percentage of c9, t11 isomer of CLA was higher in intramuscular fat of range cattle compared with that of steers fed a high-grain diet under feedlot conditions.

French et al. (2000) determined that with increasing grass intake, the intramuscular fat of steers (in longissimis dorsi muscle) had consistent increasing CLA contents. Levels of 5.4, 6.6, and 10.8 mg CLA/g fatty acid methyl esters (FAME) were detected in grazing steers with increasing grass intake compared with 3.7 mg/g FAME in animals fed concentrate. Grass silage also positively influenced CLA content (4.7 g/g FAME), but not to the same extent. Poulson et al. (2004) reported 6.6 times higher CLA content in the longissimis and semitendinosus muscle from steers raised only on forages compared with steers fed a common high grain feedlot diet (13.1 vs. 2.0 mg/g FAME). Steers fed a grain based diet in the growing period and grazed on pasture during the finishing period still had tissue CLA content 4 times higher than those fed only the grain based diet (8.0 vs. 2.0 mg/g FAME). Finishing steers on pasture increases CLA concentrations in intramuscular fat (5.3 vs. 2.5 mg/g FAME) and was confirmed in another study (Realini et al., 2004). Grazing tall fescue pasture for about 200 days before feeding a drylot diet for about 60 days also increased CLA concentrations in steers and heifers compared with animals offered only the drylot diet (Sonon et al., 2004). Contrary to these results, Nuernberg et al. (2002) did not observe any effect of grass (non-specified) feeding on the CLA content in bulls and steers compared with concentrate feeding (5.6/5.2 vs. 6.0/5.5 mg/g FAME). However, in a subsequent study Nuernberg et al. (2004) reported significantly higher proportions of the c9,t11-18:2 isomer in bulls and lambs after pasture (non-specified) feeding compared with concentrate

feeding. Santos-Silva et al. (2002) reported higher CLA concentrations in the longissimis muscle of lambs raised on ryegrass pasture than of lambs fed a concentrate diet (7.1 vs. 3.2 mg/g FAME). Aurousseau et al. (2004) noted that CLA content in muscle triglycerides was dependent on diet and on the growth rate. The CLA concentrations were higher at higher growth rates in lambs possibly because of the higher daily grass (non-specified) intakes of these lambs.

The increased CLA content in meat from animals grazing on pasture is attributed to the high PUFA content of grass (especially n-3 18:3 with a n-6:n-3 ratio of approximately 1:3-5). The amount of dietary PUFA determines the rate of generation of *trans* fatty acids by rumen bacteria (Lawson et al., 2001). This alone does not explain why hay and grass silage differ in the magnitude of CLA production. This may be related to the reduction of sugar and soluble fiber through the ensiling process which may influence the ruminal environment of the animals consuming the silage (French et al., 2000).

Pasture feeding also influences fatty acid composition. A decrease in the n-6:n-3 PUFA ratio as well as an increase in the PUFA:SFA was shown in beef adipose and muscle tissue by inclusion of grass in the diet (French et al., 2000; Nuernbberg et al., 2002; Realini et al., 2004). In lambs a decrease in n-6:n-3 PUFA ratio has been documented as well (Aurousseau et al., 2004; Nuernberg et al., 2001; Santos-Silva et al., 2002). Cattle with a high potential for lean beef production are frequently fattened on concentrate diets, which may be unfavorable to the ratio of n-6/n-3 polyunsaturated fatty acids in meat because the fat in concentrates contains higher levels of C18:2n6. Including forage in the diet of beef cattle should enhance the n-3 fatty acid concentrations because forages are a good source of C1:8n-3 (Scollan et al., 2001a).

The increase in c9, t11 CLA content in beef is not as dramatic as the increase seen in milk from cows grazed on pasture. This difference is probably the result of differences in CLA

production in the rumen or endogenous synthesis of CLA in intramuscular fat of beef cattle fed high-forage diets.

Conjugated linoleic acids in dairy production

Milk fat consists predominantly of triglycerides (over 95%) in all mammals, but actual fat content of milk varies widely among species. For many species, the fatty acid composition of milk fat strongly reflects the fatty acid composition of the diet. Ruminants are an exception because dietary lipids are extensively altered by bacterial metabolism in the rumen, and one of the major changes is the biohydrogenation of PUFA. Diet can markedly affect the bacterial population and rumen microbial processes, and as a consequence diet and nutrition have major effects on the fat content and fatty acid composition of milk, even in ruminants.

The fatty acids in milk arise from two sources, absorbed from blood and de novo synthesis within the mammary epithelial cells. Short-chain fatty acids (4 to 8 carbons) and medium chain fatty acids (10 to 14 carbons) arise almost exclusively from de novo synthesis. Long chain fatty acids (>16 carbons) are derived from the uptake of circulating lipids, and fatty acids of 16 carbons in length originate from both sources.

In ruminants, about one-half of the milk fatty acids (molar percentage) are derived from de novo synthesis. Whereas glucose is used for de novo synthesis by nonruminants, ruminants utilize acetate produced in rumen fermentation of carbohydrates as the major carbon source. In addition beta-hydroxybutyrate, produced by the rumen epithelium from absorbed butyrate, provides about one half of the first four carbons of de novo synthesized fatty acids in the ruminant.

Preformed fatty acids taken up by the mammary gland and directly used for milk fat synthesis are derived from circulating lipoproteins and nonesterified fatty acids (NEFA) that

originate from the absorption of lipids from the digestive tract and from the mobilization of body fat reserves, respectively. In ruminants, fatty acids in milk fat that are taken up from circulation are derived predominantly from the intestinal absorption of dietary and microbial fatty acids. Typically, lipolysis and the mobilization of body fat account for < 10% of the fatty acids in milk fat. When cows are in negative energy balance, the contribution from mobilized fatty acids increase in direct proportion to the extent of the energy deficit (Bauman and Griinari, 2001).

The predominant CLA isomer in milk originates from endogenous synthesis from *trans*-11 C18:1 via the enzyme delta-9 desaturase and to a lesser extent from CLA produced in the rumen (Bauman et al., 2000b; 2001). Given the importance of endogenous synthesis, it is possible delta-9 desaturase is limited in body fat; an increase in the activity or amount of delta-9 desaturase would favor formation of CLA. If this was the major reason, then the ratio of *cis*-9, trans-11 CLA to trans-11 C18:1 would be much lower in body fat than in milk fat. Madron et al. (2002) observed a ratio of 0.23, and Enser et al. (1999) reported a ratio of 0.28 for beef fat. Studies with dairy cows have observed ratios of *cis*-9, *trans*-11 CLA/*trans*-11 C18:1 in milk fat ranging from 0.25 to 0.46 (Jiang et al., 1996; Jahreis et al., 1997; Lawless et al., 1998; Griinari and Bauman, 1999).

Rumen fermentation can be altered by environmental changes resulting in the production of unique biohydrogenation intermediates are produced, and these intermediates affect CLA production. This occurs in classical milk fat depression, wherein increases in the *trans*-10, *cis*-12 CLA and *trans*-10 C18:1 content of milk fat are observed (Bauman and Griinari, 2001). *Trans*-10, *cis*-12 CLA is a potent inhibitor of delta-9 desaturase, which would result in a reduction in endogenous synthesis of *cis*-9, *trans*-11 CLA. Work with lactating cows indicates that

steers. This would mean that rumen production of *trans*-11 C18:1, as well as delta-9 desaturase, is crucial for the production of trans-11 C18:1 and to understand the regulation of delta-9 desaturase in adipose tissue (Madron et al., 2002).

The content of CLA in milk fat is affected by a number of factors, including forage to concentrate ratio (Griinari et al., 1998), level of intake (Jiang et al., 1996; Timmen and Patton, 1988), and intake of unsaturated fatty acids, especially plant oils that are high in linoleic acid (Griinari et al., 1998; Kelly et al., 1998a; McGuire et al., 1996). Timmen and Patton (1988) showed higher concentrations of CLA in milk fat of cows grazing pasture, and Dhiman et al. (1999) recently demonstrated that concentrations of CLA in milk increased as consumption of pasture increased. Banni et al. (1996) also showed that concentrations of CLA in the milk fat of sheep were greater when lush pasture was consumed.

Latham et al. (1972) found that switching lactating dairy cows from a high (44%) to a low (20%) roughage diet resulted in lower levels of lipolytic activity and biohydrogenation of unsaturated fatty acids in ruminal fluid as measured by in vitro experiments. Kalscheur et al. (1997) reported increased flow of linoleic acid to the duodenum in low fiber (25%) compared with high-fiber (60%) diets of lactating dairy cows as a result of lower biohydrogenation levels of unsaturated fatty acids. Bauman et al. (1999) has shown that the trans-10, cis-12 isomer of CLA increases in concentration when lactating dairy cows are consuming a low-fiber/high-concentrate diet.

Muller and Delahoy (2004) reported a two-fold increase in CLA with pasture (5.4 to 10.9 mg/g of fat). This increase has been attributed to increased supply of fatty acids, and to potential changes in the rumen environment and synthesis in the mammary gland. Adding supplements to the ration of grazing cows may diminish this effect. Replacing conserved forages with fresh

pasture clearly increases CLA concentrations in milk. A study comparing confinement feeding of a TMR to pasture + TMR (pTMR) and pasture plus concentrate (PC) clearly shows that feeding pasture elevated CLA in milk (Bargo et al., 2002). CLA in the milk of cows fed a TMR was constant at 6 mg/g of fat for the 18 week study. Cows fed pasture plus concentrate had elevated CLA in milk by week 4 and 6, and the concentration peaked at 18 mg/g fat at week 18. Cows fed pasture plus a TMR had CLA concentrations closer to that of cows fed TMR in confinement (Bargo et al., 2002).

Substantial increases in milk fat concentration of CLA occur when a dietary supplement contains full-fat seeds that have been processed. These investigations have included rapeseeds, soybeans, and cottonseeds, and the processed seeds have been ground, roasted, micronized, flaked, and extruded. In studies with lactating cows, the increase in milk fat CLA observed with plant oils can be transitory (Bauman et al. 2000a), and this may be related to alterations in rumen microflora resulting from the toxic effects of polyunsaturated fatty acids (Jenkins, 1993). Increases in milk fat CLA appear to be constant when processed full-fat seeds are used. Fat supplementation and feed sources richer in unsaturated fatty acids have increased CLA in milk. Unsaturated plant oils increase CLA in milk more than feeding saturated animal fat sources. This is caused by the lipid substrate available in the plant oils for biohydrogenation to CLA, and from CLA precursors in the rumen. It follows then that increasing levels of plant oil and feeding calcium salts of plant oils will increase levels of CLA in milk (Schroeder et al., 2004).

Feeding plant seed oils, such as sunflower, soybean, peanut, canola, and linseed increased CLA content in milk (Kelly et al., 1998a; Dhiman et al., 1999; Gonzalez et al., 2003; Loor et al., 2003; Loor and Herbein, 2003). These oils are rich in C18:2 and C18:3 FA. Studies have reported that high levels of C18:2 and C18:3 increased production of CLA and TVA, with the

TVA potentially being additional substrate for the endogenous synthesis of c9, t11 CLA (Harfoot et al., 1988; Polan et al., 1964; Harfoot et al., 1973). Besides directly increasing the yield of CLA and TVA, it is likely that C18:2 inhibit the final reduction of TVA, thus increasing its accumulation in the rumen (Griinari et al., 1999) and subsequent availability to the animal.

Plant oils high in linoleic acid have been shown to increase milk fat CLA by several-fold when supplemented to cows consuming a typical forage/concentrate based TMR (Chouinard et al., 1998; Kelly et al., 1998a; Dhiman et al., 2000). Oils rich in C18:2 are more effective at increasing CLA in milk fat compared with oils rich in C18:3 or C18:1. Dhiman et al. (2000) reported that linseed oil was not as efficient at increasing CLA content in milk fat as was soybean oil. Feeding soybean oil at 4% of diet DM resulted in a higher CLA content of milk fat (2.08% of FA) than supplementing linseed oil at 4.4% of diet DM (1.63% of FA). However, Kay et al. (2004) reported that sunflower oil at 2.8% DMI had no affect on milk fat cis-9, trans-11 CLA concentration.

Previous attempts to manipulate milk fat cis-9, trans-11 CLA concentration in pasture-fed cows have met with mixed success. Lawless et al. (1998) supplemented grazing (unspecified) cows with full fat soybeans (high in linoleic acid) and full fat rapeseed (high in oleic acid), and they observed increased milk fat CLA concentrations with both treatments. Conversely, Dhiman et al. (2002) found no change in the milk fat content of cis-9 trans-11 CLA when extruded full fat soybeans (high in linoleic acid) were added to a pasture diet. Stanton et al. (1997) reported mixed results when grazing (unspecified forage) cows were supplemented with rapeseed; a high rate of rapeseed supplementation (1650 g/d) resulted in increased milk fat CLA, but a lower feeding rate (825 g/d) of rapeseed produced no effect. Kelly et al. (1998a) reported that milk yield of CLA was greatest when cows received sunflower oil, (17.8 g CLA/d) compared with

10.2 and 13.5 g CLA/d for peanut oil and linseed oil, respectively. McGuire et al. (1996) reported that increasing dietary unsaturated fatty acids by adding corn oil, which contains ~50% linoleic acid, increased milk CLA levels with the magnitude of the increase directly related to the dietary level of corn oil. Griinari et al. (1996) also observed that changing forage concentrate ratios and the addition of dietary unsaturated fatty acids such as corn oil also enhanced milk fat concentrations of CLA.

Researchers have reported large variability in CLA concentrations in milk fat when cows consumed fresh-pasture (Kelly et al., 1998b) or extruded soybean supplements (Peterson et al., 2002). Peterson et al. (2002) reported the source of the variation in milk cis-9, trans-11 CLA levels may be related to ruminal biohydrogenation and SCD activity in the mammary gland. Several authors (Madron et al., 2002; Daniel et al., 2004; Noci et al., 2005a) have reported a high positive linear relationship among cis-9, trans-11 CLA and TVA concentrations in adipose tissues, suggesting that CLA content is dependent on TVA concentrations.

A study was conducted to increase the levels of CLA in milk by affecting rumen biohydrogenation and supplying lipid substrate (Muller and Delahoy, 2004). Cows were fed a TMR with the addition of corn oil, fish oil, or both. Including fish oil has been shown to inhibit biohydrogenation allowing more intermediate products of biohydrogenation, including CLA and CLA precursors, to escape the rumen and be incorporated into milk. Lipid substrates such as corn oil have increased CLA content of milk by providing more unsaturated fatty acids for biohydrogenation. When cows were fed both corn oil and fish oil in combination, CLA content in milk increased ten-fold. Keys to increase milk fat content of cis-9, trans-11 CLA include increasing rumen production of VA and increasing the mammary activity of delta-9-desaturase.

Effects of oil supplementation on conjugated linoleic acids

The PUFA, linoleic (18:2n6) and linolenic (18:3n3) are essential components of cattle diets because they cannot be derived metabolically from oleic acid (18:1c9; Gurr and Harwood, 1991). Despite their importance in metabolic processes, the contents of C18:2 and C18:3 in forages and cereals account for only 3-4% of DM, and both acids are extensively hydrogenated (80-95%) in the rumen (Doreau and Ferlay 1994). The CLA and n-3 PUFA concentrations in tissue are already relatively high, as in the case in pasture-fed beef cattle (French et al., 2000; Engle and Spears, 2004; Noci et al., 2005a). Inclusion of PUFA-rich plant oil or whole seeds in ruminant rations was shown to increase the concentration of CLA and PUFA in meat in several studies (Scollan et al., 2001a; Mir et al., 2002; Noci et al., 2005b), despite extensive biohydrogenation of dietary lipids within the rumen.

