ENHANCING BEEF CONJUGATED LINOLEIC ACID CONTENT THROUGH OIL SUPPLEMENTATION TO GRAZING STEERS

by

ENRIQUE PAVAN

(Under the Direction of Michael J. Azain)

ABSTRACT

Research was conducted to evaluate effect of increasing dietary linoleic acid through corn oil supplementation to grazing steers on beef conjugated linoleic acid (CLA) *cis*-9, *trans*-11 isomer content and on animal performance and carcass traits. First study: eighteen grazing Angus steers were supplemented with varying levels of corn oil (0% BW, **NONE**; 0.075% BW, **MED**; 0.15% BW, **HI**). Pelleted cottonseed hulls were used as a carrier for the corn oil. Oil supplementation had negative effects on pasture intake and digestibility, but not on animal performance. Oil supplementation increased carcass weight and fatness. Oil supplementation increased both CLA *cis*-9, *trans*-11 isomer and *trans*-11 vaccenic acid (TVA), except in the i.m. fat with HI where no differences were observed. Oil supplementation decreased myristic and palmitic acid proportions. Stearoyl-CoA desaturase activity and expression was not altered by oil level. Second study: 28 Angus steers grazing an endophyte-free tall fescue were supplemented with isoenergetic quantities of either corn grain (0.52% BW; **PC**) or soybean hulls plus corn oil (0.45 + 0.10% BW; **PO**). Negative (pasture only; **P**) and positive (85% concentrate: 15% roughage; **C**) control diets were included. PO, but not PC reduced pasture DMI; none affected total DMI. PC, but not PO affected NDF digestibility; none affected DM digestibility. PO and

PC carcasses were heavier than P, but lighter than C. Carcass fatness was greatest in C; were fatter in PO than in P, being PC intermediate. Intramuscular fat was greater in C than in the grazing treatments; PO tended to be greater than P. CLA cis-9, trans-11 and TVA content were increased by PO, but decreased by PC relative to P; C had the lowest content. Stearoyl-CoA desaturase expression was greatest in C; greater in PO, but not in PC than in P. Total CLA cis-9, trans-11 and TVA content in LM were similar for P, PC and C, but higher in PO; myristic and palmitic acids were greater in C than in the grazing treatments. Corn oil supplementation to grazing steers not only has positive effects on performance and carcass traits, but also enhances beef CLA *cis*-9, *trans*-11 and TVA content.

INDEX WORDS: Forage, Oil, Beef, Digestibility, Carcass, Fatty Acids, CLA

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ENRIQUE PAVAN

Ingeniero Agrónomo, Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Argentina, 1996

Magister Scientiae, Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Argentina, 2000

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ENRIQUE PAVAN

Major Professor:	Michael J. Azain
Committee:	Susan K. Duckett John G. Andrae Mark A. Froetschel Gary M. Hill

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
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DEDICATION

To my parents, for their unconditional love and support.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

ROLE OF CONJUGATED LINOLEIC ACID ON HUMAN HEALTH

Conjugated linoleic acid (CLA) refers to a family of positional and geometrical isomers of linoleic acid (C18:2 *cis*-9, *cis*-12) in which the double bond is separated by a carbon-carbon linkage rather than being separated by a methylene group. Pariza et al. (1979) were the first to report that the lipid extract from fried ground beef contained anticarcinogenic properties; later on, Pariza et al. (1986) reported that similar effects were also observed using extracts from raw meat. Ha et al. (1987) were able to demonstrate that the anticarcinogenic effect was triggered by the presence of a conjugated linoleic acid in beef fat.

In addition to the anticarcinogenic effect described initially, several other physiological effects of CLA have since been reported, and their mechanism(s) of action proposed:

- Anticarcinogenic (reviewed in Field and Schley, 2004; Lee et al., 2005; Bhattacharya et al., 2006)
- Anti-obesity (reviewed in Wang and Jones, 2004; Tricon et al., 2005; Bhattacharya et al., 2006; Salas-Salvadó et al., 2006);
- Antiatherogenic (reviewed in Tricon et al., 2005; Bhattacharya et al., 2006)
- Improved insulin sensitivity (reviewed in Aminot-Gilchrist and Anderson, 2004; Taylor and Zahradka, 2004; Tricon et al., 2005; Bhattacharya et al., 2006);
- Improved immune system status (reviewed in Tricon et al., 2005; Bhattacharya et al., 2006);
- Improved bone mineralization (reviewed in Watkins et al., 2004; Bhattacharya et al., 2006).

Although results on humans are not consistent, the evidence from animal studies strongly support the anti-carcinogenic effect of CLA (Field and Schley, 2004; Lee et al., 2005). That is

also the case for the other proposed physiological effects of CLA; all reviews concluded that more human studies are needed to validate the anticipated effects of CLA on human health based on research findings from laboratory animal species or cell cultured studies. According to Wahle et al. (2004) and to Bhattacharya et al. (2006) the inconsistent effects observed in human studies relative to the results observed in studies with laboratory animal species would be the consequence of a relatively lower CLA dose used in human studies, the lack of control of base diets, differences in metabolic state of the subjects used for evaluation, and the length of the evaluation period, among others.

In addition, another potential source of variation among studies could be the use of different mixes of CLA isomers. Recent studies with purified CLA isomers, show that CLA isomers *cis*-9, *trans*-11 and *trans*-10, *cis*-12 could act similarly or antagonistically to modify cellular function or metabolism, and could also act through different signaling pathways (Wahle et al., 2004; Bhattacharya et al., 2006). Thus, although both *trans*-10, *cis*-12 and *cis*-9, *trans*-11 isomers had antiproliferative effects on human prostate cancer cells these act through different mechanisms (Ochoa et al., 2004); CLA *trans*-10, *cis*-12 isomer would act through modulation of genes involved in apoptosis and cell cycle control; whereas *cis*-9, *trans*-11 isomer modulates the expression of genes involved in the arachidonic pathway and eicosanoid synthesis. Evidence exists that these isomers have antagonistic effects on insulin sensitivity; CLA *cis*-9, *trans*-11 ameliorates insulin resistance, whereas CLA *trans*-10, *cis*-12 increases insulin resistance (Taylor and Zahradka, 2004). Similarly, deleterious effects of CLA on oxidative process, on eicosanoid production and on carcinogenesis were mainly, but not exclusively, observed with CLA *trans*-10, *cis*-12, but not *cis*-9, *trans*-11 or with their mixture (Wahle et al., 2004). Finally, whereas CLA isomer *trans*-10, *cis*-12 would be responsible for the anti-obesity effects (Wang and Jones,

2004), the isomer *cis*-9, *trans*-11 seems to be the more effective in improving cardiovascular health (Bhattacharya et al., 2006). Although there is sufficient evidence that each isomer has specific functions, very few studies have been reported with purified isomers, making it difficult to define the protective effect of the individual biologically active isomers.

Due to these difficulties there is not yet a recommended level of CLA intake. From the human studies reviewed by Bhattacharya et al. (2006) the minimum CLA level that significantly affected serum lipids content or fat mass were 0.7 or 1.8 g/d. However, the minimum effective CLA dose needed to help prevent the incidence of cancer in animal models is 0.05% of the diet (Ip et al., 1994). For an average human daily food intake of 600 g, this percentage would represent a daily intake of 300 mg of CLA.

CONJUGATED LINOLEIC ACID IN FOODS

Chin et al. (1992) evaluated the CLA content of different food items including meat, poultry, seafood, dairy products, plant oils, and infant and processed foods. They observed that meat from ruminant and dairy products contained significantly greater CLA amounts than food derived from non-ruminant animals or from vegetable oils. In addition, 75 to 90% of the total CLA in ruminant products was the CLA *cis*-9, *trans*-11 isomer, but less than 50% in vegetable oils. In the american diet 60% of total dietary CLA or 68% of total dietary CLA *cis*-9, *trans*-11 comes from dairy products; whereas ruminant meats contributes with an another 32 and 25%, respectively (Ritzenthaler et al., 2001). Ritzenthaler et al. (2001) estimated total CLA intake to be 212 and 151 mg/d for men and women in the USA, respectively, and CLA *cis*-9, *trans*-11 intake 193 and 140 mg/d, respectively. Wahle et al. (2004) suggested that dietary contribution of CLA in the UK could be 200 mg greater than the actual CLA intake as a result of *trans*-11 vaccenic acid (**TVA**) intake. Turpeinen et al. (2002) has estimated that on average, 19% of the

dietary **TVA** could be converted to CLA *cis*-9, *trans*-11 in human tissues via the stearoyl-CoA desaturase enzyme.

Dhiman et al. (2005a) calculated that one normal serving of cheese, milk, lean beef and chicken would provide a total CLA intake of 127 mg/d, but that total CLA intake could rise to 441 mg/d when replacing low-CLA products by CLA-enriched products. For this estimation Dhiman et al. (2005a) used grain-fed beef as low-CLA beef and pasture-fed beef as the CLA-enriched beef. Thus a further increase of CLA, in particular isomer *cis*-9, *trans*-11, or TVA content in pasture-fed beef would enhance its nutraceutical properties and reduced the need for supplements with synthetic CLA.

CONJUGATED LINOLEIC ACID IN BEEF MEAT

Research in lactating dairy cows (Griinari et al., 2000; Corl et al., 2001; Kay et al., 2004; Mosley et al., 2006) has shown over 85% of CLA, *cis*-9 *trans*-11 isomer present in mammalian adipose tissues is produced via stearoyl Co-A desaturase enzyme (SCD) (Ntambi, 1995). Santora et al. (2000) showed that adipose tissue CLA *cis*-9, *trans*-11 content in rodents is increased by dietary supplementation of TVA, remarking the importance of SCD in CLA accumulation. Duckett et al. (2002) suggested that conversion of TVA to CLA *cis*-9, *trans*-11 by SCD in the adipose tissue also serves as major source of CLA in beef fat based on duodenal outflows of TVA and CLA *cis*-9, *trans*-11. Furthermore, based on ratios of TVA: CLA *cis*-9, *trans*-11 in duodenal and adipose tissues Gillis et al. (2003) suggest that over 86% of tissue CLA in beef originates from endogenous desaturation of TVA. The rest of the CLA *cis*-9, *trans*-11 present in beef tissues originates as an intermediate product of linoleic acid ruminal biohydrogenation. Thus the amount of CLA *cis*-9, *trans*-11 in the adipose tissues depends upon the availability of TVA and the activity of SCD.

Both TVA and CLA *cis*-9, *trans*-11 are produced during ruminal biohydrogenation of linoleic acid (Hartfoot and Hazlewood, 1997). Ruminal biohydrogenation of dietary unsaturated fatty acids occurs as a result of fatty acid utilization by fatty acid-auxotrophic bacteria, as a way to dispose reducing power or to reduce the toxicity of fatty acids (Hartfoot and Hazlewood, 1997). Biohydrogenation of linoleic acid to stearic acid occur in three steps: isomerization of linoleic acid to CLA *cis*-9, *trans*-11 and hydrogenation of the two double bonds (Hartfoot and Hazlewood, 1997). The double bond in the *cis*-9 position is hydrogenated first producing TVA as the second intermediate product before complete hydrogenation to stearic acid (Hartfoot and Hazlewood, 1997). Although the first two steps take place rapidly, the third bio-hydrogenation step, hydrogenation from TVA to stearic acid, occurs at a slower rate producing a net accumulation of TVA in the rumen (Hartfoot and Hazlewood, 1997). As a result of the ruminal accumulation of TVA, duodenal flow of CLA *cis*-9, *trans*-11 is negligible when compared to that of TVA (Duckett et al., 2002; Sackmann et al., 2003).

In rodents, stearoyl-CoA desaturase (SCD) is primarily found in the liver (Ntambi, 1995); whereas in growing ruminants it is found primarily in the adipose tissue (St John et al., 1991; Martin et al., 1999). Stearoyl-CoA desaturase is key-limiting enzyme in the production of monounsaturated fatty acids. Although, palmitic and stearic acids are the preferred substrates, *trans*-11 vaccenic acid can also be desaturated (Ntambi, 1999). Thus its activity and expression could alter the fatty acid composition of phospholipids and triglycerides. In rodents, diets rich in PUFA have reduced hepatic abundance of SCD (Ntambi, 1992; Jump and Clarke, 1999; Ntambi, 1999) and decrease dietary TVA conversion to CLA *cis*-9, *trans*-11 (Santora et al., 2000). However, the basal transcriptional activity of bovine SCD was not affected by PUFA (Keating et al., 2006). Furthermore, neither SCD activity in the adipose tissue from growing steers (Page et

al., 1997) or its expression in the mammary gland (Delbecchi et al., 2001) of dairy cattle was affected when increasing dietary PUFA. Keating et al. (2006) also observed a reduced activity expression of bovine SCD by CLA *cis*-9, *trans*-11 and *trans*-11, *cis*-12 isomers. Although, Lee et al. (1998) observed that dietary CLA reduced hepatic SCD expression in rodents and that the effect was not due to the CLA *cis*-9, *trans*-11.

INCREASING CONJUGATED LINOLEIC ACID IN BEEF PRODUCTS

In order to enhance CLA *cis*-9, *trans*-11 content in beef meat, different studies evaluated the use of vegetable oils or oil seeds to increase dietary linoleic acid content in high-corn diets, but increases were only marginal when significant (Beaulieu et al., 2002; Madron et al., 2002; Gillis et al., 2004; Dhiman et al., 2005b). No effects were observed in the proportion of CLA *cis*-9, *trans*-11 in muscle or adipose tissue by adding 5% (Beaulieu et al., 2002) or 2 and 4% (Dhiman et al., 2005b) of soybean oil (DM basis) to the basal diet. When Madron et al. (2002) added 25.6% of extruded full-fat soybean (ESB; 48.6% linoleic acid) the basal diet CLA *cis*-9, *trans*-11 increased by 0.11 percentage units from a 0.66 % of total FA in muscles tissues (rib longissimus, eye round and chuck tender) from steers fed the basal diet, but no effects were observed when 12.7% ESB were added. Gillis et al. (2004) added 4% corn oil (58% linoleic acid) to the basal diet CLA *cis*-9, *trans*-11 increased by 0.07 percentage unit on average of three adipose tissues (intramuscular, perirenal and s.c). Madron et al. (2002) observed similar increases in TVA and *trans*-10 octadecenoic acid; from 1.33 to 1.71% and from 0.85% to 1.20% of total FA, respectively. Whereas Gillis et al. (2004) observed a greater increased in *trans*-10 octadecenoic acid than in TVA. Corn oil supplementation increased TVA from 0.84% in the basal diet to 0.98%, *trans*-10 octadecenoic acid from 1.4 to 1.78% of total FA when oil was fed for 32 d and from 1.25 to 1.78% when fed for 60 d (Gillis et al., 2004). Sackmann et al. (2003)

showed that in a typical high-corn diet (12% forage) duodenal flow of *trans*-10 octadecenoic acid was 12.2 fold greater than TVA flow. Furthermore, Duckett et al. (2002) showed that increasing dietary linoleic acid in high-corn diets increase duodenal flow of *trans*-10 octadecenoic acid as proportion of total linoleic acid intake, but not TVA flow.

It was observed that as the proportion of forage in the diet is increased, duodenal flow of *trans*-10 octadecenoic acid as proportion of total linoleic acid intake decreases whereas that of TVA increases (Sackmann et al., 2003; Loor et al., 2004). Thus, in high-forage diets, the proportion of TVA availability in the small intestine for absorption and later endogenous desaturation to CLA *cis*-9, *trans*-11 is greater than in high-corn diets. The proportion of intramuscular CLA *cis*-9, *trans*-11 from grazing cattle was greater than from cattle fed a high-concentrate diet (French et al., 2000; Rule et al., 2002; Poulson et al., 2004; Realini et al., 2004). Additionally, increasing days on pasture before slaughter from 0 to 40, 99 or 158d linearly increased both TVA and CLA *cis*-9, *trans*-11 in i.m. and s.c. fat from beef heifers (Noci et al., 2005a). Intramuscular TVA and CLA *cis*-9, *trans*-11 proportion increased from 1.35 and 0.50% with 0 d to 3.01% with 0 to 0.71% with 158 days on pasture, respectively; whereas in the s.c. fat these isomers increased from 1.34 and 0.66% to 4.1 and 1.64%, respectively. When dietary linoleic acid was increased in cattle fed grass-silage based diets by gradually replacing lard with a linoleic-rich sunflower oil, the i.m. proportion of CLA *cis*-9, *trans*-11 linear increased from 0.43 to 0.91% of the total FA (Noci et al., 2005b). This data suggest that CLA *cis*-9, *trans*-11 and TVA proportion in the adipose tissues from beef cattle fed high-forage diets could be increased when high-linoleic acid vegetable oil is supplemented; though limited information is available to confirm this assumption from studies with grazing beef cattle in forage based systems.

OIL SUPPLEMENTATION IN HIGH-FORAGE DIETS

Previous research has shown that oil supplementation greater than 3-5% can negatively impact fiber digestion, intake, and animal performance. Brokaw et al. (2001) evaluated the effect of lipid supplementation on *in vivo* digestibility of grazing beef cattle; supplementation with 0.035% of BW of soybean oil did not affect NDF digestibility. Others have evaluated the effects of lipid supplementation on *in vivo* digestibility for high-forage diets (≥ 50%) by using hay as the forage source (Hardin et al., 1989; Hall et al., 1990; Patil et al., 1993; Scholljegerdes et al., 2004). Hardin et al. (1989) and Hall et al. (1990) observed a reduction in NDF digestibility of bermudagrass hay with lipid supplementation compared to non-supplementated cattle. Total tract NDF digestibility of bromegrass hay was reduced 17% when 5% high-linoleate or high-oleate safflower cracked seeds were supplemented (Scholljegerdes et al., 2004). In contrast, Patil et al. (1993) reported no differences in NDF digestibility of bermudagrass hay with the addition of 0.33 or 0.67% BW of partially hydrogenated tallow. According to Palmquist (1984) and Jenkins (1993) the effects of lipid supplementation on fiber digestion depend on the oil source, its fatty acid composition, quantity of lipid supplemented and proportion of forage in the diet.

Hardin et al. (1989) and Patil et al. (1993) did not observe any effect on average (ADG) when grazing steers were supplemented with soybean oil or partially hydrogenated tallow, respectively. Whitney et al. (2000) observed a quadratic increase in ADG with soybean oil supplementation to heifers consuming hay; however, no differences were observed in a companion trial. In addition, Patil et al. (1993) observed an increased in marbling score and hot carcass weight when partially hydrogenated tallow was supplemented to grazing steers.

CONCLUSIONS

Though still under study, conjugated linoleic acids have potential for beneficial effects on human health. At present, the available CLA in the typical human diet is presumed to be bellow the minimum level required to elicit a response. However, dietary CLA intake could be increased by either increasing total intake of ruminant meat or dairy food products and by increasing their CLA and TVA content. Although beef CLA or TVA content were not enhanced when dietary level of linoleic acid was increased in animals fed high-grain diets, a different response in grazing cattle could be expected when linoleic acid is increased, through vegetable oil supplementation. However, vegetable oil supplementation could negatively affect productive performance of grazing steers in forage-finishing systems through its effects on fiber digestibility. Thus, the effects of supplementation with oils rich in linoleic acid to grazing beef cattle needs to be evaluated with special consideration to the effects on fatty acid composition, animal performance, and carcass quality.

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CHAPTER 2

CORN OIL SUPPLEMENTATION TO STEERS GRAZING ENDOPHYTE-FREE TALL FESCUE. I. EFFECTS ON *IN VIVO* DIGESTIBILITY, PERFORMANCE, AND CARCASS TRAITS[1]

[1] Pavan, E., S. K. Duckett, and J. G. Andrae. Submitted to J. Anim. Sci., 09/11/2006.

ABSTRACT: Eighteen Angus steers (438 ± 4 kg) were supplemented with varying levels of corn oil (0 g/kg BW, **NONE**; 0.75 g/kg BW, **MED**; 1.5 g/kg BW **HI**) on rotationally stocked endophyte-free tall fescue to determine the effect of supplemental oil level on *in vivo* digestibility, intake, performance, and carcass traits. Pelleted cottonseed hulls were used as a carrier for the oil supplements and all supplements were offered to steers using Calan gate feeders for individual intake determination. On d 49, each steer was dosed with a controlled release capsule containing chromium sesquioxide and fecal samples obtained 12 d later over a 7 d period to estimate fecal output that, with forage, supplement, and fecal INDF concentration, was used to estimate DMI and *in vivo* total diet digestibility. Steers were slaughtered at the end of the 116-d grazing period and carcass data collected at 24 h postmortem. Data were analyzed as a completely randomized design using the MIXED procedure of SAS with animal as experimental unit. Total fatty acid intake linearly increased with corn oil supplementation, and forage DMI, total DMI, and total DE intake were linearly decreased ($P < 0.01$). The decrease in total DMI was reflected in forage substitution rates greater ($P \leq 0.01$) than one, with a trend ($P = 0.09$) for a greater substitution rate in HI than in MED. In vivo DM, OM and NDF digestibility were linearly decreased ($P < 0.01$) by corn oil supplementation. Average daily gain and final BW tended ($P = 0.09$) to increase linearly in response to oil level. Oil conversion (0.36 kg BW gain/kg corn oil) was higher ($P \leq 0.05$) than zero and did not differ ($P = 0.15$) between MED and HI. Dressing percentage ($P = 0.09$), carcass weight ($P = 0.01$) and carcass backfat thickness ($P = 0.01$) increased linearly with oil supplementation. No treatment effect was observed for carcass LM area, KPH fat percentage, marbling score, or yield grade ($P > 0.10$). Oil supplementation to grazing steers linearly reduced forage DMI intake; however, animal performance was maintained

and tended to be greater for oil supplemented cattle. Oil supplementation increased carcass fat thickness and weight without altering other carcass quality parameters.

Key Words: Beef, Carcass, Digestibility, Forage, Corn Oil Supplementation

INTRODUCTION

Recent interest in enhancing fatty acid composition of beef, particularly the cis-9 trans-11 isomer of CLA, has resulted in utilization of vegetable oils as linoleic acid sources for ruminant animals. Oil supplementation to concentrate-finished animals has resulted in small changes to tissue CLA or trans-11 vaccenic acid (TVA) levels (Beaulieu et al., 2002; Madron et al., 2002; Gillis et al., 2004). Previous 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.

Previous research has shown that oil supplementation can impact fiber digestion and potentially lower animal performance. The 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 total dietary lipid levels less than 1.6 g/kg BW (Zinn, 1994). Limited information is available on the supplementation of unsaturated plant oils to grazing steers in a forage finishing system. Therefore, the objective of this study was to determine the effect of corn oil supplementation level to steers grazing endophyte-free tall fescue on performance, *in vivo* digestibility, and carcass quality.

MATERIALS AND METHODS

Study site. The experiment was conducted at University of Georgia Wilkins Beef Unit (Rayle, GA) between October 2003 and April 2004. The soil types present in the utilized plot are Enon (EnB) and Mecklenburg (MeB) fine sandy loam, with 2 to 6 percent slopes. Pasture consisted of a 21-ha endophyte-free tall fescue (*Festuca arundinacea* Shreb cv. Jesup) plots subdivided into 27 paddocks of approximately 0.77-ha each for rotational stocking. Pasture was fertilized with 336 kg/ha of 20-5-10 in September, but because of a drought during September and October pasture was fertilized in November an additional 168 kg/ha. Animals were rotated from grazed paddocks when pasture height was reduced to approximately 6 cm. Steers had ad libitum access to the paddock, fresh water and to a vitamin-mineral premix except for 2 h when supplements were offered (0800-1000). Initial and final forage availabilities were estimated, every three paddocks, by harvesting 10 random samples (0.09 m^2 frame) at 1 cm height. The 10 samples from each cutting time were weighed and pooled, and a sub-sample was frozen at -20ºC for subsequent chemical analysis. An additional sub-sample was oven dried at 60ºC for 48 h to estimate DM content.

Animals. Eighteen Angus steers (438 ± 4 kg; 16 mo of age) obtained from the Northwest Georgia Experiment Station in Calhoun, GA, were randomly assigned to one of three supplementation treatments. Steers were treated for internal and external parasites (Unimectrin; Universal Co-op., Eagan, MI) on d -29, 21 and 61. The three supplementary treatments consisted of three corn oil supplementation levels: 0 g/kg BW (NONE), 0.75 g/kg BW (MED) and 1.5 g/kg BW (HI). Pelleted cottonseed hulls (CSH) were used as a carrier for the oil supplement and were fed at equal amounts to all steers regardless of treatment throughout the experiment. Levels of CSH fed to steers (0.7 to 1 % BW) were adjusted across treatments during the experiment

according to forage availability. Samples of CSH and corn oil were taken on a monthly basis and frozen at -20ºC for subsequent analyses.

Steers were trained to use Calan gate feeders (American Calan, Inc., Northwood, NH) and then adapted to supplement treatments for 29 d prior to the start of trial. During adaptation, steers were allowed to graze the same pasture that was used for the experimental period. At the beginning (d 0) and end (d 116) of the experimental period steers full weights were recorded on two consecutive days at 0800 and averaged. Live weights were recorded before supplementation at d 21, 42, 69, 85, and 104, and used to determine gain and adjust the amount of supplement to the appropriate BW level. Real-time ultrasound measures of s.c. fat thickness and LM area were collected on d 116 using an Aloka 500-V ultrasonograph (Corometrics Medical Systems, Wallingford, CT) equipped with a 17-cm, 3.5-MHz linear probe. Images were interpreted using Beef Information Manager software, Version 3.0 (Critical Vision, Inc., Atlanta, GA).

