

# Carcass, Sensory, and Adipose Tissue Traits of Brangus Steers Fed Casein-formaldehyde-protected Starch and(or) Lipid

C. D. Gilbert, D. K. Lunt, R. K. Miller, and S. B. Smith  
Texas A&M University, Department of Animal Science, 2471 TAMU, College Station, TX 77843-2471

## ABSTRACT

Eighteen Brangus steers of similar live weight were assigned randomly to one of three dietary treatment groups: cracked corn (Corn), casein-formaldehyde-protected Canola Lipid (CL), or casein-formaldehyde-protected Marble Plus (MP). The purpose of the study was to determine if feeding protected starch and/or lipid increased marbling scores without increasing carcass fat. All diets were equally balanced for ME (2.91 Mcal/kg), crude protein (12.5%), and dry matter (89%). Either extract was 3.7, 6.9, and 6.9% for the Corn, CL, and MP diets, respectively. The CL and MP diets provided equal amounts of protected lipid (3.3%). The MP also contained 3.7% protected starch. Steers were fed their respective diets for 126 to 130 d before slaughter. Average daily gain and feed efficiency did not differ among treatments ( $P > 0.05$ ). Carcasses from steers fed Corn, CL, or MP did not differ in adjusted fat, ribeye area, hot carcass weight, or yield grade ( $P > 0.23$ ). Yield grades were 3.3, 3.9, and 3.4 for steers fed the Corn, CL, and MP diets, respectively. Percentage KPH fat was higher ( $P < 0.05$ ) for carcasses from CL (3.2%) and MP-fed (2.5%) steers than for carcasses from Corn-fed (2.1%) steers. There were no differences ( $P > 0.02$ ) in marbling score or quality grade for carcasses from steers fed Corn (small02; high Select), CL (small02; low Choice), or MP (small02; high Select). Warner-Bratzler shear force, meat palatability, and sensory flavor attributes of steaks did not differ among treatment groups ( $P > 0.13$ ). Subcutaneous and i.m. adipose tissue explains were increased with 5 mM [ $U$ - $^{14}C$ ]glucose  $\pm$  0.1 mU/mL insulin. Glucose incorporation into total lipids (TL), glyceride-glycerol (GG), and fatty acid (FA) fractions was highest ( $P < 0.05$ ) in s.c. adipose tissue from steers fed MP, but was unaffected by diet in i.m. adipose tissue. Insulin did not affect ( $P > 0.05$ ) glucose incorporation into TL, GG, or FA for s.c. or i.m. adipose tissue. Percentages of 18:2 and 18:3 were higher ( $P < 0.05$ ) in tissues from CL and MP-fed steers. Mean cell volume was greater (811 pL vs 319 pL) and cells/g were less (1.6 vs 2.3 million) in s.c. than in i.m. adipose tissue ( $P < 0.05$ ). Peak diameter (154 vs 98  $\mu$ m) and volume (684 vs 2182 pL) were greater in s.c. than in i.m. adipose tissue ( $P < 0.05$ ). There were no treatment  $\times$  tissue interactions for mean cell volume or cells/g ( $P > 0.20$ ). Intramuscular adipocyte peak volume was greater ( $P < 0.05$ ) in CL-fed steers (848 pL) than in Corn-fed steers (536 pL). The activities of 6-phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase, NADP-malate dehydrogenase, and fatty acid synthetase did not differ among treatment groups for either s.c. or i.m. adipose tissue ( $P > 0.05$ ). These data indicate that the increased availability of glucose to the small intestine modified s.c., but not i.m. adipocyte glucose metabolism. The inclusion of protected lipid in both the CL and MP diets decreased ruminal biohydrogenation and modified the fatty acid composition of both s.c. and i.m. adipose tissue. We were unable to demonstrate a direct effect of protected starch on i.m. cellularity. The CL supplement increased i.m. adipocyte peak volume, which may have been related to the numerically higher marbling scores of the CL-fed steers.