In addition to CLA content, modifications in fatty acid composition in muscle and adipose tissues of beef cattle and lambs have been reported when diets were supplemented with unsaturated fatty acids (Bolte et al., 2002; Enser et al., 1999; Kott et al., 2003; Mir et al., 2000; Mir et al., 2003). Although isomerization and biohydrogenation of unprotected unsaturated fatty acids takes place in the rumen, resulting in a higher ratio of saturated fatty acids in the adipose tissues of cattle than expected from the dietary fatty acid profile, a certain amount of unsaturated fatty acids escapes microbial modification. Therefore, the fatty acid composition in meat is altered according to dietary fatty acid supplementation (Casutt et al., 2000; Raes et al., 2004).

The CLA and trans-11 C18:1 concentration in muscle tissue from cattle can be increased by feeding diets supplemented with linoleic-acid-rich oil. In cattle fed linseed and fish oils, concentrations of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and C18:3 increased in muscle phospholipids, but the oil supplementation did not affect growth rate or feed

intake compared with the control group (Scollan et al. 2001b). Addition of 3% or 6% sunflower oil to a barley based finishing diet resulted in increased the CLA content in longissimis muscle (Mir et al., 2003). A greater increase in the CLA concentration occurs when sunflower oil is added to both the growing and finishing diet of beef cattle. Added to a barley and hay-based diet sunflower oil supplementation increased the CLA content in the lipids of the longissimis muscle (Mir et al., 2002). Noci et al. (2005b) documented 4.3, 6.3, and 9.1 mg CLA/g FAME in longissimis dorsi muscle lipids of heifers after supplementing the feed with 0, 55 and 110 g of sunflower oil per kg of the diet for 142 days before slaughter.

Oil supplementation to concentrate-finished animals resulted in small changes in tissue CLA or trans-11 vaccenic acid (TVA) levels (Beaulieu et al., 2002; Madron et al., 2002; Gillis et al., 2004). Research (Duckett et al., 2002; Sackmann et al., 2003) has shown that plant oil supplementation to high-concentrate diets favors a greater predominance of the trans-10 biohydrogenation pathway and increases ruminal outflow of trans-10 octadecenoic acid. Highconcentrate diets favor production of trans-10, cis-12 CLA and trans-10 octadecenoic acid as an intermediate of linoleic acid biohydrogenation as opposed to the cis-9, trans-11 CLA and TVA produced with high-forage diets (Bauman and Griinari, 2003). Several studies have been conducted to increase beef CLA *cis*-9, *trans*-11 proportion through supplementation of high concentrate diets with plant oils rich in linoleic acid; however, responses have been limited. Others (Beaulieu et al., 2002; Griswold et al., 2003; Hristov et al., 2005) did not observe changes in TVA or CLA cis-9, trans-11. Similarly, Madron et al. (2002) did not observe any change in TVA or CLA cis-9, trans-11 proportions of longissimis muscle when cattle were supplemented with 12.7% extruded full-fat soybeans in a high-concentrate diet. However when inclusion of extruded full-fat soybeans increased to 25.6% of diet, TVA and CLA cis-9, trans-11 content

increased with respect to the control diet. Trans vaccenic acid and CLA *cis-9*, *trans-11* content in 3 different adipose tissues (i.m., s.c., and perirenal adipose) increased when 4% corn oil was added to high-concentrate diet (Gillis et al., 2004).

Other studies in which steers were fed diets containing high-oil corn (McGuire et al., 1998) or supplemented with soybean oil (Beaulieu et al., 2000), resulted in little or no change in the CLA content of body fat. Enser et al (1999) fed steers diets supplemented with 6% linseed oil and fish oil, and they observed a twofold increase in the adipose tissue content of CLA. Mir et al. (2000) observed a similar increase in body fat of lambs fed a diet supplemented with 6% safflower oil. Engle and Spears (2004) also reported 6.1% for total C18:1 trans in muscle fat when feeding a high oil corn to finishing steers. Supplementing a corn-based diet fed to Angus-Wagyu heifers with 5% (DM) soybean oil had no effect on the proportion of c9,t11-18:2 in muscle tissue (Beaulieu et al., 2002). Wagyu were derived from native Asian cattle which were crossed with British and European breeds in the late 1800s. Wagyu cattle are renowned for their marbling. However, in some of the tissues analyzed the t10,c12-18:2 was increased after the soybean feeding. In a study with steers by Griswold et al. (2003), supplementation of 4% soybean oil to a finishing diet (corn/SBM) based on concentrate and corn silage (80:20) resulted in a depression of the CLA deposition in muscle tissues compared with the same diet without soybean oil. Comparing 4% with 8% added soybean oil in a 60:40 concentrate:forage diet showed a numerical increase of the CLA content with the higher soybean oil supplementation.

Pavan et al. (2007b) reported a linear decrease in myristic and palmitic acids with increasing corn oil supplementation of cattle grazing tall fescue. These two SFA are considered to have hypercholesterolemic effects in humans, whereas stearic acid, the other predominant SFA in beef, is considered neutral in this regard (Ulbricht and Southgate, 1991). Pavan et al.

(2007b) reported that high corn oil supplementation generated an 18 and 8% reduction in myristic acid and palmitic acid proportions, respectively. Reductions in palmitic acid proportions were observed when lipid intake was increased in lambs (Bolte et al., 2002) or beef cattle diets (Andrae et al., 2001; Beaulieu et al., 2002; Madron et al., 2002). Mir et al. (2002) had previously suggested the occurrence of a feedback inhibition of lipogenesis by adding 6% of sunflower oil to the diet. Linear reductions in tissue myristic and palmitic acids result from a reduction in de novo FA synthesis caused by an increase in exogenous dietary FA (Vernon, 1981). The reduction in the percentage of odd chain FA percentage with corn oil supplementation by Pavan et al. (2007b) would suggest lower ruminal propionate production, or lower de novo FA synthesis. Odd-chain FA are produced when propionate is substituted for acetate in de novo FA synthesis (Garton et al., 1972). According to Jenkins (1993), a reduction in fiber digestion would result in a lowering of the acetate to propionate ratio in the rumen. The reduction in odd chain FA percentage would agree with a reduced de novo FA synthesis with exogenous FA supplementation.

The effect of increasing dietary linoleic acid through vegetable oil supplementation on tissue stearic and oleic acid content is variable across studies. Pavan et al. (2007a) and Andrae et al. (2001) did not observe changes in stearic or oleic acid percentage when feedlot diets containing typical corn were replaced by high-oil corn. Madron et al. (2002) observed an increase in stearic acid and decrease in oleic acid percentage in longissimis muscle when extruded full-fat soybeans were included in high-concentrate diet at increasing levels. In contrast, Gillis et al. (2004) detected similar proportions of stearic acid and lower oleic acid when heifers were fed a high-concentrate corn diet for 60 d with 4% corn oil.

Dietary oil affects the ruminal ecosystem, especially protozoa, which in turn can impact the biohydrogenation and isomerization that can occur to the fatty acids in the oil. Ciliate protozoa, together with bacteria and fungi, are an integral part of the rumen ecosystem and they contribute to the fermentative degradation of feed in the rumen. However, protozoa engulf bacteria (Jouany 1996), and the ciliate protozoa contribute significantly to ruminal production of methane (Hegarty 1999) because methanogenic bacteria are associated with ciliate protozoa (Newbold et al. 1995). Plant oils, milk fat (Kreuzer and Kirchgessner 1987; Machmuller and Kreuzer 1999) and unsaturated C18 fatty acids (Newbold and Chamberlain 1988) in ruminant diets are toxic to protozoa. The toxicity results from protozoa having a limited ability to absorb, assimilate, and transform dietary lipids (Williams 1989), which cause swelling, and consequent disruption of protozoa (Girard and Hawke 1978). But the unsaturated C18 fatty acids are H₂ sinks and are biohydrogenated in the rumen.

Isomerization is the first critical step in biohydrogenation which leads to formation of CLA. Conjugated linoleic acids are produced in the rumen by bioconversion of linoleic acid by the bacterium *Butyrivibrio fibrosolvens* (Kepler et al. 1966) to TVA. Because the ruminal protozoa population affects the ruminal bacteria population (Williams and Coleman 1992), it could be speculated that a decrease in the ruminal protozoa population would affect the population of *B. fibrosolvens* and, indirectly, the ruminal synthesis of CLA. Dietary supplementation with linoleic-acid rich sunflower seed oil (6% of dietary DM) in barley silage based diets resulted in both massive reduction in ruminal protozoa population and increased concentration of CLA in tissues of sheep (Ivan et al. 2001). Such a dual effect of the dietary oil supplement is of great interest to ruminal production, utilization and the quality of animal products for human consumption.

Free plant oils with high PUFA concentrations are normally not included in ruminant diets as high levels of dietary fat disturb the rumen environment and inhibit microbial activity (Lawson et al., 2001; Raes et al., 2004a). Additionally, vegetable oils are rather expensive for dietary supplementation of ruminants, and they are more susceptible to oxidation than whole oil seeds. An alternative approach is to feed full fat processed seeds, which allows the oil to become more gradually available in the rumen without adverse effects on microbial growth (Griinari and Bauman, 1999). This approach markedly increases milk fat content of CLA in dairy cows (Lawless et al., 1998; Dhiman et al., 1999; Chouinard et al., 2001). Aharoni et al. (2005) compared soybean oil with full fat soybeans as supplements over five months in a high forage (wheat and safflower silages) fattening diet of Friesian bull calves. Extruded full fat soybeans were about 20% more efficient than free oil in increasing the CLA concentration in intramuscular fat. The full fat soybean supplement also resulted in higher PUFA and lower SFA and MUFA content in the intramuscular fat than supplementation with soybean oil. This may cause a partial protection of the oils against ruminal biohydrogenation by roughly crushed seeds (Casutt et al., 2000).

Oil supplementation can impact fiber digestion and potentially reduce animal performance. Effects of oil supplementation on fiber digestion depend on the oil source and fatty acid composition, quantity of lipid supplemented, and proportion of forage in the diet (Palmquist, 1984; Jenkins, 1993). For high-concentrate diets, optimal growth performance is often obtained at a total dietary lipid level less than 1.6 g/kg of BW (Zinn, 1994). Brokaw et al. (2001) evaluated the effect of lipid supplementation on in vivo digestibility of beef cattle grazing bromegrass; however, the level of soybean oil supplemented was relatively low (0.35 g/kg of BW), and no effect was observed. Others have evaluated effects of lipid supplementation on in

vivo digestibility for high forage (>50%) diets using bermudagrass hay (Hardin et al., 1989; Hall et al., 1990; Patil et al., 1993) or bromegrass hay (Scholljegerdes et al., 2004) as the forage source. Pavan et al. (2007a) reported that corn oil supplementation on tall fescue grazing decreased NDF digestibility by 6 and 12% respectively, for 0.75 g/kg of BW and 1.5 g/kg of BW. Hristov et al. (2005) reported no difference in ruminal true nutrient digestibilities when beef cattle were finished on barley grain, wheat silage and alfafa hay diets with 5% dietary supplement of safflower oil. However, the linoleic acid-rich oil (safflower) decreased NDF digestibility.

Over the last decade fat supplementation has become a common practice to increase the energy density of the diets fed to high producing dairy cows and finishing beef steers. Inclusion of plant oil or whole seeds in ruminant rations reported (Scollan et al., 2001b; Mir et al., 2002; Noci et al., 2005b) to increase the concentration of CLA and polyunsaturated fatty acids (PUFA) in meat. Conjugated linoleic acid and n-3 PUFA concentrations in tissue have been reported to be relatively high in pasture-fed beef cattle (French et al., 2000; Engle and Spears, 2004; Noci et al., 2005a). Pavan et al. (2007b) reported an increase in TVA and CLA when steers grazing endophyte-free tall fescue were supplemented with corn oil at 0.75 g/kg of BW. The cis-9, trans-11 isomer of CLA was increased when 0.75 g/kg of BW of corn oil was supplemented to grazing steers. Feeding higher levels of forage in finishing diets apparently alter ruminal biohydrogenation of linoleic acid, resulting in greater outflow of intermediates via the trans-11 pathway.

Fatty acids and CLA in Pigeon Peas

Pigeon pea [*Cajanus cajan* (L.)] is a drought tolerant legume originating in India and ranking sixth in production worldwide, when compared with other grain legumes. They are an

important grain legume crop grown in tropical and subtropical regions (Nene and Sheila, 1990). Indian farmers have used pigeon pea plants and grain as animal feed for centuries (Pathak, 1970; Wallis et al., 1986). It is not uncommon for plants to be left in the field to be grazed by animals after the seed have been harvested and all other crops have been harvested. Pigeon peas have attracted attention in several other countries as a crop capable of feeding animals because of its perennial nature, large potential biomass production, and the relatively high nitrogen content of the plant (Whiteman and Norton, 1981; Whyte et al., 1953; Pathak, 1970). In addition, pigeon pea can reduce soil erosion (Morton, 1976; Sheldrake and Narayanan, 1979; Ong and Daniel, 1990).

Pigeon pea is remarkably drought resistant, tolerating dry areas with less than 65 cm annual rainfall, even producing seed profusely under dry zone conditions, as the crop matures early and the incidence of pest damage is low. Pigeon pea is somewhat photoperiod sensitive; short days decrease time to flowering. Under humid conditions pigeon pea tends to produce luxuriant vegetative growth, rain during the time of flowering causes defective fertilization and permits attack by pod-caterpillars. Annual precipitation of 6-10 cm is most suitable, with moist conditions for the first two growing months, drier conditions for flowering and harvest. Growing best under temperatures of 18-29 °C, some cultivars will tolerate 10°C under dry conditions and 35 °C under higher moisture conditions. The plant is sensitive to excessive moisture and frost. It will grow in all types of soils, varying from sand to heavy clay loams, well-drained medium heavy loams being best (Duke, 1981).

In recent years, early maturing lines were developed at the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT). These new lines are relatively photoperiod insensitive and mature in 125 to 140 d (Singh et al., 1990). Ali (1990) reported that in northern

India, early maturing Pigeon pea lines can be successfully grown in rotation with winter wheat. Early and medium-maturing Pigeon peas were successfully used for cattle grazing as well as forage and seed production (Akinola and Whiteman, 1975). It was recently demonstrated that Pigeon pea grain from early maturing line GA-2 can be used as a protein supplement for livestock (Phillips and Rao, 2001).

By far the greatest use of pigeon pea grain is for human food. In 1987, pigeon pea as a vegetable was an important market commodity in a few areas of India (Faris et al., 1987). As a vegetable, pigeon peas are usually less expensive and contain more protein, solids and minerals than green peas. In regards to human consumption, the green seed is more nutritious than the dry seed because it has more digestible protein, sugar, and fat than the mature seed. Green seeds have less starch, considerably lower quantities of gas producing sugars and fewer minerals than mature seeds. On a fresh weight basis, vegetable pigeon pea has a great edible portion, more protein, carbohydrates, crude fiber and fat than green peas. Pigeon peas have a very high level of Vitamin A, Vitamin C and other vitamins and minerals.

Watt (1966) reported that in India dry pigeon pea leaves were valued as fodder for cattle. Pigeon pea as fodder alone may be too low in energy (Patel et al., 1972). The leaves can provide a good substitute for alfalfa in animal feed formulations, especially in areas that are not suitable for growing alfalfa. Results in Hawaii suggested that pigeon peas could potentially produce 10 times the DM yield of alfalfa (Krauss, 1921; Embong and Ravoof, 1978). The DM yield of pigeon pea pods is approximately equal to seed yield. The feed value of the pods is limited by its low protein and high fiber contents. Pods are recommended for use as a roughage source for cattle when supplemented with other forage and minerals (Jayal et al., 1970; Kumar et al., 1978).

In India almost all pigeon pea is milled to produce dhal (a preparation of dried beans which have been stripped of their outer hulls). In this process the recovery of dhal varies from around 65 to 75%; the remainder being the by-product known locally as chuni. At 25% of the total amount of seed that passes through India's dhal mills the amount of by-product is approximately 500,000 tons per year. This by-product consists of about 3-8% broken peas, 15% powder, and 10% husks. Chuni is usually sold to dairy producers or feed mills. The husks can be aspirated off and sold at a lower price for cattle feed. The powder and broken seeds are a valuable source of protein for cattle feed, are sold at a higher price (Kurien and Parpia, 1968) and are a favored supplement of dairy cattle (Pathak, 1970; Jain et al., 1987).