Intake and digestibility. Forage DM intake and total DM *in vivo* apparent digestibility were estimated using chromium sesquioxide as an external marker and indigestible NDF (INDF) as an internal marker of the digesta (Lippke et al., 1986). On d 49, a controlled release fecal marker capsule (CAPTEC, Cattle Chrome MCM for 300-700 kg Cattle, active constituent: chromium sesquioxide 65% w/w; release rate 1.7 g Cr_2O_3 daily; NUFARM Ltd., Auckland, NZ) was bolused to each steer. Twelve days later, steers were moved to a new paddock and fecal collection started one day later. Fecal samples were collected from each steer at 0700 and 1700 for 7 d. Fecal samples (25 g wet weight) were pooled for each animal and frozen at -20ºC for subsequent analyses. Supplement refusals were recorded daily and samples collected for DM determination. Pre- and post-grazing pasture samples from each paddock were collected and stored at -20ºC for subsequent analyses of DM composition. Samples were used to determine

DM composition of consumed forage in each paddock during fecal collection period by herbage mass difference (Burns et al., 1994) assuming that treatment did not affect forage selection.

Forage, CSH, and fecal 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 8 h 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). Nutrient and fatty acid composition of forage, CSH, and corn oil are shown in Table 2.1. Forage, CSH, and fecal samples collected during the fecal collection period were also analyzed for gross energy using a Parr bomb calorimeter (Parr Instrument Company, Moline, IL, USA) and for INDF. For INDF analysis, samples were incubated *in situ* for 7 d (Lippke et al., 1986) in the rumen of two Holstein steers with ad libitum access to tall fescue hay. Residual samples were analyzed for NDF concentration as described previously. Chromium concentration was measured via spectrophotometry in the fecal samples (Fenton and Fenton, 1979). Forage DMI was calculated based on fecal output and indigestibility (Reis and Combs, 2000) with modifications. Fecal output was calculated based on chromium concentration in the feces, whereas indigestibility was determined based on forage, CSH, and feces INDF concentrations. Total forage INDF excreted was estimated by difference between total INDF excreted and total CSH INDF. Forage DMI was then calculated as the ratio between total forage INDF excreted

and INDF concentration in the forage sample. The DMI for each component of the diet was multiplied by the corresponding concentration of OM, CP, FA, NDF, and ADF that then were added to obtain total DMI and total OM, CP, FA, NDF, and ADF intakes. Total tract DM, OM, FA, NDF, and ADF digestibilities were estimated as the coefficient of the total tract disappearance (intake minus excretion) and intake. The proportion calcium soaps in total fecal lipid excreted was estimated as the ratio of the difference in lipid content of fecal acidified and non-acidified samples, and lipid content in acidified samples. Acidified samples were pretreated with 0.5 N HCL for 8 h (Bohman and Lesperance, 1962) before the lipid extraction to account for any fecal calcium soap present in the sample. Lipid extraction was performed using the chloroform:methanol procedure (Folch et al., 1957) instead of ether as suggested by Andrae et al. (2000).

Carcass traits. On d 117, steers were transported 45 km to the University of Georgia Meat Science and Technology Center in Athens and fasted live animal weights were obtained following an overnight feed withdrawal. After slaughter, HCW was recorded and carcasses chilled at -1ºC for 24 h. At 24 h postmortem adjusted s.c. fat thickness, LM area, marbling score, percentage of KPH fat, and skeletal maturity were determined on the left side of each carcass.

Supplement conversions. Total diet conversion efficiency was calculated as the ratio between total BW gain (final BW minus initial BW) and total DMI throughout the experiment. Total DMI throughout the experiment was calculated by adding daily CSH and corn oil intake per animal and the total forage DMI, which was estimated from the average BW of each animal during the supplementation period and the forage DMI as a percent of BW estimated using chromium sesquioxide and INDF. Individual oil conversions (OC) for steers in MED and HI

were calculated as follows: [(individual BW gain, kg)$_{\text{MED or HI}}$ − (average BW gain, kg)$_{\text{NONE}}$] / (Corn oil intake, kg)$_{\text{MED or HI}}$.

The substitution rate of forage DMI with corn oil supplementation was calculated as the difference between average forage DMI in NONE and individual forage DMI in MED and HI relative to the corn oil intake (kg/d; Bargo et al., 2003). The relative stocking density was defined as the approximate number of animals in MED and HI treatments needed to utilize equal amounts of forage relative to NONE. Relative stocking density was calculated as the ratio between the average forage DMI for NONE and the individual forage DMI for either MED or HI.

Statistical analysis. Fecal chromium concentration for one steer was three standard deviations below the overall mean, resulting in an extremely high DMI (28 kg) when compare to the overall mean (13.4 kg and SD 0.97). Therefore, data from this animal was removed from the dataset due to an apparent malfunction of the controlled release chromium sesquioxide capsule. Intake, digestibility and carcass variables were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) with animal as the experimental unit and dietary treatment as a fixed effect. Initial weight (d 0) was used as a covariate when $P \leq 0.05$. Differences in corn oil level were compared using orthogonal polynomial contrasts for linear and quadratic effects. When significant ($P < 0.05$), linear or quadratic equations were predicted using REG procedure of SAS (SAS Inst., Inc., Cary, NC). The relationship between carcass and real time ultrasound measures taken just before slaughter was determined using the regression procedure of SAS. For substitution rate and relative stocking density, values for MED, and HI were tested to determine significance from one.

RESULTS

Intake and digestibility. During the intake evaluation period, CSH intake was similar among supplementation treatments either when expressed as kg DM (3.5 $kg \cdot hd^{-1} \cdot d^{-1}$; $P \geq 0.12$) or as % of BW (0.73% BW, $P \geq 0.39$). Corn oil intake increased linearly ($P < 0.01$) with higher levels of corn oil supplementation (Table 2.2). As corn oil intake increased there was a linear decrease in forage (-2.42 kg × g/kg BW of corn oil; $P < 0.01$) and total DMI (-1.76 × g/kg BW of corn oil; $P < 0.01$). Total fatty acid intake increased ($P < 0.01$) with corn oil supplementation level. The proportion of total lipid excreted as calcium salts was not altered by oil supplementation (16.2 ± 4.6%, linear effect, $P = 0.99$; quadratic effect, $P = 0.88$). These changes in DMI with oil supplementation resulted in greater proportions of supplement contributing to total dietary DMI (Supplement, % of DMI = 24.6 + 10.5 × g oil /kg BW; $P < 0.01$). The proportion of OM and ADF in the diet increased linearly ($P < 0.01$) with oil supplementation. In contrast, the calculated dietary proportion of crude protein decreased linearly ($P < 0.01$) with oil supplementation. Dietary NDF concentration was not altered with oil supplementation. Gross energy concentration of the diet increased linearly ($P < 0.01$); however, DE concentration was not changed ($P = 0.42$) by oil level. Thus in accordance with total DMI, total DE and total GE intake linearly decreased with oil supplementation ($P < 0.01$).

Apparent *in vivo* DM, OM, and NDF digestibility decreased linearly ($P < 0.01$) with increasing level of oil supplementation (-3.21, -3.96, and -4.11% units per g/kg BW of oil supplemented, respectively; Table 2.3). Oil supplementation did not alter the apparent *in vivo* digestibility of dietary ADF or total fatty acids. The proportion total lipid excreted as calcium salts was not affected by the level of corn oil supplemented, averaging 16.3% ($P > 0.87$).

Performance and carcass traits. Pre- and post-grazing forage DM throughout the study averaged 3,216 ± 191 kg/ha and 2,069 ± 122 kg/ha, respectively. Each paddock was grazed for 3.9 ± 0.2 d. By d 61 of supplementation there was a trend ($P = 0.10$) to a linear BW increase with corn oil supplementation that was sustained ($P = 0.09$) at end of supplementation (d 116; Table 2.4). Thus there was a trend ($P = 0.08$) for total BW gain to increase with corn oil supplementation. During the first 61 d, ADG tended ($P = 0.10$) to increase linearly with oil supplementation level. From d 61 to 116, there was a trend for a quadratic response ($P = 0.07$) in ADG with MED having the highest rate of gain. Overall, ADG tended to increase ($P = 0.09$) 0.12 ± 0.07 kg per each unit (g/kg BW) of added corn oil. Similarly, final BW tended ($P = 0.09$) to increase linearly with corn oil supplementation. Ultrasound s.c. fat thickness tended to increase linearly ($P = 0.08$; 0.10 ± 0.05 cm) with oil supplementation. Oil supplementation did not alter LM area as measured by real-time ultrasound. The standard errors of prediction for fat thickness, and LM area with ultrasound (0.11 cm and 6.0 cm^2, respectively) were within the range required for certification by the Beef Improvement Federation (0.30 cm and 7.74 cm^2). Oil supplementation increased HCW and fat thickness linearly ($P < 0.01$); whereas no differences in carcass LM area were observed among treatments. For both LM area and fat thickness, ultrasound measures overestimated actual carcass measures of fat thickness (carcass fat thickness cm = 0.002 + 0.699 x ultrasound fat thickness; $R^2 = 0.54$) and LM area (carcass LM area cm^2 = 5.43 + 0.84 ultrasound LM area; $R^2 = 0.38$). Oil supplementation did not alter other carcass traits of KPH percent, marbling score, quality grade, or yield grade. Carcass price ($/kg) did not differ among treatments; however, carcass value was higher ($P = 0.05$) with oil supplementation because of heavier carcass weights.

Oil supplementation linearly increased ($P < 0.01$) the conversion efficiency of the total diet from 0.049 kg gain/ kg total DMI in NONE to 0.062 and 0.082 in MED and HI, respectively. Substitution rate effects for MED and HI were significant ($P \leq 0.01$ for significance from 1; Table 2.5). These results show that each kg of corn oil supplement substituted for significantly more than one kg of forage DM. As corn oil supplementation increased from 0.75 to 1.5 g/kg BW, the substitution rate tended to be higher ($P = 0.09$) for HI than MED. This increase in substitution rate resulted in a higher ($P < 0.01$ for significance from 1) calculated relative stocking density for HI but not for MED ($P = 0.20$ for significance from 1). According to these calculations, 1.6 more animals could be stocked to obtain the same forage utilization when supplemented with corn oil at 1.5 g/kg BW. The relative stocking density was also greater ($P < 0.01$) for HI than MED. Oil conversion did not differ between MED and HI ($P = 0.81$); on average, BW gain of supplemented steers was increased by 0.36 kg per kg of corn oil supplemented with respect to BW gains of NONE.

DISCUSSION

The range of total DMI (% BW) are in accordance to the values reported in the literature for steers or heifers grazing endophyte-free (3.4%, Burns at al., 1991; 1.8-2.7% BW, McCracken et al., 1993; 2.5% BW, Judkins et al., 1997), low-endophyte (2.4-2.7% BW, Hitchcock et al., 1990), or endophyte-infected (3.2%, Elizalde et al., 1998) tall fescue without supplementation or with supplementation (3.4% BW, Elizalde et al, 1998; 2.8-3.0% BW, Judkins et al., 1997). To our knowledge, this is the first work evaluating the effects of high levels of supplemental corn oil supplementation to grazing cattle in a forage-finishing system. Recently, Schroeder et al. (2004) reviewed the literature on fat supplementation to grazing dairy cattle. In this review, the effects of fat supplementation on DMI were non-significant; however, only 8 of 25 comparisons

evaluated the effect of supplementation on DMI. In the reviewed studies, lipid sources supplemented were of calcium salts of fatty acids or hydrogenated oils. According to Jenkins (1993), supplementation of calcium salts or hydrogenated oils would have a lower negative effect on ruminal fermentation than unsaturated plant oils. Brokaw et al. (2001) observed no changes in DMI when heifers were supplemented with low levels (0.375 g/kg BW) of vegetable oil while grazing a summer pasture (75% *Bromus biebersteinii*). Others (Hardin et al., 1989; Hall et al., 1990; Patil et al., 1993) have reported reductions in DMI when lipids were supplemented to high-forage diets when hay was used as the forage source.

In this study, oil supplementation linearly reduced NDF in vivo digestibility. Jenkins (1993) also reported reductions in OM and NDF digestibilities with oil supplementation in a review of the literature; however limited research is available on the effects of lipid supplementation to grazing cattle. Brokaw et al. (2001) evaluated the effect of lipid supplementation on *in vivo* digestibility of grazing beef cattle; however, the level of soybean oil supplemented was relatively low (0.35 g/kg BW) and no effect was observed. Others have evaluated the effects of lipid supplementation on *in vivo* digestibility for high-forage ($\geq$ 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. Hardin et al. (1989) and Hall et al. (1990) observed a reduction in NDF digestibility of bermudagrass hay with lipid supplementation compared to non-supplementated. Total tract NDF digestibility of bromegrass hay was reduced 17% when 5% high-linoleate or high-oleate safflower cracked seeds were supplemented (Scholljegerdes et al., 2004). In contrast, Patil et al. (1993) reported no differences in NDF digestibility of bermudagrass hay with the addition of partially hydrogenated tallow. In

our study, corn oil supplementation decreased NDF digestibility by 6 and 12%, respectively, for 0.75 g/kg BW and 1.5 g/kg BW.

Both OM and NDF digestibilities were lower than those reported by Judkins et al. (1997) and Elizalde et al (1998) in steers grazing tall fescue without supplementation. This lower digestibility could, in part, be due to the supplementation of cottonseed hulls to all steers in this study. Lippke et al. (2000) reported reduced OM digestibility in steers grazing a high-quality wheat pasture (*Triticum aestivum*) supplemented with cottonseed hulls. These authors estimated the digestibility of cottonseed hulls to be 21.5%. In addition, the increased proportion of CSH in the diet as forage DMI declined with oil supplementation could, as suggested by Scholljegerdes et al. (2004), confound the direct effect of corn oil level on NDF digestion. In our trial, CSH INDF was 43.5%, whereas forage INDF was 31.6%. The reduction in forage intake with increasing level of corn oil supplementation also decreased dietary CP content. However, according to Satter and Roffler (1975), dietary CP content with oil supplementation in this study should still be adequate for ruminal function and would not be responsible for the observed depression in NDF digestibility. In addition, an excess of ruminal degradable protein would not increase NDF degradability (Hristov et al., 2004).

Research has shown that lipid supplementation levels of 10.7% of dietary DM for steers using yellow grease (Plascencia et al., 2003) or 12.5% of dietary DM with soybean oil for lambs (Kucuk et al., 2004) in high-concentrate diets linearly reduced postruminal FA digestion. Palmquist (1991) observed that apparent digestibility of FA remained constant when FA intake increased up to 5% of dietary DM but that it was reduced when FA intake was further increased to 8% of dietary DM. The reduction in FA digestion in these trials was largely explained by a reduction in C18:0 digestion (Plascencia et al., 2003; Kucuk et al., 2004). Apparent FA

digestibility increased when Wu et al. (1991) increased dietary FA content from 2.5% of dietary DM to 4.5 or 5.2% using animal-vegetable blend fat or calcium soaps, respectively, but declined when dietary content was further increased to 6.5 or 7.9%, respectively. In contrast, others have not observed any change in apparent fatty acid digestibility with supplementation of different fat sources to dairy (Palmquist, 1991; Palmquist et al., 1993; Kalscheur et al., 1997) or beef cattle (Zinn and Plascencia, 1993, 1996; Scholljegerdes et al., 2004) which is in agreement with our study.

The ADG observed in this study (0.81 ± 0.07 kg) is similar to those reported by Thompson et al. (1993) for steers grazing low endophyte infected (<5%) tall fescue in southeastern United States during spring (0.84 ± 0.06 kg). Others (Hess et al., 1996; Judkins et al., 1997; Elizalde et al., 1998) have also reported similar gains (0.82, 0.84, and 0.69 kg/d) for steers grazing endophyte-free tall fescue without supplementation. In all three studies, energy (corn grain) or protein (wheat bran or corn gluten meal) supplementation increased ADG to 1.04, 1.11, and 0.74 kg, respectively. Similarly, oil supplementation in this study tended to increase ADG. In contrast, others (Hardin et al., 1989; Patil et al., 1993) have reported no change in ADG with either soybean oil or partially hydrogenated tallow supplementation to grazing steers. Whitney et al. (2000) observed a quadratic increase in ADG with soybean oil supplementation to heifers consuming hay; however, no differences where observed in a companion trial.

Corn oil supplementation to grazing steers increased diet GE concentration, but the negative effect of oil supplementation on DM intake and digestibility resulted in a reduction of DE intake. Similar results were observed by Scholljegerdes et al. (2004) and McGinn et al. (2004) who found that the decrease in NDF digestibility offset the additional DE supplied by the lipid supplement. Corn oil supplementation increased the efficiency of energy utilization as fat

deposition and ADG increased when DE intake was reduced. According to the NRC (2000), the relationship between DE and ME as well as that of ME and NE can vary considerably among diets with different composition (fiber, starch, fat). One of the forms of energy lost from DE to ME is as methane produced during ruminal fermentation; Zinn and Plascencia (1996) and McGinn et al. (2004), either indirectly or by direct determination, observed reductions in methane production with lipid supplementation. Because methane production is greater in high-fiber than high-concentrate diet, the reduction in methane production with lipid supplementation would have a greater impact on a high-fiber diet (Zinn and Plascencia, 1996). Another factor that may have been involved in an increase in the efficiency of energy utilization by oil supplementation could have been by its effect on DMI. Caton and Dhyvetter (1997) suggested that energy supplementation would reduce maintenance energy cost by reducing grazing and ruminating energy expenditures if forage intake is reduced. In addition, the reduction in forage intake could reduce the maintenance energy costs by reducing gastrointestinal tract mass (Sainz et al., 1995; Sainz and Bentley, 1997). The trend for a linear increase in dressing percentage with oil supplementation in our study supports this theory.

Lipid supplementation increased external fat deposition but did not alter marbling deposition in our study. Patil et al. (1993) observed a numerical increase in fat thickness (1.4 mm) and a significant increase in marbling deposition with lipid supplementation. The lack of oil effect on marbling score with corn oil supplementation observed in this study may be the result of the relatively low amount of reserves in that depot. Duckett et al. (1993) observed in a serial slaughter trial that a significant increase in marbling score for steers in a high concentrate diet occurs after fat thickness had reach 6.8 mm. In our trial, the maximum fat thickness at slaughter was 5.1 mm for HI; whereas the fat supplemented treatment of Patil et al. (1993) reached 6.6

mm. The trend for a linear increase in ADG was reflected in the linear increase of HCW with oil supplementation. Patil et al. (1993) observed an increase in HCW with supplementation of grazing steers; however, supplemental fat addition did not further increase HCW.

Animal response to supplementation is subject to the forage substitution rate (Bargo et al., 2003). In grazing dairy cattle the substitution rate for fiber- or starch-rich supplements typically ranges between 0 and 1 (Bargo et al., 2003; Doyle et al., 2005). However, when 0.5 or 1 kg of partially hydrogenated oil was added to the concentrate of grazing dairy cattle the substitution rate was 7 and 4.1 kg/kg, respectively (Schroeder et al., 2002). Suggesting, in agreement with our results, that the substitution rate observed with oil supplementation could be considerably higher than 1. According to our results with non-limiting forage availability, oil supplemented steers consumed less forage and produced heavier carcasses than unsupplemented steers. Supplement conversion improved when forage substitution rates were reduced by lower forage availability (Beretta et al., 2006). In our study, oil conversion was within the range (0.12 to 0.77) observed for fiber- and starch-rich supplements when offered to grazing beef cattle (Grigsby et al., 1991; Horn et al., 1995; Bodine et al., 2001). According to our data when supplementing with 1.5 g/kg BW of corn oil, stocking density could be increased by 62% relative to the stocking density without oil supplementation.

IMPLICATIONS

Dietary oil supplementation to grazing steers in a forage-finishing system reduced forage intake and improved animal performance. Carcass weights and fatness were increased with increasing levels of oil supplementation. Due to the reduction in forage intake with oil supplementation, stocking density could be increased by 62% relative to the stocking density without oil supplementation.

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Table 2.1. Mean DM chemical composition and fatty acid composition of the different dietary component through the 116 d of the trial.

	Forage		Cottonseed hulls	Corn oil
	Pre-grazing	Post-grazing		
Component, %DM				
OM	92.7 ± 0.14	91.5 ± 0.56	96.9 ± 0.02	--
CP	15.5 ± 0.49	14.8 ± 0.58	7.5 ± 0.18	--
NDF	60.7 ± 1.16	62.9 ± 1.04	80.9 ± 1.01	--
ADF	30.2 ± 0.75	32.0 ± 0.80	62.2 ± 1.14	--
Total FA	1.7 ± 0.11	1.6 ± 0.10	3.3 ± 0.27	91.9 ± 1.97
FA composition, mg/100 mg of FA				
C14:0	0.7 ± 0.05	0.6 ± 0.03	0.7 ± 0.03	<0.1 ± 0.00
C16:0	14.2 ± 0.28	14.7 ± 0.35	23.2 ± 0.58	10.8 ± 0.01
C18:0	1.8 ± 0.13	1.9 ± 0.08	2.58 ± 0.05	1.96 ± 0.02
C18:1	4.1 ± 0.34	5.1 ± 0.56	15.0 ± 0.21	28.5 ± 0.09
C18:2	16.9 ± 0.78	19.2 ± 1.16	54.9 ± 0.91	55.7 ± 0.16
C18:3	37.6 ± 2.33	34.0 ± 2.03	0.6 ± 0.02	1.3 ± 0.13
Others [a]	5.7 ± 0.38	5.9 ± 0.19	2.15 ± 0.06	0.75 ± 0.03
Unidentified	19.0 ± 0.83	18.6 ± 0.83	0.93 ± 0.13	0.95 ± 0.09

[a] Sum of C12:0, C15:0, C16:1, C17:0, C20:0, C21:0, C22:0.

Table 2.2. Effect of corn oil supplementation to grazing steers on DMI, fatty acid intake, and dietary DM composition.

	Corn oil level, g/kg BW			Pooled SEM	Oil effect	
	0 (NONE)	0.75 (MED)	1.5 (HI)		Linear	Quad.
N	6	5	6			
Intake						
DMI, kg·an^{-1}·d^{-1}						
Total diet	13.1	12.7	10.5	0.43	<0.01	0.15
Forage	9.9	8.7	6.3	0.43	<0.01	0.25
Cottonseed hulls	3.30	3.63	3.66	0.16	0.12	0.46
Corn Oil	0.00	0.34	0.62	0.00	<0.01	0.11
DMI, % BW	2.79	2.67	2.16	0.13	0.01	0.24
Fatty acid						
kg·an^{-1}·d^{-1}	0.34	0.63	0.84	0.02	<0.01	0.08
g/kg BW	0.71	1.32	1.71	0.05	<0.01	0.11
Energy, Mcal						
GE	58.5	57.8	49.6	1.95	<0.01	0.15
DE	34.4	32.6	26.9	1.44	<0.01	0.31
Diet Composition (DM basis)						
OM, %	94.36	94.65	95.08	0.06	<0.01	0.34
CP, %	16.04	15.19	13.89	0.18	<0.01	0.34
NDF, %	54.38	54.12	54.42	0.41	0.94	0.59
ADF, %	30.73	31.29	32.71	0.50	<0.01	0.50
FA, %	2.55	4.94	7.99	0.16	<0.01	0.12
GE, Mcal/kg DM	4.44	4.56	4.71	0.01	<0.01	0.11

	Corn oil level, g/kg BW			Pooled SEM	Oil effect	
	0 (NONE)	0.75 (MED)	1.5 (HI)		Linear	Quad.
DE, Mcal/kg DM	2.60	2.57	2.55	0.05	0.42	0.93

Table 2.3. Apparent in vivo digestion of diet chemical components from forage-finished steers supplemented with increasing level of corn oil.

	Corn oil level, g/kg BW			Pooled SEM	**Oil effect**	
	0 (NONE)	0.75 (MED)	1.5 (HI)		Linear	Quad.
N	**6**	**5**	**6**			
DM, %	57.65	55.14	51.24	0.94	<0.01	0.57
OM, %	59.68	57.24	53.75	0.88	<0.01	0.64
NDF, %	49.77	46.64	43.60	0.85	<0.01	0.97
ADF, %	40.06	39.16	38.67	1.14	0.39	0.89
Fatty acids, %	71.10	78.32	75.66	3.29	0.33	0.25

Table 2.4. Performance, feed efficiency, and carcass traits for steers grazing an endophyte-free tall fescue supplemented with increasing levels of corn oil

	Corn oil level, g/kg BW			SEM	Oil effect	
	0 (NONE)	0.75 (MED)	1.5 (HI)		Linear	Quad.
N	6	6	6			
Body weight, kg						
d 0	439.5	433.9	440.6	13.9	0.78	0.96
d 61 [a]	482.7	479.6	495.1	4.95	0.10	0.15
d 116 [a]	519.9	534.0	541.5	8.39	0.09	0.75
Total body weight gain, kg	82.0	95.6	103.7	8.20	0.08	0.79
ADG, kg [a]						
d 0 – 61	0.73	0.68	0.94	0.08	0.10	0.15
d 61 – 116	0.67	0.98	0.83	0.10	0.25	0.07
d 0 – 116	0.71	0.83	0.89	0.07	0.09	0.75
Real-Time Ultrasound, d 116						
s.c. fat, cm	0.53	0.55	0.68	0.06	0.08	0.50
LM area, cm^2	66.9	67.4	67.7	2.26	0.81	0.97
Carcass traits						
Hot carcass weight, kg [a]	268.4	281.4	286.5	4.59	0.01	0.50
Dressing, %	56.2	56.8	58.0	0.67	0.09	0.74
Fat thickness, cm [a]	0.30	0.42	0.51	0.02	0.01	0.73
LMA, cm^2 [a]	60.7	62.6	61.8	2.90	0.80	0.72
KPH, %	1.4	1.5	1.4	0.11	0.80	0.47
Marbling score [b]	432	428	435	17.6	0.90	0.79

	Corn oil level, g/kg BW			SEM	Oil effect	
	0 (NONE)	0.75 (MED)	1.5 (HI)		Linear	Quad.
Quality grade [c]	3.3	3.2	3.3	0.41	0.99	0.75
Yield grade	2.3	2.4	2.6	0.17	0.23	0.89
Carcass Price, $/45.4 kg [c]	120	119	121	1.89	0.67	0.50
Carcass Value, $/carcass [c]	708.61	737.83	763.54	18.57	0.05	0.94

[a] Initial weight used as covariate ($P \leq 0.05$).