## INTRODUCTION

According to the National Beef Quality Audit-1995 (NBAQ-1995), insufficient marbling along with excessive external, seam, and beef-trim fat were among the top 10 "quality" concerns in cattle. Over the last three decades, USDA yield grades have improved slightly due to an increase in leaner, more muscular cattle, however, USDA quality grades and marbling scores have decreased during this same period. The international market considers U.S. Choice to be the benchmark for quality in beef, and the demand for Choice and Prime beef greatly exceeds current production; however, the chief complaint among international consumers regarding U.S. beef was excessive external fat (NBAQ-1995).

The production of cattle with inadequate marbling and excessive subcutaneous fat continues to be a problem for the beef industry. The decline in carcass quality combined with higher numerical yield grades due to increased subcutaneous fat is detrimental to the competitiveness of beef in the U.S. and international market. U.S. beef cattle have traditionally been fed grain diets for more than 100 d to increase marbling, but this also results in excessive external fat in many cattle.

A better understanding of the mechanisms by which intramuscular (i.m.) and subcutaneous (s.c.) adipose tissue accumulate may make it possible to manipulate their growth. Previous research indicated that i.m. and s.c. adipose tissue represent different cell lines and utilize different precursors for fatty acid biosynthesis. Intramuscular adipose tissue uses a higher proportion of glucose for fatty acid biosynthesis, whereas s.c. adipose tissue primarily utilizes acetate. Casein-formaldehyde-protected starch and/or lipid is resistant to digestion at rumen pH (5 to 7), but the protective coating of protein and formaldehyde is readily digested in the abomasum (pH 2 to 3), allowing for subsequent digestion of the starch and lipid in the small intestine. Increased availability of glucose via protected starch may increase the deposition of i.m. fat without increasing s.c. and internal fat.

This study proposed to determine the effectiveness of feeding casein-formaldehyde-protected starch in increasing marbling scores in beef cattle without increasing fat accumulation in other deposits. The protected starch product to be fed contained 3.7% protected starch, therefore, the study also established the effect of protected lipid on adipose tissue growth and fatty acid composition.

## OBJECTIVES

- 1) To determine the effect of protected starch and/or lipid on carcass fat development and sensory characteristics of beef.
- 2) To determine the effect of protected starch and/or lipid on fatty acid biosynthesis, lipogenesis from glucose, lipogenic enzyme activity, and cellularity in i.m. and s.c. adipose tissue.
- 3) To determine the effect of protected starch on insulin stimulation of lipogenesis from glucose in i.m. and s.c. adipose tissue.

## MATERIALS AND METHODS

Eighteen Brangus steers of similar live weight were assigned randomly to one of three dietary treatment groups: cracked corn (Corn), casein-formaldehyde-protected Canola Lipid (CL), or casein-formaldehyde-protected Marble Plus (MP). The diets were equally balanced for ME (2.91 Mcal/kg), crude protein (12.5%), and dry matter (89%). Either extract was 3.7, 6.9, and 6.9% for the Corn, CL, and MP diets, respectively. The CL and MP diets provided equal amounts of protected lipid (3.3%). The MP also contained 3.7% protected starch. The steers were fed their respective diets for 126 to 130 d before humane slaughter.

After hide removal, the 5th to 8th lumbar vertebrae region of the longissimus dorsi muscle was removed and transported to the laboratory in Krebs-Henseleit bicarbonate buffer with 5 mM glucose (pH 7.4) at 37°C. Pieces of s.c. and i.m. adipose tissue were dissected from the longissimus muscle and incubated for 2 h in 3 mL of incubation media containing Krebs-Henseleit buffer (pH 7.4), 5 mM sodium acetate, 5 mM glucose, 10 mM HEPES,  $\pm$  0.1 mU/mL insulin, and 0.5 mCi [ $U$ - $^{14}C$ ]glucose. Lipids were extracted using standard techniques and glucose incorporation expressed as nmoles per 100,000 cells.

USDA yield and quality grades were determined by trained Texas A&M personnel after 48 h chilling. Three 2.54-cm steaks were removed from the left carcass side, vacuum packaged, and held at 2°C for 7 d. One steak was used to determine Warner-Bratzler shear force values. The other two steaks were evaluated for descriptive meat attributes by a trained sensory panel.