The major market for good-quality pigeon peas is for human consumption, but cracked and pinched grain and by-products may be available for incorporation into animal feeds (Whiteman and Norton, 1981). Febles and Padilla (1970) reported yields of >7.8 Mg/ha of green pods, roughly equivalent to 3.9 Mg/ha of grain, from high-yielding cultivars in Puerto Rico. Phatak et al. (1993) evaluated over 60 early and medium-maturing lines in Georgia and Mississippi that were acquired from ICRISAT. Six of the lines produced >4 Mg of grain per ha. Sheldrake and Narayanan (1979) reported pigeon pea grain yields of 0.5 to 1 Mg/ha/yr under limited rainfall, and 1.6 to 2.5 Mg/ha under more favorable growing conditions.

In the southern portion of the United States, both warm and cool season forages are used as pasture for grazing livestock. Growing beef cattle that graze warm season grasses need supplemental protein during the last half of the grazing season in order to continue gaining because forage crude protein levels usually have declined. Producers can utilize off-farm sources of protein such as by products from the food and fiber industry (cottonseed meal, soybean meal, corn gluten feed, brewers grain, etc.) or dehydrated forage legumes (alfalfa pellets), but the cost

of these products vary and require the investment of additional capital. Forage legumes such as lespedeza (Rao and Phillips, 1999) and grain legumes such as pigeon pea have been explored as potential new crops. These crops can utilize land resources during the normally unproductive summer period between annual crops such as winter wheat.

Phillips and Rao (2001) evaluated the dry matter and protein digestibility and N balance of a diet containing pigeon peas in comparison with diets containing more traditional protein sources (cottonseed and alfalfa meal). They reported decreased N digestibility when pigeon peas were used as a protein source for diets fed to lambs. Pigeon peas contain trypsin inhibitors and tannins which lower DM and protein digestion. Ene-Obong (1995) reported that the in vitro protein digestibility was 76.3% for pigeon peas and that the tannin concentration was 7.5 to 14.4 mg/g, which was slightly lower than the tannin concentration of cowpeas. Reed et al. (2000) concluded that the presence of condensed tannins in the diet can lower apparent and true digestibility of protein. However, in small quantities tannins can reduce unnecessary proteolysis and deamination of amino acids in the rumen resulting in increased post-ruminal non-ammonia N flow. In some cases the increase in fecal N losses due to tannins was offset by a decrease in urinary N, resulting in no change in N retention. A three year study evaluating the performance of cattle grazing pigeon peas reported a definite, sustained reluctance of growing calves and of mature cows to graze the forage, resulting in large body weight losses, presumably as a result of higher tannin content of varieties grazed (Hill unpublished). Rao et al. (2002) reported that three pigeon pea ecotypes (ICP8151, ICPX910007, and PBNA) produced large quantities of high quality forage during the summer fallow period when other available forages are inadequate. Pigeon peas have the potential to provide abundant, high quality forage for grazing livestock when other summer forages are unproductive.

Pigeon peas contain (% DM) 9.9% moisture, 19.5% protein, 1.3% fat, 65.5%

carbohydrate, 1.3 % crude fiber, 3.8 % ash, 0.16 % Ca, 0.29 % P, and 0.15 % Fe. The oil of the seeds contains 5.7% linolenic acid, 51.4% linoleic, 6.3% oleic, and 36.6% saturated fatty acids. Seeds are reported to contain trypsin and chymotrypsin inhibitors. Fresh green forage contains 70.4% moisture, 7.1 % crude protein, 10.7 % crude fiber, 7.9 % N-free extract, 1.6 % fat, and 2.3 % ash (Duke 1981).

Pigeon pea has potential to produce high biomass yields ranging from 40 to 57.6 ton/ha (Akinola et al., 1975; Singh and Kush, 1981). About 50% of this yield is edible forage and the rest is wood (Whiteman and Norton 1981). Grazing of pigeon pea is either by vegetative growth at intervals or by using pigeon pea grown as a stand over forage for the dry season when there is a deficit energy and protein for the growing animal. Pigeon pea forage possesses a high nutritive index (Whiteman and Norton 1981). Pigeon pea forage is useful as a protein supplement when pasture quality is low. Dry leaves of pigeon pea provide a good substitute for alfalfa in animal feed. In India it is mixed with wheat straw to feed cattle. Dry leaves were found to be a useful replacement for alfalfa as a source of carotene and other essential nutrients in chicken rations (Squibb et al., 1950). The leaf is a main component in the vegetative phase; however, the nutritive value of forage is improved if pods and seeds are also available (Henke 1943).

There has been a focus on using pigeon peas as a feed supplement since high yields can be obtained even under relatively adverse conditions. In areas where soybeans do not grow well this characteristic of pigeon peas is particularly attractive (Wallis et al., 1988). Pigeon peas have the potential to be an excellent calf creep feed since the grain is high in protein but low in fat. Spring calves in South Georgia which were creep fed pigeon peas on bermudagrass pastures had 15% greater adjusted weaning weights compared with oats and no creep feed. Calves had 49%

greater average daily gains compared with no creep feeding and 14% greater average daily gains than oats (Hill unpublished, 2004).

Steers fed pigeon peas or pearl millet supplements with Tifton 85 bermudagrass hay had higher apparent digestion of CP on pigeon pea diets. Digestibility of diets supplemented with pigeon peas was equal to or higher than corn-soybean meal. Pigeon peas are highly digestible and supply high quality protein to cattle (Hill et al., 2006).

Yearling beef heifers were fed supplements including whole cottonseed, corn gluten feed, pigeon peas, or ground corn plus soybean meal with free-choice corn silage in a feedlot. The average daily gain for heifers tended to be higher for pigeon peas compared with corn plus soybean meal, whole cottonseed and corn gluten feed (1.01, 0.93, 0.79 and 0.89 kg respectively). However, dry matter intake per gain tended to be higher with corn gluten feed compared with other supplements (Corriher et al., 2007).

In a related study (Corriher et al., 2007), yearling beef steers fed the same supplements as in the heifer experiment had higher total dry matter intake when fed whole cottonseed as a supplement with Tifton 85 bermudagrass hay. Crude protein, ADF and OM digestibility were higher for corn gluten feed which suggests greater nutrient availability from corn gluten feed for cattle. Digestibility of hay-based diets supplemented with pigeon peas by steers was equal to or higher than a corn plus soybean meal supplement. Stocker heifers verified increased gains for supplemental pigeon peas compared with corn plus soybean meal, whole cottonseed (Corriher et al., 2007).

Because pigeon peas can be produced under relatively adverse climatic conditions, they may be grown in the warmer and dryer regions of the Southeastern United States. With 21-24%

crude protein (DM basis) pigeon peas could be a suitable supplement for growing or finishing beef cattle.

Overview

Though still under study, CLA may have potential positive benefits for human health. Available human dietary CLA is typically below the minimum level required to elicit a response. However, dietary CLA could be increased by either increasing total intake of ruminant products or by increasing the CLA and TVA content of ruminant products. Although beef CLA or TVA content were not enhanced with increased dietary supplementation of linoleic acid was increased in animals fed high-grain diets, a different response could be expected when linoleic acid is increased through vegetable oil supplementation to grazing cattle. However, this type of supplementation could negatively affect productive performance of grazing steers in foragefinishing systems by reducing fiber digestibility. Thus the effects of supplementation with richlinoleic oil to grazing beef cattle needs to be evaluated considering the effect on fatty acid composition, on animal performance and carcass quality.

Pigeon peas, though typically used for human consumption, can be used as an alternative protein supplement for growing cattle. Even though low in dietary fat, pigeon peas may alter the rumen environment ultimately enhancing the concentration of conjugated linoleic acids in ruminant products. Therefore, research is needed to determine effects of pigeon pea supplementation of beef and dairy cattle on fatty acid composition, on animal performance and milk quality.

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CHAPTER THREE

PERFORMANCE OF FINISHING STEERS IN DRYLOT OR ON RYEGRASS PASTURES WITH CORN OIL $^{\rm 1}$

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ABSTRACT:

Beef steers were finished on ryegrass pasture or in drylot with corn oil supplementation to determine effects on conjugated linoleic acid (CLA) production, especially *cis*-9, *trans*-11. Steers for both Exp.1 and 2 were implanted with Component[®] on d1 of each experiment. Steer BW were means of two consecutive daily unshrunk BW. Rib sections and subcutaneous fat samples were retained for fatty acid composition. Exp.1: Steers were backgrounded on rye pasture (initial BW 369.4 \pm 29.1 kg) for 71d (ADG: 1.78 \pm 0.11 kg). Following rye grazing, beef steers (n=16; initial BW 424.3 ± 27.8 kg; age 18 mo.; Angus and Angus-X) were fed ground corn supplement (SUP: 1% BW; Rumensin 200 mg/d) without and with corn oil (0.075% BW) while grazing ryegrass pastures (cultivar "Big Daddy," 4 total pastures, 1.62 ha each). Steers were ranked by BW and randomly assigned to dietary treatments for 83 d. Steer supplement DMI on ryegrass was higher for corn than corn + oil treatments (P < 0.053). Despite increased supplement DMI, steer ADG, HCW, YG and QG (12 = US Choice -) were similar (P > 0.10) for both treatments. Supplemental corn oil tended to decrease (P > 0.10) the fatty acid profile of LD, with c9, t11 increasing from 0.49 to 0.63 % of total FA (P > 0.10). The fatty acid profile for s.c. lipids tended to be altered (P > 0.10); however, c9,t11 was increased (P < 0.05) with corn oil supplementation. Exp. 2: Two-year-old beef steers (n=20; initial BW 488.2 \pm 42.4 kg; British crossbreed and Brahman derivative) were fed a free-choice TMR consisting of 55% corn silage (DM 35.6%, CP 11%) and 45% concentrate mix (88% ground corn, 10% SBM, 0.019% mineral/Rumensin/vitamin premix) on DM basis, without (Control) and with corn oil (Control + Oil; 7% of DMI) while in dry lot. Steers were ranked by BW and randomly assigned to dietary treatments. Steer DMI, 86-d ADG, QG, YG and HCW respectively, were Control = 24.43, 2.41, 381.9, 1.97, 11.6; Control + Oil = 24.30, 2.41, 401.3, 2.8, 11.9. Performance and carcass traits

were unaffected (P > 0.10) by treatments, except for yield grade, which was higher (P < 0.05) for Control + Oil than Control. The Control + Oil had decreased palmitic (C16:0; P < 0.01) and myristic (C14:0; P < 0.05) acid concentrations in longissimis dorsi (LD), and Control + Oil had decreased overall fatty acid content for LD. Oil supplementation decreased c9,t11 concentration, and had no effect on t10,c12 in LD samples. The overall fatty acid profile for s.c. lipids were decreased. A trend was observed for increased c9,t11 with corn oil supplementation in s.c. lipids. Feeding corn oil decreased SUP intake for grazing steers (Exp1) and tended to increase ADG, HCW, YG and QG. Steers fed corn oil in drylot (Exp2) had similar DMI and ADG but higher HCW, YG and QG.

Key words: Beef, forage, corn oil supplementation, carcass, fatty acids

INTRODUCTION

Conjugated linoleic acid (CLA) is a collective term used to describe one or more positional and geometric isomers of linoleic acid (cis-9, cis-12-octadecadienoic acid). Enhancing the content of CLA, the cis-9, trans-11 isomer, has acquired attention in the beef industry because of its anticarcinogenic and antiatherogenic effects (Scollan et al., 2006). Milk and beef represent the major sources of CLA in the human diet (Ritzenthaler et al., 2001). Although CLA are produced in the rumen by incomplete biohydrogenation of dietary C18:2n-6, it is also synthesized in adipose tissue and in the mammary gland by desaturation of C18:1trans-11 (Griinari et al., 2000).

Fat supplementation has become a common practice to increase dietary energy density for high producing dairy cows and finishing steers. Forages and concentrates are the primary sources of lipid in the ruminant diet. Forages typically contain 2 to 3 % of the DM of the leaf as lipid. Generally forage sources contain a higher concentration of linolenic acid (18:3) whereas linoleic acid (18:2) is the predominant fatty acid in cereal grains and seeds. Inclusion of plant oil or whole seeds in ruminant rations was shown in several studies (Scollan et al., 2001; Mir et al., 2002; Noci et al., 2005b) to increase the concentration of CLA and polyunsaturated fatty acids (PUFA) in meat.

The CLA and n-3 PUFA concentrations in tissue are relatively high in pasture-fed beef cattle (French et al., 2000; Engle and Spears, 2004; Noci et al., 2005a). Pavan et al. (2007b) reported increase vaccenic acid (VA) and CLA concentrations when steers grazing endophyte-free tall fescue were supplemented with corn oil at 0.75 g/kg of BW. In that study the cis-9, trans-11 isomer of CLA was increased when corn oil was supplemented to grazing steers. Feeding higher levels of forage in finishing diets appears to alter ruminal biohydrogenation of

linoleic acid, resulting in greater outflow of intermediates via the trans-11 pathway. Therefore our objective was to determine the effect of corn oil supplementation on performance and carcass quality in steers finished on ryegrass pasture and in steers finished in dry lot.

MATERIALS AND METHODS

Two experiments were conducted at The University of Georgia Tifton Beef Unit (Tifton, GA) between February and May, 2007. Steers for both experiments were implanted with Component ® (Ivy Animal Health, Overland Park, KS; Trenbolone acetate, estradiol, tylosin tartrate) on d1 of each experiment. All cattle were managed under procedures approved by the University of Georgia Institutional Animal Care and Use Committee.

Backgrounding of Steers:

During a 71-d preliminary growing period, beef steers (n=18, initial BW 369.4 ± 29.1 kg; age 15 mo; Angus and Angus-X; 2.0 hd/ha stocking rate) grazed rye pasture and had ADG of 1.78 ± 0.11 kg. Pastures (4 total pastures; 1.62ha each) were harrowed September 21, 2006 and drilled with Wrens Abruzzi Rye on October 28, 2006 at 1.5 bu/acre.

Grazing Experiment:

Following rye grazing, beef steers (n=16; initial BW 424.3 \pm 27.8 kg; age 18 mo; stocking rate 2.24 hd/ha) were fed ground corn supplement (SUP: 1% BW; Rumensin[®] 200mg/d; Table 3.1) without and with corn oil (0.075% BW) while grazing ryegrass pastures (cultivar "Big Daddy," 4 total pastures, 1.62ha each). Ryegrass pastures were fertilized with a blended fertilizer (24-6-12, N-P2O5-K2O, at 280 kg/ha, on November 27, 2006, January 11, 2007, and March 7, 2007). Steers were ranked by BW and randomly assigned to dietary treatments for 83 d. Supplements were fed free-choice daily at 0800h. Supplement refusals were weighed daily to determine intake. At 14-d intervals beginning in February, forage mass was estimated using a double sampling procedure. Four quadrants (0.1m²) were clipped to ground level in each pasture and clipped samples were oven-dried. Pooled estimates were converted to a moisture-free basis using pooled DM values. Steers were weighed at 28-d intervals and initial and final BW were means of consecutive daily full weights. A commercial mineral (NaCl [maximum] 16.8%; Ca [maximum] 19.20%; P [minimum] 8.0%; Mg [minimum] 1.00%; Cu [minimum] 0.15%; Zn [minimum] 0.27%; Se [minimum] 0.003%; Beef 8 ™, W.B. Fleming Co., Tifton GA) was provided free choice along with water in each pasture.

Drylot Experiment:

Two-year-old beef steers (n=20; initial BW 488.2 \pm 42.4 kg; British crossbreed and Brahman derivative) were fed a free-choice total mixed ration in diet for 86 d. Diets consisted of 55% corn silage (DM 35.6%, CP 11%) and 45% concentrate mix (88% ground corn, 10% SBM, 0.019% mineral/Rumensin/vitamin premix) on DM basis, without and with corn oil (7% of DMI) while in drylot (Table 3.2). Steers were ranked by BW and randomly assigned to four pens in a completely random design. Steers were weighed at 28-d intervals and initial and final BW were means of two consecutive daily full weights. Steers were provided water in each pen. Diets were fed free choice daily at 0800h. Refusals were weighed daily to determine DMI.