[b] Marbling score code: 400 to 499 = slight, 500 to 599 = small; Quality grade code: 2 = Standard+; 3 = Select-, 4 = Select+, 5 = Choice-.

[c] Base carcass price (Choice-, YG 3) = $129.85/45.5 kg; Premiums: YG 1 $ 3.09/45.5 kg; Discounts: Select -$ 7.28/45.5 kg; Standard -$ 16.50/45.5 kg; Lightweight carcass (< 250 kg) - $8.83/45.4 kg (AMS, 2004).

Table 2.5. Effect of level of corn oil supplementation to grazing steers on substitution rate, stocking density, and corn oil conversion efficiency

	Corn oil level, g/kg BW			***P value***		
				0.75 vs 1.5	**LSMean Different from 1**	
	0.75 (MED)	**1.5 (HI)**	**SEM**		**0.75 (MED)**	**1.5 (HI)**
Substitution Rate, kg/kg [b]	3.6	5.8	0.80	0.09	0.01	<0.01
Relative stocking density, an/an in NONE [c]	1.14	1.62	0.10	0.01	0.20	<0.01
Corn oil conversion, kg /kg [d]	0.38	0.33	0.15	0.81	nd[a]	nd[a]

[a] nd = non determined

[b] Substitution rate = (Average forage DMI_{NONE} – Individual forage DMI $_{MED\ or\ HI}$) / (Corn oil intake) $_{MED\ or\ HI}$

[c] Relative stocking density = Average forage DMI_{NONE} / $(Individual\ forage\ DMI)_{MED\ or\ HI}$

[d] Corn oil conversion efficiency = $[(Individual\ BW\ gain)_{MED\ or\ HI} - (Average\ BW\ gain)_{NONE}]$ / $(Corn\ oil\ intake)_{MED\ or\ HI}$

CHAPTER 3

CORN OIL SUPPLEMENTATION TO STEERS GRAZING ENDOPHYTE-FREE TALL FESCUE. II. EFFECTS ON LM AND S.C. FATTY ACID COMPOSITION AND STEAROYL-COA DESATURASE ACTIVITY AND EXPRESSION[1]

[1] Pavan, E. and S. K. Duckett. Submitted to J. Anim. Sci., 6/11/2006.

ABSTRACT: Eighteen steers were used to evaluate the effect of increasing level of corn oil supplementation to steers grazing endophyte-free tall fescue on fatty acid (FA) composition in LM and s.c. adipose tissues, stearoyl Co-A desaturase(SCD) activity and expression in s.c. fat, and adipose cellularity in s.c fat. Corn oil was supplemented at 0 (**NONE)**, 0.75 (**MED**) and 1.5 (**HI**) g / kg of BW. Cottonseed hulls were used as a carrier for the corn oil and supplemented according to pasture availability (0.7 to 1% BW). Steers were finished on a rotational grazed tall fescue pasture for 116 d. Fatty acid (FA) composition was determined by GLC. Fatty acid intake was analyzed as a complete randomized design and tissue fatty acid profile as a split-plot design using the MIXED procedures. Linear and quadratic oil effects were tested. Total linoleic acid intake increased linearly ($P < 0.01$) with corn oil supplementation (90.7, 265.1 and 406.7 g in NONE, MED and HI, respectively). Oil supplementation linearly reduced ($P < 0.05$) myristic, palmitic and linolenic acids content in the adipose tissues. *Trans*-11 vaccenic acid (TVA) was 46% greater in MED and 32% in HI than in NONE; resulting in linear and quadratic ($P < 0.01$) oil level effects. Effect of oil supplementation on CLA *cis*-9, *trans*-11 was affected by adipose tissue ($P < 0.01$). In the i.m. fat CLA cis-9, trans-11 isomer was 25% greater for MED than for NONE, and intermediate for HI; whereas CLA cis-9 trans-11 CLA isomer was 48 and 33% greater in s.c. fat for MED and HI than for NONE, respectively. Corn oil linearly increased ($P \leq 0.01$) *trans*-10 octadecenoic acid and CLA *trans*-10, *cis*-12; however, values were low (< 0.35% and < 0.035% of total fatty acids, respectively). Oil supplementation did not alter ($P > 0.05$) total SFA, MUFA, PUFA nor total n-6 or n-3 FA, but linearly increased ($P = 0.03$) n-6: n3 ratio from 2.4 to 2.9 in NONE and HI, respectively. Among adipose tissues, total SFA and MUFA were greater in s.c. than i.m. fat; whereas total PUFA, n-6, n-3 FA and the n-6: n-3 ratio were lower. *Trans*-10 octadecenoic acid, TVA, CLA *cis*-9, *trans*-11 and CLA *trans*-10, *cis*-12 were

greater ($P \leq 0.05$) in s.c. than in i.m. fat. Oil supplementation did not affect ($P > 0.05$) stearoyl-CoA desaturase activity or mRNA expression. Corn oil supplementation to grazing steers reduced the percentages of highly atherogenic fatty acids, myristic and palmitic acids, and increased the percentages of antiatherogenic and anticarcinogenic fatty acids, trans-11 vaccenic acid and CLA *cis*-9, *trans*-11.

Key Words: Beef, Forage, Fatty Acid, CLA

INTRODUCTION

Increasing attention has been placed on enhancing the content of conjugated linoleic acid (**CLA**), *cis*-9, *trans*-11 isomer, in beef as a result 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). 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 stearoyl Co-A desaturase enzyme present in mammalian adipose tissues (Ntambi, 1995). In beef, TVA is present in adipose tissues at levels 1.4-fold higher than CLA cis-9 trans-11 isomer (Gillis et al., 2004) due to greater formation of TVA during ruminal biohydrogenation than CLA (Gillis et al., 2004). Because the majority of CLA in beef fat originates from TVA, enhancing the concentration of both TVA and CLA in beef products is of importance for potential human health implications.

Research directed at enhancing CLA content of beef fat has utilized supplementation of plant oils or oilseeds to increase linoleic acid content of high concentrate, finishing cattle diets; however, results have been limited (Beaulieu et al., 2002; Gillis et al., 2004; Madron et al., 2002). In grazing cattle, higher levels of CLA *cis*-9, *trans*-11 and TVA content in milk or beef fats have been reported (Dhiman et al., 1999; Realini et al., 2004; Scollan et al., 2001). 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 36% in finishing cattle diets. The proportion of CLA *cis*-9, *trans*-11 in the i.m. fat of heifers fed a silage-based diet increased linearly when the fat source in the concentrate was gradually changed form lard to a linoleic-rich sunflower oil (Noci et al., 2005b). However there is limited information on the effect of increasing fat supplementation, in particular plant oils, to grazing

beef cattle in a forage based system and its effect on TVA, CLA and stearoyl-CoA desaturase. Thus our objective was to determine changes in fatty acid composition, adipose cellularity, and stearoyl-CoA desaturase activity and expression with oil supplementation to steers grazing endophyte-free tall fescue.

MATERIALS AND METHODS

Animals, management, pasture and treatments. Eighteen Angus steers (438 ± 4 kg) were finished on a rotational grazed 21-ha endophyte free tall fescue pasture (*Festuca arundinacea*) for 116 d. Three dietary treatments were defined by the level of corn oil supplementation: 0 g/kg BW (**NONE**), 0.75 g/kg BW (**MED**) and 1.5 g/kg BW (**HI**). Pelleted cottonseed hulls (**CSH**) were used as a carrier for the oil supplement and were fed at equal amounts to all steers regardless of treatment throughout the experiment. Levels of CSH fed to steers were adjusted across treatments during the experiment according to pasture availability (0.7 to 1 % BW). Additional information regarding in vivo digestibility, performance and carcass quality are available in Pavan et al. (2006).

Sample collection. Animals were transported (45 km) to the University of Georgia Meat Science and Technology Center in Athens and slaughtered following an overnight feed withdrawal. Within 30 minutes of exsanguination, a sample of s.c. fat from the tail head region was removed from each carcass and rinsed with sterile saline solution. Approximately, 10 g of s.c. fat was immediately frozen in liquid nitrogen and stored at -80ºC for subsequent RNA extraction. Samples of s.c. fat (10 g) were obtained for determination of stearoyl-CoA desaturase enzyme activity according to Smith et al. (2002). The remaining s.c. adipose tissue from the tail head region was stored frozen at -80ºC for adipose cellularity. During evisceration, samples of digesta from the abomasum (aprox. 250 mL) were obtained to estimate the proportion of

conjugated linoleic acid coming from endogenous desaturation of trans-11 vaccenic acid. The conversion of TVA to CLA was calculated based on the differential in the ratios of CLA to TVA in abomasum compared to s.c. or i.m. fat. Abomasal samples were immediately stored at -20ºC, freeze dried, and ground through a Wiley mill equipped with a 1-mm screen for subsequent fatty acid analyses.

At 24 h postmortem, samples of s.c. adipose tissue and a 2.54 cm thick LM steak were removed from the left half of each carcass at the 13th rib region. All external fat and connective tissue were removed from the LM. Both s.c. fat and LM samples from each carcass were stored at −20°C; before analyses samples were pulverized in liquid nitrogen.

Proximal analyses. Duplicate samples were analyzed for nitrogen content by the combustion method using a Leco FP-2000 N analyzer (Leco Corp., St. Joseph, MI) and multiplied by 6.25 to determine crude protein content. Moisture content was determined by weight loss after drying at 100°C for 24 h. Total ash content was determined by ashing at 600°C for 8 h (AOAC, 1990). Total lipids were extracted in duplicate from LM and s.c. fat samples according to the procedures of Folch et al.(1957). Lipid extracts from the s.c. and from the LM (i.m. fat) samples were stored at -80°C for subsequent fatty acid determination.

Fatty acid composition. Subcutaneous and i.m. lipid extracts containing approximately 4 mg of total lipids, were transmethylated according to the method of 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 (0.25 mm i.d. and 0.20 μm film thickness). 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 1 mL per min. Individual FA were identified by comparison of retention times with standards (Sigma, St. Louis, 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 a percentage of total FA. Abomasal FAME were obtained by direct transmethylation of lyophilized samples according to Park and Goins (1994) and analyzed as s.c. and i.m. FAME. Cholesterol content of LM was determined according to Du and Ahn (2002) and quantified by incorporating an internal standard, stigmasterol, into each sample.

Stearoyl-CoA Desaturase Activity and Gene Expression. Samples of s.c. adipose tissue (5 g) collected at slaughter were immediately processed for stearoyl-CoA desaturase enzyme activity according to Smith et al. (2002) in duplicate. Enzyme activity was determined according to St. John et al. (1991) with the following modifications: unlabeled stearic acid was used in the assay, extracts were transmethylated (Park and Goins, 1994) to quantify the amount of stearic and oleic acids by GLC as described above, and blanks were also included in the assay to determine purity of stearic acid. The activity values were calculated as a ratio among oleic acid and remaining stearic acid.

Samples of s.c. adipose tissue were also collected and immediately frozen in liquid nitrogen for storage at -80°C. Total RNA was extracted from the s.c. samples according the TRIzol procedure (Invitrogen, Carlsbad, CA), which included an initial centrifugation step to remove lipids from the extract according to the manufacturers directions. RNA was quantified using Quant-iT RNA assay kit (Invitrogen, Carlsbad, CA). RNA (5 ug) was separated in an

agarose gel (Pellé and Murphy, 1993) and transferred to nylon membranes downward capillary transfer. Northern blots were performed by hybridization with ^{32}P-labeled SCD probes (5' to 3', Forward: GATATAGGTGTATATCTTGCAGGTGG; Reverse: ATCTCTAGCTCCTACACAACCACC) and exposed to film for 38 h. Gene expression was quantified by densitometry the SCD bands and was normalized to the 18S ribosomal RNA bands.

Adipose cellularity. Adipose cellularity was determined in duplicate by osmium tetroxide fixation according to Mersmann and MacNeil (1986). Adipocyte number and size distribution in the range of 20 to 240 μm were measured electronically using Coulter Counter (Coulter Electronics, Hialeah, FL). Only counts in the 30 μm and above were included in calculations of cell number, diameter and volume (Lee et al., 1994). Peak cell diameter specifies the diameter of cell occurring most frequently, and peak cell volume specifies the volume of cell that contributes the most to total adipocyte volume.

Statistical analysis. Data were analyzed by ANOVA as a complete randomized design with three corn oil levels and with individual animal serving as the experimental unit (n = 6) with the exception of the fatty acid data. Tissues fatty acid profile data were analyzed by ANOVA according to a split-plot design with the three corn oil levels as the main plots and the two adipose tissues as the subplots treatments. The effect of corn oil level was tested using the variance between animals within corn oil level (n = 6) as the error term. Effect of adipose tissue and corn oil level × adipose tissue interaction was tested against the residual error. MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) was used for all analyses. To analyze differences among corn oil levels for all variables the sum of squares for the corn oil level effect was further partitioned by preplanned linear and quadratic orthogonal contrasts. If the overall F-test of corn

oil level × adipose tissue was significant ($P \leq 0.05$), a t-test was performed to discern differences among corn oil level within adipose tissue and among adipose tissue within corn oil level. For total CLA and CLA *cis*-9, *trans*-11 content analyses the model was adjusted to account for heterogeneous variances among dietary treatments. Least square means are reported. The PROC REG function of SAS was used to compute the regression equation and determination coefficient between *trans*-11 vaccenic acid or CLA *cis*-9, *trans*-11 and total linoleic acid intake.

RESULTS

Daily fatty acid intake of total diet, supplement plus forage, is shown in Table 1. Total fatty acid intake increased ($P < 0.01$) as expected with corn oil supplementation. Corn oil supplementation also linearly increased ($P < 0.01$) intake of palmitic (C16:0), stearic (C18:0), oleic (18:1) and linoleic (C18:2) acids; whereas linolenic (C18:3) acid intake was linearly decreased ($P = 0.03$). Myristic acid (C14:0) was unaffected by oil supplementation. Linoleic acid intake increased by 174 g in MED and 316 g in HI with respect to NONE. Corn oil supplementation to grazing steers did not alter ($P > 0.05$) LM proximate composition (Table 2). The average moisture, crude protein, ash and total lipid content across dietary treatments were: 74.1, 23.3, 1.4 and 2.5%, respectively. Cholesterol content in the LM was unchanged by level of corn oil supplemented to the steers ($P > 0.05$; 58.3 mg/100 g). Total lipid content of s.c. was also unchanged ($P > 0.05$) and averaged 66.7%.

Corn oil supplementation and adipose tissue effects on fatty acid profile are presented in Table 3. The two-way interaction between corn oil supplementation and adipose tissue was non-significant ($P > 0.05$) for the majority of fatty acids; however, the interaction was significant ($P < 0.05$) for odd chain fatty acids, total CLA, CLA cis-9 trans-11 isomer, and CLA cis-11 trans-13 isomer (Table 4). Increasing corn oil supplementation to grazing cattle resulted in a linear

decrease ($P < 0.01$) in myristic and palmitic acids, two atherogenic fatty acids. Myristic acid was reduced by 0.30 ± 0.14 percentage units with each g/kg BW increment of corn oil supplementation, and palmitic acid by 1.27 ± 0.42. Thus, HI generated an 18% and 8% reduction in myristic acid and palmitic acid proportions, respectively. Concentrations of other saturated fatty acids (lauric and stearic acids) as well as total SFA were unchanged with corn oil supplementation. Corn oil supplementation did not alter ($P > 0.05$) myristoleic (C14:1) acid, oleic (C18:1) or total monounsaturated (MUFA) fatty acid concentrations.

Trans-10 octadecenoic acid and CLA isomer *trans*-10, *cis*-12 linearly increased ($P \leq 0.01$) with oil supplementation. Both, linear ($P = 0.02$) and quadratic ($P = 0.01$) corn oil level effects were significant for TVA proportion. Compare with NONE, MED increased TVA acid by 46% and HI by 32%. Oil supplementation did not alter ($P > 0.05$) the proportion of total PUFA nor linoleic, EPA, DHA, or DPA acids. Linolenic acid proportion linearly decreased ($P < 0.01$) with corn oil supplementation. The lack of effect of corn oil supplementation on both total SFA and total PUFA resulted in similar ($P > 0.10$) PUFA: SFA ratio across dietary treatments. Likewise, total n-6 and total n-3 FA did not differ ($P > 0.05$) across dietary treatments. Nonetheless, n-6: n-3 ratio was increased ($P = 0.03$) by 0.35 ± 0.15 units per g/kg BW of corn oil supplemented.

Total saturated fatty acids, myristic acid, and arachidic (C20:0) acid proportions were greater ($P \leq 0.05$) in s.c. than LM fat; whereas the proportions of the major SFA in beef fat, palmitic (C16:0) and stearic (C18:0) acids, as well as that of lauric (C12:0) acid were similar ($P > 0.05$) among adipose tissues. Total MUFA proportion was 15% greater ($P < 0.01$) in s.c. than in LM fat. In addition, *trans*-10 octadecenoic acid and TVA, intermediates products of C18:2 ruminal biohydrogenation, were in greater ($P < 0.01$) proportions in the s.c. than LM fat. Cis-

octadecenoic acids formed during ruminal biohydrogenation were present in greater ($P < 0.01$) proportions for LM than s.c fat.

Intramuscular fat had greater ($P < 0.01$) total PUFA, n-6 and n-3 fatty acids proportions than s.c. fat. Linoleic acid, C18:3 and longer chain PUFA (C20:3 n-6; C20:4 n-6, C20:5 n-3, C22:4 n-6, C22:5 n-3, and C22:6 n-3) were in greater ($P < 0.01$) proportions in the i.m. than in the s.c. fat. Intramuscular fat n-6: n-3 ratio was greater ($P < 0.01$) than that observed in the s.c. fat. Total content of isomers *trans, trans* of CLA were also greater ($P = 0.04$) in the i.m. than in the s.c. fat. In contrast, total content of isomers *cis*, *cis* and isomer *trans*-10, *cis*-12 were greater ($P < 0.01$) in the s.c. than in the i.m. fat.

For pentadecyclic (C15:0) acid, margaric (C17:0) acid, and total odd chain fatty acids (**OCFA**) the response to corn oil supplementation varied with tissue (Table 4). In the s.c. fat, pentadecyclic acid concentration decreased ($P < 0.05$) with increasing oil supplementation level; whereas pentadecyclic acid concentration was lower ($P = 0.04$) in the LM for HI than NONE with MED being intermediate ($P > 0.05$). The proportion of pentadecyclic acid in s.c. fat from HI was similar to those in the LM from NONE and MED ($P > 0.05$). In the s.c. fat, total OCFA and margaric acid concentrations decreased ($P < 0.01$) with increasing oil supplementation level; in the LM, total OCFA and margaric acid concentrations were greater ($P < 0.01$) in NONE than MED and HI. Proportion of total OCFA in the s.c. fat from MED was similar ($P > 0.05$) to that in the LM from NONE, and that in the s.c. fat from HI was similar ($P > 0.05$) to the proportions in the LM from NONE and MED.

The interaction effect between corn oil level and adipose tissue was also significant ($P \leq 0.05$) for total CLA, *cis*-9, *trans*-11 CLA and *cis*-11, *trans*-13 CLA proportions. Isomer *cis*-11, *trans*-13 of CLA was detected ($P < 0.01$) only in the s.c. fat of carcass from animals fed HI.

Within each dietary treatment, total CLA and CLA *cis*-9, *trans*-11 were greater ($P \leq 0.05$) in the s.c. than in the i.m. adipose tissue. However, the proportion of both total CLA and CLA *cis*-9, *trans*-11 in LM from MED and HI were similar ($P > 0.05$) to those in the s.c. fat from NONE. In LM, total CLA and *cis*-9 *trans*-11 isomer of CLA were higher ($P < 0.01$) for MED than NONE with HI being intermediate ($P > 0.05$). In s.c. fat, total CLA and *cis*-9 *trans*-11 isomer of CLA were higher ($P < 0.01$) for MED and HI than NONE. Irrespective of tissue, total CLA and CLA *cis*-9, *trans*-11 were greater ($P \leq 0.01$) in MED than in NONE.

The effects of total linoleic acid intake (g/d) on LM and s.c. adipose tissues TVA and CLA *cis*-9, *trans*-11 proportions is presented in Figure 3.1. Quadratic equations better explained the variation observed in longissimus muscle TVA proportion ($R^2 = 0.53$, $P = 0.005$) and in s.c. fat TVA ($R^2 = 0.42$, $P = 0.02$) and CLA *cis*-9, *trans*-11 ($R^2 = 0.35$, $P = 0.05$) proportions than the respective linear equations. Whereas neither the linear ($R^2 = 0.15$, $P = 0.12$) or quadratic ($R^2 = 0.18$, $P = 0.24$) regression were significant for the CLA *cis*-9, *trans*-11 in the LM and total linoleic acid intake.

Stearoyl-CoA desaturase activity in the s.c. fat from the tail head region did not differ ($P = 0.35$) among corn oil supplementation levels (Figure 3.2). However, there was a numerical increased in MED than in NONE or HI. Similarly, SCD mRNA level was neither affected ($P = 0.60$) by oil supplementation levels; but, as for SCD activity, mRNA level was numerically greater in MED than in either NONE or HI.

Corn oil supplementation to grazing steers did not affect ($P > 0.10$) adipocytes per mg of s.c. adipose tissue (Table 5). Neither mean and peak cell diameters nor cell volumes were affected by corn oil supplementation ($P < 0.10$). A biphasic adipocyte cell diameter distribution was observed for all dietary treatments (data not shown); on average, a first peak cell diameter

was observed at 30 to 40 μm and second larger peak was observed at 112 μm. No differences ($P > 0.10$) in cell frequency were observed across dietary treatment at the different diameter classes (10 μm increment).

DISCUSSION

Conjugated linoleic acid and TVA are intermediates formed during ruminal biohydrogenation of dietary linoleic acid (Bauman et al., 1999). Several studies have been conducted to increase beef CLA *cis*-9, *trans*-11 content through supplementation of high concentrate diets with plant oils rich in linoleic acid; however, responses have been limited. Beaulieu et al. (2002), Griswold et al. (2003), and 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 content of LM, eye of round, or chuck tender samples when supplementing 12.7% extruded full-fat soybeans in a high-concentrate diet. However when extruded full-fat soybean supplementation increased to 25.6% of diet, TVA and CLA *cis*-9, *trans*-11 content increased 0.38 and 0.11 percentage units with respect to the basal diet. Gillis et al. (2004) added 4% corn oil to the basal high-concentrate diet, TVA and CLA *cis*-9, *trans*-11 content in three different adipose tissues (i.m., s.c. and perirenal) increased 0.14 and 0.06 percentage units, respectively. In this study, corn oil supplementation to steers grazing endophyte-free tall fescue increased TVA and CLA by 1.88 and 0.45 percentage units, respectively, when supplemented at 0.75 g/kg of BW and CLA *cis*-9, *trans*-11 by 0.17 and 0.45 percentage units in the i.m. and s.c. adipose tissue, respectively, when 0.75 g/kg BW of corn oil was supplemented to grazing steers. These changes represent a 5-fold greater increase for TVA than the greatest increased reported for high-concentrate diets, and a 4-fold greater increase CLA *cis*-9, *trans*-11 in the s.c. fat.

The greater response to dietary linoleic observed in our study compared to those that utilized high-concentrate diets would be the related to the differences in ruminal environment generated by the basal diets. High-concentrate diets favor production of *trans*-10, *cis*-12 CLA and *trans*-10 C18:1 as intermediates of linoleic acid biohydrogenation as opposed to the *cis*-9, *trans*-11 CLA and TVA produced with high-forage diets (Bauman and Griinari, 2003). With an 86% concentrate diet, Sackmann et al. (2003) observed that ruminal outflow of *trans*-10 C18:1 was almost 12-fold that of TVA; whereas *trans*-10 C18:1 ruminal outflow decreased and that of TVA increased linearly as forage in the diet increased from 12% to 36%. Increasing dietary linoleic acid in high-concentrate diets increased ruminal outflow (Duckett et al., 2002; Sackmann et al., 2003) and adipose tissue content (Gillis et al., 2004; Hristov et al., 2005) of *trans*-10 C18:1 more than those of TVA. In the present study, *trans*-10 C18:1 acid was increased by increasing dietary linoleic acid, but it did not represent more than 6% of TVA content in both adipose tissues evaluated. The increase observed in TVA content when 0.75 g/kg BW of corn oil was supplemented was more than 12-fold that of *trans*-10 C18:1.