Samples of 500 mg of s.c. and i.m. adipose tissues were immediately homogenized in 2 volumes (wt/vol) of 0.1 M  $K_2HPO_4$  (pH 7.4). The homogenates were centrifuged and the supernate transferred to 2-mL microfuge tubes before snap-freezing in liquid nitrogen and storage at -80°C. Lipogenic enzyme activities were measured using spectrophotometry.

Total lipids were extracted from 100 mg of s.c. and i.m. adipose tissue using 20 mL of chloroform:methanol (2:1, v/v). The total lipid extracts were methylated with boron trifluoride-methanol. Fatty acids were determined by gas chromatography using a 30 m capillary column. Subcutaneous and i.m. adipocytes were fixed using osmium tetroxide and filtered onto 250-, 62-, and 20- $\mu$ m nylon mesh screens. Cell fractions collected from the 62- and 20- $\mu$ m mesh screens were used to determine cell size, volume, and cells per g of tissue with a Coulter Counter, Model ZM and Coulter Channelyzer Z56.

Data was analyzed by analysis of variance using the SuperAnova program (Abacus Concepts, Inc., Berkeley, CA). When treatment effects were significant ( $P < 0.05$ ), means were separated by the Fisher's Protected LSD method contained in the SuperAnova program.

Table 1. Carcass Traits of Steers Fed Cracked Corn, Canola Lipid, or Marble Plus

Trait	Corn	Canola Lipid	Marble Plus
Hot carcass wt, kg	300.0 $\pm$ 9.5	313.9 $\pm$ 13.7	322.5 $\pm$ 16.9
Adjusted fat thickness, cm <sup>a</sup>	1.4 $\pm$ 0.15	1.8 $\pm$ 0.1	1.5 $\pm$ 0.23
Longissimus muscle area, cm <sup>2</sup>	68.7 $\pm$ 4.6	70.0 $\pm$ 3.8	73.3 $\pm$ 3.7
Kidney, pelvic, and heart fat, %	2.1 $\pm$ 0.0 <sup>b</sup>	3.2 $\pm$ 0.17 <sup>c</sup>	2.5 $\pm$ 0.34 <sup>d</sup>
USDA yield grade	3.3 $\pm$ 0.21	3.9 $\pm$ 0.25	3.4 $\pm$ 0.27
USDA marbling score <sup>a</sup>	400.0 $\pm$ 38.5	430.0 $\pm$ 27.8	401.7 $\pm$ 19.2
USDA quality grade <sup>a</sup>	679.2 $\pm$ 24.6	703.3 $\pm$ 12.8	687.2 $\pm$ 12.5

<sup>a</sup>300 = Slight<sup>®</sup> and 400 = Small<sup>®</sup>

<sup>b</sup>600 = Select<sup>®</sup> and 700 = Choice<sup>®</sup>

<sup>c</sup>Means within a row lacking a common superscript differ ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

Carcasses from steers fed Corn, CL, or MP did not differ in adjusted fat, ribeye area, hot carcass weight, or yield grade (Table 1). Carcasses of CL-fed cattle had higher percentage kidney, pelvic, and heart fat (Table 1). There were no differences in marbling score or quality grade among treatments (Table 1). The numerically lower adjusted fat thickness and yield grade and significantly lower percentage KPH fat in CL-fed steers, even though the MP diet contained the same level of protected lipid as the CL diet, may have been the result of lower acetate production in the rumen due to the effect of the protected starch.

Fatty acid composition of s.c. and i.m. adipose tissue of beef carcasses can affect the palatability of meat. Oleic (18:1) acid has been associated with increased flavor acceptance in beef, but linoleic (18:2) acid has been associated with decreased juiciness and linolenic (18:3) acid with decreased flavor acceptance. Warner-Bratzler shear force and descriptive meat attributes did not differ in steaks from steers fed one of the three diets (Table 2). Juiciness was numerically lower in the steaks from CL- and MP-fed cattle, but the differences were not significant. Our main concern in feeding the protected lipid was the potential development of off-flavors associated with lipid oxidation as the result of increased levels of 18:2 and 18:3. The levels of aromatics associated with lipid oxidation (cardboardy, painty, and fishy) in steaks were not different (data not shown). Increased concentration of 18:2 and 18:3 in adipose tissue of CL- and MP-fed cattle did not increase lipid oxidation or development of off-flavors in steaks from these animals.