Chemical and Fatty Acid Composition Analysis:

Forage, corn grain and silage samples were lyophilized, ground through a Wiley mill equipped with a 1-mm screen, and stored at -20°C for subsequent analysis of OM, NDF, ADF, CP, total fatty acid percentage, and fatty acid profile. Organic matter was measured as the weight loss following combustion for 8h at 500°C. Neutral detergent fiber and ADF were sequentially determined using an Ankom 200 fiber extractor (Ankom Technologies, Fairport, NY) according

to the method of Van Soest et al. (1991). Crude protein concentration was determined by the combustion method using a Leco FP-2000 N analyzer (Leco Corp., St. Joseph, MI). Total fatty acid percentage and fatty acid profile were also determined for corn oil samples.

Steers from both experiments were transported (1609 km) to Cargill Taylor Beef, Wyalusing, PA for harvest. Rib sections were collected from each steer and shipped to The University of Georgia Meat Science and Technology Center in Athens. Samples of s.c. adipose tissue and a 2.5 cm LD steak, which corresponded to a ribeye steak, were removed from the sections at the 13th rib. Both s.c. and LD samples from each carcass were stored at -20° C; and, before analysis samples were pulverized in liquid nitrogen. Total lipids were extracted in duplicate from LD and s.c. samples according to the procedures of Folch et al. (1957). Lipid extracts from s.c. and LD samples were stored at -80°C for subsequent FA determination.

For wet tissue lipid, 1 g of ground muscle tissue or 0.4 g of ground s.c. fat were extracted. The s.c. and LD lipid extracts containing approximately 2 mg of total lipids, based on the calculated percent lipids on a wet tissue basis, were transmethylated (Park and Goins, 1994). Fatty acid methyl esters (FAME) were analyzed using a HP6850 (Hewlett-Packard, San Fernando, CA) gas chromatograph equipped with a HP7673A (Hewlett-Packard, San Fernando, CA) automatic sampler. Separations were accomplished using a 100-m Sp2560 (Supelco, Bellefonte, PA) capillary column (0.25mm i.d. and 0.20 µm film thickness) according to Duckett et al. (2002). Column oven temperature increased from 150 to 160 C at 1 C per min, from 160 to 167 C at 0.2 C per min, from 167 to 225 C at 1.5 C per min, and then held at 225 C for 16 min. The injector and detector were maintained at 250 C. Sample injection volume was 1 µL. Hydrogen was the carrier gas at a flow rate of 1mL per min. Individual FA were identified by comparison of retention times with standards (Sigma, St. Loui, MO; Supelco, Bellefonte, PA;

Matreya, Pleasant Gap, PA). The FA were quantified by incorporating an internal standard, methyl heptacosanoic (C27:0) acid; into each sample during methylation and expressed as g/100g of tissue. The FA composition of forage, corn oil, silage, and corn grain was determined by direct transmethylation of lyophilized samples according to Park and Goins (1994) and analyzed as s.c. and i.m. FAME.

Statistical analyses:

Intake, gain, carcass variables and long chain fatty acid analyses were statistically analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC, 2003) with pen of cattle as the experimental unit and dietary treatment as a fixed effect. Treatment means were compared using satterthwaite test of SAS (SAS Inst., Inc., Cary, NC, 2003). Least squares means are presented for main effects when interactions were not significant (P > 0.10), and, in addition, treatment means for the individual factors are presented in tables.

RESULTS AND DISCUSSION:

Grazing Experiment:

Pasture quality: Forage available DM throughout the study averaged 1224.8 ± 252 kg/ha. Ryegrass DM production was not different among treatments or sampling dates (Table 3.3). Average DM production tended to increase during the experimental period, therefore forage availability was not a limiting factor. Early rainfall and late drought may have resulted in an increasing trend in DM production. Steers were grazed on ryegrass pastures using set stocking rates of 2.24 steers/ha while being fed corn or corn with corn oil at 1% BW. This reduced forage DMI and allowed forage DM/ha to remain fairly constant throughout the experiment. *Performance and carcass traits:* Steer supplement DMI on ryegrass was higher for corn than corn + oil treatments (Table 3.4, P < 0.05) but total DMI of forage and supplements was not determined because forage intake was not measured.. Supplement DMI/gain tended to be lower for corn oil supplementation treatments. Assuming that cattle consume DM to meet their energy requirements (Mertens, 1987), less DM would be required when fat replaces carbohydrates as an energy source in diets (Gagliostro and Chilliard, 1991). Fats may decrease ruminal fermentation and digestibility of fiber (Palmquist and Jenkins, 1980), contributing to rumen fill and decreasing rate of passage. Brokaw et al. (2001) observed no changes in DMI when heifers were supplemented with low levels (0.375 g/kg of BW) of vegetable oil while grazing a summer pasture (75% Bromus Biebersteinii). Other researchers (Hardin et al., 1989; Hall et al., 1990; Patil et al., 1993) reported reductions in DMI when lipids were supplemented to high-forage diets when hay was used as the forage source. Pavan et al., (2007a) reported a linear decrease in forage and total DMI as supplemental corn oil intake increased on tall fescue pastures.

Despite increased supplement DMI on the corn treatment, steer ADG, HCW, YG and QG (Table 3.4; 12=US Choice -) were similar for both treatments. Animal response to supplementation is thought to be subject to the forage substitution rate (Bargo et al., 2003). According to our results with nonlimiting forage availability, oil-supplemented steers consumed less supplement and still produced similar carcass characteristics and quality compared with unsupplemented steers. Allen (2000) the addition of oilseeds and hydrogenated fatty acids (5 to 6 percent total fatty acids) to diets resulted in a quadratic effect on DMI with minimums occurring at 3 and 2.3 percent added fatty acids, respectively. Addition of tallow, grease or calcium salts of palm fatty acids to diets resulted in a general negative linear decrease in DMI. Smith et al. (1993)

reported ruminally active fats have a greater negative effect on DMI, ruminal fermentation, and digestibility of NDF when diets are high in corn silage than when they are high in alfalfa. Pavan et al. (2007a) reported that oil-supplemented steers consumed less forage and produced heavier carcasses than unsupplemented steers with unlimited forage. Supplement conversion rates were improved when forage substitution rates were reduced by lower forage availability (Beretta et al., 2006). Andrae et al. (2000) reported decreased DMI when Angus steers were fed rations containing high-oil corn, regardless of whether the high-oil corn diet was isocaloric with the control or had increased energy density. The increased dietary lipid resulted in increased marbling score and quality grade, although other quality parameters were unaffected (Andrae et al., 2000). In contrast Engle et al. (2000) observed decreased DMI, marbling, dressing percentage, KPH%, yield grade, longissimis muscle area, and quality grade when Angus steers were fed a high-concentrate diet supplemented with 4.0% soybean oil.

A three year study evaluating finishing steer performance on corn silage and small grain pasture resulted in no difference in ADG (Utley et al., 1973). However, their steers that were fed only corn silage and cottonseed meal had lower carcass weight than steers grazing oat and rye pastures. Steer prices were in the \$92-96/cwt (45 kg) on live weight basis in May, 2007 when steers in the present study were harvested. United States finished beef quality grades (prime, choice, select and standard) are based mostly on the marbling in the rib eye. Carcass prices are based on these grades and decline with decreasing grades. Most finished beef in the USA grades low choice to high select. Of the 16 steers finished on ryegrass 13 carcasses graded US choice, four steers received Certified Angus Beef[®] premiums and three carcasses graded US select. Ultimately, with improved genetics, Angus steers may reach acceptable market weight and quality grade on forage.

Fatty acid composition: Pasturing animals had a positive effect on concentrations of beneficial fatty acids in beef (Laborde et al., 2002; Rule et al., 2002), while maintaining carcass quality. Generally forages contain a higher concentration of linolenic acid (18:3), whereas linoleic acid (18:2) is the predominant fatty acid in cereal grains and seeds. The top round of grass-fed cattle contained higher concentrations of CLA, compared with those supplemented with 8.5 kg of cracked corn (Shantha et al., 1997). Supplemental corn oil numerically decreased the overall fatty acid profile for LD for steers in our study (Table 3.4); however, differences were not significant (P > 0.10). The overall FA profile for s.c. lipids were decreased with corn oil supplementation (Table 3.4; P < 0.05). However, C18:0, C18:1c9, and both CLA isomers (c9, t11 and t10, c12) for s.c. lipids were increased with corn oil supplementation (Table 3.4; P <0.05). French et al. (2000) reported that diets based on pasture or hay supported high amounts of CLA deposition in tissues, but silage did not, which leads to the conclusion that dietary use of both oil and the hay or pasture synergistically increased CLA content of muscle. Pavan et al. (2007b) reported an increase in TVA and CLA for corn oil supplementation (0.75 g/kg BW) to steers grazing endophyte-free tall fescue.

Drylot Experiment:

Performance and carcass traits: The objective was to determine the effect of corn oil supplementation on performance and carcass quality in steers finished on corn silage based diets. Total DMI was not affected by corn oil supplementation (Table 3.5). Total DMI/gain tended to be lower for the corn oil supplemented treatment. Hill et al. (1999) fed either pearl millet silage or corn silage to growing heifers for 38 d, in which DMI and ADG were higher for heifers fed corn silage diets. Utley et al. (1996) reported that steers fed temperate corn silage consumed

17.4% more DM/d and gained 17.6% faster than steers fed tropical corn silage. A study by Hill et al. (2004) evaluated the performance of steers fed 3 different levels (0, 4.7, and 7% of DMI) of corn oil supplementation with high-concentrate corn silage. Steer ADG and DMI were reduced when steers were supplemented with corn oil at 7% of DMI. Increasing proportion of vegetable oil in the diet did not affect yield or quality grade even when it reduced performance. In the present study, steer 86-d ADG, QG and HCW were similar for both treatments; however, YG was higher for the corn oil treatment (Table 3.5; P < 0.01). Steer 86-d ADG and HCW were greater for Angus-X steers compared with the Brangus and Braford steers (P < 0.01; Table 3.6). Of the 20 steers finished in drylot, 11 carcasses graded US Choice, four steers received Certified Angus Beef[®] premiums and seven carcasses graded US Select. Inclusion of steers with Brahmanderivative breeding contributed to the increased incident of U S Select carcasses in this relatively short duration feeding experiment.

Fatty acid composition: The effect of increasing dietary linoleic acid through vegetable oil supplementation on tissue stearic (18:0) and oleic acid (18:1) content is variable across studies. Pavan et al. (2007b) and Andrae et al. (2001) did not observe changes in stearic or oleic acid percentage when feedlot diets containing typical corn were replaced by high-oil corn. Madron et al. (2002) observed an increase in stearic acid and decrease in oleic acid percentage in LM when extruded full-fat soybeans were included in high-concentrate diet at increasing levels. In contrast, Gillis et al. (2004) detected similar proportions of stearic acid and lower oleic acid when heifers were fed a high-concentrate diet for 60 d with 4% corn oil. In our study, stearic and oleic acid were numerically decreased in LD with the supplementation of corn oil (Table 3.5). While stearic acid followed a trend to increase in s.c. with oil supplementation in this experiment.

Supplemental corn oil altered the fatty acid in LD (Table 3.5). Oil supplementation decreased c9, t11 (P < 0.05) and had no effect (P > 0.10) on t10, c12 in LD samples. The profile for s.c. lipids were numerically lower for most fatty acids with corn oil supplementation. A trend was observed for increased c9tll with corn oil supplementation. The CLA isomer t10, c12 was not affected by corn oil supplementation in s.c. lipids (P > 0.10; Table 3.5)

Oil supplementation decreased palmitic (C16:0; P < 0.01) and myristic (C14:0; P < 0.05; Table 3.5) acids in LD samples. These fatty acids are considered to have hypercholesterolemic effects on humans. Compared with palmitic and myristic, stearic acid is considered neutral in regards to cholesterol levels in humans. In a review examining the regulation of human plasma low-density lipoprotein (LDL) cholesterol concentrations by dietary cholesterol and fatty acids, Spady et al. (1993) concluded that intakes of 14:0 and 16:0 were positively correlated with increased plasma LDL-cholesterol and thus were risk factors for cardiac disease. The fatty acids C18:1 *cis* 9 and linoleic acid tended to decrease plasma LDL-cholesterol; stearic acid was neutral in effect. According to this scheme, supplemental corn oil in our study resulted in changes of LCFA in edible tissues that would result in a healthier product.

Beaulieu et al. (2002) reported that dietary soybean oil only had modest effects on proportions of the major LCFA in various tissues, in agreement with conclusions of others (Brandt and Anderson, 1990; Kimura, 1997; Engle et al., 2000), that the LCFA composition of ruminant tissues is relatively insensitive to dietary lipid. An exception to this is when the dietary lipid demonstrates some resistance to ruminal biohydrogenation (Rule et al., 1994; Andrae et al., 2001), illustrating the importance of ruminal biohydrogenation on tissue LCFA. Treatment effects may be more pronounced if imposed during the growing phase. When Angus crossbred steers were finished on pasture or high-concentrate diets, the *cis-9*, *trans-*11 content of the loin

and round was increased only when the backgrounding ration also included pasture (Poulson et al., 2001). Steers in Exp. 1 of the present study that were finished on ryegrass were backgrounded on rye for 71 d, possibly contributing to the increase in the CLA isomer, c9,t11 in the longissimis dorsi. Tissue LCFA composition was altered when calves were placed on a finishing diets at 6 mo of age rather than 18 mo (Rule et al., 1997).

The use of improved forages is common and critical for improving beef cattle production. Either grazing animals on pasture, feeding fresh forages, or increasing the amount of forage in the diet may elevate the percentage of CLA as a proportion of total FA in meat from ruminants. The increase in beef CLA content varies with the quality and quantity of forage consumed by cattle. Corn oil supplementation on grazing steers increased the concentration of the CLA isomer, c9, t11 in the longissimis dorsi. Corn oil supplementation tended to increase CLA isomers (c9, t11; t10, c12) in adipose tissue for steers fed corn silage diets in drylot. The increase in c9, t11 CLA content in beef is not as dramatic as the increase often observed in milk from cows grazed on pasture. This difference results from differences in CLA production in the rumen or endogenous synthesis of CLA in intramuscular fat of beef cattle fed high-forage diets (French et al., 2000).

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	Forage	Corn	Corn Oil	Corn + Oil
Component			% DM basis	
DM	20	89.1	-	73.5
СР	19.1	11.8	-	8.9
NDF	48.3	8.0	-	7.6
ADF	29.6	2.9	-	3.0
Total FA, %	1.19	3.78	91.9	5.17
Fatty acid			% of total FA	۹
C14:0	0.4	0.07	<0.1	0
C16:0	14.6	13.36	10.8	10.59
C18:0	1.2	2.31	1.96	1.96
C18:1	1.7	23.10	28.5	27.27
C18:2	10.6	54.06	55.7	57.47
C18:3	68.4	1.30	1.3	0.97
Others ^a	1.7	0.67	0.75	1.34
Unidentified	1.4	0.0	0.95	0.22

Table 3.1: Mean DM chemical and fatty acid composition of the different dietary components for steers grazing ryegrass pastures.

^a Sum of C12:0, C15:0, C16:1, C17:0, C20:0, C21:0, C22:0

	Corn Silage	Corn	Corn + Oil
Component		% DM	
DM	41.2	89.1	73.5
СР	11	11.8	8.9
NDF	26.6	8.0	7.6
ADF	14.3	2.9	3.0
Total FA, %	4.62	3.78	5.17
Fatty acid		% of total FA-	
C14:0	0.70	0.07	0
C16:0	27.87	13.36	10.59
C18:0	3.85	2.31	1.96
C18:1	25.09	23.10	27.27
C18:2	39.26	54.06	57.47
C18:3	2.00	1.30	0.97
Others ^a	1.23	0.67	1.34
Unidentified	0.0	0.0	0.22

Table 3.2: Mean DM chemical and fatty acid composition of the different dietary components for steers in drylot.