The lack of an additional increase in TVA content by doubling the amount of corn oil supplemented from MED to HI, might be explained by the lower increase in dietary C18:2 proportion between MED and HI than between NONE and MED. When corn oil supplementation was increased from MED to HI total linoleic intake increased 57%, but the proportion of linoleic acid in the dietary lipid only increased 15.2%. Dietary linoleic proportion was 27% of total FA in NONE, 42.2% in MED and 48.6% in HI. In addition, an increase in ruminal biohydrogenation in HI could have contributed to obtain a similar TVA between MED and HI. Duodenal flow of TVA was reduced from 78% of linoleic acid intake when dairy cows were fed *ad libitum* to only 47% when DMI was restricted to 80% of ad libitum (Qiu et al.,

2004). In our study, total DMI for HI was 83% lower than that in MED (Pavan et al., 2006). Ruminal biohydrogenation was also increased when dietary fatty acids were increased by supplementation with 2.37 % of corn oil (Duckett et al., 2002) or 2 % of soybean oil (Qiu et al., 2004) . In our study, total dietary FA content from 4.94 % of DM in MED to 7.99% in HI with minimal effect on dietary linoleic acid proportion.

The interaction between dietary treatment and adipose tissue on *cis*-9, *trans*-11 CLA content resulted from a lower response in the i.m. than in the s.c. fat with oil supplementation and to the high variation in *cis*-9, *trans*-11 CLA content observed in HI. With MED the CLA *cis*-9, *trans*-11 content was increased 27 and 48% with respect to NONE in the i.m. and s.c. fat, respectively. As suggested by Noci et al (2005a), the lower level of response in the i.m. fat could be the result of the low level of total intramuscular fat. According to Noci et al. (2005a) *cis*-9, *trans*-11 CLA would be preferentially accumulated in the neutral lipid fraction; fraction that is positively associated with the level of fat in the intramuscular fat (Duckett et al., 1993). In addition, working with mice, Santora et al. (2000) observed that although absorbed *cis*-9, *trans*-11 CLA was accumulated in both phospholipids and triacylglycerols, endogenously generated *cis*-9, *trans*-11 CLA was only stored in triacylglicerols. According to Gillis et al. (2003), based on TVA: *cis*-9, *trans*-11 CLA ratios in the duodenal content and in adipose tissues, endogenously generated *cis*-9, *trans*-11 CLA represents 86% of the total tissue content. In our study, based on similar calculations but using ratio from abomasum content obtained at during harvest instead of the duodenal content ratio, endogenously generated *cis*-9, *trans*-11 CLA represented 83% in the i.m. and 85% in the s.c. adipose tissue. The slightly, but significant, lower proportion coming from desaturation in the i.m. than in the s.c. fat would also be a reflection of the lower proportion of triacylglycerols in that tissue.

The high animal variability for *cis*-9, *trans*-11 CLA content in HI may be related to the animal's ability to respond to high fat supplementation levels. Response to fresh-pasture intake (Kelly et al., 1998) or to extruded soybeans supplementation (Peterson et al., 2002) on milk fat content of CLA was very variable between animals; some cows had minimal or no response, whereas others had a substantial response. According to Peterson et al. (2002) the major cause of this variation would be the individual variation in ruminal production of TVA, though also individual variation in stearoyl-CoA desaturase (**SCD**) activity would be a key factor. Several authors had reported high positive linear relationships among *cis*-9, *trans*-11 CLA and TVA concentrations in adipose tissues suggesting that CLA content is dependent on TVA concentrations (Daniel et al., 2004; Madron et al., 2002; Noci et al., 2005a). In our study, the linear regression within adipose tissue including all dietary treatments were significant ($P < 0.01$) with R^2 of 0.37 and 0.54 for for the s.c. and i.m. tissue, respectively. However, the relationship within dietary treatments did not exist ($P > 0.05$); suggesting again the existence of individual variation in ruminal biohydrogenation production of TVA and in SCD activity. Tissue concentration of TVA could had been greater for a given animal, because less was converted to *cis*-9, *trans*-11 CLA due to a lower SCD activity rather than a greater ruminal TVA outflow.

Despite the increase in dietary linoleic proportion with corn oil supplementation, tissue linoleic acid or total PUFA content were not affected. Similarly, no effect on tissue content of linoleic acid was observed by Engle et al. (2000) and Beaulieu et al. (2002) when dietary linoleic acid was increase through 4 and 5% soybean oil supplementation to high-concentrate beef cattle, or by Santos-Silva et al. (2003) when added 30% of expanded sunflower seed to the supplement (2% BW) of grazing lambs. In contrast, Andrae et al. (2001) and Gillis et al. (2004) used high-oil corn grain or corn oil in high-concentrate diets observed an increase in tissue linoleic acid

content. Differences in the results may be related to differences in the degree of ruminal biohydrogenation obtained with the different diets or to different FA composition of the base diets. Ruminal biohydrogenation extent was increased when dietary forage (Kucuk et al., 2001) or dietary lipid content increased, and when oil was fed instead of oilseed (Duckett et al., 2002). The reduction observed in linolenic acid with corn oil supplementation would mainly be the consequence of the reduction of linolenic acid intake by a depression in pasture intake (Pavan et al., 2006). Despite no significant effects on total n-6 and n-3, corn oil supplementation increased the n-6: n-3. However, the highest value (3.6), obtained in the i.m. fat animals fed HI, was still bellow the maximum recommended level of 4 for human consumption (Enser, 2001).

Although corn oil supplementation did not have any effect on total SFA or PUFA: SFA ratio, the negative effect of corn oil supplementation on myristic and palmitic acids is noteworthy. These two SFA are considerated to have hypercholesterolemic effects on humans; whereas stearic acid, the other predominant SFA in beef, is considered neutral in this regard (Ulbricht and Southgate, 1991). 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 when palmitic was reduced by adding 6% sunflower oil to the diet. In our study, the linear reduction in tissue myristic and palmitic acid content would be the result of the reduction of their proportions in the diet, but also by the reduction in *de novo* fatty acid synthesis by an increase of exogenous fatty acid (Vernon, 1981). The absence of corn oil effect on s.c. tail head adipose cellularity would suggest that lipogenic activity was not different across treatment; though as suggested by Azain (2004) there was a switch from endogenous synthesized FA for those coming from the diet.

The decrease in OCFA in adipose tissues when corn oil was supplemented would suggest a reduced *de novo* fatty acid synthesis with corn oil supplementation. Odd-chain fatty acids are synthesized when methyl-malonate accumulates as a consequence of an increase of ruminal production of propionate (Garton et al., 1972). In our study, ruminal production of propionic acid would have been higher with corn oil supplementation. The glycerol produced shortly after consumption by the extensive hydrolysis of acylglycerol is rapidly fermented yielding propionic acid (Jenkins, 1993). In addition, as suggested by Jenkins (1993), the lower fiber digestibility observed when corn oil was supplemented (Pavan et al., 2006) would have decreased the ratio acetate to propionate produced in the rumen. The greater reduction observed in s7.c. than in i.m. adipose tissue would be the consequence of a higher lipogenic activity in the former tissue.

A decrease in *de novo* fatty acid synthesis with increasing corn oil supplementation would have offset an increase of stearic absorption, resulting in the similar proportion of stearic and oleic acids observed in the adipose tissues across dietary treatments. The effect of increasing dietary linoleic acid through vegetable oil supplementation on tissue stearic and oleic acid content is variable across studies. In agreement with our results, Andrae et al. (2001) did not observed changes in either FA when they replaced typical corn by a high-oil corn in a high-concentrate diet. Madron et al. (2002) observed an increase in stearic acid, but a decrease in oleic acid when added of 12.7 and 25.6% of extruded full-fat soybean to beef steers high-concentrate diet in. In contrast, Gillis et al. (2004) detected similar proportions of stearic acid, but lower of oleic acid when fed heifers a high-concentrate diet for 60 days with 4% of corn oil. The reason of this variability among studies would be the several factors affecting the concentration of this two fatty acids; level of 18-carbon fatty acid in the diet, level of biohydrogenation, intestinal absorption, level of *de novo* synthesis, and SCD activity.

Stearoyl-CoA desaturase (SCD) is located in the adipose tissues of growing ruminants (Martin et al., 1999), and it is responsible for the rate-limiting step in the biosynthesis of monounsaturated fatty acids (MUFA). Stearoyl-CoA desaturase, also known as the Δ^9 desaturase, catalyzes the synthesis of monounsaturated fatty acids by inserting a cis double bond between the 9^{th} and 10^{th} carbon (counting from the carboxyl terminal) in long-chain fatty acids of 12 to 19 carbons (Ntambi, 1999). Palmitoyl- and stearoyl-CoA are the major substrates for SCD and are converted to palmitoleoyl- and oleoyl-CoA, respectively. Trans-11 vaccenic acid is also a substrate and can be converted to cis-9 trans-11 conjugated linoleic acid (CLA) by SCD. Results from this study shows that tissue CLA concentrations are dependent upon SCD activity, because 83% of CLA in adipose tissues results from desaturation of TVA. Thus, SCD is an important component of de novo lipogenesis to store excess energy. The activity and expression of SCD in ruminant adipose tissues directly impacts the fatty acid composition of adipose and cell membranes, thereby regulating membrane fluidity. In rodents, diets rich in PUFA have been shown to reduce hepatic abundance of SCD (Ntambi, 1992, Ntambi, 1999; Jump and Clark, 1999). If dietary oil supplementation reduces adipose SCD activity or expression, then the conversion of TVA to CLA may be negatively impacted. Results from this study indicate that SCD activity or expression in s.c. adipose tissues was not negatively impacted with oil supplementation. This was also confirmed indirectly from the measurement of abomasal and tissue CLA:TVA ratios, which did not differ among dietary treatments. In addition, these results suggest a parallel response between activity and expression of SCD in s.c. adipose tissues.

IMPLICATIONS

Corn oil supplementation to grazing steers reduced the percentages of highly atherogenic fatty acids, myristic and palmitic acids, and increased the percentages of antiatherogenic and anticarcinogenic fatty acids, trans-11 vaccenic acid and CLA *cis*-9, *trans*-11.

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Table 3.1. Daily fatty acid intake of total diet, supplement plus pasture, for steers grazing endophyte-free tall fescue supplemented with increasing levels of corn oil.

	Corn oil level, g/kg BW			Oil effect	
	0 (NONE)	**0.75 (MED)**	**1.5 (HI)**	**Linear**	**Quad.**
Intake, g/d					
C14:0	1.7 ± 0.05	1.7 ± 0.06	1.6 ± 0.05	0.09	0.13
C16:0	51.5 ± 2.19	83.9 ± 2.40	105.9 ± 2.19	<0.01	0.09
C18:0	4.6 ± 0.29	10.6 ± 0.32	15.3 ± 0.29	<0.01	0.10
C18:1 *cis-9*	21.8 ± 3.21	110.3 ± 3.52	184.6 ± 3.21	<0.01	0.11
C18:2 *cis-9, cis-12*	90.7 ± 7.48	265.1 ± 8.19	406.7 ± 7.48	<0.01	0.12
C18:3 *cis-9, cis-12, cis-15*	120.0 ± 5.01	109.6 ± 5.49	83.0 ± 5.01	<0.01	0.23
Unidentified	46.1 ± 1.83	46.2 ± 2.00	39.8 ± 1.83	0.03	0.19

Table 3.2. Effect of corn oil supplementation to grazing steers on LM and s.c. adipose tissue proximate composition.

	Corn oil level, g/kg BW			**SEM**	**Oil effect**	
	0 (None)	**0.75 (MED)**	**1.5 (HI)**		**Linear**	**Quad.**
Longissimus dorsi						
Moisture, %	74.16	73.98	74.09	0.28	0.87	0.69
Crude protein, %	23.04	23.40	23.32	0.26	0.46	0.51
Ash, %	1.42	1.36	1.43	0.04	0.77	0.16
Total lipid, %	2.55	2.47	2.40	0.24	0.65	0.98
Cholesterol, mg/100g	58.59	57.24	59.03	0.96	0.75	0.20
Subcutaneous fat						
Total lipid, %	65.82	65.16	68.96	3.76	0.56	0.64

Table 3.3. Effect of corn oil supplementation to grazing steers on fatty acid composition of LM or s.c. adipose tissue.

	Corn oil level, g/kg BW				**Oil effect**		**Adipose tissue**				
	0 (NONE)	**0.75 (MED)**	**1.5 (HI)**	**SEM**	**Linear**	**Quad.**	**i.m.**	**s.c.**	**SEM**	**Adipose Tissue effect**	**Interaction effect**
FA, mg/100 mg											
C12:0	0.07	0.07	0.06	0.02	0.72	0.93	0.05	0.08	0.01	0.06	0.31
C14:0	2.48	2.12	2.03	0.14	0.04	0.45	1.82	2.60	0.09	<0.01	0.25
C14:1 *cis-9*	0.59	0.49	0.50	0.06	0.28	0.43	0.34	0.71	0.04	<0.01	0.92
C16:0	23.71	22.35	21.80	0.44	<0.01	0.47	22.51	22.73	0.28	0.31	0.14
C16:1 *cis-9*	3.42	2.90	2.95	0.18	0.08	0.22	2.54	3.64	0.11	<0.01	0.79
C18:0	18.15	17.80	18.38	1.12	0.88	0.74	17.73	18.49	0.70	0.16	0.28
C18:1 *cis-9*	32.75	32.33	33.52	1.04	0.61	0.54	31.35	34.38	0.66	<0.01	0.21
C18:1 *cis-11*	1.01	0.93	0.94	0.04	0.25	0.32	1.08	0.85	0.03	<0.01	0.60
C18:1 *cis-12*	0.12	0.08	0.11	0.03	0.83	0.44	0.13	0.08	0.02	0.04	0.85
C18:1 *trans-10*	0.17	0.32	0.32	0.03	<0.01	0.07	0.20	0.35	0.03	<0.01	0.44
C18:1 *trans-11*	4.07	5.95	5.36	0.36	0.02	0.01	4.25	6.01	0.22	<0.01	0.16

	Corn oil level, g/kg BW				**Oil effect**		**Adipose tissue**				
	0 (NONE)	**0.75 (MED)**	**1.5 (HI)**	**SEM**	**Linear**	**Quad.**	**i.m.**	**s.c.**	**SEM**	**Adipose Tissue effect**	**Interaction effect**
C18:2 *cis-9, 12*	2.42	2.67	2.55	0.22	0.69	0.50	4.15	0.95	0.17	<0.01	0.59
C18:3 *cis- 9,12,15*	0.76	0.69	0.60	0.03	<0.01	0.83	0.93	0.44	0.03	<0.01	0.62
C20:0	0.14	0.14	0.15	0.01	0.52	0.76	0.11	0.18	0.01	<0.01	0.58
C20:3 n-6	0.21	0.23	0.23	0.02	0.54	0.72	0.44	0.002	0.02	<0.01	0.66
C20:4 n-6	0.69	0.70	0.77	0.08	0.48	0.77	1.41	0.04	0.06	<0.01	0.70
C20:5 n-3	0.24	0.25	0.24	0.03	0.95	0.77	0.48	0.01	0.02	<0.01	0.96
C22:4 n-6	0.72	0.73	0.81	0.09	0.48	0.78	1.51	0.00	0.07	<0.01	0.75
C22:5 n-3	0.41	0.41	0.41	0.04	1.00	1.00	0.77	0.04	0.03	<0.01	0.96
C22:6 n-3	0.04	0.04	0.04	0.005	0.55	0.88	0.08	0.00	0.004	<0.01	0.93
CLA											
Trans-10, *cis*-12	0.020	0.026	0.033	0.003	0.01	0.97	0.019	0.033	0.002	<0.01	0.46
cis, *cis*	0.071	0.074	0.063	0.004	0.16	0.11	0.056	0.083	0.003	<0.01	0.66
Trans, *trans*	0.044	0.021	0.024	0.006	<0.01	0.04	0.035	0.025	0.004	0.04	0.35

	Corn oil level, g/kg BW				**Oil effect**		**Adipose tissue**				
	0 (NONE)	**0.75 (MED)**	**1.5 (HI)**	**SEM**	**Linear**	**Quad.**	**i.m.**	**s.c.**	**SEM**	**Adipose Tissue effect**	**Interaction effect**
Unidentified	5.10	6.05	5.71	0.52	0.43	0.33	5.86	5.38	0.41	0.40	0.36
Total SFA	44.54	42.48	42.42	1.17	0.22	0.49	42.22	44.07	0.72	<0.01	0.24
Total MUFA	42.14	42.99	43.70	1.19	0.37	0.96	39.87	46.01	0.73	<0.01	0.12
Total PUFA	5.50	5.72	5.64	0.48	0.83	0.79	9.76	1.48	0.38	<0.01	0.78
PUFA:SFA ratio	0.13	0.14	0.13	0.01	0.66	0.57	0.23	0.03	0.01	<0.01	0.66
n-6 fatty acids	4.05	4.34	4.36	0.40	0.58	0.78	7.51	0.99	0.32	<0.01	0.70
n-3 fatty acids	1.45	1.39	1.29	0.10	0.24	0.88	2.27	0.49	0.08	<0.01	0.94
n-6: n-3 ratio	2.41	2.75	2.93	0.16	0.03	0.71	3.31	2.08	0.09	<0.01	0.65

Table 3.4. Effects of corn oil supplementation to grazing steers within adipose tissue on odd-chain fatty acids and total CLA content

Tissue	Oil level × adipose tissue						SEM	Oil level effect	Adipose tissue effect	Interaction effect
	LM			s.c. fat						
Oil level, g/kg BW	0 (NONE)	0.75 (MED)	1.5 (HI)	0 (NONE)	0.75 (MED)	1.5 (HI)				
FA, mg/100 mg										
OCFA	1.56 ^{cd}	1.35 ^{ab}	1.26 ^{a}	2.00 ^{e}	1.69 ^{d}	1.48 ^{bc}	0.05	< 0.01	<0.01	< 0.01
C15:0	0.52 ^{b}	0.45 ^{ab}	0.43 ^{a}	0.80 ^{d}	0.68 ^{c}	0.59 ^{b}	0.03	< 0.01	<0.01	< 0.01
C17:0	1.04 ^{b}	0.89 ^{a}	0.84 ^{a}	1.21 ^{c}	1.00 ^{b}	0.88 ^{a}	0.03	< 0.01	<0.01	< 0.01
Total CLA isomers	0.80 ± 0.034 ^{a}	0.96 ± 0.049 ^{b}	0.91 ± 0.119 ^{ab}	1.09 ± 0.034 ^{b}	1.53 ± 0.049 ^{c}	1.41 ± 0.119 ^{c}	-	<0.01	<0.01	<0.01
cis-9, trans-11	0.68 ± 0.033 ^{a}	0.85 0.048 ^{b}	0.81 ± 0.120 ^{ab}	0.94 0.033 ^{b}	1.39 ± 0.048 ^{c}	1.25 ± 0.120 ^{c}	-	0.02	< 0.01	<0.01
cis-11, trans-13	0.000 ^{a}	0.000 ^{a}	0.000 ^{a}	0.000 ^{a}	0.008 ^{a}	0.027 ^{b}	0.004	0.01	<0.01	0.01

$^{a, b, c, d, e}$ within a row least square means without a common superscripts letter within adipose tissue differ at $P < 0.05$

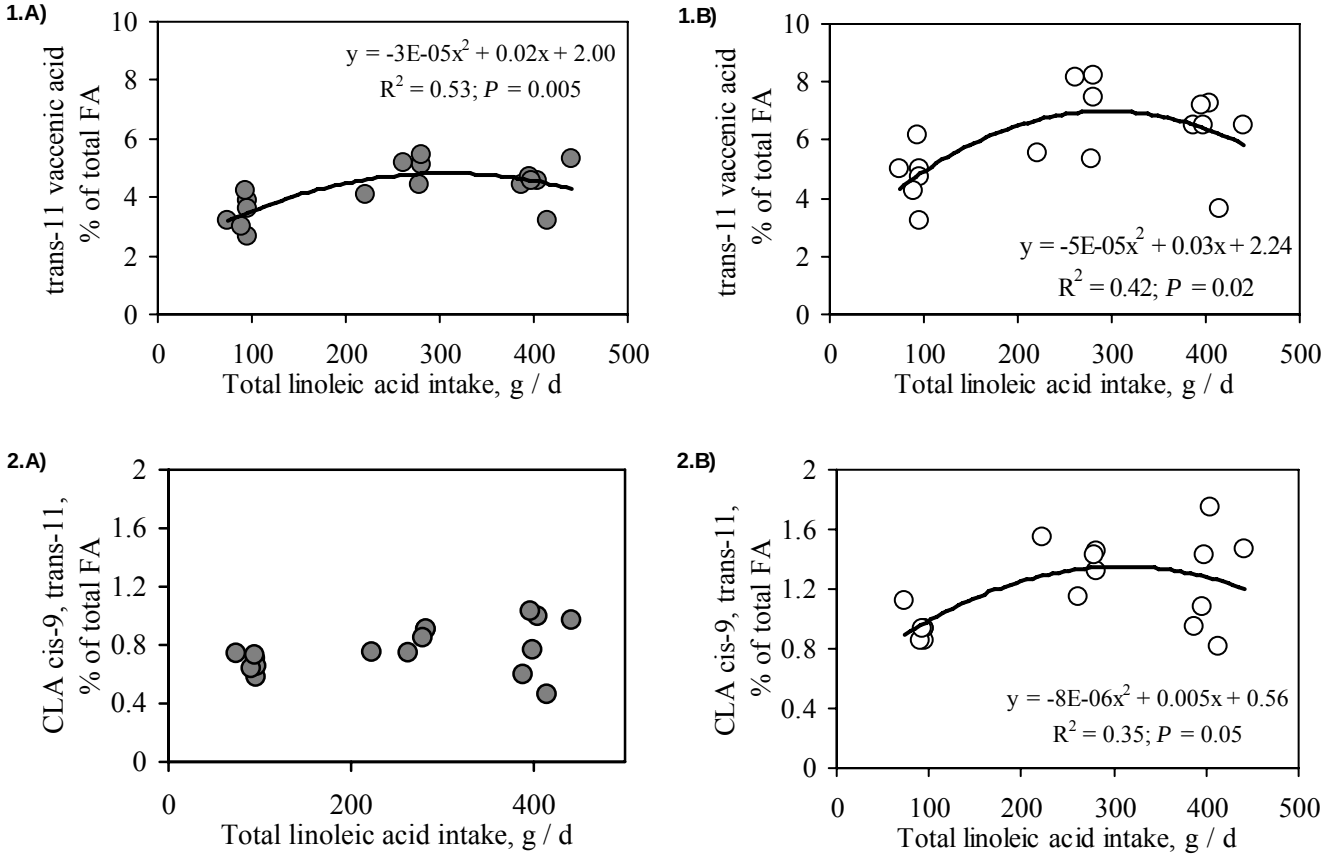

Figure 3.1. Effect of linoleic acid intake (g/d) in grazing steers supplemented with increasing corn oil supplementation on trans-11 vaccenic acid (**1**) and CLA cis-9, trans-11 (**2**) content in the i.m. (**A**) or s.c. (**B**) adipose tissue. Dots represent the individual trans-11 vaccenic CLA cis-9, trans-11 content, whereas the line connects the predicted content.

Table 3.5. Cellularity of s.c. adipose tissue from grazing steers supplemented with increasing amount of corn oil.

Item	Corn oil level, g/kg BW [1]				Oil effect	
	0 (NONE)	0.75 (MED)	1.5 (HI)	SEM	Linear	Quad
N^o cell per mg tissue	1401	1248	1448	134	0.81	0.30
Mean cell diameter, μm	87.5	99.3	90.0	5.4	0.75	0.13
Peak cell diameter, μm	103.3	120.0	113.3	9.5	0.47	0.33
Mean cell volume, μm^3 (10^4)	50.5	67.5	55.9	8.0	0.64	0.17
Peak cell volume, μm^3 (10^4)	79.8	106.6	102.3	16.2	0.34	0.44

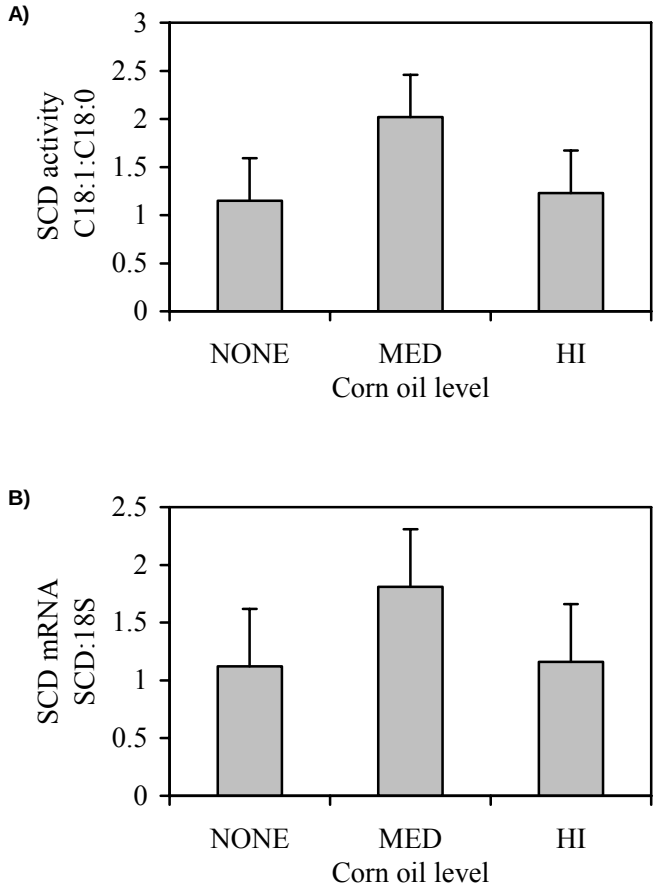

Figure 3.2. Effect of linoleic acid intake (g/d) in grazing steers supplemented with increasing corn oil supplementation on s.c. fat stearoyl-CoA desaturase (SCD) activity (**A**) and mRNA level (**B**).