The Corn diet was substantially higher in 18:2 (Figure 1); however, the subcutaneous (Figure 2) and i.m. (Figure 3) adipose tissues from steers fed CL or MP exhibited increased relative percentages of 18:2 and 18:3. This indicated that the casein-formaldehyde encapsulation of lipids was effective in reducing ruminal biohydrogenation of fatty acids, allowing absorption of unsaturated fatty acids in the small intestine and subsequent incorporation into the adipose tissues of cattle. The lack of change in 18:1 concentration in s.c. (Figure 2) and i.m. (Figure 3) adipose tissues reflects the composition of 18:1 in the diet (Figure 1), which was close to the maximal endogenous synthesis level.

Cells per g of tissue did not differ among treatments in s.c. or i.m. adipose tissue (Figure 4). Peak diameter (Figure 5) and volume (Figure 6) were not affected by diet in s.c. adipocytes. Peak diameter of i.m. adipocytes tended ( $P = 0.07$ ) to be larger in steers fed CL (101.7  $\mu$ m) or MP (101.7  $\mu$ m) than in steers fed Corn (87.7  $\mu$ m; Figure 7). Peak volume of i.m. adipocytes was larger ( $P = 0.03$ ) in steers fed CL (847.5 pL) than in steers fed MP (644.9 pL) or Corn (536.2 pL; Figure 8). The s.c. (Figure 5) and i.m. (Figure 7) adipose tissues of cattle fed Corn, CL, or MP all had biphasic adipocyte diameter distributions; however, cellularity was only determined on adipose tissues from animals of the same age so we cannot confirm the occurrence of secondary hyperplasia. Adipocyte hyperplasia likely was not affected by the inclusion of protected lipid and/or starch because the number of cells per g of tissue in the s.c. or i.m. adipose tissues of cattle fed Corn, CL, or MP was not different among treatments. Subcutaneous adipose tissue contained fewer cells per g of tissue and larger peak diameters and peak volumes than i.m. adipose tissues. This is consistent with the conclusion of numerous researchers that i.m. adipose tissue is a later maturing depot than s.c. adipose tissue.

There were no differences in lipogenic enzymatic rates among treatments in s.c. or i.m. adipose tissue (Figures 9 and 10). The numerically higher activities of the pentose cycle dehydrogenases and NADP-malate dehydrogenase in the s.c. adipose tissue of cattle fed MP may have stimulated glucose incorporation into lipids in s.c. adipose tissue.

Glucose incorporation into total lipids and glyceride-glycerol was higher in s.c. adipose tissue of steers fed MP than Corn; however, they were not different than steers fed CL (Figure 11). Glucose incorporation into fatty acids was higher in s.c. adipose tissue of steers fed MP than steers fed either Corn or CL (Figure 11). There were no differences among treatments for glucose incorporation in i.m. adipose tissue (Figure 12). The significantly greater utilization of glucose for lipid synthesis by the s.c. adipose tissue of cattle fed MP was not expected; however, it corresponded with the numerically higher lipogenic enzyme rates in these same tissues. Insulin did not affect glucose incorporation for s.c. or i.m. adipose tissue (data not shown).