^a Sum of C12:0, C15:0, C16:1, C17:0, C20:0, C21:0, C22:0

Item ^a	Pasture 1	Pasture 2	Pasture 3	Pasture 4	Avg of 4
	Trt 1	Trt 2	Trt 2	Trt 1	Pastures
			kg/ha		
Feb 9	937	818	890	867	878
Mar 7	1169	1178	1534	1199	1270
Mar 23	924	1002	1158	1226	1078
Apr 6	1205	1377	1511	1435	1382
Apr 20	1617	1284	1300	1864	1516

Table 3.3: Available ryegrass DM in pastures being grazed by finishing steers.

^aEach value is the mean of 4 ground-level forage samples.

steers grazing ryegrass with	Corn	Corn + Oil	SE	<i>P</i> <
Steer Performance				
Initial BW, kg	422.96	424.69	22.41	0.91
SUP DMI, kg ^a	4.63	4.33	0.01	0.03
83-d ADG, kg	1.78	1.89	0.19	0.40
SUP DMI/gain, kg	2.64	2.33	0.10	0.12
HCW, kg	347.90	354.44	6.95	0.17
YG ^b	2.38	2.75	0.13	0.16
QG ^c	12.27	12.61	0.39	0.55
LD: FA composition		% of Te	otal FA	
C16:0	30.36	29.43	0.82	0.28
C16:1	4.16	3.93	0.22	0.31
C14:0	3.80	3.42	0.27	0.19
C14:1	0.85	0.82	0.06	0.58
C18:0	19.50	15.73	3.26	0.27
C18:1 t11	2.07	1.67	0.44	0.38
C18:1 c9	37.76	38.66	1.10	0.43
c9t11	0.49	0.63	0.11	0.23
t10c12	0.03	0.04	0.01	0.26
Total, mg/g	96.14	140.10	4.46	0.36
s.c.: FA composition				
C16:0	29.69	28.31	0.55	0.03
C16:1	5.69	5.10	0.26	0.04
C14:0	4.90	4.16	0.29	0.02
C14:1	1.86	1.55	0.16	0.08
C18:0	13.26	13.60	0.69	0.63
C18:1 t11	-	-	-	-
C18:1 c9	39.09	40.71	1.07	0.16
c9t11	0.77	1.15	0.16	0.04
t10c12	0.04	0.05	0.01	0.22
Total, mg/g	81.98	77.47	7.24	0.62

Table 3.4: Performance, carcass characteristics, and fatty acids in muscle and external fat of steers grazing ryegrass with oil supplementation.

- ^a Abbreviation: SUP = supplement, corn or corn plus corn oil.
 ^b YG = Yield grade (scale: 1 to 5).
 ^c Quality grade: 11 = US Select +, 12 = US Choice -, 13 = US Choice.

Item ^a	Control	Control + Oil	SE	<i>P</i> <
Steer Performance				
Initial BW, kg	467.47	496.01	27.04	0.12
Total DMI, kg ^a	24.43	24.30	0.71	0.92
86-d ADG, kg	2.41	2.41	0.35	0.99
Total DMI/gain	10.88	10.57	0.95	0.79
HCW, kg	381.88	401.31	21.24	0.43
YG^{b}	1.97	2.81	0.17	0.01
QG ^c	11.63	11.92	0.75	0.74
LD: FA composition		% of Tota	al FA	
C16:0	27.86	26.30	0.94	0.12
C16:1	4.59	4.28	0.29	0.31
C14:0	3.29	2.97	0.27	0.26
C14:1	0.96	0.75	0.15	0.20
C18:0	14.31	14.79	0.70	0.51
C18:1 t11	-	-	-	-
C18:1 c9	42.18	41.98	1.64	0.91
c9t11	0.31	0.32	0.02	0.74
t10c12	0.02	0.02	0.004	0.35
Total, mg/g	112.08	93.56	12.08	0.25
s.c.: FA composition				
C16:0	28.05	25.86	0.76	0.02
C16:1	4.73	4.49	0.30	0.46
C14:0	3.64	3.15	0.37	0.23
C14:1	1.14	1.01	0.22	0.56
C18:0	14.60	15.38	1.26	0.56
C18:1 t11	-	-	-	-
C18:1 c9	42.71	32.56	7.24	0.20
c9t11	0.48	0.66	0.15	0.27
t10c12	0.02	0.02	0.01	0.73

Table 3.5: Performance, carcass characteristics, and fatty acids in muscle and external fat of steers in drylot with oil supplementation.

Total, mg/g	84.47	88.86	9.18	0.74

a Treatments: Control = Control without oil added; Control + Oil = Control plus 7% corn oilb YG = Yield grade (scale: 1 to 5).c Quality grade: 11 = US Select +, 12 = US Choice -, 13 = US Choice.

Item ^a	Angus-X	Braford	Brangus	SE	<i>P</i> <
86-d ADG, kg	2.71	2.12	2.40	0.35	0.007
HCW	434.61	343.23	396.69	35.1	0.0005
YG^b	2.42	2.34	2.42	0.40	0.97
QG^{c}	12.86	11.20	11.75	1.02	0.26

Table 3.6: Breed differences of steers finished in drylot.

^a Breeds: Angus-X = Angus X P. Hereford cross ; Braford = Brahman X P. Hereford; Brangus = Breeds: Angus-X = Angus X P. Hereford cross ; Braford = Brahman . Brahman X Angus. ^b YG = Yield grade (scale: 1 to 5). ^c Quality grade: 11 = US Select +, 12 = US Choice -, 13 = US Choice.

CHAPTER FOUR

PERFORMANCE OF FINISHING STEERS FED CORN SILAGE OR FORAGE SORGHUM SILAGE WITH CORN OIL SUPPLEMENTATION.²

² Corriher, V.A., G.M. Hill, and B.G. Mullinix, Jr. To be submitted to Journal of Animal Science.

ABSTRACT:

Beef steers (n=32; initial BW 524.9 \pm 63.3 kg; age 24 mo.; Angus-X) were fed a freechoice TMR consisting of 55% corn silage (CS) or low-grain sorghum silage (SS) and 45% concentrate mix (88% ground corn, 10% SBM, 0.019% mineral/Rumensin/vitamin premix) on DM basis, without and with corn oil (7% of DMI). Low grain forage sorghum silage was used to simulate a grazing environment compared with high corn grain silage diet. Steers were ranked by BW, randomly assigned to dietary treatments in a 2 X 2 factorial arrangement, and individually fed with Calan ® gates for 78d. Steers were implanted with Component ® on d 1, and initial and final BW were means of two consecutive daily unshrunk BW. Steers were harvested in a commercial abattoir at the conclusion of the experiment. Rib sections and subcutaneous fat samples were retained for FA analysis. Steer 78-d ADG exhibited a silage x treatment interaction (P < 0.01), in which the addition of corn oil depressed ADG for steers fed corn silage, but increased ADG for steers fed sorghum silage. Steer DMI was higher for sorghum silage diets than corn silage diets (25.99 vs. 22.10 kg; P < 0.01), however DMI was not affected (P > 0.10) by corn oil supplementation. Despite increased DMI for steers receiving sorghum silage treatments, 78-d ADG was higher (P < 0.01) for corn silage treatments (ADG: SS = 3.11; CS = 3.85 kg; P < 0.01). Steers fed corn oil had greater (P > 0.10) concentrations of CLA isomer *cis*-9 trans-11 in longissimus dorsi (LD) and subcutaneous (s.c.) fat samples. Steers fed corn oil had greater (P > 0.10) concentrations of CLA isomer *trans*-10, *cis*-12 in s.c. tissue. Steer HCW, YG and QG (12=US Choice-) respectively, were CS: 370.21, 1.50, 9.44; SS: 354.25, 1.44, 11.13; Oil: 354.34, 1.63, 10.94; No Oil: 370.13, 1.31, 9.63. Carcass traits were unaffected (*P* >0.10) by treatments, except for quality grade, which was greater (P > 0.10) for SS than CS. Corn oil

supplementation tended to increase YG and QG, however it tended to decrease HCW of steers. Corn oil supplementation had no affect on steer performance; however, it increased CLA isomer concentrations.

Key words: Beef, corn oil supplementation, carcass, fatty acids, silage

INTRODUCTION

North American beef production has included the use of high concentrate diets with limited amounts of roughage during the finishing phase. High grain diets maximize growth performance (gain, feed intake, feed conversion), limit time on feed, and reduce problems associated with feeding roughages, including availability, transportation costs, storage and processing requirements, and variation in quality. Research has shown that comparable growth performance and similar times on feed can be attained by feeding diets that contain high amounts of corn silage (Vance et al., 1972a; Brennan et al., 1987; Loerch and Fluharty, 1998) without negatively impacting carcass traits and meat quality. Tropical corn hybrids are more acclimated to the environmental conditions of the summer in the southeast region of the United States. Forage sorghum is more suitable for comparison with tropical corn for silage production during summer in the southeast. Forage sorghum can produce silage that has a similar yield of digestible DM compared with tropical corn silage (Black et al., 1980). The energy level of forage sorghum silage is proportional to the amount of grain in the silage. Grain sorghum silages have feed value similar to corn silage. Forage sorghums typically have slightly lower energy values than corn silage, but are similar in protein.

Hay production strongly decreases the FA concentration and the α -linolenic proportion in grass FA, whereas silage production, when carried out properly, does not (Doreau and Poncet, 2000). Although ensiling grass releases free FA from grass glycerides, there is no apparent interconversion of FA (Steele and Noble, 1984). However, FA and especially α -linolenic acid in silage may decrease when undesirable fermentations occur (Lough and Anderson, 1973) or when silage is wilted (Dewhurst and King, 1998), so the variability of FA content and composition is high. In several studies, lipid metabolism of complete forage diets has indicated extensive biohydrogenation of C18:2n-6 and C18:3n-3 (Outen et al., 1975; Bauchart et al., 1984; Scollan et al., 2003). Lee et al (2003a) investigated the effect of clover silages (red vs. white and in combination with grass) on ruminal metabolism of forage lipids. Red clover supported a greater flow of C18:3n-3 to the duodenum of beef steers per unit DMI than grass silage, which resulted from reduced biohydrogenation of C18:3n-3. Dairy cows fed red clover silage had increased duodenal flow (Dewhurst et al., 2003a) and milk levels (Dewhurst et al., 2003b) of C18:3n-3.

Oil supplementation to concentrate-finished animals has resulted in small changes in tissue CLA or trans-11 vaccenic acid (TVA) levels (Beaulieu et al., 2002; Madron et al., 2002; Gillis et al., 2004). Research (Duckett et al., 2002; Sackmann et al., 2003) has shown that plant oil supplementation to high-concentrate diets favors a greater predominance of the trans-10 biohydrogenation pathway and increases ruminal outflow of trans-10 octadecenoic acid. Beef and milk produced from cattle grazing forages have greater concentrations of TVA and CLA (Dhiman et al., 1999; Scollan et al., 2001; Realini et al., 2004). Sackmann et al. (2003) reported a linear increase in duodenal outflow of TVA and linear decrease in trans-10 octadecenoic acid when dietary forage level increased from 12 to 32% in finishing cattle diets. Thus oil supplementation to grazing animals has the potential to increase CLA and TVA to a greater extent than in grain-fed cattle (Pavan et al., 2007).

If α -linolenic acid in silage are not altered during the ensiling process and oil supplementation favors the trans-10 biohydrogenation pathway it may be feasible to alter the CLA composition of meat in ruminants. Therefore our objective was to determine the effect of corn oil supplementation level on performance and carcass quality in steers finished on corn silage and low grain sorghum silage without and with corn oil supplementation.

MATERIALS AND METHODS

Beef steers (n=32; initial BW 524.9 \pm 63.3 kg; age 24 mo.; Angus-X) were fed a freechoice TMR consisting of 55% corn silage (CS) or low-grain sorghum silage (SS) and 45% concentrate mix (88% ground corn, 10% SBM, 0.019% mineral/Rumensin/vitamin premix) on DM basis, without and with corn oil (7% of DMI). Steers were trained to use Calan® gate feeders (American Calan, Inc., Northwood, NH) using corn silage for 28 d. Following training, steers were weighed on two consecutive days, blocked by initial BW, randomly assigned to dietary treatments for 78 d. Steers were implanted with Component ® (Ivy Annal Health; Trenbolone acetate, estradiol, tylosin tartrate) on d 1 of the experiment. During the experimental period steers had access to a pasture of dormant bermudagrass for exercise, and water was provided free-choice. Diets were mixed daily and fed to steers at 1200h using a Data Ranger® (American Calan, Inc., Northwood, NH). Refusals were weighed daily to determine intake. Low grain sorghum silage was used to simulate a grazing environment compared with a high grain corn silage diet. Steers were weighed at 28-d intervals and initial and final BW were means of two consecutive daily full BW. The level of corn oil fed to steers (7% of DMI) were adjusted across treatments during the experimental period according to daily intake. Samples of supplements and silages were taken on a monthly basis and frozen at -20° for subsequent analyses. All cattle were managed under procedures approved by the University of Georgia Animal Care and Use Committee.

Chemical and Fatty Acid Composition Analyses:

Supplement and silage samples were lyophilized, ground through a Wiley mill equipped with a 1-mm screen and stored at -20°C for subsequent OM, NDF, ADF, CP, total fatty acid percentage, and fatty acid profile. Organic matter was measured as the weight loss following combustion for 8h at 500°C. Neutral detergent fiber and ADF were sequentially determined using an Ankom 200 fiber extractor (Ankom Technologies, Fairport, NY) according to the method of Van Soest et al. (1991). Crude protein concentration was determined by the combustion method using a Leco FP-2000 N analyzer (Leco Corp., St. Joseph, MI). Total fatty acid percentage and fatty acid profile were also determined for corn oil samples. Fatty acids were methylated according to Park and Goins (1994) and separated by GLC according to Duckett et al. (2002).

Steers were transported (1609 km) to Cargill Taylor Beef in Wyalusing, PA for harvest. Rib sections were collected from each steer and shipped to the University of Georgia Meat Science and Technology Center in Athens. Samples of s.c. adipose tissue and a thick longissimus dorsi (LD) steak, which corresponds to a ribeye steak, were removed from the sections at the 13th rib region. Both s.c. and LD samples from each carcass were stored at -20° C; before analysis samples were pulverized in liquid nitrogen. Total lipids were extracted in duplicate from LD and s.c. samples according to the procedures of Folch et al. (1957). Lipid extracts from the s.c. and from the LD samples were stored at -80°C for subsequent fatty acid determination.

For wet tissue lipid, 1 g of ground muscle tissue or 0.4 g of ground s.c. fat were extracted. Subcutaneous and LD lipid extracts containing approximately 2 mg of total lipids, based on the calculated percent lipids on a wet tissue basis, were transmethylated (Park and Goins, 1994). Fatty acid methyl esters (FAME) were analyzed using a HP6850 (Hewlett-Packard, San Fernando, CA) gas chromatograph equipped with a HP7673A (Hewlett-Packard, San Fernando,

CA) automatic sampler. Separations were accomplished using a 100-m Sp2560 (Supelco, Bellefonte, PA) capillary column (0.25mm i.d. and 0.20 μm film thickness) according to Duckett et al. (2002). Column oven temperature increased from 150 to 160 C at 1 C per min, from 160 to 167 C at 0.2 C per min, from 167 to 225 C at 1.5 C per min, and then held at 225 C for 16 min. The injector and detector were maintained at 250 C. Sample injection volume was 1 μL. Hydrogen was the carrier gas at a flow rate of 1mL per min. Individual FA were identified by comparison of retention times with standards (Sigma, St. Loui, MO; Supelco, Bellefonte, PA; Matreya, Pleasant Gap, PA). Fatty acids were quantified by incorporating an internal standard, methyl heptacosanoic (C27:0) acid, into each sample during methylation and were expressed as g/100g of tissue. Fatty acid composition of forage, corn oil, and corn grain was determined by direct transmethylation of lyophilized samples according to Park and Goins (1994) and analyzed as s.c. and i.m. FAME.