CHAPTER 4

CORN OIL OR CORN GRAIN SUPPLEMENTATION TO STEERS GRAZING ENDOPHYTE-FREE TALL FESCUE. I. EFFECTS ON IN VIVO DIGESTIBILITY, PERFORMANCE AND CARCASS TRAITS[1]

[1] Pavan, E. and S. K. Duckett. To be submitted to J. Anim. Sci.

ABSTRACT. Angus steers (n=28; 288.5 ± 4.4 kg) were used in a completely randomized design to evaluate the effect of isocaloric supplementation of two different energy sources to steers rotationally grazing tall fescue pastures for 197 d. Steers were supplemented with either corn grain (0.52 % BW; **PC**) or soybean hulls plus corn oil (0.45% BW plus 0.10 % BW, respectively; **PO**) using Calan gates for individual intake measurement. Negative (pasture only; **P**) and positive (85% concentrate: 15% roughage; **C**) control diets were also included in the study. Steers on PC, PO and P treatments were managed together under a rotational grazing system; whereas, C steers were maintained on an adjacent tall fescue pasture and then fed a high-concentrate diet for the last 92 d using Calan gates. Forage intake, DM and NDF apparent digestibility was evaluated using Cr_2O_5 and indigestible NDF as digesta flow markers. Data were analyzed using Mixed procedure of SAS. Regardless of the energy source, supplementation reduced ($P = 0.02$) forage DMI (% BW) with respect to P, but not ($P = 0.58$) total DMI. There were no differences ($P = 0.53$) between grazing treatments on apparent total DM digestibility, though NDF digestibility was lower ($P \leq 0.05$) in PC than in PO and P, which were similar ($P > 0.05$). Despite energy source ($P > 0.05$), periodic and overall ADG were greater ($P < 0.01$) in supplemented than in non-supplemented grazing treatment. Last 92 d and overall ADG was greater in C than in any of the grazing treatments ($P < 0.01$). Supplement conversion did not differ ($P > 0.05$) among supplement type and were similar ($P > 0.01$) to the high-concentrate diet conversion. Carcass traits were not ($P > 0.05$) affected by supplement type. Regardless of supplement type, dressing percentage and hot carcass weight were greater in supplemented treatment than in P; whereas fat thickness and KPH fat percentage were greater ($P < 0.01$) in PO but not ($P > 0.05$) in PC. Ribeye area, marbling score and quality grade did not differ ($P > 0.05$) across grazing treatments. Hot carcass weight for C was 67.5 and 104 kg heavier ($P < 0.001$)

than supplemented (PO or PC) and pasture only (P), respectively. Quality and yield grade of C carcasses were also higher ($P < 0.001$) than carcasses from supplemented or pasture only steers. Energy supplementation, regardless of energy source, to grazing steers increased performance and carcass weight but did not alter carcass quality with respect to non-supplemented grazing steers.

Key Words: Beef, Pasture, Supplementation, Carcass

INTRODUCTION

Ruminant products, meat and milk, are the major natural sources of CLA *cis*-9, *trans*-11 in human diet (2001). Given its potential anticarcinogenic and antiatherogenic properties, an increase of its proportion in bovine adipose tissue would be greatly desirable. Particularly, it has been observed that the proportion of both CLA *cis*-9, *trans*-11 and its precursor, trans-11 vaccenic acid (TVA), is greater in the adipose tissue from forage- than from grain-finished beef (Poulson et al., 2004; Realini et al., 2004), and that it can be further increased by corn oil supplementation of grazing steers (Pavan and Duckett, 2006). However, the opposite effect could be obtained when corn grain is supplemented to grazing cattle to meet the energy production requirements. Presumably changes in microbial population generated by the reduction in ruminal pH (Piperova et al., 2002) or by the increased availability of highly degradable carbohydrates (Loor et al., 2004) are responsible for lower production of TVA as an intermediate product of linoleic acid biohydrogenation when corn grain is supplemented.

The use of concentrates as energy supplement for grazing cattle often improves animal performance (Caton and Dhuyvetter, 1997), although negative associated effects could exist between concentrates and forage that reduce fiber digestion and, in consequence, forage intake (Doyle et al., 2005). In a previous study we observed that despite reducing fiber digestibility and intake, corn oil supplementation to grazing steers increased carcass weight and fatness (Pavan et al., 2006). Thus, oil could be used as a substitute for traditional high-starch energy supplements, to not only meet production requirements in grazing systems, but enhance CLA and TVA content. The objective of this study was to evaluate the effect of supplementing with iso-caloric amounts of corn grain or an oil supplement to grazing steers on *in vivo* digestibility, animal performance and carcass quality.

MATERIALS AND METHODS

Study site. The experiment was conducted at University of Georgia Wilkins Beef Unit (Rayle, GA) between November 2004 and June 2005. The soil types present in the utilized plot are Enon (EnB) and Mecklenburg (MeB) fine sandy loam, with 2 to 6 percent slopes. Pasture consisted of 11.5-ha endophyte-free tall fescue (*Festuca arundinacea* Shreb cv. Jesup) plot subdivided into 15 paddocks of approximately 0.77-ha each for rotational stocking. Pasture was fertilized with 280 kg/ha of 20-5-10 in September, and in March with 224 kg/ha of ammonium nitrate. Animals were removed from grazed paddocks when pasture height was reduced to approximately 6 cm. Steers had ad libitum access to the paddock. Pre- and post-grazing forage availabilities were estimated, every three paddocks, by harvesting 10 random samples (0.09 m^2 frame) at 1 cm height. The 10 samples from each cutting time were weighed and pooled, and a sub-sample was frozen at -20ºC for subsequent chemical analysis. An additional sub-sample was oven dried at at 60ºC for 48 h to estimate DM content.

Animals. Twenty eight yearlings Angus steers (288.5 ± 4.4 kg) obtained from the Northwest Georgia Experiment Station in Calhoun, GA, were randomly assigned to one of four dietary treatments. All steers were treated for internal parasites with ivermectin (Ivermectin Pour-On, Durvet Inc., Blue Spring, MO) on d 0, 42, and 84 and with doramectin (Dectomax, Pfizer Inc., Expon, PA) on d 105 and 147.

Two isoenergetic supplementation treatments were evaluated: corn grain (**PC**) or soybean hulls and corn oil (**PO**). The energy level was defined by setting corn oil level at 0.10% BW and soybean hulls at 0.45% BW (DM basis), resulting in 3.05 Mcal ME/kg DM of supplement according to the beef NRC (1984) values. Cracked corn grain (3.2 Mcal ME /kg DM) was supplemented at 0.52% BW to match the total amount of ME energy supplemented in

PO. Corn oil and SBH were mixed daily on individual basis before feeding. Negative (pasture only, **P**) and positive (high concentrate diet (C): 85% concentrate: 15% chopped bermudagrass hay on DM basis) controls were included in the study. Concentrate used in C was formulated to contain 94.11% rolled corn, 2.91% soybean meal, 1.50% limestone, 0.95% urea and 0.53% of trace minerals on DM basis. Supplements (SBH, corn oil, corn grain), concentrate and hay samples were taken at monthly intervals, frozen at -20ºC, and pooled for subsequent analyses.

Steers assigned to PO and PC were trained to use Calan gate feeders (American Calan, Inc., Northwood, NH) for 21 d prior to the start of trial and in the last 5 days steers were adapted to supplements. During adaptation, steers assigned to the grazing treatments (P, PO and PC) were allowed to graze the same pasture that was used for the experimental period. Steers assigned to C were hold in confinement (drylot) and fed the high-concentrate diet during the last 92 d of the trial (d 105 to d 197); prior to that they were allow to graze an adjacent endophyte-free tall fescue pasture similar to that used by the grazing treatments. Twenty one days before starting their feeding period (d 84 – d 105) steers were trained to use the Calan gate feeders and adapted to the high-concentrate diet. Initial and final live weights were recorded on two consecutive days at 0800 and averaged at the beginning of grazing (d 0) or feeding period (d 105) for grazing steers of steers fed the high-concentrate diet, respectively, and all at the end of the experimental period (d 197). Individual live weights were recorded before supplementation every 21 days and used to adjust the supplement level to the treatment live weight average the following day.

When present, supplement orts were removed and weighed daily to determine supplement intake (as fed basis) by difference with total amount offered. The high-concentrate

diet was offered to allow approximately a 10% refusal, orts were removed weekly, and daily intake (as fed basis) was then calculated by weight difference with the total amount offered. .

Intake and digestibility. Forage DM intake and total dietary DM and NDF *in vivo* apparent digestibility were estimated using chromium sesquioxide as an external marker and indigestible NDF (INDF) as an internal marker of the digesta (Lippke et al., 1986). On d 158, a controlled release fecal marker capsule (CAPTEC, Cattle Chrome MCM for 300-700 kg Cattle, active constituent: Chromium sesquioxide 65% w/w; release rate 1.7 g Cr_2O_3 per day; NUFARM Ltd., Auckland, NZ) was bolused to five steers in P and to three steers in either PO and PC with ADG closest to the treatment mean. At the time of the study, the number of CAPTEC Cattle Chrome MCM controlled release fecal marker capsule available for purchase were because of environmental restrictions in NZ than discontinued production with chromium. Therfore, we selected five steers from P and three steers from each of the PO and PC that were closest to the treatment mean to use in the digestion evaluation phase. Ten days later, fecal samples were collected from each steer twice daily at 0700 and 1700 for 7 d. Fecal samples (25 g wet weight) were pooled for each animal and frozen at -20ºC for subsequent analyses. Supplement refusals were recorded daily and samples collected for DM determination. During the seven days of fecal sample collection forage samples were obtained by observing grazing behavior and simultaneously hand plucking plant parts comparable to the ones selected by the animals. Samples were pooled on fresh weight basis and stored at -20ºC for subsequent analyses of DM composition.

Forage, SBH, corn grain and fecal 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 8 h 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). Forage, SBH corn grain and fecal samples collected during the fecal collection period were also for INDF. For INDF analysis, samples were incubated *in situ* for 7 d (Lippke et al., 1986) in the rumen of a Hereford steers with ad libitum access to tall fescue hay. Residual samples were analyzed for NDF content using an Ankom 200 fiber extractor (Ankom Technologies, Fairport, NY) according to the method of Van Soest et al.(1991). Chromium concentration was measured via spectrophotometry in the fecal samples (Fenton and Fenton, 1979). Forage DMI was calculated based on fecal output and indigestibility (Reis and Combs, 2000) with modifications. Fecal output was calculated based on chromium concentration in the feces, whereas indigestibility was determined based on forage, SBH, corn grain and feces INDF concentrations. Total forage INDF excreted was estimated by difference between total INDF excreted and total SBH or corn grain INDF. Forage DMI was then calculated as the ratio between total forage INDF excreted and INDF concentration in the forage sample. The DMI for each component of the diet was multiplied by the corresponding concentration of OM, CP, FA, NDF and ADF that then were added to obtain total DMI and total OM, CP, FA, NDF and ADF intakes. Total tract DM, OM, FA, NDF and ADF digestibilities were estimated as the coefficient of the total tract disappearance (intake minus excretion) and intake.

Carcass traits. Steers were transported 45 km to the University of Georgia Meat Science and Technology Center in Athens and fasted live animal weights were obtained following an overnight feed withdrawal. After slaughter, HCW was recorded and carcasses chilled at -1ºC for 24 h. At 24 h postmortem adjusted s.c. fat thickness, LMA, marbling score, percentage of kidney, pelvic, and heart fat (KPH) and skeletal maturity were determined on the left side of each carcass.

Calculations. Mean forage allowance per paddock was estimated as 0.5 × (pre-graze + post-graze forage mass, kg DM/ha)/(average BW, kg/steer × steer/ha) according to Fike et al. (2003). The added BW gain by the supplements (PO and PC) relative to BW gain for P was calculated as follows: [(BW d 197 – BW d 0) $_{PO\ or\ PC}$ – (BW d 197 – BW d 0)$_{P}$]; whereas the added BW gain by C was equal to the change in BW during the period when the high-concentrate diet was fed (BW d 105 – BW d 197). Individual supplement conversions (SC) for steers calculated as follows: [(added BW change) / (total supplement intake for PO and PC or total diet intake for C)].

The substitution rate of forage DMI by the supplements was calculated as the difference between average forage DMI in P and individual forage DMI in PO or PC relative to the supplement intake (Bargo et al., 2003). The relative stocking density was defined as the approximate number of animals in PO and PC treatments needed to utilize equal amounts of forage relative to P. Relative stocking density was calculated as the ratio between the average forage DMI for P and the individual forage DMI for either PO or PC.

Statistical analysis. Performance, intake, digestibility and carcass variables were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) with animal as the experimental unit and dietary treatment as a fixed effect. If

the overall F-test for the treatment effect was significant ($P \leq 0.05$) means differences were separated using a t-test ("lsmeans/pdiff" command in SAS). Substitution rate and relative stocking density values for PO and PC were also tested to determine significance from one. A substitution rate higher than one means that each kg of corn oil supplement substituted for significantly more than one kg of forage DM; whereas a relative stocking density higher than one means that more supplemented animals are needed in turn to obtain a similar forage utilization relative to unsupplemented animals.

RESULTS

Forage quality during forage DM intake and in vivo digestibility determination phase was: 12.6% CP; 57.4% NDF and 29.5% ADF content (DM basis). Total DM intake did not differ ($P = 0.65$) across grazing treatments (Table 4.1). However, forage DM intake (% BW) decreased ($P < 0.05$) 34% with supplementation, regardless of supplementation type, compared to forage DM intake of P. Forage DM intake did not ($P = 0.34$) differ between the two types of supplement evaluated. Similarly, forage substitution rate with supplementation was not affected by the type of supplement ($P = 0.25$) and levels were above 0.73. Relative stocking density did not differ ($P = 0.18$) between supplements, but values were greater ($P < 0.05$) than one for both supplements. In vivo apparent digestibility of DM was similar ($P = 0.53$) across grazing treatments; however, NDF and ADF digestibility were lower ($P < 0.05$) for PC than PO or P, which did not differ ($P > 0.10$).

Pre-grazing forage mass averaged 3,333 ± 373 kg/ha and the post-grazing forage mass averaged 1,892 ± 121 kg/ha. On average, each paddock was grazed for 6.6 days with a mean forage mass of 25 kg DM/ 100 kg BW. The average dry matter chemical composition from pre-

and post-grazing forage samples, and for the pooled soybean hulls, corn grain, concentrate, and bermudagrass hay samples are presented in Table 4.2.

Neither the overall ($P = 0.81$) nor periodic ADG (d 0 to d 105, $P = 0.11$ or d105 to d 197, $P = 0.35$) were affected by the type of supplement used (Table 3). During the first 104 d, the ADG from the supplemented treatment was similar ($P > 0.05$) to that on C, which were grazing during this phase of the experiment, but 0.13 kg greater ($P < 0.01$) than that observed for forage only (P) treatment. During the second part of the study (d 105 to d 197), ADG was greater for C, when the high concentrate diet was fed, ($P < 0.001$) than in any of the grazing treatments. Average daily gain in the second period (d 105 – 197) was also greater ($P = 0.008$) in PO than P, with PC being intermediate. Overall ADG was also greater ($P < 0.001$) in C than in any grazing treatments, and also for supplemented (PO and PC) than P ($P < 0.01$). Body weight did not differ ($P = 0.18$) across dietary treatments at d 104 or d 197. Body weight was 68 and 110 kg heavier ($P < 0.001$) for C than supplemented (PC and PO) or pasture only (P), respectively.

The daily or total amount of supplement consumed during the 197 d of supplementation did not differ between PO and PC ($P > 0.05$) and was lower ($P < 0.001$) than the daily or total amount of high-concentrate diet (concentrate plus bermudagrass hay) consumed for C during 92 d (Table 4.4). The cost ($/steer) of total amount of supplement consume during the 197 d of supplementation was also similar ($P > 0.05$) between PO and PC, and lower ($P < 0.001$) than the feed cost per steer in C. However, the additional BW gain (kg) obtained when supplementing grazing steers for 197d relative to BW gain of non-supplemented steers was also lower ($P < 0.001$) than the total BW gain obtained by feeding a high-concentrate diet for 92d (C), with no differences ($P > 0.05$) between the supplements. Thus, supplement conversion efficiency was

not affected by the type the supplement and was similar to the conversion efficiency of the high-concentrate diet ($P = 0.51$).

Hot carcass weight (**HCW**) was 64 or 104 kg greater ($P < 0.001$) for C than supplemented (PO and PC) or P, respectively. Hot carcass weight did not differ (P = 0.51) among supplement types. Dressing percentage did not differ between type of supplement ($P = 0.51$; 56.4%); however, dressing percentage from supplemented steers was 3.4 percentage units greater ($P < 0.01$) and 4.9 lower ($P < 0.001$) than that from non-supplemented and high-concentrate fed steers, respectively. No treatment effect was observed for skeletal maturity ($P = 0.11$). The rest of the carcass traits evaluated ribeye area, fat thickness, marbling, and the percentage of kidney-pelvic-heart fat, were greater in C than in the grazing treatments ($P < 0.01$) and did not differ between type of energy source used in the supplement of grazing steers ($P > 0.05$). Fat thickness and KPH fat percentage obtained with PO, but not PC ($P = 0.15$), was greater than in P ($P = 0.01$); neither LMA nor marbling score from PO and PC differ from those in P ($P > 0.16$). When LMA was expressed on a HCW basis, P had the greatest value ($P < 0.05$); PO and PC did not differ ($P = 0.24$), though PC was greater than C ($P = 0.005$), PO did not differ from C ($P = 0.07$). Yield grade was lower for all three grazing treatments than in C ($P < 0.001$), and in P or PC than in PO ($P < 0.05$). Quality grade was also lower in the grazing treatments than in C ($P < 0.001$), but no differences were observed across grazing treatments ($P > 0.15$). Regardless of the type of energy used, supplementation to grazing steers increased ($P < 0.05$) carcass price ($/45.4 kg) and carcass value ($/carcass) relative to non-supplemented grazing steers. However, both carcass price and value were greater ($P < 0.001$) for steers finished on the high concentrate diet than on grazing regardless of supplementation.

DISCUSSION

The depression in forage DM intake (% BW) when cracked corn grain was supplemented agrees with the results of others (Hess et al., 1996; Elizalde et al., 1998). Forage intake was reduced when steer grazing an endophyte-free (Hess et al., 1996) or endophyte-infected (Elizalde et al., 1998) tall fescue were supplemented with 0.34 or 0.75% BW of cracked corn, respectively; or increasing levels (0.4, 0.8 and 1.2% BW) of cracked corn were supplemented to fresh alfalfa fed steers (Elizalde et al., 1999). In contrast, Brokaw et al. (2001) observed no changes in forage DMI when steers grazing a summer pasture were supplemented with lower levels of oil (0.0375% BW) and cracked corn (0.35% BW) or by Judkins et al. (1997) when supplemented steers grazing an endophyte-free tall fescue with 0.4% BW of ground corn. Similarly, others have reported reductions in forage intake with supplementation of SBH plus corn oil (Pavan et al., 2006) or high-fiber supplements (Hess et al., 1996; Elizalde et al., 1998; Richards et al., 2006) to grazing steers. The reduction in forage intake with supplementation resulted in high substitution rates. In grazing dairy cattle, the substitution rate for fiber- or starch-rich supplements typically ranges between 0 and 1 (Bargo et al., 2003; Doyle et al., 2005). The mean substitution rate over a wide range of grazing situations was 0.69 kg of forage per kg of supplement as reviewed by Minson (1990). Stocking density must be adjusted with supplementation in order to optimize forage utilization and maximize animal response. In an earlier study (Pavan et al., 2006), stocking density could be increased by 62% relative to the stocking density without oil supplementation when supplementing steers with 1.5 g/kg BW of corn oil. According to the present study, stocking density can be increased 52% when corn oil and soybean hulls are supplemented or 29% when corn grain is supplemented.

The reduction in forage intake with corn grain supplementation would be associated with the observed NDF digestibility decline. Reduction on forage intake when supplementing would result from negative associative effects between forage and supplement that would reduce fiber digestion (Doyle et al., 2005) or by a reduction of the grazing time (Bargo et al., 2003). In our study, total tract NDF digestibility was reduced with corn grain supplementation but not with SBH plus corn oil. The lack of oil supplementation effect on NDF digestibility contrasts with the linear decrease observed when increasing corn oil levels (0.075 and 0.15% BW) were supplemented to grazing steers in a previous experiment (Pavan et al., 2006). The difference between studies could be partially explained by the different fiber source used as oil carrier; low-degradable cottonseed hulls in the first study versus highly-degradable soybean hulls in this study. Cottonseed hulls depressed OM digestibility when supplemented to steers grazing wheat pasture (Lippke et al., 2000); whereas SBH did not alter (Richards et al., 2006) or increased NDF digestibility when supplemented to steers fed freshly clipped endophyte-infected tall fescue (Faulkner et al., 1994). Fieser and Vanzant (2004) observed that SBH supplementation improved total dietary NDF digestibility as tall fescue maturity advanced from vegetative to boot-, heading-stage or mature stage; whereas, corn grain supplementation reduced NDF digestibility. Similar results were observed by Matejovsky and Sanson (1995) with lambs. In our study, the period of forage intake and digestibility determination was conducted while the pasture was in heading-stage. Thus, a higher NDF digestibility of the SBH with respect to the forage may have counter balanced a possible negative effect of the corn oil. A decrease in NDF digestibility was also observed when lipid was supplemented to bermudagrass hay (Hall et al., 1990; Patil et al., 1993) or bromegrass hay based-diets (Scholljegerdes et al., 2004). Others have shown no changes in total dietary NDF digestibility when 0.0375% BW of soybean oil was

supplemented to grazing heifers (Brokaw et al., 2001) or when 300 mL of soybean oil were infused intraruminally to heifers fed a grass-hay diet (Krysl et al., 1991).

The overall ADG for grazing steers without supplementation was within the 0.52 to 0.84 kg/d range reported in the literature for steers grazing endophyte-infected (Thompson et al., 1993; Elizalde et al., 1998; Beck et al., 2006) or endophyte-free (Hess et al., 1996; Judkins et al., 1997; Pavan et al., 2006) tall fescue pastures. In accordance with the forage intake and digestibility results, SBH plus corn oil supplement provided a gain response similar to that observed with corn supplementation when supplemented at isocalcoric levels. Greater ADG were obtained with corn grain than with wheat bran (Hess et al., 1996) or corn gluten fed (Elizalde et al., 1998), respectively when both supplements type were supplemented at 0.34 or 0.5% BW, respectively. Likewise, ADG did not differ when three iso-nitrogenous and isocaloric concentrates containing different carbohydrate sources (starch, starch + fiber or fiber-based) where supplemented to grazing steers (French et al., 2001). In our previous study (Pavan et al., 2006), corn oil supplementation to grazing steers increased ADG by 0.12 kg/d for each 0.10% BW of added oil. The lower response to oil supplementation in the previous study could have been due to an indirect effect of oil supplementation on diet digestibility through an increase in the proportion of cottonseed hulls as mentioned above. When 3 and 6% of soybean oil (about 0.083 and 0.166% BW, respectively) partially replaced corn grain in isocaloric formulated concentrate supplemented to prepuberal heifers fed a hay-based diet, no change in the ADG was observed (Whitney et al., 2000). In a second trial using the same diets, ADG increased by 0.10 kg with the 3% oil inclusion, but no effect was observed with 6% inclusion (Whitney et al., 2000). Differences in ADG were not observed when 8.2% (DM basis) of corn supplemented to

steers grazing common bermudagrass (*Cynodon dactylon*) was replaced by a 6.85% vegetable oil (0.056% BW) plus calcium carbonate (Hall et al., 1990).

As a similar added BW gain per steer was obtained similar total amounts of supplement between PO and PC resulted in also similar supplement conversions. However, the supplement conversion observed in the present study were lower than those (0.20 and 0.16 kg/kg supplement) observed by Hess et al. (1996) and Judkins et al. (1997) when supplementing corn grain (0.34 and 0.40% BW, respectively) to steers grazing endophyte-free tall fescue pastures. This difference could be the result of the use of lighter animals (final live wt < 350 kg) by them than in the present study. In addition, variation in supplement conversion efficiency among studies could be the result of differences of forage availability during supplementation. Supplement conversion decrease as forage availability increased (French et al., 2001; Beretta et al., 2006). In our study, each paddock was grazed for an average of 6.6 d with mean forage mass during that period of 25 kg DM /100 kg BW. This could have resulted in high substitution rates throughout the grazing period limiting supplement conversions. Thus, if the stocking density is corrected in order to improve forage utilization as mention above, supplement conversion will improve. According to our results, although finishing steers on a high concentrate diet allows to obtain greater carcass weight than supplementing while grazing, that additional weight is at the expense of greater consumption of a high-concentrate diet and not to greater conversion efficiency. In our study the conversion efficiency of the supplements offered during the grazing period were similar to the conversion efficiency of the high concentrate diet. According to French et al. (French et al., 2001) if the stocking density is adjusted when supplementing reduce substitutive effects, supplement conversion efficiency could be greater than that obtained with a

high-concentrate diet as a result of positive associative effects between the pasture and the supplement.