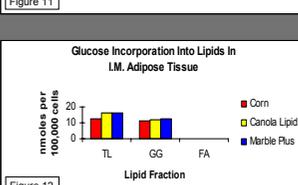
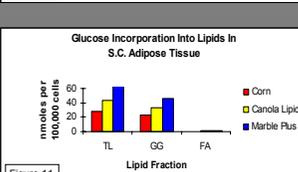
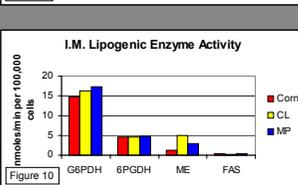
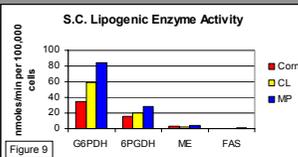
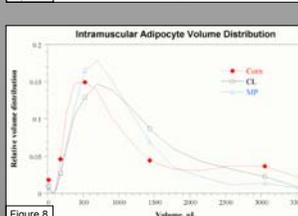
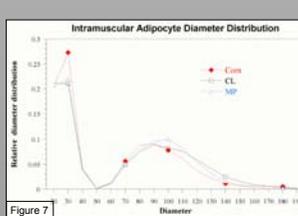
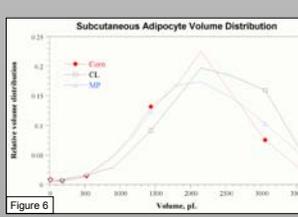
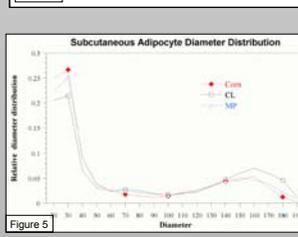
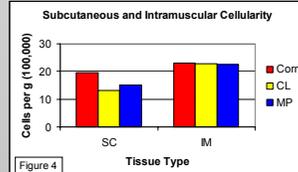
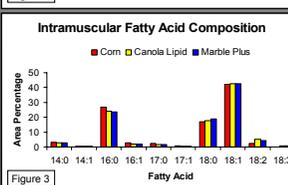
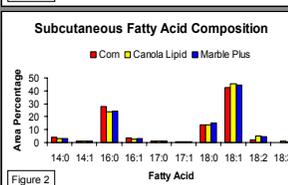
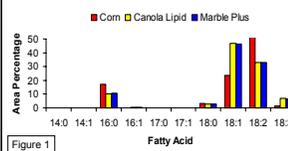
Table 2. Warner-Bratzler Shear Force Values and Descriptive Meat Sensory Traits of Steers Fed Cracked Corn, Canola Lipid (CL), or Marble Plus (MP)<sup>a</sup>

Item	Corn	CL	MP
Warner-Bratzler shear force	2.97 $\pm$ 0.39	3.39 $\pm$ 0.61	2.92 $\pm$ 0.4
Descriptive meat sensory attributes <sup>b</sup>			
Juiciness	6.07 $\pm$ 0.21	5.88 $\pm$ 0.14	5.80 $\pm$ 0.34
Muscle fiber tenderness	5.68 $\pm$ 0.34	5.52 $\pm$ 0.25	5.98 $\pm$ 0.43
Connective tissue amount	6.55 $\pm$ 0.19	6.48 $\pm$ 0.16	6.97 $\pm$ 0.16
Overall tenderness	5.68 $\pm$ 0.34	5.52 $\pm$ 0.25	6.02 $\pm$ 0.42
Overall flavor intensity	5.75 $\pm$ 0.07	5.75 $\pm$ 0.07	5.85 $\pm$ 0.06

<sup>a</sup>Means within a row were not different ( $P > 0.1$ ).

<sup>b</sup>Sensory attributes were scored using the following scales for juiciness, muscle fiber tenderness, connective tissue amount, overall tenderness and flavor intensity: 8 = extremely juicy, tender, none, tender, intense; 1 = extremely dry, tough, abundant, tough, bland.

### Diet Fatty Acid Composition



## CONCLUSIONS

Casein-formaldehyde-protected starch and lipid did not increase marbling scores or reduce overall carcass fatness.

Changes in s.c. and i.m. fatty acid composition indicated that protected lipids decreased ruminal biohydrogenation of fatty acids.

Cell number and size were unchanged by treatment.

Lipogenic enzyme activities were not affected by protected starch and lipid.

Protected starch increased lipogenesis from glucose in s.c., but not i.m. adipose tissue.

Insulin did not affect lipogenesis from glucose in bovine adipose tissue.