Statistical analysis:

Intake, gain, carcass variables, and long chain fatty acid analyses were statistically analyzed as a 2 X 2 factorial using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) with pen of cattle as the experimental unit and dietary treatment as a fixed effect. Treatment means were compared using satterthwaite test of SAS (SAS Inst., Inc., Cary, NC). Least squares means are presented for main effects when interactions were not significant (P > 0.10), and, in addition, treatment means for the individual factors are presented in tables.

RESULTS AND DISCUSSION:

Steer 78-d ADG exhibited a silage by oil interaction (P < 0.01), in which ADG was higher for corn silage without corn oil then sorghum silage without oil (Figure 4.1). Ten years of

research conducted at Auburn Agricultural Experiment Station, Auburn; AL, demonstrated that yearling steers wintered on forage sorghum silage consistently gained less than those fed corn silage (1.25 vs. 1.50 lbs ADG; Ball, 1998). In the present study, the DMI was higher (P < 0.05) for sorghum silage treatments; however, corn oil supplementation did not affect DMI (P > 0.10; Table 4.3). In contrasts, Lusk et al. (1984) observed an increase in consumption of corn silage over sorghum silage by dairy heifers, and they noted a similar trend in lactating cows. An increase in sorghum silage DMI resulted from the difference in NE_{M} (corn vs. sorghum: 0.82 vs. 0.64 Mcal/kg DM; NRC, 2000). Despite increased DMI for steers fed sorghum silage treatments (Table 4.3), 78-d ADG was higher with corn silage treatments (Figure 1). Cattle fed a highenergy corn silage diets in drylot gained more when compared with similar animals finished on other forages (Coombs et al., 1990). In an 84-d study, Utley et al. (1997) reported that steers fed temperate corn silage had 16.9% higher DMI and 17.6% higher ADG than steers fed tropical corn silage. O'Connor et al. (2002) reported higher DMI for temperate corn silage compared with tropical corn, sorghum and pearl millet grain hybrid silages. Steer HCW, yield grade (YG; scale 1-5) and quality grade (QG; 12=US Choice-) tended to be higher for steers for corn silage than sorghum silage. Corn oil supplementation increased QG and YG; however, it decreased HCW of steers. Presumably, higher ADG and QG would have been realized if diets had been higher in energy. However, the objectives of this experiment were to evaluate finishing steer performance on a higher forage diets and to observe differences in FA and CLA production resulting from corn oil supplementation.

Quality forage sorghum silage is a useful feed for dairy and beef cattle. According to Grant et al. (1995) use of sorghum silage in the diet of lactating cows resulted in a similar performance of milk yield when fed up to 65% DM of the diet. Studies indicate that when silage

constitutes a considerable portion of the diet in beef production, there is potential for increasing returns by increasing the quality of silage. In feedlot diets for growing cattle, sorghum silage can be used as a substantial portion of the diet. Results of feedlot studies using silage have been variable, with some studies reporting improvements (Freckle et al., 1985; Young, 1998), whereas others have found no effect (Rojas et al., 1987) on total DM digestibility or animal performance. In a study where sorghum silage compared with maize silage in a ration for calves, the digestibility and protein efficiency were higher in sorghum diets (Adewakun et al., 1989). The agronomic performance and nutritive value of forage are significantly influenced by the variety of forage and stage of maturity at harvest (Smith et al., 1984; Harrison et al., 1996; Sonon et al., 1996; Sutton et al., 2000). In the present study steer performance was decreased for sorghum silage treatments because of the low grain content and decreased NE_g compared with corn silage, and diets were not formulated to provide equal concentrations of energy.

Steers supplemented with corn oil had greater concentrations of CLA isomer *cis-9 trans*-11 in *longissimus dorsi* (LD) and subcutaneous (s.c.) fat samples (P > 0.10; Table 4.3). Corn oil supplementation increased *trans* 10, *cis* 12 concentration for both corn silage and sorghum silage treatments; however, the increase was greater for corn silage treatments. Corn oil supplementation did not affect (P > 0.10) steer performance, but CLA concentrations were higher with corn oil supplementation (P < 0.01; Table 4.3).

Feeding hay along with corn oil increased CLA content in beef (Mir et al. 2002a,b). Similarly, French et al. (2000) reported higher concentrations of CLA in tissue when pasture or hay was fed compared with silage. Oil supplementation with silage may synergistically increase CLA content of muscle; however, this may occur at a lower rate than on pasture or hay.

Corn and grass silages have had high concentrations of C18:2 (41% of FA) and C18:3 (46% of FA), and most plant seeds and oils are rich in C18:2, accounting for 53 to 69% of total FA (Dhiman et al. 2005). When consumed by ruminants, the lipid proportions of these feeds undergo two major processes in the rumen (Dawson et al., 1977; Demeyer et al., 1999). In the first process, esterified plant lipids or triglycerides are quickly hydrolyzed to free FA by microbial lipases (Jenkins, 1993). In the second process, the unsaturated free FA is rapidly hydrogenated by microorganisms in the rumen to produce more highly saturated end products.

Production costs for finishing cattle may be lowered by incorporating more corn silage in finishing diets, provided gain performance, time on feed, and carcass traits are not compromised. Corn oil supplementation had no significant affect on steer performance however it did increase the concentration of CLA isomers. Cost of corn oil supplementation may prohibit widespread use of this product in finishing beef production, even though it has potential to create a healthier product for consumers.

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Item ^a	Corn Silage	Sorghum Silage	Corn	Corn + Oil
Component		% of	DM	
DM	41.2	40	89.1	73.5
CP	11.2	11.5	11.8	8.9
NDF	26.6	34	8.0	7.6
ADF	14.3	21.1	2.9	3.0
Total FA, %	4.62	2.45	3.78	5.17
FA composition		% of Tot	tal FA	
C14:0	0.70	0.58	0.07	0.0
C16:0	27.87	25.75	13.36	10.59
C18:0	3.85	3.85	2.31	1.96
C18:1	25.09	24.16	23.10	27.27
C18:2	39.26	38.25	54.06	57.47
C18:3	2.00	5.76	1.30	0.97
Others ^a	1.23	1.65	0.67	1.34
Unidentified	0.0	0.0	0.0	0.22

Table 4.1: Mean DM chemical and fatty acid composition of the dietary components for steers.

^a Sum of C12:0, C15:0, C16:1, C17:0, C20:0, C21:0, C22:0

Item"	CS w/ Oil	SS w/ Oil	CS w/o Oil	SS w/o Oil
-				
Component		DM ba	sis, %	
DM	73.5	71.5	71.2	72
СР	11.1	10.4	13.5	11.6
NDF	27.9	43.2	23.5	38.0
ADF	16.8	27.6	15.2	22.6
Total FA, %	9.79	7.62	8.40	6.23

Table 4.2: Mean DM chemical and fatty acid composition of the different diets fed to steersItemaCS w/OilSS w/OilCS w/OilSS w/Oil

^aAbbreviations: CS = corn silage; SS = sorghum silage

steers.		Treat	ment			Р	<
Item	Corn	Sorghum	Oil	No Oil	SE	Silage	Oil
Steer Performanc	e						
Initial BW, kg	524.68	525.68	522.94	526.76	36.67	0.99	0.87
Total DMI, kg	22.10	25.99	23.61	24.47	0.94	0.05	0.65
Total DMI/gain	6.07	8.63	7.34	7.35	0.53	0.002	0.98
HCW, kg	370.21	354.25	354.34	370.13	11.48	0.33	0.34
QG ^a	9.44	11.13	10.94	9.63	0.63	0.07	0.15
YG^b	1.50	1.44	1.63	1.31	0.31	0.89	0.48
LD: FA composition			% 0	f Total FA-			
C16:0	28.27	28.26	27.96	28.57	0.53	0.99	0.26
C16:1	3.75	3.81	3.93	3.63	0.20	0.75	0.14
C14:0	2.70	2.80	2.78	2.71	0.15	0.50	0.61
C14:1	0.52	0.53	0.58	0.47	0.05	0.83	0.03
C18:0	17.71	18.88	18.08	18.52	0.75	0.14	0.57
C18:1 t11	-	-	-	-	-	-	-
C18:1 c9	38.30	35.94	36.71	37.53	1.35	0.09	0.55
c9t11	0.40	0.47	0.45	0.42	0.04	0.05	0.49
t10c12	0.04	0.05	0.03	0.05	0.008	0.21	0.08
Total, mg/g	89.50	74.87	83.24	81.13	5.62	0.08	0.80
s.c.: FA composition			% o	f Total FA-			
C16:0	28.53	27.36	27.29	28.59	0.80	0.16	0.12
C16:1	4.42	4.92	4.81	4.53	0.46	0.28	0.55
C14:0	3.65	3.79	3.78	3.67	0.12	0.43	0.55
C14:1	0.85	0.98	0.97	0.87	0.09	0.18	0.35
C18:0	17.71	18.40	18.50	17.61	1.02	0.51	0.39
C18:1 t11	-	-	-	-	-	-	-

Table 4.3: Effect of corn oil supplementation and silage on performance and carcass qualities of steers.

C18:1 c9	10.0	10.15	40.21	20.04	0.01	0.07	0.60
0101107	40.0	40.15	40.31	39.84	0.91	0.87	0.62
c9t11	0.51	0.54	0.53	0.52	0.06	0.68	0.79
t10c12	0.015	0.012	0.014	0.013	0.002	0.16	0.87
Total, mg/g	86.81	87.48	88.83	85.45	4.20	0.91	0.58

^a YG = Yield grade (scale: 1 to 5). ^b Quality grade: 11 = US Select +, 12 = US Choice -, 13 = US Choice.

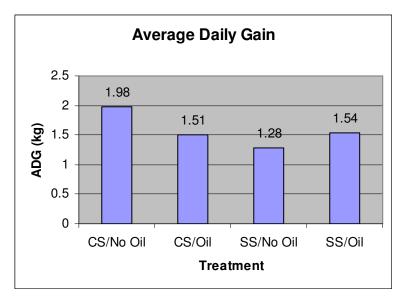


Figure 4.1: The ADG (kg/d) of steers fed diets based on either corn (CS) or sorghum silage (SS) with oil or without corn oil resulted in an interaction (P < 0.0019; SE =0.24).

CHAPTER FIVE

PIGEON PEAS AS A SUPPLEMENT FOR LACTATING DAIRY COWS FED CORN SILAGE BASED DIETS 3

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ABSTRACT:

Holstein rumen canulated cows (n=7; initial BW 640.56 ± 71.43 kg) were fed a corn silage basal diet with one of three concentrate treatments (C=control; P10=10% pigeon peas; P20=20% pigeon peas). Cows were randomly assigned to treatments in a replicated 3 x 3 Latin square design and individually fed using Calan® gates. Each experimental period was 21 d with 7 d for adaption and 14 d for sample collection. Ruminal fluid samples were taken the last day of the each experimental period, which were analyzed for pH, ammonia, long chain fatty acids (LCFA) and volatile fatty acids (VFA). Consecutive a.m. and p.m. milk samples were taken from each cow during the last 2 wk of the 21d period and analyzed for fat, protein, LCFA and SCC. Dry matter intake (kg/d) was lower during the second period and higher for P10 diets (P < 0.05). Somatic cell count in milk samples tended to be greater for cows fed the control diet compared with 10% pigeon peas (P > 0.10). Milk protein was higher for cows fed the 20% pigeon pea diet compared with the 10% diet (P < 0.05). Milk ECM was higher for cows fed the control diet compared with 10% pigeon peas (P < 0.05). Treatment had no effect on milk yield (P > 0.10) but milk yield tended to be higher for control cows than for cows on P10 or P20 diets. Ruminal fluid pH decreased over sampling times during the day (P < 0.01); however, the pH remained at or above 5.5. Diets did not affect ruminal fluid pH (P > 0.10); however, pH was different for sampling periods (P < 0.01). There was no relationship for ruminal ammonia between treatment and dates. Ruminal ammonia decreased until 8h post-feeding at which time it peaked consistent with changes in ammonia concentrations that usually peak 3 to 5h post-feeding on diets high in plant proteins. Dietary treatments altered ruminal fluid VFA with lower concentrations of acetate and higher concentrations of propionate for the control diet, resulting in a lower acetate: propionate ratio (P < 0.05). There was an hour by treatment interaction for isobutyrate (P < 0.05).

0.05), in which isobutyrate decreased until 8 h post-feeding and then isobutyrate tended to be higher for P10 than other treatments. Diets of P10 had higher concentrations of ruminal isovalerate (P < 0.05). The ruminal *cis* 9, *trans* 11 and *trans* 10, *cis* 12 CLA isomers were not affected by dietary treatments (P > 0.10). The P10 diet had the highest ruminal synthesis of c9, t11, but control cows had the highest ruminal synthesis of t10, c12 (P > 0.10). The CLA isomers (c9, t11 and t10, c12) in milk samples were similar (P > 0.10) among treatments. Trends were observed for greater (P > 0.10) c9, t11 and t10, c12 for the 10% pigeon pea treatment. Pigeon peas may be used as a protein supplement in dairy diets without affecting milk production, DM intake or the ruminal environment when they replace corn and soybean meal.

Key words: Dairy, pigeon peas, fatty acids, milk

INTRODUCTION

Pigeon pea [*Cajanus cajan* (L.)] is a drought tolerant legume originating in India and ranks sixth in grain production worldwide compared with other grain legumes. It is an important grain legume crop grown in tropical and subtropical regions (Nene and Sheila, 1990). The major use of good-quality pigeon peas is for human consumption, but cracked and pinched grain and by-products are available for incorporation into animal feeds (Whiteman and Norton, 1981). Pigeon peas typically contain (Duke, 1981; DM basis) 9.9% moisture, 19.5 % protein, 1.3 % fat, 65.5 % carbohydrate, 1.3 % fiber, 3.8 % ash, 0.16 % Ca, 0.29 % P, and 0.015 % Fe. Steers fed pigeon peas or pearl millet grain supplements with Tifton 85 bermudagrass hay had higher apparent digestion of CP for pigeon pea diets which supplied high quality protein to cattle (Hill et al., 2006). The ADG of yearling heifers tended to be higher when fed pigeon peas compared with corn/soybean meal, whole cottonseed or corn gluten feed (Corriher et al., 2007).

The oil in pigeon peas contains 5.7% linolenic acid, 51.4% linoleic, 6.3% trans vaccenic, and 36.6% saturated fatty acids. Despite having only approximately 2% total fat, the high concentration of linoleic acid (51.4%) suggests that pigeon peas could be an important dietary factor for increasing CLA concentrations in ruminant products.

For many species, the fatty acid composition of milk fat strongly reflects the fatty acid composition of the diet. Ruminants are an exception because dietary lipids are extensively altered by ruminal bacterial metabolism, and one of the major changes is the biohydrogenation of polyunsaturated fatty acids (PUFA). Diet can markedly affect the bacterial population and ruminal microbial processes; therefore, diet and nutrition may have major effects on fat content and fatty acid composition of milk in many species, including ruminants. Research indicates that changes in the ruminal environment will lead to changes in microbial activity that corresponds to

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altered end-product formation. Because the ruminal environment is subject to change, it is possible that these changes (pH, substrate types and concentrations, dilution rates) will have an effect on CLA formation by the ruminal microbial population (Martin and Jenkins, 2002).

The CLA content of milk fat is affected by a number of factors, including forage to concentrate ratio (Griinari et al., 1998), DMI (Jiang et al., 1996; Timmen and Patton, 1988), and intake of unsaturated fatty acids (Griinari et al., 1998; Kelly et al., 1998a; McGuire et al., 1996). Timmen and Patton (1988) reported higher CLA concentrations in milk fat of cows grazing pasture, and Dhiman et al. (1999) demonstrated that CLA concentrations in milk increased as pasture DMI increased. Banni et al. (1996) reported that concentrations of CLA in the milk fat of sheep were greater when lush pasture was consumed. Latham et al. (1972) found that switching lactating dairy cows from a high (44%) to a low (20%) roughage diet resulted in lower levels of lipolytic activity and biohydrogenation of unsaturated fatty acids in ruminal fluid as measured by in vitro experiments. Kalscheur et al. (1997a) reported increased flow of linoleic acid to the duodenum in low fiber (25%) compared to high-fiber (60%) diets of lactating dairy cows as a result of lower biohydrogenation levels of unsaturated fatty acids. Bauman et al. (1999) has reported that concentrations of *trans*-10, *cis*-12 isomer of CLA increase when lactating dairy cows are fed a low-fiber, high-concentrate diet.