Carcasses from steers fed the high-concentrate diet were fatter than those from grazing treatments. Similar results were reported for fat score by French et al. (French et al., 2001) and for intramuscular fat by French et al. (2003). The main reason for the inclusion of the high-concentrate treatment was to compare with the carcass traits obtained by the supplemented grazing treatments. Energy supplementation to grazing steers resulted in heavier carcasses than in unsupplemented grazing steers with relatively slight differences in carcasses traits when compared to grazing steers. Andrae et al. (2001)observed no changes in hot carcass weight, fat thickness, longissimus area, kidney, pelvic, and heart fat (%) or yield grade when high-oil corn grain was used instead of conventional corn in a high concentrate diet. Marbling scores and quality grades increased when the caloric density of the diet was increased by feeding high-oil corn but not when diets were offered in isocaloric quantity (Andrae et al., 2001). Others have shown no changes in carcass quality or yield with oil supplementation using 4% of corn oil (Gillis et al., 2004) or 5% of soybean oil (Beaulieu et al., 2002) to high-concentrate diets. Engle et al. (2000) reported reductions in HCW, fat thickness, KPH, marbling score and quality grade when 5% SBO was added to a high-concentrate diet. In contrast, carcass weight and fat thickness were linearly increased when grazing steers were supplemented with increasing levels of corn oil (Pavan et al, 2006). Our present results, confirm the positive effect of corn oil supplementation to grazing cattle on carcass fatness. Carcass weight was increased by energy supplementation, regardless of energy source used, to grazing steers; however, fat thickness and KPH also increased with corn oil plus SBH supplementation but not with corn grain supplemented at isocaloric levels. This would suggest greater energy efficiency of the

supplements when corn oil and SBH were supplemented rather than corn grain. The lack of effect on marbling score would be the result of the relatively low amount of reserved in that fat depot. As in our previous study (Pavan et al. 2006), fat thickness in the grazing treatments was below the minimum level (6.8 mm) upon which marbling would increase (Duckett et al., 1993). Patil et al. (1993) observed increased fat thickness and marbling scores with fat supplementation, (0.98% BW of concentrate and 0.98% BW concentrate plus 0.33 or 0.67% BW of partially hydrogenated fat). French et al. (2001) reported increased KPH with increased concentrate supplementation but no changes in fat thickness or intramuscular fat content. The greater carcass value obtained when finishing steers on high-concentrate than when grazing steers were supplemented was obtained at the expense of a greater cost of feed per animal. The ratio between the added carcass value (P) and the added feed cost relative to pasture-only was 2.6, 4.8 and 3.2 ($/$) for C, PO and PC, respectively; being 1.8- and 1.5- folds greater for PO than for C and PC, respectively, and 1.2-folds greater for PC than C. Suggesting that corn oil supplementation to grazing steers could be a supplement alternative to corn grain as a supplement for grazing-systems were energy supplementation is required to meet production demands. Furthermore, the cost of the supplements used for finishing steers in forage-systems is fully recovered by the added value obtained in the carcass.

IMPLICATIONS

Energy supplementation in pasture-finishing systems improves animal performance and carcass weight. Due to the reduction on forage intake with supplementation, stocking density could be increased by an average of 40% relative to stocking density without supplementation. Supplement conversion efficiency did not differ between supplements and was similar to the conversion efficiency obtained with the high-concentrate diet.

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Table 4.1. Effect of supplementation and type of supplement on total and forage DMI, substitution rate, relative stocking density and in vivo apparent total DM, NDF, and ADF digestibility

	Dietary treatments [1]			
	PO	*PC*	*P*	***P-value***
N	**3**	**3**	**5**	
DM intake, %BW				
Total	1.61 ± 0.18	1.83 ± 0.18	1.84 ± 0.13	0.58
Forage	1.09 ± 0.17[a]	1.33 ± 0.17[a]	1.84 ± 0.13[b]	0.02
Supplement	0.53	0.50	-	
Substitution rate [2]	1.07 ± 0.18	0.73 ± 0.18	-	0.25
Relative stocking density [3]	1.52 ± 0.10	1.29 ± 0.10	-	0.18
In vivo apparent digestibility, %				
DM	67.2 ± 1.3	66.7 ± 1.3	68.5 ± 1.3	0.53
NDF	69.8 ± 1.5[a]	64.2 ± 1.5[b]	69.5 ± 1.0 [a]	0.046
ADF	88.5 ± 1.4[a]	72.6 ± 1.4[b]	83.6 ± 1.1[a]	<0.001

[1] Dietary treatments: PO = steers grazing tall fescue supplemented with 0.1% BW corn oil and 0.45% BW soybean hulls; PC = steers grazing tall fescue supplemented with 0.52% BW corn grain; P = steers grazing tall fescue only

[2] Substitution rate = $[(\text{Average forage DMI})_{P} - (\text{Individual forage DMI})_{PO \text{ or } PC}]$/ Individual supplement DMI

[3] Relative stocking density = $(\text{Average forage DMI})_{P} / (\text{Individual forage DMI})_{PO \text{ or } PC}$

	Dietary treatments [1]			
	PO	*PC*	*P*	***P-value***

[a, b] Within a same row, LSmeans without a common superscripts letter differ ($P \leq 0.05$)

Table 4.2. Chemical composition (% of DM) of pre- and post-grazed forage, and foods offered

	Forage					
	Pre-grazing	**Post-grazing**	**Soybean hulls**	**Corn Grain**	**Concentrate**	***Bermudagrass hay***
Component, %DM						
OM	92.63 ± 0.19	91.33 ± 0.70	94.68	98.62	96.74	93.87
CP	15.73 ± 0.65	15.05 ± 0.53	13.48	11.36	12.74	15.22
NDF	56.06 ± 1.05	61.18 ± 0.73	68.41	18.29	15.19	68.81
ADF	27.15 ± 0.68	30.27 ± 0.50	47.44	0.69	0.62	32.65
Total FA	2.08 ± 0.10	1.73 ± 0.09	1.89	3.78	3.97	1.34

Table 4.3. Body weights and average daily gains (ADG) of steers grazing endophyte-free tall fescue pasture supplemented with either cracked corn or soybean hulls plus corn oil

	Dietary treatments [1]				*SEM*	*P-value*
	C	**PO**	**PC**	**P**		
Body weight, kg						
d 0	293	289	283	290	7.7	0.85
d 105	387	374	379	355	10.5	0.18
d 197	550[a]	484[b]	481[b]	440[c]	11.7	<0.001
ADG, kg/d						
d 0 - 104	0.90[a]	0.81[a]	0.91[a]	0.62[b]	0.04	< 0.001
d 105 - 197	1.76[a]	1.19[b]	1.11[bc]	0.93[c]	0.06	< 0.001
d 0 – 197	1.30[a]	0.99[b]	1.00[b]	0.76[c]	0.04	< 0.001

[1] Dietary treatments: PO = steers grazing tall fescue supplemented with 0.1% BW corn oil and 0.45% BW soybean hulls; PC = steers grazing tall fescue supplemented with 0.52% BW corn grain; P = steers grazing tall fescue only; C = steers finished on high concentrate diet.

[a, b, c] Within a row means without a common superscript letter differ ($P < 0.05$)

Table 4.4. Daily and total supplement or high-concentrate diet intake (DM basis), cost and conversion

	Dietary treatments [1]				
	C	**PO**	**PC**	**SEM**	**P-value**
Supplement intake					
Daily, kg DM.d^{-1}.steer^{-1}	13.00^{a}	1.93^{b}	1.94^{b}	0.26	<0.001
Total, kg DM/steer	1173.2^{a}	380^{b}	382^{b}	23.2	<0.001
Added feed cost, $/steer [2]	168.4^{a}	32.9^{b}	42.4^{b}	3.30	<0.001
Added BW gain, kg/steer [3]	162.3^{a}	47.2^{b}	44.1^{b}	6.14	<0.001
Conversion [4]	0.140	0.124	0.116	0.014	0.51

[1] Dietary treatments: PO = steers grazing tall fescue supplemented with 0.1% BW corn oil and 0.45% BW soybean hulls; PC = steers grazing tall fescue supplemented with 0.52% BW corn grain; P = steers grazing tall fescue only; C = steers finished on high concentrate diet.

[2] Extra ingredient cost, DM basis: Corn grain $111.83/Tn; corn oil $28.75/Tn; Soybean hulls $99.08/Tn; Concentrate $148.41/Tn; bermudagrass hay $115.80/Tn;

[3] Added BW gain: for C = BW d 105 – BW d 197; for PO and PC = [(BW d 197 – BW d 0) $_{PO\ or\ PC}$ – (BW d 197 – BW d 0)$_{P}$]

4 Conversion = [(added BW change) / (total supplement intake for PO and PC or total diet intake for C)]

[a, b, c] Within a row means without a common superscript letter differ ($P < 0.05$)

Table 4.5. Carcass traits of steers grazing endophyte-free tall fescue pasture supplemented with either cracked corn (PC) or soybean hulls plus corn oil (PO) compared to positive control (C; high concentrate diet) or negative control (P, pasture only)

	Dietary treatments [1]				SEM	P-value
	C	*PO*	*PC*	*P*		
Carcass traits						
HCW, kg	319[a]	255[b]	248[b]	215[c]	7.1	< 0.001
Dressing, %	61.4[a]	56.8[b]	56.1[b]	53.1[c]	0.7	< 0.001
REA						
cm^2	72.8[a]	62.3[b]	63.4[b]	59.4	2.0	< 0.001
cm^2:HCW	0.23[a]	0.25[bc]	0.26[b]	0.28[a]	0.01	< 0.001
FT, cm	1.25[a]	0.53[b]	0.41[bc]	0.26[c]	0.07	< 0.001
Marbling[2]	587[a]	424[b]	397[b]	367[b]	30.7	< 0.001
KPH, %	1.9[a]	1.3[b]	1.0[bc]	0.8[c]	0.1	< 0.001
Skeletal Maturity [3]	161	166	154	159	3.2	0.11
Yield grade	3.2[a]	2.3[b]	2.0[c]	1.8[c]	0.1	< 0.001
Quality grade [4]	5.6[a]	3.3[b]	2.7[b]	2.4[b]	0.4	< 0.001
Carcass price, $/45.4 kg [5]	134.9[a]	116.9[b]	116.4[b]	105.8[c]	3.11	<0.001
Carcass value, $/carcass	948.5[a]	659.7[b]	639.8[b]	502.3[c]	31.4	< 0.001

[1] Dietary treatments: PO = steers grazing tall fescue supplemented with 0.1% BW corn oil and 0.45% BW soybean hulls; PC = steers grazing tall fescue supplemented with 0.52% BW corn grain; P = steers grazing tall fescue only; C = steers finished on high concentrate diet.

	Dietary treatments [1]				SEM	P-value
	C	*PO*	*PC*	*P*		

[2] Marbling score: 500 = small, 400= slight, 300 = traces.

[3] Skeletal maturity: 100-199 = A

[4] Quality grade = 5 = Choice^{-}, 4 = Select^{+}, 3 = Select^{-}, 2 = Standard^{+}

[5] Base carcass price (Choice-, YG 3) = $133.32/45.5 kg; Premiums: YG 1 $ 3.57/45.4 kg; Discounts: Select -$ 7.41/45.4 kg; Standard -$ 17.09/45.4 kg; Lightweight carcass (< 250 kg) -$12.87/45.4 kg

[a, b, c] Within a row means without a common superscript letter differ ($P < 0.05$)

CHAPTER 5

CORN OIL OR CORN GRAIN SUPPLEMENTATION TO STEERS GRAZING ENDOPHYTE-FREE TALL FESCUE. II. EFFECTS ON LM AND S.C. FATTY ACID COMPOSITION AND STEAROYL-COA DESATURASE GENE EXPRESSION[1]

[1] Pavan, E. and S. K. Duckett. To be submitted to J. Anim. Sci.

ABSTRACT: Samples of s.c. and i.m. fat were obtained from 28 Angus steers (n = 28; 288.5 ± 4.4 kg) after slaughter to determine the effect of energy supplementation of steers grazing tall fescue pastures on fatty acid composition and stearoyl-CoA gene expression. Steers were supplemented with either corn grain (0.52% BW; PC) or corn oil plus soybean hulls (0.10 plus 0.45% BW, respectively; PO). Negative (pasture only; P) and positive (85% concentrate/15% roughage; C) controls were also included in the study. Steers were slaughtered at similar time-on-feed endpoint. Fatty acid (FA) composition from the s.c. and LM fat were determined by GLC and analyzed with dietary treatment in the model by fat depot. Total fatty acid content of LM was 123% greater ($P < 0.01$) for C than PC, PO, or P. Longissimus muscle from all steers grazing forages (PO, PC and P) contained lower ($P < 0.01$) percentages of myristic and palmitic acids. Concentrations of *trans*-11 vaccenic acid (TVA) were 90% greater ($P < 0.01$) for PO than P. For PC, trans-11 vaccenic acid levels were 114% greater than for C ($P < 0.01$). Concentration of conjugated linoleic acid (CLA) *cis*-9 *trans*-11 was 24% greater (P < 0.01) for PO than P; for PC was 33% lower than P, but 72% greater than for C ($P < 0.01$). In s.c. fat, PO reduced (P < 0.01) palmitic acid percentages compared to PC and P; however, all forage-finished beef were lower ($P < 0.01$) in palmitic acid than C. Oil supplementation increased ($P < 0.01$) concentration of TVA and CLA *cis*-9, *trans*-11 by 71% and 27%, respectively, compared to P; they were reduced by 33% and 18%, respectively, in PC compared to P, but increased by 184% and 122%, respectively, compared to C. Levels of SCD mRNA in the s.c. fat were greatest ($P < 0.05$) in C and greater ($P = 0.02$) in PO than in P; PC did not differ from either PO or P ($P > 0.05$). Only 32 and 38% of the myristic and palmitic acids contained in a LM serving from C was contained in a LM serving from grazing steers. A LM serving from P, PC or C had similar ($P > 0.05$) amounts of CLA *cis*-9, *trans*-11 and TVA, but their amounts were enhanced ($P < 0.01$) in PO by 98 and

209%, respectively. The type of energy supplement used in grazing systems can alter the fatty acid composition, corn oil supplementation could be used to enhance CLA *cis*-9, *trans*-11 and TVA content in meat form grazing-beef cattle.

Key words: Forage-finished beef, Supplementation, Fatty acid

INTRODUCTION

Conjugated linoleic acid (CLA) refers to a family of linoleic acid (C18:2 *cis*-9, *cis*-12) isomers with potential anticarcinogenic and antiatherogenic properties (Scollan et al., 2006). Milk and beef represent the major sources of CLA in human diet, being CLA *cis*-9, *trans*-11 the principal isomer (Ritzenthaler et al., 2001). Increasing beef CLA *cis*-9, *trans*-11 content will permit to offer an enhance nutraceutical product to consumers who have increasing interests in health attributes of food products (Grunert et al., 2004).

Research in lactating dairy cows (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 stearoyl Co-A desaturase (**SCD**), enzyme that is present in mammalian adipose tissues (Ntambi, 1995). In beef, TVA is present in adipose tissues at higher levels than CLA *cis*-9, *trans*-11 isomer (Gillis et al., 2004) because of greater formation of TVA during ruminal biohydrogenation than CLA (Duckett et al., 2002). Although SCD expression is greater in concentrate-based than in forage-based diets (Daniel et al., 2004), increasing dietary concentrate proportion reduce ruminal TVA outflow (Sackmann et al., 2003; Loor et al., 2004). Levels of CLA *cis*-9, *trans*-11 and TVA content in beef fats have been reported to increase when forage-based diets were fed instead of concentrated-based diets (Rule et al., 2002; Realini et al., 2004), and to decrease when a low-linoleic concentrate was supplemented (French et al., 2000), but to further increase when a high-linoleic oil was supplemented to grazing steers (Pavan and Duckett, 2006).

High-starch supplements, such as corn grain, are used in grazing beef systems to offset energy deficiencies or to meet production demands. Thus, the aim of the present study is to

evaluate the isocaloric substitution of corn grain by corn oil plus soybean hulls as supplement for grazing steer on their fatty acid composition and stearoyl-CoA desaturase expression.

MATERIALS AND METHODS

The experiment was conducted at University of Georgia Wilkins Beef Unit (Rayle, GA) between November 2004 and June 2005. Pasture consisted of 11.5-ha endophyte-free tall fescue (Festuca arundinacea Shreb cv. Jesup) plot subdivided into 15 paddocks of approximately 0.77-ha each for rotational stocking. Pre- and post-graze forage samples were collected during the grazing period, freeze dried and stored at -20º C for fatty acid analysis.

Angus steers (n=28; 288.5 ± 4.4 kg) obtained from the Northwest Georgia Experiment Station in Calhoun, GA, were randomly assigned to one of four dietary treatments. All steers were treated for internal parasites with ivermectin (Ivermectin Pour-On, Durvet Inc., Blue Spring, MO) on d 0, 42, and 84 and with doramectin (Dectomax, Pfizer Inc., Expon, PA) on d 105 and 147. Two supplementation treatments were defined by the type of supplement offered in isocaloric amounts to grazing steers: corn grain (0.52% BW DM basis; **CG**) or corn oil plus soybean hulls (0.10 and 0.45% BW, respectively; **PO**). Negative (pasture only, **P**) and positive (**C** high-concentrate diet: 85% concentrate: 15% chopped bermudagrass hay on DM basis) control diets were included in the study to compare with data from the supplemented treatments. Corn oil and soybean hulls (**SBH**) were mix daily on individual basis before feeding. Concentrate used in C was formulated to contain 94.11% rolled corn, 2.91% soybean meal, 1.50% lime stone, 0.95% urea and 0.53% of trace minerals on DM basis. Samples of the different dietary components were collected every three weeks during the grazing period, pooled, freeze dried and stored at -20º C for fatty acid analysis.

Steers assigned to PO and PC were trained to use Calan gate feeders (American Calan, Inc., Northwood, NH) for 21 d prior to the start of trial and in the last 5 days steers were adapted to supplements. During adaptation, steers assigned to the grazing treatments (P, PO and PC) were allowed to graze the same pasture that was used for the experimental period. Steers assigned to C were hold in confinement (drylot) and fed the high-concentrate diet during the last 92 d of the trial (d 105 to d 197); prior to that they grazed an adjacent endophyte-free tall fescue pasture similar to that used by the grazing treatments. Twenty one days before starting their feeding period (d 84 – d 105) steers were trained to use the Calan gate feeders and adapted to the high-concentrate diet. Additional information regarding in DMI, vivo digestibility, performance and carcass quality are available (Pavan et al., 2006).

Sample collection. Animals were transported (45 km) to the University of Georgia Meat Science and Technology Center in Athens and slaughtered following an overnight feed withdrawal. Within 30 min. of exsanguination, a s.c. tail head fat sample (5 g) was removed from each carcass, rinsed with sterile saline, frozen in liquid nitrogen and stored at -80ºC for subsequent RNA extraction. After 24 h at -2.2 ºC, s.c. adipose tissue sample and a 2.54-cm-thick **LM** steak were removed from the left side of each carcass at the 12th rib. All external fat and connective tissues were removed from the LM steak. Both s.c. and LM samples from each carcass were stored at −20°C. Adipose and LM tissues samples were frozen in liquid nitrogen and pulverized using a Waring blender prior to analysis.

Analyses. Duplicate LM samples were analyzed for nitrogen by the combustion method using a Leco FP-2000 N analyzer (Leco Corp., St. Joseph, MI) and multiplied by 6.25 to determine crude protein content. Moisture content was determined by weight loss after drying at 100°C for 24 h and mineral content by ashing at 600°C for 8 h (**AOAC, 1984**). Cholesterol

content of LM was determined according to Du and Ahn (2002) and quantified by incorporating an internal standard, stigmasterol, into each sample.

Total lipids were extracted in duplicate from LM and s.c. samples using organic solvents according to the procedures of Folch et al.(1957), using a solvent to sample ratio of 10:1. Lipid extracts from the s.c. and from the LM samples were stored at -80°C for subsequent FA composition determination. Subcutaneous and LM lipid extracts containing approximately 4 mg of total lipids, were transmethylated according to the method of 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 (0.25 mm 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 1 mL per min. Individual FA were identified by comparison of retention times with standards (Sigma, St. Louis, MO; Supelco, Bellefonte, PA; Matreya, Pleasant Gap, PA). Fatty acids were quantified by incorporating an internal standard, methyl heptacosanoic (C27:0), into each sample during methylation and expressed as a percentage of total FA. Fatty acid composition of forage, corn oil, corn grain, soybean hulls, concentrate and bermudagrass hay was determined by direct transmethylation of lyophilized samples according to Park and Goins (1994) and analyzed as s.c. and i.m. FAME (Table 5.1).

Total RNA was extracted from the s.c. samples according the TRIzol procedure (Invitrogen, Carlsbad, CA), which included an initial centrifugation step to remove lipids from

the extract according to the manufacturers directions. RNA was quantified using Quant-iT RNA assay kit (Invitrogen, Carlsbad, CA). RNA (5 ug) was separated in an agarose gel (Pellé and Murphy, 1993) and transferred to nylon membranes downward capillary transfer. Northern blots were performed by hybridization with ^{32}P-labeled SCD probes (5' to 3', Forward: GATATAGGTGTATATCTTGCAGGTGG; Reverse: ATCTCTAGCTCCTACACAACCACC) and exposed to film for 38 h. Gene expression was quantified by densitometry the SCD bands and was normalized to the 18S ribosomal RNA bands.

Statistical analyses. Data were analyzed by ANOVA as a complete randomized design with four dietary treatments, except for fatty acid intake were only three dietary treatments were used, and with individual animal serving as the experimental unit. MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) was used for all analyses. If the overall F-test for the dietary treatment effect was significant ($P \leq 0.05$), a t-test was performed to discern the differences among treatments using the pdiff option of the LS-means statement.

RESULTS

Fatty acid composition of the forages, supplements, and concentrate diet is shown in Table 5.1. The predominant fatty acids in the forage samples were: linolenic (C18:3), linoleic (C18:2), and palmitic (C16:0) acids and comprised 64 to 71% of total fatty acids. Forage fatty acid content and composition varied across growing season (Figure 5.1). Fatty acid content of the forage was the lowest (1.5%) in late May and highest (3.0) in early April. Corn oil and corn grain both contained 54% of total fatty acids as linoleic acid. Soybean hulls contained low levels of fatty acids (1.89%) with linoleic acid as the major fatty acid. Daily fatty acid intake of total diet is shown in Table 5.2. Linoleic acid intake was 247, 205, and 30 g/d greater ($P < 0.001$) for PO than P, PC or C respectively. Linoleic acid intake was also 175 g/d greater ($P < 0.001$) for C

than in PC, and 42 g/d for PC than P. Total myristic (C14:0) acid intake was greatest for PO, lowest for C, and intermediate for PC and P ($P < 0.001$). Intake of palmitic and oleic (C18:1) was greatest for PO and C, and greater for PC than P ($P < 0.001$). Total stearic (C18:0) acid intake was greatest for PO, lowest for P, and greater for C than PC ($P < 0.001$). Linolenic acids intake was greatest for P, lowest for C, and intermediate for PO and PC ($P < 0.001$).

Moisture content of the LM was lower ($P < 0.001$) for C than the grazing treatments, regardless of supplementation (Table 5.3). Ash content was lower in C and PO than in PC and P ($P \leq 0.05$). Crude protein and cholesterol contents in the LM did not differ between the dietary treatments ($P > 0.05$). Total i.m. FA content in the LM was 123 % greater ($P < 0.001$) for C than the grazing treatments, regardless of supplementation. Among the grazing treatments, total i.m. FA content tended to be greater ($P = 0.068$) for PO than P. Total FA in the s.c. adipose tissue was lowest in P ($P < 0.05$) and greatest in C.

The fatty acid profile (g/100 g of total FA) of the LM is presented in Table 5.4. Lauric (C12:0) acid did not differ ($P = 0.54$) among dietary treatments. Myristic acid percentage was greater for C than for PO, PC, and P. Palmitic acid concentration was greater ($P < 0.001$) for C than the three grazing treatments (PO, PC, P). Palmitic acid concentration was also greater ($P < 0.001$) for supplementation treatments (PO and PC) than pasture only (P). In contrast, oil supplementation increased ($P < 0.001$) stearic acid concentration compared to PC and P, which were also greater than C. Based on these changes in individual saturated fatty acid concentrations, the overall saturated fatty acid content of the LM was greater ($P < 0.001$) for C and PO than P with PC being intermediate. Pentadecyclic (C15:0) acid concentration was greater for P ($P < 0.05$) than C, PO or PC, which did not differ ($P > 0.05$). Margaric (C17:0) acid proportions were greater for C than all grazing treatments. Concentrations of margaric acid were

also greater for P and PC than PO. Overall odd chain fatty acid concentrations were greater ($P < 0.001$) for C and P than PO and PC.