Pigeon peas, which contain up to 60% unsaturated fatty acid, could increase the concentration of CLA in milk. Therefore our objective was to determine the effect of pigeon pea supplementation on milk production and ruminal characteristics in dairy cows fed a TMR with 0, 10 and 20% pigeon peas.

MATERIALS AND METHODS:

Cows and sampling methods:

Seven primiparous ruminally canulated Holstein cows ($640.6 \pm 71.4 \text{ kg BW}$) were used in a 3 X 3 Latin square with 21 d periods. Cows were housed in a free stall barn and averaged 89 \pm 6.7 DIM and 40.1 \pm 4.0 kg/d of milk at the beginning of the trial. Treatments diets included three levels of pigeon peas, which included none (Control), 10% (P10) or 20% (P20) of pigeon peas. Pigeon peas were incorporated into rations (Table 5.1) that were formulated to be isocaloric and isonitrogenous, and were individually fed once daily behind Calan® doors (American Calan Inc., Northwood, NH). Each cow was offered each treatment for 21d and ruminal fluid samples were taken the last day of the experimental period, which were analyzed for pH, ammonia, VFA and LCFA. Cows were milked twice daily at approximately 0400h and 1500h. Consecutive a.m. and p.m. milk samples were taken from each cow the last two wk of the 21 d period and analyzed for fat, protein, SCC, and LCFA. Samples of diets and ingredients were taken every 14 d and frozen at -20° for subsequent analyses. All cattle were managed under procedures approved by the University of Georgia Animal Care and Use Committee Guidelines. Diets were mixed daily and fed individually to cows at 1200h daily, with amounts of feed offered and refused recorded daily. Amounts of feed offered and refused were recorded daily. Individual milk yield was recorded electronically (Alpro, Delaval, Kansas City, MO) at each milking and summed for each day. Cows were trained to use Calan® gate feeders (American Calan, Inc., Northwood, NH), and then fed supplement treatments.

Chemical analyses of feeds and samples:

Forage and silage samples were lyophilized, ground through a Wiley mill (1-mm screen), and stored at -20°C for subsequent chemical analyses. Organic matter was measured as the

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weight loss following combustion for 8h at 500°C. Neutral detergent fiber and ADF were sequentially determined using an Ankom 200 fiber extractor (Ankom Technologies, Fairport, NY) according to the method of Van Soest et al. (1991). Crude protein concentration was determined by the combustion method using a Leco FP-2000 N analyzer (Leco Corp., St. Joseph, MI). On the last day of the experimental period, ruminal fluid samples were collected at -2, 0, 2, -2, 0, 24, 6, 8 and 10 h post-feeding. Approximately 50 mL of ruminal fluid was collected and strained through 3 layers of cheesecloth, analyzed for pH, ammonia and stored frozen for subsequent analyses of LCFA. A 40-mL subsample was strained through 3 layers of cheesecloth and immediately mixed with 10 mL of metaphosphoric acid (25% wt/vol). The sample was frozen for later analyses of VFA (Erwin et al., 1961). These samples were later thawed and centrifuged at 10,000 x g for 10 min and the supernatant collected for VFA analysis using a Hewlett-Packard gas chromatograph (GLC; Varian 3400 Instrument Group; Hewlett Packard Gas Chromatograph, Hewlett-Packard Company, Avondale, PA.)fitted with a nitroterephthalic acid modified polyethylene glycol megabore column (30 m x 0.53 mm i.d. with 1-µm film; J & W Scientific, Folsom, CA). Initial oven temperature was 130°C for 7 min, and helium flow was 7mL/min. The oven temperature was increased at the rate of 2.9°C/min over 7 min to a final temperature of 150°C. Helium flow was increased to 9 mL/min. Airflow was 400 mL/min, and hydrogen flow was 29 mL/min. Heptanoic acid was used as an internal standard.

Total fatty acid percentage and fatty acid profile were determined for forage and supplement samples. Fatty acids were methylated, methods described by Park and Goins (1994) and separated by GLC procedures, described by Duckett et al. (2002). For wet tissue lipid, 1 g of milk lipids were extracted. Milk lipid extracts containing approximately 2 mg of total lipids, based on the calculated percent lipids on a wet tissue basis, were transmethylated (Park and

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Goins, 1994). Fatty acid methyl esters (FAME) were analyzed using an HP6850 (Hewlett-Packard, San Fernando, CA) gas chromatograph equipped with an HP7673A (Hewlett-Packard, San Fernando, CA) automatic sampler. Separations were accomplished using a 100m Sp2560 (Supelco, Bellefonte, PA) capillary column (0.25mm i.d. and 0.20 µm film thickness) according to Duckett et al. (2002). Column oven temperature increased from 150 to 160 C at 1 C per min, from 160 to 167 C at 0.2 C per min, from 167 to 225 C at 1.5 C per min, and then held at 225 C for 16 min. The injector and detector were maintained at 250 C, with sample injection volume of 1 µL. Hydrogen was the carrier gas at a flow rate of 1mL per min. Individual FA were identified by comparison of retention times with standards (Sigma, St. Loui, MO; Supelco, Bellefonte, PA; Matreya, Pleasant Gap, PA). Fatty acids were quantified by incorporating an internal standard, methyl heptacosanoic (C27:0) acid; into each sample during methylation and expressed as g/100g of tissue.

Statistical analysis:

Data were statistically analyzed as a replicated 3 X 3 Latin Square using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) with animal as the experimental unit and dietary treatment and experimental period blocked. Ruminal pH, ammonia and VFA data were subjected to ANOVA using PROC MIXED procedures of SAS (2003). The model included cow, hour, period, treatment, hour × treatment, and error.

RESULTS AND DISCUSSION:

Pigeon peas replaced corn and soybean meal in dairy diets at two levels (P10 = 10%Pigeon peas; and, P20 = 20% Pigeon peas; Tables 5.1 and 5.2). Dry matter intake for treatment periods 1, 2 and 3 were 23.39, 22.76 and 23.20 kg/d, respectively. The DMI (kg/d) was lower during the second period, and higher for P10 diets (P < 0.05; Table 5.3). Increasing pigeon pea supplementation did not decrease DMI for cows. Treatment diets were balanced for CP and fiber content, and they were isocaloric and isonitrogenous

The SCC in milk samples tended to be higher for cows on the control diet compared with 10% pigeon peas diet (P > 0.10; Table 5.3). General agreement rests on the values of less than 100,000 cells/ml for uninfected cows, and greater than 300,000 for cows infected with significant pathogens. During treatment periods, SCC was never greater than 300,000 cells; therefore, the possible occurrence of a pathogen never affected experimental results. Diets do not generally affect SCC, but environment may affect SCC in dairy cows. The environment of tester animals was never altered during the experimental period. Milk protein percent was higher for cows on P20 compared with the P10 diet (P < 0.05; Table 5.3). The ECM accounts for the true protein content of milk and it was calculated using the equation: ECM = (0.3246 x kg of milk) + (12.86 x kg fat) + (7.04 x kg protein). Milk ECM was higher for cows when on the control diet compared with P10 (P < 0.05; Table 5.3). The cows fed control diet used more tissue energy to support milk production. Treatment had no effect on milk production (P > 0.10; Figure 5.7), but control cows tended to have higher milk production.

Ruminal fluid pH decreased during the 12 h period of sampling (P < 0.01; Figure 5.3); however, the pH never fell below 5.5. No effect of treatment on pH (P > 0.10; Figure 5.2) was observed. With typical diets fed to lactating dairy cows, the pH of ruminal fluid is usually between 5.5 and 6.5, whereas high forage diets support a pH in the range from 6.2 to 7. Inclusion of pigeon peas in the TMR did not have an effect on ruminal pH because it remained between 5.5 and 6.5 throughout the sampling interval. Cellulose digestion is inhibited when pH drops below 6 (Owens and Goetsch, 1988). Depressed fiber digestion has been attributed to low ruminal pH associated with feeding high levels of concentrate (Orskov and Fraser, 1975). They attributed this to the sensitivity of cellulolytic microbes to low ruminal pH. Depressed starch digestion has been attributed to an increased rate of flow through the rumen or small intestine, providing less time for digestion and absorption of starch (Zinn and Owens, 1980). With continuous feeding of high concentrate diets, animals tend to consume many meals per day and ruminal pH never rises sufficiently to initiate substantial cellulose digestion. The time after feeding when pH is lowest is usually between 0.5 to 4 h after a meal (Zinn and Owens, 1980). The lowest pH in the present study occurred at 8-10 h after the AM feeding (Figure 5.3); however, pH did decrease at 4 hours post-feeding. This drop in pH reflects the balance between rates of acid production, input of dietary buffers from saliva and presence or release of buffers or bases from the feed.

When pH falls from 7 to between 5 and 5.5, many ruminal microbes cease growing, despite an ability to survive even higher concentrations of H⁺. Most acids, like lactic acid, which inhibit microbes at high concentrations, are more effective at high concentrations and when pH is low. If microbes are inhibited by pH, a lower pH could ultimately decrease biohydrogenation in the rumen. Decreased rumen pH often results in bacterial population shifts and consequent changes in the pattern of fermentation end products (Van Soest, 1994). Leat et al. (1977) provided evidence showing that changes in ruminal bacterial populations are associated with modifications in the biohydrogenation pathways consistent with the altered trans-octadecenoic acid profile found in ruminal digesta and tissue lipids.

Ruminal ammonia decreased linearly during the first 6 h post-feeding, but at 8 h post-feeding it increased to concentrations similar to 2 h prior to feeding (Figure 5.4). Ammonia concentrations usually peak about 1-2 h after a meal, whereas with diets high in plant proteins

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high level peaks are usually at 3-5 h post feeding. Ammonia concentrations increased 2 h postfeeding, and decreased from the 2h concentration at 4h and 6 h post-feeding. At 8h post-feeding a second increase in ammonia concentration was observed, which probably resulted from a second meal taken by the cows after they returned from the 1500h milking. Ammonia disappears from the rumen pool because of uptake by microbes, absorption through the rumen wall and flushing to the omasum. Changes in any of these factors will alter ammonia concentration in the rumen. The decrease in ammonia can be attributed to an increase in ammonia uptake by microbes, or an increase in microbe numbers. An increase in microbe numbers could modify the biohydrogenation pathways within the rumen.

Volatile fatty acids are produced by specific microbial pathways and absorbed continually from the rumen. Ruminal concentrations represent the balance between rates of production and of removal for each VFA, as well as their interconversions. Total ruminal VFA were affected by dietary treatments (P < 0.05; Table 5.4). Acetate was higher in ruminal fluid when cows were fed both 10% and 20% pigeon peas. The control diet resulted in the highest concentration of propionate, leading to a lower acetate: propionate ratio. The control diet resulted in the lowest amount of butyrate but the highest concentrations of valerate. The acetate:propionate ratio was highest (P < 0.05; Table 5.4) for the P10 diet and the acetate:propionate ratio did not fall below 2.5. An acetate:propionate ratio below 2.5 has been associated with milk fat depression in lactating dairy cows (Woodford et al., 1986), a syndrome that has been attributed to increased ruminal synthesis of 18:1 *trans*-10 (Griinari et al., 1998) and CLA 10,12 (Baumgard et al., 2000). The P10 diet resulted in higher concentrations of ruminal synthesis of 18:1 *trans*-10 (Griinari et al., 1998) and cLA 10,12 (Baumgard et al., 2000). The P10 diet resulted in higher concentrations of ruminal isovalerate (P < 0.05; Table 5.4). Isobutyrate exhibited a sampling time x treatment interaction

(P < 0.05; Table 5.5). The control diet resulted in the lowest levels of isobutyrate, regardless of sampling time in relation to feeding.

The ruminal concentration of propionate increased with time following feeding (P < 0.01; Table 5.6). The VFA concentrations were not different by sampling time (P > 0.10). The acetate: propionate ratio decreased linearly from 4 h post feeding to 10 h post feeding (P < 0.05). The changes in pH, and ruminal ammonia occurring 1-2 h post feeding and then again at 8 h post feeding may have resulted in similar changes in the ruminal VFA synthesis.

Trans vaccenic acid (C18:1 *trans* 11; TVA) was not affected by dietary treatments (Table 5.7; P > 0.10). The *cis* 9, *trans* 11 and *trans* 10, *cis* 12 CLA isomers were not different among dietary treatments (P > 0.10; Table 5.8). The increase in ruminal concentrations of c9, t11 is reflective of higher concentrations of linoleic acid found in pigeon peas (Table 5.2). Trans vaccenic acid was not different among sampling times (P > 0.10; Table 5.9). However, ruminal concentrations of TVA increased linearly from 2h post feeding to 6 h and then decreased linearly. The *cis* 9, *trans* 11 increased linearly from 2 h post feeding to 6 h and then decreased linearly. The *trans* 10, *cis* 12 increased from 2 h post feeding to 4 h and then peaked at 8 h.

Concentrations of *trans*-10, *cis*-12 CLA increase under certain dietary conditions, which may including feeding high levels of unsaturated fatty acids (Bauman and Griinari, 2001). The two main CLA isomers (c9, t11 and t10, c12) in milk fat were not different among treatments in the current trial (P > 0.10; Table 5.10). The *trans*-10, *cis*-12 CLA isomer inhibits the activity of delta-9 desaturase (Lee et al., 1998; Bretillon et al., 1999), ultimately reducing endogenous synthesis of *cis*-9, *trans*-11 CLA. An increase in *trans*-10, *cis*-12 could have resulted in the decrease in *cis*-9, *trans*-11 in dietary treatments of 10 and 20% pigeon peas (Table 5.9). Under certain dietary conditions, such as high-concentrate, low-fiber diets, the profile of CLA can be altered so the concentration of the *trans*-10, *cis*-12 isomer increases in milk fat (Griinari et al., 1999). Diets low in roughage decreased ruminal lipolysis and biohydrogenation (Latham et al., 1972; Gerson et al., 1985). Therefore, it has been suggested that the main ruminal biohydrogenating bacteria are cellulolytic (Latham et al., 1972; Harfoot and Hazlewood, 1997). In addition, Griinari et al. (1998) demonstrated that an altered rumen environment induced by feeding high-concentrate, low-fiber diets is associated with a change in the *trans*-octadecenoic acid profile of milk fat. In this situation, *trans*-10 octadecenoic acid replaced *trans*-11 C18:1 as the predominant *tran*- C18:1 isomer in milk fat. Further evidence in support of a specific bacterial *cis*-9, *trans*-10 isomerase is provided by observations that low-fiber diets increase the proportion of *trans*-10, *cis*-12 CLA isomer in milk fat (Griinari et al., 1999). The diet is known to strongly influence the CLA content of milk and includes feedstuffs such as pasture, conserved forages, plant seed oils, cereal grains, marine oils and feeds and animal fat.

Studies with lactating dairy cows have demonstrated that even in herds in which all cows were managed similarly and fed the same diet, there may still be a threefold variation in the milk fat content of CLA (Jiang et al., 1996; Kelly et al., 1998a,b). These results suggest that it may be possible to see a difference in LCFA if the amount of pigeon peas was increased in the diet well above 20%. The inclusion rates of pigeon peas at 10% and 20% of the diets were apparently too low to effect significant differences in LCFA in milk, or in the rumen environment. Additional research is needed to evaluate the effect of higher levels of pigeon peas on the rumen environment and the concentration of conjugated linoleic acids in muscle, s.c. fat and/or milk.

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		P20
	% DM basis	
36.51	36.51	36.51
7.69	7.69	7.69
8.65	8.65	8.65
	% DM basis	
0	9.99	19.98
5.76	5.76	5.76
8.65	6.72	5.19
12.30	6.92	1.15
6.72	4.03	1.34
1.34	1.34	1.34
12.38	12.39	12.39
	7.69 8.65 0 5.76 8.65 12.30 6.72 1.34	36.51 36.51 7.69 7.69 8.65 8.65 % DM basis 0 9.99 5.76 5.76 8.65 6.72 12.30 6.92 6.72 4.03 1.34 1.34 12.38 12.39

Table 5.1: Composition of the different total diets for dairy cows.

^a Abbreviations: C = Control; P10 = 10% Pigeon Peas; P20 = 20% Pigeon Peas. ^b Ingredients were mixed together prior to blending the TMR.

^cConcentrate premix = citrus pulp, urea, Prolak, bicarbonate sodium, Dynamate, Limestone, Magox, salt, potassium carbonate, phosphate defluorinate, ZinproAvalia-4, Diamond V XP, Minvit2.

	•		Exp	perimental Die	ts
em ^a	Corn Silage	PP	С	P10	P20
			-% DM basis		
М	41.2	88.4	52.4	52.3	52.3
Р	11.0	24.0	19.6	18.2	19.1
DF	26.6	12.8	28.3	26.3	24.9
DF	14.3	12.1	18.8	19.1	17.9
otal FA	3.62	2.5	4.9	4.8	4.6
A composition		1	ng/100 mg of I	FA	
14:0	0.70	0.02	2.02	5.16	2.20
16:0	27.87	0.23	67.57	199.08	88.02
18:0	3.85	0.043	12.51	28.71	12.03
18:1	25.09	0.75	8.89	15.47	13.99
18:2	39.26	0.57	46.09	33.80	40.19
18:3	2.00	0.046	22.95	14.02	48.37
thers ^b	1.23	0.019	7.01	5.30	5.70
nidentified	0.0	0.8	30.20	22.79	24.48
nidentified	0.0	0.8	0%	30.20	

Table 5.2: Mean DM chemical and fatty acid composition of the total diets for dairy cows.

ige peas. ^b Sum of C12:0, C15:0, C16:1, C17:0, C20:0, C21:0, C22:0

	J			FF · · · · · F	
Item ^a	С	P10	P20	SE	<i>P</i> <
DMI, kg/d	22.92	23.38	22.87	1.40	0.04
Fat,%	3.24	2.92	3.29	0.12	NS
Protein, %	2.83 ^c	2.57 ^b	2.90°	0.10	0.05
ECM, %	35.02 ^c	29.41 ^b	33.37 ^c	3.67	0.02
lnSCC	1.72	1.60	1.38	0.10	0.07

Table 5.3: Dairy cow DMI and milk composition when fed supplemental pigeon peas.

^aAbbreviations: BFT = butter fat; PRT = protein; lnSCC = natural log of SCC; C = control; P10 = 10% Pigeon peas; P20 = 20% Pigeon peas. ^{bc}Means on same line with different superscript letters differ (P < 0.05).

Item ^a	С	P10	P20	SE	P<
Ruminal VFA		% of Total V	/FA		
Acetate	0.60	0.61	0.61	0.004	0.03
Propionate	0.24	0.23	0.23	0.005	0.02
Isobutyrate*	-	-	-	-	-
Butyrate	0.112	0.114	0.114	0.002	0.85
Isovalerate	0.014	0.016	0.014	0.0008	0.05
Valerate	0.021	0.021	0.020	0.0008	0.29
Total, mMol	95.84	98.50	100.18	1.26	0.57
Acetate:Propionate	2.55	2.74	2.66	0.19	0.03

Table 5.4: Ruminal fluid volatile fatty acids of dairy cows fed varying levels of supplemental pigeon peas.

^aAbbreviations: C = Control; P10 = 10% Pigeon peas; P20 = 20% Pigeon peas. *Isobutyrate exhibited an hour by treatment interaction, see Table 5.6

Item ^{ab}	Sample Time	С	P10	P20	SE
Isobutyrate			% of Tota	al VFA	
	- 2 h	0.01	0.01	0.008	0.002
	0*	-	-	-	-
	2 h	0.008	0.01	0.007	0.002
	4 h	0.008	0.007	0.008	0.002
	6 h	0.005	0.005	0.006	0.002
	8 h	0.003	0.004	0.007	0.002
	10 h	0.004	0.008	0.006	0.002

Table 5.5: Isobutyrate x treatment interaction in ruminal fluid of dairy cows fed supplemental pigeon peas.

^a Abbreviations: C = Control; P10 = 10% Pigeon peas; P20 = 20% Pigeon peas. ^b Sampling time X treatment interaction (P < 0.01) * 0 h = time of feeding. No samples were taken at 0 h.

<u>Sampling Time</u>									
Item	- 2 h	0 h	2 h	4 h	6 h	8 h	10 h	SE	P<
Ruminal VFA			(% of Tot	al VFA				
Acetate	0.61		0.62	0.62	0.62	0.59	0.60	0.01	0.13
Propionate	0.23		0.23	0.22	0.24	0.25	0.25	0.006	0.0008
Butyrate	0.11		0.11	0.12	0.11	0.12	0.11	0.008	0.52
Isovalerate	0.016		0.014	0.015	0.012	0.014	0.015	0.002	0.68
Valerate	0.02		0.02	0.02	0.02	0.021	0.021	0.0008	0.86
Total, mMol	97.69		113.06	82.14	91.90	106.70	97.55	13.37	0.05
Acetate:Propionate	2.70		2.76	2.85	2.68	2.46	2.45	0.20	0.04

Table 5.6: Ruminal fluid volatile fatty acids at different sampling times relative to feeding for dairy cows fed supplemental pigeon peas.

Item ^a	С	P10	P20	SE	P<
LCFA			% of Total FA		
C14	1.93	2.38	2.16	0.30	0.06
C14:1	3.02	3.46	3.62	0.40	0.31
C16	30.65	31.11	30.83	0.57	0.74
C16:1	0.41	0.43	0.54	0.07	0.08
C18:0	40.99	41.63	41.09	0.90	0.86
C18:1t11	4.43	4.80	3.96	0.46	0.04
C18:1c9	6.74	5.63	6.20	0.74	0.34
c9t11	0.21	0.24	0.22	0.04	0.74
t10c12	0.30	0.23	0.23	0.11	0.74
Total, mg/g	22.33	21.81	21.64	1.45	0.94

Table 5.7: Selected fatty acid composition of ruminal fluid of dairy cows fed varying levels of supplemental pigeon peas.

^a Abbreviations: C = Control; P10 = 10% Pigeon peas; P20 = 20% Pigeon peas.

	<u>Sampling Time</u>									
Item ^a	-2 h	0 h	2 h	4 h	6 h	8 h	10 h	SE	P<	
LCFA	LCFA% of Total FA									
C14	2.30		2.01	1.97	2.14	2.11	2.42	0.31	0.46	
C14:1	3.46		2.98	3.00	3.55	3.39	3.80	0.50	0.65	
C16	29.66		31.85	31.37	31.02	30.10	31.15	0.70	0.12	
C16:1	0.50		0.59	0.43	0.42	0.46	0.37	0.09	0.37	
C18:0	41.40		40.89	40.82	38.84	45.04	40.43	1.25	0.03	
C18:1t11	4.56		4.52	4.52	4.52	3.95	4.31	0.50	0.78	
C18:1c9	4.43		7.93	.46	6.46	5.23	6.63	0.90	0.04	
c9t11	0.19		0.20	0.30	0.25	0.20	0.21	0.05	0.51	
t10c12	0.20		0.22	0.20	0.42	0.26	0.22	0.13	0.66	
Total, mg/g	24.63		20.04	23.62	21.45	21.67	20.17	2.01	0.56	

Table 5.8: Fatty acid composition in ruminal fluid of dairy cows fed supplemental pigeon peas.

^a Abbreviations: C = Control; P10 = 10% Pigeon peas; P20 = 20% Pigeon peas.

Item ^a	С	P10	P20	SE	<i>P</i> <
LCFA	% of Total FA				
C14	10.97	10.64	10.57	0.35	0.48
C14:1	0.82	0.75	0.73	0.05	0.15
C16	33.90	34.99	36.04	1.35	0.29
C16:1	1.81	2.40	1.70	0.58	0.44
C18	18.76	12.36	13.05	4.41	0.29
C18:1t11	1.99	1.81	1.58	0.28	0.33
C18:1 c9	12.10	10.97	11.34	1.90	0.83
c9t11	0.48	0.78	0.57	0.19	0.29
t10c12	0.25	0.65	0.33	0.17	0.06

Table 5.9: Fatty acid composition in milk of dairy cows fed supplemental pigeon peas.

^a Abbreviations: C = Control; P10 = 10% Pigeon peas; P20 = 20% Pigeon peas.

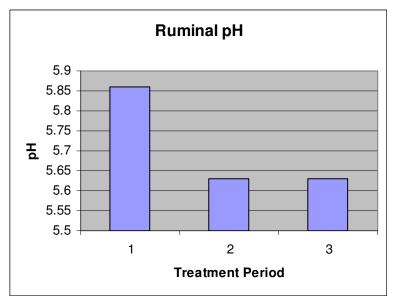


Figure 5.1: Ruminal fluid pH of dairy cows fed supplemental pigeon peas by treatment periods. (P < 0.0008; SE = 0.07)

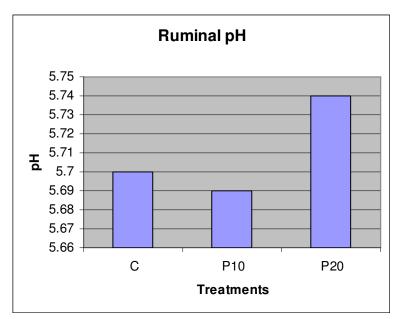


Figure 5.2: Ruminal fluid pH of dairy cows fed supplemental pigeon peas by dietary treatments. (P < 0.59; SE = 0.07) Abbreviations: C = Control; P10 = 10% Pigeon peas; P20 = 20% Pigeon peas.

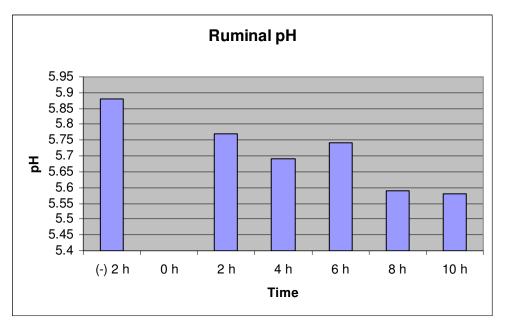


Figure 5.3: Effect of sampling time on ruminal fluid pH relative to time of feeding for dairy cows fed supplemental pigeon peas. (P < 0.002; SE = 0.08).

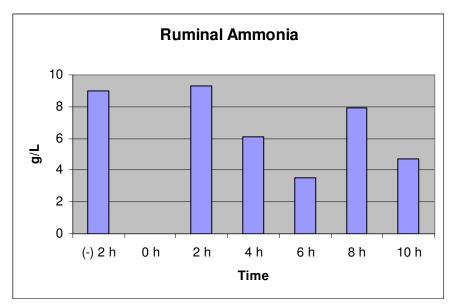


Figure 5.4: Effect of sampling time on ruminal fluid ammonia concentration relative to time of feeding for dairy cows fed supplemental pigeon peas. (g/L; P < 0.002; SE = 1.07).

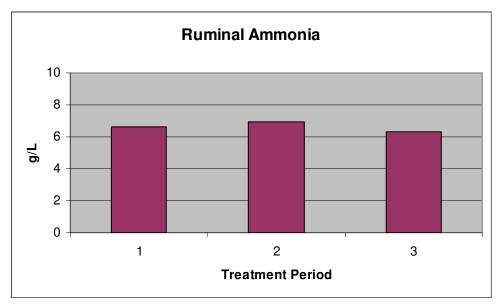


Figure 5.5: Effect of treatment period on ruminal fluid ammonia of dairy cows fed supplemental pigeon peas. (g/L; P < 0.85; SE = 0.08)

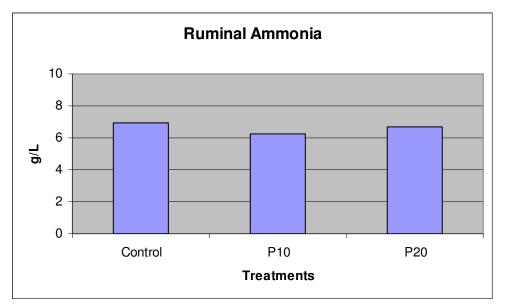


Figure 5.6: Ruminal fluid ammonia of dairy cows fed supplemental pigeon peas by dietary treatments. (g/L; P < 0.80; SE = 0.08) Abbreviations: C = Control; P10 = 10% Pigeon peas; P20 = 20% Pigeon peas.

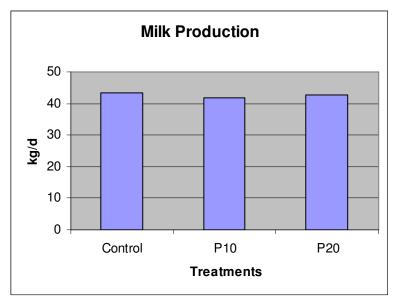


Figure 5.7: Milk production of dairy cows fed supplemental pigeon peas by dietary treatments. (P < 0.34) Abbreviations: C = Control; P10 = 10% Pigeon peas; P20 = 20% Pigeon peas.

CHAPTER SIX

CONCLUSIONS

The trend for North American beef production has included the use of high concentrate diets with limited amounts of roughage during the finishing phase. High grain diets maximize growth performance, limit time on feed, and eliminate problems associated with feeding roughages, including availability, transportation costs, storage and processing requirements, and variation in quality. With increasing cost of fuel, grain, and fertilizer and increased consumer demand, producers are using more forage for finishing cattle. Fat supplementation has become a common practice to increase dietary energy density for high producing dairy cows and finishing steers. Forages and concentrates are the primary sources of lipid in the ruminant diet. Forages typically contain 4 to 6 % of the dry weight of the leaf as lipid. Enhancing the content of conjugated linoleic acid (CLA), the cis-9, trans-11 isomer, has acquired attention, in the beef industry because of its anticarcinogenic and antiatherogenic effects.

Results from the first three studies demonstrate that beef nutraceutical properties could be enhanced by increasing its CLA *cis*-9, *trans*-11 isomer content through supplementation of grazing steers, drylot steers or finishing steers on low grain sorghum with corn oil. The positive effects that oil supplementation had on animal performance and carcass traits could facilitate the future use of these types of supplementation in forage-finishing beef systems. According to our results finishing steers on forages could enhance CLA *cis*-9, *trans*-11 isomer content. Corn oil supplementation in the first three experiments tended to increase c9, t11 proportions in the longissimis dorsi (LD) to a larger extent than in subcutaneous lipids. Steers on corn silage with oil supplementation diets had higher concentrations of *trans*-10, *cis*-12. However, steers fed corn silage diets in drylot corn oil supplementation had decreased c9, t11 in LD. A trend was observed for increased c9t11 and t10c12 with corn oil supplementation in s.c. lipids. Corn oil supplementation decreased palmitic (C16:0) and myristic (C14:0) acids in LD. These fatty acids are considered to have hypercholesterolemic effects on humans.

Pigeon pea [*Cajanus cajan* (L.)] is a drought tolerant legume originating in India and ranking sixth in production worldwide, compared with other grain legumes. They are an important grain legume crop grown in tropical and subtropical regions. The major market for good-quality pigeon peas are for human consumption, but byproducts may be available for incorporation into animal feeds. Pigeon peas containing up to 60% unsaturated fatty acids could increase the concentration of conjugated linoleic acids in milk. Results from the fourth study demonstrate that pigeon peas may be used as a protein supplement in dairy diets affecting neither milk production, DM intake nor the rumen environment. CLA isomers (c9, t11 and t10, c12) were not significantly different among treatments in milk samples. Supplementation rate of pigeon peas at 20% is not high enough to cause a dietary effect. Further research is needed to evaluate the effect of pigeon peas at greater supplementation rates on performance, ruminal parameters and concentration of conjugated linoleic acids in ruminant products.

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