Myristioleic (C14:1) acid concentration was greater ($P < 0.001$) for C than all grazing treatments, regardless of supplementation. Palmitoleic (C16:1) acid concentration was greatest ($P < 0.001$) in C and lowest ($P < 0.001$) in PO. Oleic acid concentrations was 5.3 percentage units greater ($P < 0.001$) for C than PC, which was 3.3 percentage units greater ($P < 0.001$) for PC than PO and P. Overall monounsaturated fatty acid content (MUFA) was 6.6 percentage units greater ($P < 0.001$) for C than PC, which was 4.5 percentage units greater ($P < 0.05$) for PC than PO and P.

Ruminal biohydrogenation of dietary unsaturated fatty acids is sometime incomplete resulting in the production of trans-octadecenoic and conjugated linoleic acids (Bauman et al., 1999). *Trans*-9 octadecenoic acid concentration in LM was greater ($P < 0.01$) for PO than all other dietary treatments. *Trans-10* octadecenoic acid concentration was greater ($P < 0.001$) for C and PO than PC and P. *Trans*-11 vaccenic acid (TVA) was increased ($P < 0.001$) with oil supplementation and concentrations in the LM for PO were 1.9-, 2.6-, and 5.7-fold greater than P, PC, and C, respectively. Corn grain supplementation to grazing cattle reduced ($P < 0.05$) TVA concentrations compared to pasture only but concentrations were still greater ($P < 0.01$) for PC than in C. In contrast, *cis*-11 octadecenoic acid (*cis*-11 C18:1) was lowest in PO ($P < 0.01$), intermediate in PC and P and highest ($P < 0.001$) in C. Concentration of conjugated linoleic acid (CLA), *cis*-9, *trans*-11 isomer, was 1.2-, 1.8-, and 3.2-fold greater for PO than P, PC, and C, respectively. Corn grain supplementation to grazing steers reduced ($P < 0.01$) *cis*-9 *trans*-11 isomer of CLA in LM compared to pasture only (P) but levels were still greater ($P < 0.05$) than C. Corn oil supplementation reduced CLA *cis*-11, *trans*-13 isomer and increased *trans*-10 *cis*-12

isomer. Concentrations of total CLA were highest ($P < 0.01$) for PO and P, and lowest ($P < 0.01$) for C.

Linoleic acid (C18:2 n-6) content of PO was 51, 70 and 151% greater ($P < 0.01$) than PC, P and C, respectively. Linoleic acid concentration was greater ($P < 0.01$) for PC than C with P being intermediate. Energy supplementation to grazing steers reduced ($P < 0.01$) linolenic acid concentration in the LM compared to P. However, differences in the response to supplementation differed among supplementation types. Linolenic acid concentration was greater ($P < 0.01$) for PC than PO, both of which were higher than C. Arachidonic (C20:4 n-6) acid concentration in LM was lower for C than all grazing treatments regardless of supplementation. The greatest proportion of eicopentasanoic acid (**EPA**; C20:5 n-3), docosapentaenoic acid (**DPA**; C22:5 n-3) and docosahexaenoic (**DHA**; C22:6 n- 3) in the LM was observed in P. Concentrations of EPA were also greater ($P < 0.01$) for PC than C, with PO being intermediate. Concentrations of DPA and DHA were greater ($P < 0.05$) in PC than in either PO or C. Total PUFA was greatest for all grazing treatments regardless of supplementation and lowest for C. Oil supplementation to grazing steers elevated omega-6 fatty acid concentration. Omega-6 fatty acid concentration was also higher ($P < 0.01$) for PC and P than C. Concentrations of omega-3 fatty acids were highest ($P < 0.01$) for P and lowest ($P < 0.01$) for C. The elevation of omega-6 fatty acids with oil supplementation resulted in an increased ratio of omega-6 to omega-3 fatty acids. Ratio of omega-6 to omega-3 fatty acids in PO was greater than C and the other grazing treatments (PC and P). Corn grain supplementation also elevated omega-6 to omega-3 fatty acids compared to P but levels were lower than C.

The fatty acid composition of s.c. fat is presented in Table 5.5. Dietary treatments did not alter ($P = 0.14$) lauric nor ($P = 0.54$) myristic acids proportions in the s.c. fat. Palmitic acid

concentration was highest ($P < 0.01$) for C and lowest ($P < 0.01$) for PO. Stearic acid concentration was greater ($P < 0.01$) for PO than PC and C with P being intermediate. Stearic acid percentage was also greater for PC than C. The total SFA content in the s.c. fat was not altered ($P = 0.23$) by dietary treatments, averaging 45.5% of total s.c. fatty acids. Pentadecyclic acid concentration was highest ($P < 0.001$) in P than in PC, PO and P. Pentadecyclic acid content was greater ($P < 0.01$) for PC than C with PO being intermediate. Margaric (C17:0) acid proportions were greatest ($P < 0.0$) in C and P, lowest ($P < 0.0$) in PO, and intermediate in PC. Overall odd chain fatty acid concentrations were greatest ($P < 0.001$) for P, lowest ($P < 0.0…$) for PO, and intermediate for PC and C.

Myristoleic acid concentration was highest ($P < 0.01$) for PC and C and lowest ($P < 0.01$) for PO and P. Oil supplementation reduced palmitoleic acid concentration in the s.c. fat compared to P, PC and C. Oleic acid was 3.8 percentage units greater ($P < 0.05$) in C than in PC, which was also 5.2 percentage units greater ($P < 0.01$) than PO and P. Similarly, the overall MUFA content was 3.8 percentage units greater ($P < 0.05$) in C than in PC, and 6.2 percentage units in PC than in PO and P ($P < 0.01$).

Of the intermediate product of ruminal biohydrogenation, *trans*-9 octadecenoic acid concentration in LM was greater ($P < 0.001$) for PO than all other dietary treatments; whereas there was a trend ($P = 0.06$) to a greater *trans-10* octadecenoic acid concentration in C and PO than in PC and P. Oil supplementation increased ($P < 0.001$) TVA content in the s.c. fat by 1.7-, 2.6-, and 7.3-fold relative to P, PC, and C, respectively. Corn grain supplementation to grazing cattle reduced ($P < 0.01$) TVA concentrations compared to pasture only but concentrations were still greater ($P < 0.001$) for PC than in C. The s.c content of *cis*-11 octadecenoic acid was greater ($P < 0.001$) in C than all grazing treatments regardless of supplementation. Concentration of

CLA *cis*-9, *trans*-11 isomer, was 1.2-, 1.6-, and 3.5-fold greater ($P < 0.01$) for PO than P, PC, and C, respectively. Corn grain supplementation to grazing steers reduced ($P < 0.05$) *cis*-9 *trans*-11 isomer of CLA in LM compared to pasture only (P) but levels were still greater ($P < 0.001$) than C. The proportion of CLA *cis*-11, *trans*-13 isomer in the s.c. fat was lowest in PC and P ($P < 0.05$), intermediate in PO and highest ($P < 0.05$) in C. Although CLA *trans*-10, *cis*-12 isomer was also lowest ($P < 0.05$) in PC and P, it was intermediate for C and highest ($P < 0.001$) with oil supplementation. Total content of CLA in the s.c. fat was lower ($P < 0.001$) for C than all grazing treatments. Compared to pasture only (P), total CLA content was increased ($P < 0.05$) by corn oil supplementation to grazing steers and decreased ($P < 0.01$) by corn grain supplementation.

Linoleic acid content in the s.c fat of PO was 66, 79 and 159% greater ($P < 0.001$) than C, PC and P, respectively. Linoleic acid concentration was greater ($P < 0.05$) for C and PC than in P. Energy supplementation to grazing steers reduced ($P < 0.01$) linolenic acid concentration in the s.c fat compared to P. Although there were no differences ($P = 0.15$) among supplementation types, linolenic content in C was similar ($P = 0.23$) to PO but lower ($P < 0.05$) than PC. Arachidonic acid concentration did not differ ($P = 0.12$) across treatments. The s.c. fat proportion of EPA was greatest ($P < 0.01$) for P and lowest ($P < 0.05$) for C, being greater ($P = 0.05$) in PC than in PO. Docosapentaenoic acid was greater ($P < 0.05$) in P and PC than in C, being PO intermediate. No differences ($P = 0.13$) were observed among treatment for the proportion of DHA in the s.c. fat. Total PUFA and the ratio PUFA to SFA fatty acids were greater ($P < 0.001$) for PO than for PC, C and P. Omega-6 fatty acid was also greatest ($P < 0.001$) in PO, but was lower ($P < 0.01$) in P than in PC and C. In contrast, concentration of omega-3 fatty acids was greatest ($P < 0.01$) in P. Omega-3 fatty acid was also greater ($P < 0.01$) in PC than in C, being

PO intermediate. These variations of omega-6 and omega-3 fatty acid content in the s.c. fat among treatments resulted in a greater ($P < 0.001$) ratio of omega-6 to omega-3 fatty acids with PO than with the other dietary treatments. Ratio of omega-6 to omega-3 fatty acids was greater ($P < 0.001$) in C and greater in PC, and lowest ($P < 0.001$) in P. Corn oil supplementation increased the ratio PUFA to SFA

Total, atherogenic (myristic and palmitic acids), anticarcinogenic (*trans*-11 vaccenic acid and CLA *cis*-9, *trans*-11) fatty acids content per serving of LM (114 g), as well as the content of the major groups of fatty acids are shown in Table 5.6. A serving from the longissimus muscle from carcass of the grazing treatments regardless of supplementation contained, on average, 55, 68, 62, 56 and 65% less total fatty acids, myrictic, palmitic, total SFA and total MUFA, respectively than from a carcass of a steer finished on high-concentrate diet (C). Total content of both anticarcinogenic fatty acids were greater ($P < 0.001$) when corn oil was supplemented to grazing steers than in the other dietary treatments, being TVA and CLA *cis*-9, *trans*-11 isomer 3.1- and 2.0-folds greater, respectively. Total PUFA content was greatest ($P < 0.01$) in PO, lowest ($P < 0.01$) in PC and P, and intermediate in C. Total omega-6 fatty acids in a LM serving was greatest ($P < 0.001$) in PO and lowest ($P < 0.001$) in P; whereas total omega-3 content was lowest ($P < 0.01$) in PO and greatest ($P < 0.01$) in P.

Levels of SCD mRNA were greater ($P < 0.03$) in the s.c. tail head fat from steers fed the high-concentrate diet than from grazing steers (Figure 5.2). Among grazing treatments, SCD mRNA levels were greater ($P = 0.02$) for oil supplemented steers than for non-supplemented, whereas the level in PC was intermediate.

DISCUSSION

Extrapolation of fatty acid intake data should be done with caution as pasture intake was determined during only 7 of the 192 days of the feeding period and in Figure 5.2 can be seen the variability not only in total pasture total fatty acid but in the proportion of the main fatty acids throughout the grazing period. Similar, Dewhurst et al. (2001) observed that total fatty acid content and the proportion of linolenic acid in three lolium species were high during the vegetative growth (late April), declining markedly as forage enter into the reproductive growth stage, and recovering by autumn (November). In our study, forage entered into the reproductive growth stage at the end of April beginning of May. Additionally, Dewhurst et al. (2001) also reported that forage fatty acids content of decline, specially the most unsaturated ones, when the regrowth period was extended from 20 to 38 days. As the forage grazed during winter was stockpiled from the autumn growth, the decline of total FA content particularly that of linolenic acid during that period could be in part attributed to a longer regrowth period. The first new regrowth was grazed the 16th of March.

The changes in total fatty acid content observed in the i.m. and s.c. fat agree with the differences in marbling score and fat thickness reported by Pavan et al. (2006) and suggested that the increased in s.c. fat occur by an increase in adipocyte cell size. The differences in i.m. fat content alter change i.m. fatty acid profile by altering the proportions of neutral and polar lipids. The changes in total i.m. lipids, are associated with changes of neutral lipids that have a greater proportion of SFA and MUFA, but lower of PUFA than polar lipids (Duckett et al., 1993). Thus, differences observed in the i.m. fatty acid profile in the present study could be attributed to the dietary effect on total FA content and to the direct effect of diets on fatty acid profile. In

contrast, as s.c. fat is mainly composed by neutral lipids changes in the fatty acid profile of this tissue could be attributed mainly to direct dietary effects on lipogenesis.

The main objective of this study was to evaluate the effect of corn oil or corn grain supplementation on tissue concentration of TVA and CLA *cis*-9, *trans*-11. In agreement with our previous study (Pavan and Duckett, 2006) corn oil supplementation to grazing steers enhanced the anticarcinogenic and antiatherogenic properties of meat, by increasing the proportion of both TVA and CLA *cis*-9, *trans*-11. In contrast, the small amount of corn grain supplemented was enough to reduce the proportion of both fatty acids with respect to the levels observed in the adipose tissues from non-supplemented grazing steers. Similar reductions of i.m. fat CLA *cis*-9, *trans*-11 content had been reported when grazing steers were supplemented with 2.5 kg of concentrate that contained 46% of ground barley and 42% unmolassed sugar beep pulp (French et al., 2000). According to Loor et al. (2004) the changes that occur in the rumen environment with the increase starch availability are associated with a shift in the biohydrogenation pathway from TVA to *trans*-10 C18:1 acid production. When the proportion of corn grain in the diet of dairy cows was increased from 22% to 52%, milk fat content of TVA and CLA *cis*-9, *trans*-11 was reduced and that of *trans*-10 octadecenoic acid and CLA *trans*-10, *cis*-12 isomer increased (Piperova et al., 2000). Likewise, as observed in our study, CLA *cis*-9, *trans*-11 content in the adipose tissue from beef cattle finished on pasture is greater than from those finished on high concentrate diets (French et al., 2000; Rule et al., 2002; Realini et al., 2004). In addition, in our study TVA was greater, and *trans*-10 octadecenoic acid lower in the adipose tissues from the steers finished on pasture than from those finished on the high-grain diet. In contrast, although corn grain supplementation reduced TVA and CLA *cis*-9, *trans*-11 neither *trans*-10 octadecenoic acid nor CLA *trans*-10, *cis*-12 were not affected when compared

to the non-supplemented steers. Suggesting that the level of corn grain supplemented was not enough to produce a significant change in ruminal biohydrogenation pathway. In addition, the greater linoleic acid content in the adipose tissues with respect to non-supplemented steers would suggest that more linoleic acid escape ruminal biohydrogenation and probably reduced the amount available for TVA production in the rumen despite greater linoleic acid intake.

As observed when dietary linoleic acid was increase through vegetable oil supplementation to high-grain diets (Gillis et al., 2004; Hristov et al., 2005) and in our previous study of corn oil supplementation to grazing steers (Pavan and Duckett, 2006), corn oil supplementation increased *trans*-10 octadecenoic acids and CLA *trans*-10, *cis*-12 proportions in the adipose tissues with respect to the levels observed in the basal diets. Oil supplementation increased *trans*-10 octadecenoic acid from grazing steers to a similar content as in high-grain finished steer, and and that of CLA *trans*-10, *cis*-12 to an even greater content. However, despite the increase observed of *trans*-10 octadecenoic acid with oil supplementation and in agreement with our previous study (Pavan and Duckett, 2006), *trans*-10 octadecenoic acid only represented 7 or 6% of TVA content in the i.m. or s.c. fat, respectively. Contrasting with 55 or 62% observed in the i.m. or s.c. fat from steers fed the high-grain or with the even greater *trans*-10 octadecenoic acids proportions reported in other studies for high-grain diets (Gillis et al., 2004; Hristov et al., 2005). Our results and those from Pavan and Duckett (2006) suggest that even with the ruminal conditions provided by forage diets with no starch supplements, significant amounts of these two fatty acids are produced in the rumen if sufficient amounts of linoleic acids are available. Moreover, they suggest that dietary starch is not required for ruminal production of *trans*-10 octadecenoic acid and CLA *trans*-10, *cis*-12 tissue, though is clear that their ruminal

outflow (Duckett et al., 2002; Sackmann et al., 2003; Loor et al., 2004) or adipose tissue content (Gillis et al., 2004; Hristov et al., 2005) are enhance in high starch diets.

The CLA *cis*-9, *trans*-11 isomer levels obtained in the i.m. fat from pasture-finished steers is within the range (0.12 to 1.25 % of total FA) given by Mir et al. (2004) when reviewed the i.m. fat CLA content reported in different studies. The results from the present study and those from a previous study (Pavan and Duckett, 2006) demonstrated CLA *cis*-9, *trans*-11 content from animals finished on pasture-based systems can be enhance through an increase of dietary linoleic acid. However, increasing dietary linoleic acid through corn oil supplementation has a greater effect on TVA than on CLA *cis*-9, *trans*-11 proportions in the adipose tissues. In both studies was observed that the increased of TVA in the i.m. fat by corn oil supplementation was approximately 11-fold greater than that of CLA *cis*-9, *trans*-11. As TVA can also be convert to CLA *cis*-9, *trans*-11 once absorbed in the human body (Turpeinen et al., 2002), that increase in i.m. TVA will also contribute to increase the total amount of CLA *cis*-9, *trans*-11 available. However, even when total available CLA *cis*-9, *trans*-11 could be double by the endogenous desaturation of 19% of the observed TVA, the ability to convert TVA to CLA is highly variable among individuals (Turpeinen et al., 2002). In addition, increasing the contribution of CLA *cis*-9, *trans*-11 through an increase in TVA would be highly inefficient; on average, 81% of the TVA in animal tissues may not converted at all. Thus, more study needs to be done to increase the desaturation of the already available TVA to CLA *cis*-9, *trans*-11 in tissues from grazing cattle through an increase in the activity and (or) expression of the enzyme stearoyl-CoA desaturase. This will also avoid the increase of other *trans* fatty acids (*trans*-9 and *trans*-10 octadecenoic acids) when high-linoleic acid is supplemented as observed in the present study.

In agreement with the results of Daniel et al. (2004), the stearoyl-CoA desaturase expression observed in the grazing treatments was lower than that observed for the high-grain diet. However, SCD mRNA levels from the s.c. fat from grazing steers were not affected with the level of corn grain supplementation used, but in contrast to what was expected, SCD mRNA levels were increased by corn oil supplementation. This increased observed with oil supplementation may had resulted from a better energy balance that would had increased lipogenesis as suggested by the increased proportion of total FA content in the adipose tissues, and of the carcass s.c. fat thickness and kidney, pelvic and heart fat with oil supplementation (Pavan et al., 2006). Our results give no indication that SCD expression in the s.c. fat was reduced by the greater content of PUFA or CLA *trans*-10, *cis*-12 in the tissue, as suggested based on rat and mice (Ntambi, 1999) or in dairy cattle studies (Baumgard et al., 2002; Peterson et al., 2004), respectively. In concordance with our results, supplementation with 30% of whole cottonseeds did not affect SCD activity in adipose tissue or in the liver of growing steers (Page et al., 1997); diets containing unprotected or protected canola seeds did not affected SCD expression levels in the mammary gland of dairy cows (Delbecchi et al., 2001). Page et al. (1997) suggested that SCD activity could increased with oil supplementation in response to greater absorption of stearic acid. In addition, sunflower oil supplementation increased and sterulic acid (potent inhibitor of SCD activity) infusions into the duodenum reduced milk fat oleic acid content, but no interaction effect (sterulic infusion × oil supplementation) was observed (Kay et al., 2004). These results from Kay et al. (2004) suggest that SCD activity was not affected by sunflower oil supplementation. Recently the activity of transcriptional promoter from the bovine SCD was evaluated (Keating et al., 2006). It was shown that neither linoleic nor linolenic acid or the enzyme substrates (TVA and stearic acid) affects the promoter activity, and

that CLA *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers or oleic acid reduced its activity. Insulin was the only hormone evaluated that increased increased the promoter activity; leptin, prolactin, dexamethasone alone or in combination with insulin did not have any effect. It has been suggested that dietary fatty acid could have a glucose spearing effect, potentially increasing gluconeogenesis and insulin secretion from the pancreas, by being use as oxidative substrate instead of glucose precursors or by reducing the novo fatty acid synthesis reducing the used of glucose to generate reducing agents (Chilliard, 1993). This glucose sparing effect may be more significant when lipids are supplemented to forage only diets, as in our trial or in that of Pavan and Duckett (2006), since the added fat do not substitute any starch source and all the glucose spare could be extra-glucose available.

Beef fat is commonly associated with coronary heart diseases due to is high SFA content (Scollan et al., 2006). However, not all the SFA present in beef fat are equally likely to produce hypercholesterolemia that is associated with coronary heart disease. Of the three more important SFA in beef fats only myristic and palmitic acids are considered cholesterol-raising, being stearic acid considered to have neutral effects (Ulbricht and Southgate, 1991). As observed earlier by Pavan and Duckett (2006), corn oil supplementation reduced s.c. palmitic acid content with respect to non-supplemented grazing steers, despite increasing palmitic acid intake. Although the trend to a greater total i.m. lipid content with oil supplementation, which may had increased i.m. palmitic acid content by increasing neutral lipids content (Duckett et al., 1993), palmitic acid content in the i.m. fat from oil supplemented steers was similar to the that observed on the non-supplemented grazing steers. Likewise, reductions in palmitic acid proportions were also observed when total lipid intake was increased in grain-concentrate diets (Andrae et al., 2001; Beaulieu et al., 2002; Madron et al., 2002). Mir et al. (2002) suggested that the reduction of

palmitic acid when sunflower oil was added to the diet was the result of a feedback inhibition of lipogenesis by the dietary fatty acids. In addition, a 29% decrease in lipogenesis was reported when 6.6% of lipids were added to the diet of growing steers (Page et al., 1997). In contrast, agreeing with previous reports (French et al., 2000; French et al., 2003), grain supplementation of grazing steers increased the proportion of palmitic acid in the i.m. fat. According to French et al. (2000) this is the result of greater palmitic acid content in the supplement than in the pasture. The lower myristic and palmitic acids content in the i.m. fat from grazing steers than from those in the high-grain diet agrees with the results observed by Realini et al. (2004). When compared at similar total i.m. fat content, French et al. (2000) observed no difference in myristic acid i.m. fat content, but a lower palmitic acid content in the grazing treatments than in the high-concentrate diet. Suggesting that the diet did not have a direct effect on myristic acid proportion, but on palmitic acid; agreeing with our results from s.c. fat.

Substitution of SFA by a greater proportion of PUFA, either n-6 or n-3, is beneficial to decrease plasma cholesterol and reduce risk of producing atheroma; however, n-3 PUFA are preferred against n-6 PUFA because of its antithrombotic effect (Ulbricht and Southgate, 1991). As expected by its lower total FA content, total PUFA content in the i.m. fat from grazing animals double the proportion observed in the i.m. fat from steers finished on the high-grain diet. Similar results were observed when comparing steers finished on pasture or on concentrate with either different (Realini et al., 2004) or similar (French et al., 2000) i.m. fat levels. Duckett et al. (1993) observed that as time on a high-grain diet increase, total PUFA proportion in the total lipid decreased. In agreement with previous results of corn oil (Pavan and Duckett, 2006) or concentrate (French et al., 2000) supplementation to grazing steers, both type of supplement used in the present study to grazing steers increased n-6: n-3 ratio. In the case of corn oil

supplementation the increase in the ratio was the result of both an increase of n-6 and a decrease of n-3 fatty acids; whereas with corn grain supplementation the increased in i.m. fat ration was the result of the decrease of n-3.

As both linoleic and linolenic acids can not be synthesis endogenously, their proportion in the tissues is highly dependent on the amount absorbed in the small intestine that in turn depends on the intake of the particular fatty acid and it biohydrogenation. The consistent increased of linoleic acid proportion in i.m. and s.c. fat with oil supplementation contrast with our previous results (Pavan and Duckett, 2006), where no differences were observed with corn oil supplementation (0.075 and 0.15 % BW). Differences in pasture quality and (or) in the type of oil carrier used in the studies could have generated different extents of ruminal biohydrogenation between studies. Different passage rates had been reported for the two types fiber sources used as oil carrier, 2.9 %/h for cottonseed hulls (Moore et al., 1990) and 4.9 to 7.6%/h for soybean hulls (Weidner and Grant, 1994). Thus the use of soybean hulls in the present study may have increased the amount of linoleic acid available for absorption in the small intestine and for deposition in the adipose tissues, contributing to the increase in total n-6 fatty acids. Different responses on the linoleic acid proportion had also been obtained when high-linoleic oil was supplemented to cattle fed high-grain diets. Linoleic acid increased when high-oil corn replaced conventional corn grain (Andrae et al., 2001) or 4% corn oil was added to the diet (Gillis et al., 2004), but no effects were observed by Engle et al. (2000) and Beaulieu et al. (2002) when added 4 and 5% soybean oil to the diet, respectively. The greater linoleic acid proportion in the s.c. fat from corn grain supplemented than from non-supplemented grazing steers indicates as mention above that a greater amount of linoleic acid escape ruminal biohydrogenation; though the difference was not enough to generate a change in the i.m. fat.

French et al. (2000) and French et al. (2003) did not observe any change in linoleic acid content in the total i.m. fat or in the i.m. triglycerides, respectively, when supplemented increasing amounts of concentrate to grazing cattle. However, linoleic acid content in concentrate and pasture used in their studies were relatively similar (14 and 16% or 8 and 17% for pasture and concentrate, respectively) when compare to the difference between the corn grain and pasture used in the present study. The decrease of linolenic acid proportion in the adipose tissues with supplementation was associated with a lower proportion of linolenic acid in the supplements. Intramuscular linolenic acid content was also reduced when French et al. (2000) and French et al. (2003) increased the level of concentrate supplemented to grazing steers or when fed a concentrate diet.

As suggested by Dhiman et al. (2005) despite the difference in CLA *cis*-9, *trans*-11 proportion in the adipose tissues the actual content of this CLA isomer per serving from pasture-finished cattle is similar to that from cattle finished on high-grain diets. Though, the difference between the two beef products (pasture- vs. grain-finished beef) is that in the pasture-finished beef the same amount of CLA *cis*-9, *trans*-11 and TVA is obtain with half the amount of total fat and with less than one third of the highly atherogenic myristic and palmitic acid present in the grain-finished beef. In addition, despite the lower total fat content, 100g of pasture-finished beef contributes with more total n-3 to the human diet than the same amount of grain-finished beef. The use of corn grain supplementation to enhance performance, does not increase the content of the atherogenic fatty acid neither decrease the content of CLA *cis*-9, *trans*-11 or TVA per serving; though it increase total n-6 and reduces total n-3 fatty acids without altering the content of total PUFA. However, when corn oil is used instead of corn grain as supplement for grazing steers the amount of myristic and palmitic per serving does not increase, but the amount of CLA

cis-9, *trans*-11 and TVA increase by 1.8 and 2.8-folds with respect to the amount present in a 100 g serving of non-supplemented pasture-finished beef. Assuming that 19% of the TVA is desaturated by human stearoyl-CoA desaturase (Turpeinen et al., 2002) the total contribution of CLA *cis*-9, *trans*-11 from 100 g of beef could be 48.5 mg with corn oil supplementation versus 20.3 mg with the other dietary treatments. Though oil supplemented beef had a lower content of n-3 and greater of n-6 fatty acids.

IMPLICATIONS

The result of this study confirms that corn oil supplementation further enhances the proportion of anticarcinogenic acids *trans*-11 vaccenic and *cis*-9, *trans*-11 CLA in fat from grazing steers and that reduces the proportion of the highly atherogenic palmitic acids. Low level corn grain supplementation (0.52 % of BW) to grazing steers generated would be enough to produce changes in the fatty acid composition of grazing steers, but that would be minimal when compare with those produced by concentrate diets. Supplementation with linoleic-rich supplement increase the n-6: n3 ratio. No evidence was found of reduction of stearoyl-CoA desaturase expression by corn oil supplementation.

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Table 5.1. Average fatty acid composition of the dietary components used during the finishing period

	Forage		Soybean hulls	Corn oil	Corn grain	Concen-trate[1]	Hay
	Pre-grazing	Post-grazing					
Total fatty acid, % of DM	2.08 ± 0.10	1.73 ± 0.08	1.89	96.66	3.78	3.97	1.35
Fatty acid, % of total FA							
C12:0	0.43 ± 0.04	0.48 ±0.03	-	-	-	-	0.64
C14:0	0.61 ± 0.05	0.67 ± 0.06	0.18	0.05	0.07	0.07	0.77
C15:0	0.16 ± 0.02	0.29 ± 0.03	0.17	-	-	-	0.14
C16:0	13.23 ± 0.20	13.57 ± 0.26	13.53	10.87	13.36	12.49	17.82
C16:1	0.74 ± 0.05	1.07 ± 0.10	0.32	0.11	0.12	0.14	0.44
C17:0	0.36 ± 0.03	1.30 ± 0.25	0.40	0.09	0.10	0.10	0.43
C18:0	1.37 ± 0.06	1.65 ± 0.09	5.01	2.21	2.31	2.38	2.40
C18:1	3.89 ± 0.29	4.34 ± 0.30	17.12	26.46	23.10	25.93	2.41
C18:2	13.68 ± 0.41	14.80 ± 0.40	44.13	54.42	54.06	51.97	12.25
C18:3	43.89 ± 1.41	36.48 ± 1.51	9.87	1.22	1.30	1.55	22.12
C20:0	0.44 ± 0.04	0.58 ± 0.04	0.53	0.43	0.38	0.43	0.98
C20:4	1.19 ± 0.07	1.33 ± 0.07	-	-	-	-	2.32
C22:5	1.09 ± 0.10	1.24 ± 0.10	0.13	0.04	0.07	0.06	1.45
Unidentified	18.93 ± 0.62	22.21 ± 0.74	8.62	4.10	5.14	4.88	35.85

[1] Concentrate composition, DM basis: 94.11% corn grain, 2.91% soybean meal, 1.50% limestone, 0.53% trace minerals, 0.95% urea.

Table 5.2. Daily fatty acid intake of total diet, pasture or supplement plus pasture, for non-supplemented (P) steers grazing endophyte-free tall fescue or supplemented with either corn oil plus soybean hulls (PO) or with corn grain (PC)

	Grazing treatments[1]				
	C	**PO**	**PC**	**P**	**P-value**
N	7	3	3	5	
Total FA Intake					
g/d	473 ± 11.2[a]	567 ± 17.1[b]	197 ± 17.1[c]	143 ± 13.3[d]	<0.001
% of BW	0.9 ± 0.03[b]	1.2 ± 0.04[a]	0.4 ± 0.04[c]	0.3 ± 0.03[c]	<0.001
% of DMI	3.57 ± 0.04[b]	7.60 ± 0.07[a]	2.40 ± 0.07[c]	1.87 ± 0.05[d]	< 0.001
Individual FA intake, g/d					
C14:0	0.5 ± 0.03[c]	0.7 ± 0.04[a]	0.5 ± 0.04[bc]	0.6 ± 0.03[ab]	0.020
C16:0	60.7 ± 1.47[a]	65.6 ± 2.25[a]	27.1 ± 2.25[b]	19.9 ± 1.74[c]	<0.001
C18:0	11.3 ± 0.24[b]	12.6 ± 0.37[a]	3.3 ± 0.37[c]	1.7 ± 0.28[d]	<0.001
C18:1	116.4 ± 2.37[a]	125.4 ± 3.63[a]	24.2 ± 3.62[b]	5.8 ± 2.81[c]	<0.001
C18:2 n-6	235.4 ± 4.83[b]	265.4 ± 7.38[a]	60.2 ± 7.38[c]	18.0 ± 5.72[d]	<0.001
C18:3 n-3	13.0 ± 2.84[c]	55.5 ± 4.34[b]	56.4 ± 4.34[b]	70.3 ± 3.37[a]	<0.001
Others[2]	5.0 ± 0.24[b]	7.1 ± 0.37[a]	4.8 ± 0.37[b]	5.4 ± 0.29[b]	0.002
Unidentified	31.6 ± 1.06[a]	34.9 ± 1.62[a]	20.9 ± 1.62[b]	21.0 ± 1.26[b]	<0.001

[1] P = Pasture only; PC = Pasture plus corn grain; PO = Pasture plus corn oil and soybean hulls. Total and individual fatty acid intake in C were calculated based on the actual daily DMI throughout the 92 d of feeding; in PO, PC and P total and individual fatty acid intake were

	Grazing treatments[1]				
	C	**PO**	**PC**	**P**	**P-value**

calculated using forage DMI estimated using chromium oxide and INDF during a 7 d period and the actual supplement DMI during that period.

[2] Others = C12:0 + C15:0 + C16:1 + C17:0 + C20:0 + 20:4 n-6 + C22:5 n-3

[a, b, c, d] Within a row means without a common superscript letter differ ($P < 0.05$)

Table 5.3. Longissimus muscle (LM) composition and s.c. adipose tissue total fatty acid content from grazing steers supplemented with either corn oil plus soybean hulls or corn grain

	Dietary treatment [1]					
	C	**PO**	**PC**	**P**	SEM	**P-value**
Longissimus dorsi						
Moisture, %	72.12[b]	74.67[a]	74.42[a]	75.20[a]	0.33	< 0.001
Crude protein, %	23.16	22.61	23.45	22.76	0.23	0.07
Ash, %	1.06[c]	1.07[c]	1.11[a]	1.11[a]	0.01	0.02
Total Fatty acids, %	4.05[a]	2.21[b]	1.71[b]	1.52[b]	0.25	<0.001
Cholesterol, mg/100g	55.88	52.40	52.65	52.61	1.12	0.11
Subcutaneous fat						
Total Fatty acids, %	81.86[a]	74.69[ab]	70.29[b]	58.47[c]	2.87	<0.001

[1] Dietary treatments: C = high-concentrate diet, PO = pasture plus corn oil and soybean hulls, PC = pasture plus corn grain, P = pasture only.

[a, b, c] Within a row means without a common superscript letter differ ($P < 0.05$)

Table 5.4. Fatty acid composition of the intramuscular fat (i.m.) from longissimus muscle of grazing steers supplemented with either corn oil plus soybean hulls or corn grain during 197 days

	Dietary treatments [1]				SEM	P-value
	C	PO	PC	P		
Fatty acid, % of total FA						
C12:0	0.020	0.036	0.013	0.019	0.012	0.54
C14:0	2.85[a]	2.03[b]	1.98[b]	1.95[b]	0.132	<0.001
C14:1	0.74[a]	0.47[b]	0.38[b]	0.35[b]	0.063	<0.001
C15:0	0.48[a]	0.44[a]	0.45[a]	0.61[b]	0.023	<0.001
C16:0	26.60[a]	22.15[bc]	23.17[b]	21.71[c]	0.483	<0.001
C16:1	3.63[a]	2.31[c]	2.74[b]	2.67[b]	0.109	<0.001
C17:0	1.26[a]	0.71[c]	0.87[b]	1.00[b]	0.053	<0.001
C18:0	13.29[c]	18.12[a]	16.21[b]	16.02[b]	0.447	<0.001
C18:1 trans-9	0.000[b]	0.382[a]	0.000[b]	0.041[b]	0.038	<0.001
C18:1 trans-10	0.523[a]	0.424[a]	0.080[b]	0.082[b]	0.084	<0.01
C18:1 trans-11	0.95[d]	5.40[a]	2.04[c]	2.83[b]	0.231	<0.001
C18:1 cis-9	38.67[a]	27.87[c]	33.30[b]	30.23[c]	1.009	<0.001
C18:1 cis-11	1.51[a]	0.98[c]	1.20[b]	1.20[b]	0.045	<0.001
C18:2 cis-9, cis-12	2.77[c]	6.95[a]	4.59[b]	4.08[bc]	0.521	<0.001
C18:3 cis-9, cis-12, cis-15	0.46[d]	0.73[c]	0.97[b]	1.56[a]	0.056	<0.001
C20:4	0.77[b]	1.93[a]	1.94[a]	2.14[a]	0.221	<0.001
C20:5	0.18[c]	0.37[bc]	0.58[b]	1.11[a]	0.084	<0.001
C22:5	0.40[c]	0.62[c]	0.93[b]	1.30[a]	0.094	<0.001

	Dietary treatments [1]				SEM	P-value
	C	PO	PC	P		
C22:6	0.050[c]	0.076[c]	0.130[b]	0.190[a]	0.017	<0.001
Total CLA	0.43[c]	1.23[a]	0.70[b]	1.05[a]	0.064	<0.001
CLA cis-9, trans-11	0.36[d]	1.14[a]	0.62[c]	0.92[b]	0.061	<0.001
CLA cis-11, trans-13	0.031[a]	0.007[b]	0.020[a]	0.031[a]	0.003	<0.001
CLA trans-10, cis-12	0.004[b]	0.013[a]	0.002[b]	0.002[b]	0.002	<0.001
CLA cis, cis	0.030[c]	0.076[b]	0.062[b]	0.091[a]	0.005	<0.001
CLA trans, trans	0.002	0.000	0.000	0.007	0.04	0.51
SFA	42.76[a]	42.34[a]	41.38[ab]	39.69[b]	0.778	0.047
MUFA	43.04[a]	30.64[c]	36.42[b]	33.25[c]	1.065	<0.001
PUFA	4.63[b]	10.66[a]	9.15[a]	10.38[a]	0.928	<0.001
Odd FA	1.74[a]	1.15[b]	1.33[b]	1.61[a]	0.070	<0.001
Unidentified FA	4.43[c]	6.79[b]	7.70[b]	9.87[a]	0.740	<0.001
n-6	3.54[c]	8.78[a]	6.53[b]	6.22[b]	0.728	<0.001
n-3	1.08[d]	1.79[c]	2.61[b]	4.16[a]	0.240	<0.001
n-6:n-3	3.27[b]	4.95[a]	2.49[c]	1.49[d]	0.151	<0.001
PUFA:SFA	0.10[b]	0.25[a]	0.22[a]	0.26[a]	0.025	<0.001

[1] Dietary treatments: C = high-concentrate diet, PO = pasture plus corn oil and soybean hulls, PC = pasture plus corn grain, P = pasture only.

[a, b, c, d] Within a row means without a common superscript letter differ ($P < 0.05$)

Table 5.5. Fatty acid composition of the subcutaneous fat (s.c.) from grazing steers supplemented with either corn oil plus soybean hulls or corn grain during 197 days

	Dietary treatment [1]				SEM	P-value
	C	PO	PC	P		
Fatty acid, % of total FA						
C12:0	0.000	0.027	0.006	0.040	0.013	0.14
C14:0	3.41	3.20	3.19	3.19	0.130	0.54
C14:1	1.07^{a}	0.63^{b}	0.93^{a}	0.71^{b}	0.062	<0.01
C15:0	0.64^{c}	0.69^{bc}	0.78^{b}	1.02^{a}	0.038	<0.001
C16:0	27.30^{a}	22.85^{c}	25.35^{b}	24.61^{b}	0.457	<0.001
C16:1	4.09^{a}	3.16^{b}	4.30^{a}	4.06^{a}	0.189	<0.01
C17:0	1.37^{a}	0.85^{c}	1.13^{b}	1.35^{a}	0.065	<0.001
C18:0	12.98^{c}	19.93^{a}	17.13^{b}	18.66^{ab}	0.946	<0.001
C18:1 trans-9	0.000^{b}	0.499^{a}	0.000^{b}	0.021^{b}	0.027	<0.001
C18:1 trans-10	0.718	0.534	0.100	0.250	0.165	0.058
C18:1 trans-11	1.15^{d}	8.36^{a}	3.27^{c}	4.90^{b}	0.342	<0.001
C18:1 cis-9	39.95^{a}	30.47^{c}	36.10^{b}	31.36^{c}	1.120	<0.001
C18:1 cis-11	1.51^{a}	0.90^{b}	0.92^{b}	0.88^{b}	0.054	<0.001
C18:2 cis-9, cis-12	1.11^{b}	1.84^{a}	1.03^{b}	0.71^{c}	0.068	<0.001
C18:3 cis-9, cis-12, cis-15	0.48^{c}	0.52^{bc}	0.59^{b}	0.68^{a}	0.029	<0.001
C20:4	0.036	0.049	0.042	0.040	0.004	0.12
C20:5	0.001^{d}	0.007^{c}	0.012^{b}	0.018^{a}	0.002	<0.001
C22:5	0.034^{b}	0.051^{ab}	0.054^{a}	0.068^{a}	0.006	0.009

	Dietary treatment [1]				SEM	P-value
	C	PO	PC	P		
C22:6	0.001	0.002	0.004	0.004	0.001	0.137
Total CLA	0.53^{d}	1.75^{a}	1.15^{c}	1.51^{b}	0.082	<0.001
CLA cis-9, trans-11	0.46^{d}	1.62^{a}	1.02^{c}	1.31^{b}	0.075	<0.001
CLA cis-11, trans-13	0.033^{a}	0.023^{b}	0.012^{c}	0.009^{c}	0.003	<0.001
CLA trans-10, cis-12	0.005^{b}	0.016^{a}	0.000^{c}	0.000^{c}	0.001	<0.001
CLA cis, cis	0.037^{c}	0.090^{b}	0.088^{b}	0.136^{a}	0.005	<0.001
CLA trans, trans	0.006^{c}	0.004^{c}	0.034^{b}	0.057^{a}	0.003	<0.001
SFA	43.69	46.01	45.67	46.49	0.992	0.23
MUFA	45.10^{a}	34.26^{c}	41.34^{b}	36.12^{c}	1.238	<0.001
PUFA	1.65^{b}	2.47^{a}	1.73^{b}	1.51^{b}	0.094	<0.001
Odd FA	2.01^{b}	1.54^{c}	1.91^{b}	2.38^{a}	0.084	<0.001
Unidentified FA	3.64	3.67	3.91	5.93	0.850	0.20
n-6	1.14^{b}	1.89^{a}	1.07^{b}	0.77^{c}	0.070	<0.001
n-3	0.51^{c}	0.59^{bc}	0.66^{b}	0.77^{a}	0.033	<0.001
n-6:n-3	2.27^{b}	3.21^{a}	1.64^{c}	0.97^{d}	0.096	<0.001
PUFA:SFA	0.038^{b}	0.054^{a}	0.038^{b}	0.033^{b}	0.002	<0.001

[1] Dietary treatments: C = high-concentrate diet, PO = pasture plus corn oil and soybean hulls, PC = pasture plus corn grain, P = pasture only.

[a, b, c, d] Within a row means without a common superscript letter differ ($P < 0.05$)

Table 5.6. Total, atherogenic (myristic and palmitic acids) and anticarcinogenic (trans-11 vaccenic acid and CLA cis-9, trans-11) fatty acids content per serving of LM from grazing steers supplemented with either corn oil plus soybean hulls or corn grain during 197 days

	Dietary treatments [1]					
	C	**PO**	**PC**	**P**	**SEM**	**P-value**
FA, mg per serving, broiled						
Total	4621[a]	2524[b]	1950[b]	1730[b]	294.7	<0.001
C14:0	132[a]	52[b]	40[b]	34[b]	8.7	<0.001
C16:0	1230[a]	564[b]	458[b]	381[b]	77.7	<0.001
C18:1 *trans*-11	44[b]	138[a]	41[b]	49[b]	12.3	<0.001
CLA *cis*-9, *trans*-11	17[b]	29[a]	12[b]	16[b]	2.7	<0.001
SFA	1982[a]	1077[b]	815[b]	698[b]	139.5	<0.001
MUFA	1998[a]	799[b]	712[b]	588[b]	130.5	<0.001
PUFA	209[b]	245[a]	171[c]	164[c]	7.2	<0.001
n6	160[b]	203[a]	122[c]	98[d]	6.2	<0.001
n3	49[b]	41[c]	49[b]	66[a]	1.9	<0.001

[1] Dietary treatments: C = high-concentrate diet, PO = pasture plus corn oil and soybean hulls, PC = pasture plus corn grain, P = pasture only.

[a,b, c, d] Within a row means without a common superscript letter differ ($P < 0.05$)

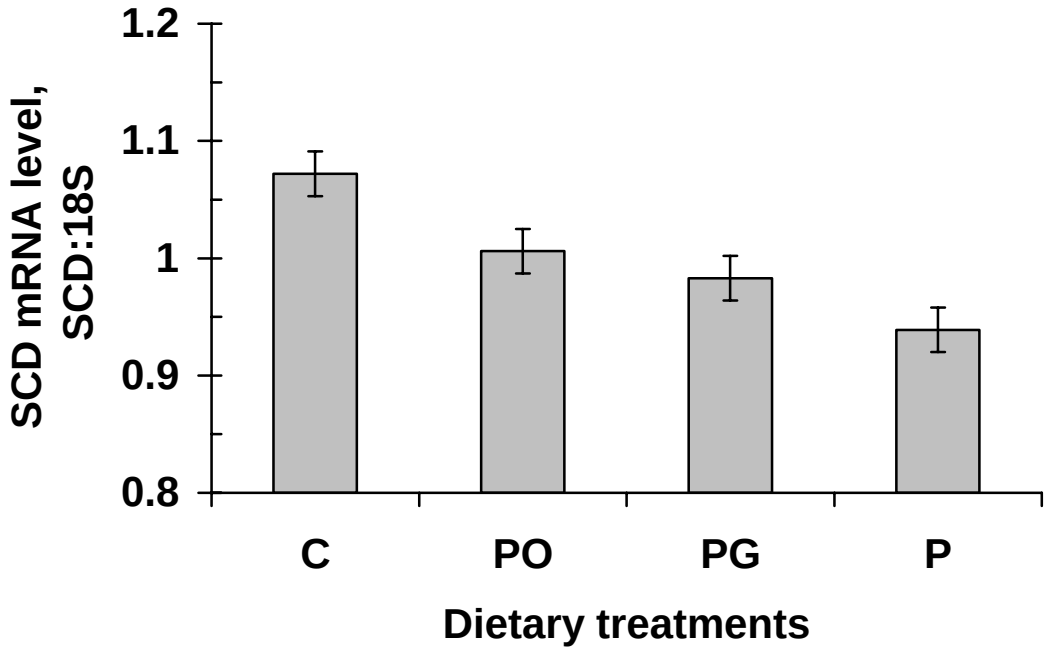

Figure 5.1. Stearoyl CoA Desaturese (SCD) mRNA levels in the s.c. fat of grazing steers supplemented with either corn oil plus soybean hulls or corn grain during 197 days (C = high-concentrate diet, PO = pasture plus corn oil and soybean hulls, PC = pasture plus corn grain, P = pasture only)

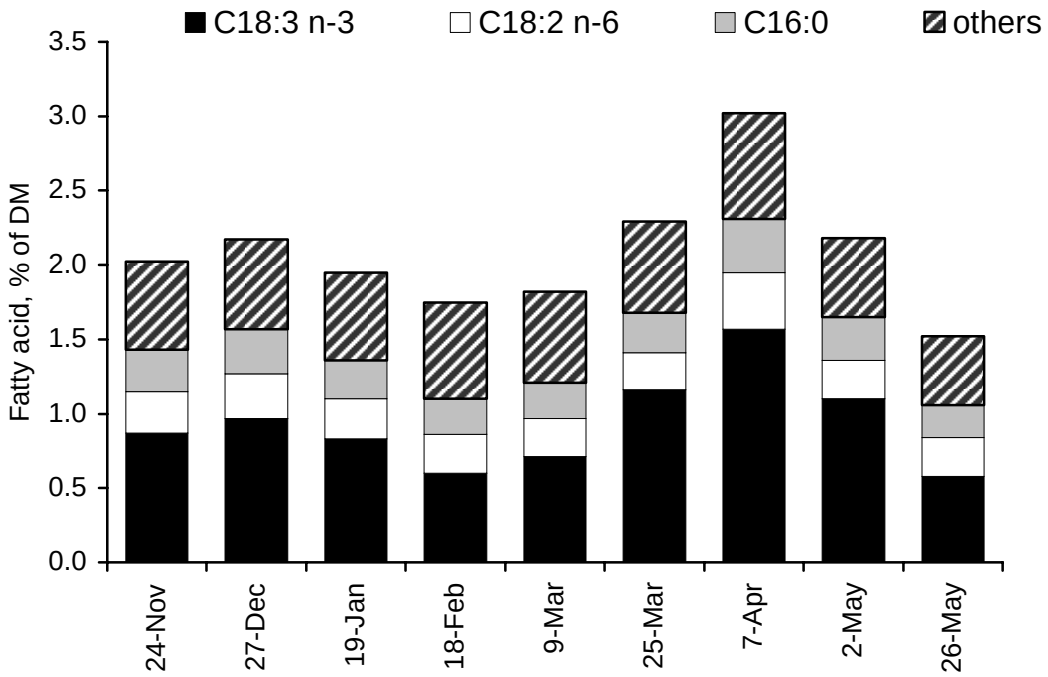

Figure 5.2. Variation of fatty acids content on the pre-grazed forage through the grazing period

CHAPTER 6

CONCLUSIONS

Results from the two studies demonstrate that beef nutraceutical properties could be enhanced by increasing its CLA *cis*-9, *trans*-11 isomer and trans-vaccenic acid (TVA) content through supplementation of grazing steers with corn oil. Furthermore, the positive effects that oil supplementation had on animal performance and carcass traits could facilitate the adoption of this type of supplementation in forage-finishing beef systems. In addition, due to the reduction on forage intake with corn oil supplementation, the stocking density of grazing systems could be increased when supplementing with oil relative to the stocking density without oil supplementation. According to our results corn oil plus soybean hulls could replace iso-caloric amounts of corn grain as supplement for grazing steers without altering performance and enhancing CLA *cis*-9, *trans*-11 isomer and TVA content.

Corn oil supplementation increased TVA acid proportions in the adipose tissues to a larger extend than CLA *cis*-9, *trans*-11 isomer proportions. However, this was not the result of a lower stearoyl-CoA activity or expression.

Despite greater myristic and palmitic acid intake with corn oil supplementation their proportion in the adipose tissues did not increase or even was reduced; suggesting that corn oil supplementation to grazing steers reduced de novo fatty acid synthesis.

As suggested by the different response on linoleic acid adipose tissues proportion of among studies, ruminal biohydrogenation of the supplemented oil was affected by forage and (or) oil carrier variations. Regardless of the effect on total omega-6 fatty acid proportion in the adipose tissues, corn oil supplementation reduced linolenic acid (amega-3) proportion and increased the ratio of total omega-6 to omega-3 fatty acids.

Corn oil supplementation increased adipose tissue proportion of both *trans*-10 octadecenoic acid and CLA *trans*-10, *cis*-12 isomer to similar proportions as in the adipose tissue from steers finished on high-grain diet. This suggests that the main limitation on ruminal production of these two intermediate fatty acids of linoleic acid biohydrogenation is the dietary level of linoleic acid.