UNIVERSITY OF MANITOBA

THE EFFECT OF ANATOMICAL LOCATION, CROSSBREED, AND SEX ON THE FATTY ACID COMPOSITION OF THE NEUTRAL, PHOSPHOLIPID AND FREE FATTY ACID FRACTIONS OF BOVINE LIPIDS

by

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ABSTRACT

The neutral, phospholipid and free fatty acid fractions from the lipids of twenty-four Limousin and Simmental crossbreeds were examined to determine the effects of breed, sex and anatomical location on the fatty acid composition. Phospholipids were separated from the biceps femoris and longissimus dorsi lipid extracts using a modification of the method of Choudhury and Arnold. A simple quantitative procedure was employed to partition the neutral lipids from the free fatty acids. A 98.0% recovery of the free fatty acids from the other lipid fractions was confirmed using ^{14}C-labeled palmitate. Significant (P 0.05) differences in fatty acid composition due to anatomical location were observed in all fractions. The neutral and free fatty acid fractions of the biceps femoris intramuscular and subcutaneous lipids were less saturated than the corresponding fractions of the longissimus dorsi. The percentages of C14:0, C16:0, C16:1 and C18:0 differed in the neutral fraction of both biceps femoris and longissimus dorsi, as well as those of C18:1 and C18:2 in the neutral fraction of the subcutaneous lipid. The neutral and free fatty acid fractions from the exterior layer of the longissimus dorsi were less saturated than those from the interior layer. Levels of C18:0 and C18:2 in the neutral fraction of the subcutaneous lipid were affected by crossbreed as were levels of C18:2 in the subcutaneous free fatty acid fraction. The fatty acid composition of the phospholipid extracts was influenced by both crossbreed and sex. Breed differences were observed for C18:1 and sex differences for C18:0.

Sex x muscle, breed x sex, and breed x sex x muscle interactions also occurred for C18:0 in the phospholipid fraction. A sex x muscle interaction was also observed for C18:1 in the free fatty acid fraction of the subcutaneous lipid.

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GENERAL INTRODUCTION

Lipid components determine many of the organoleptic and biochemical properties of meat; and the color and quantity of fat on roasts and steaks is a decisive factor in consumer meat selection. During storage, oxidative and enzymatic changes in the complex lipids influence flavor, tenderness and juiciness. The involved interrelationships between lipid and other chemical constituents of meat are not well understood, and the exact mechanisms responsible for differences in sensory parameters need much more elucidation. It is hoped that the information reported here on the fatty acid composition of bovine lipids will contribute to a better understanding of those factors which influence the quality of meat.

Animal breeders, responding to consumer demands for leaner beef at reasonable prices, are continually developing beef cattle which convert food to body tissue with increased efficiency. Productivity may be increased through selective breeding of superior animals, or by the cross-breeding. In a program to evaluate foreign cattle breeds, the Canada Department of Agriculture imported Limousin and Simmental bulls to crossbreed with Aberdeen Angus, Hereford and Shorthorn cows. Detailed carcass data on the hybrid male offspring have been collected by the Canada Department of Agriculture Research Station in Brandon. Evaluation of the sensory parameters of two muscles from these bulls and steers, as well as analyses of the total lipids from similar

anatomical locations were carried out in this laboratory by McLandress (1972) and Gillis (1972) respectively.

The following study was undertaken to determine the fatty acid composition of the neutral, phospholipid, and free fatty acid fractions of intramuscular lipid from two muscles, two sexes, and six bovine crossbreeds; and to determine the fatty acid composition of the neutral and free fatty acid fractions of associated subcutaneous lipid.

REVIEW OF LITERATURE

Introduction

The quantity and composition of bovine lipids have been related to the organoleptic and biochemical properties of meat (Hornstein et al., 1961; Terrell et al., 1969b; and Dryden and Marchello, 1970). Increased tenderness in beef has been correlated with higher lipid levels (Dryden and Marchello, 1970); although when factors such as age and sex were controlled such differences in tenderness and flavor were no longer apparent (Reddy et al., 1970; Waldman et al., 1968; Martin et al., 1971). In several of these studies, there were small significant correlations between individual fatty acids and flavor scores, but these results followed no consistent pattern. However, an increase in the total free fatty acid content of aged meat has proved to be a reliable indicator of aroma change (Pearson, 1968). The susceptibility to oxidation of the polyunsaturated fatty acids from the phospholipid fraction appears to be responsible for some of the undesirable flavor changes associated with stored meat (Hood and Allen, 1971). The relationship between lipid composition and the factors affecting beef quality are still little understood, and will undoubtedly receive more attention in the future.

This review will discuss the influence of anatomical location, breed and sex on the fatty acid composition of the neutral, phospholipid and free fatty acid fractions of bovine lipids.

The Nature of Lipids

Naturally occurring lipids of animal origin consist of polar and nonpolar components. After removal of the polar compounds, the remaining neutral fraction is mainly composed of triglycerides, with mono- and

diglycerides, together with free and esterified cholesterol accounting for only about ten percent of the total. Polar lipids can themselves be conveniently separated into phospholipids and free fatty acids. A brief discussion of the origin and function of these individual components is essential to an understanding of the mechanisms responsible for the differences observed in animal tissues.

Triglycerides. Triglycerides are stored in the adipose tissue of the animal body to provide a reserve energy supply. When a high-carbohydrate diet is ingested by the animal, fatty acids for triglyceride formation arise from de novo biosynthesis. When fat is ingested, it is hydrolysed to free fatty acids and glycerol in the intestinal tract, and these products are absorbed by the intestinal lumen. The longer chain fatty acids enter the lymphatic system in the form of chylomicrons, largely reconstituted triglycerides in composition, which pass via the thoracic duct into the blood. These chylomicrons are then transported to adipose tissue sites where it appears that the triglycerides are again enzymatically broken down to free fatty acids prior to their incorporation into the cell. Within the cell itself the fatty acid is converted to fatty acyl-CoA, three molecules of which combine with one of L-glycerophosphate to form the triglyceride molecule. It thus appears that triglycerides are not incorporated into the cell intact, but are hydrolysed before, or at the time of, uptake (Masoro, 1968).

The mobilization of fat during fasting or in the postabsorptive state results from the hydrolysis of triglycerides to free fatty acids and glycerol by hormone-sensitive lipase. The degree of mobilization is determined by the relative rates of lipolysis and triglyceride biosynthesis, and is further controlled by the availability of plasma albumin for

transportation. Consequently, adipose tissue, once considered inert, is now believed to undergo constant metabolic activity (Jensen, 1971).

Phospholipids. Phospholipids are an integral part of all biological membranes, and are the major lipid components of myelin, as well as of mitochondrial and microsomal membranes. They represent a much smaller proportion of intramuscular lipid, and usually account for much less than 2 percent of adipose tissue lipids. As essential components of membranes, it has been suggested that phospholipids are involved in energy transfer, triggering mechanisms, nerve impulses, protein synthesis, and cell adheviseness; and that they may be implicated in cancer and atherosclerosis (Williams and Chapman, 1970).

Many permutations are possible in phospholipid structure, because of the large number of polar head groups which may associate with the saturated or unsaturated fatty acids. The chain length of these fatty acids generally ranges from 12 - 26 carbon atoms. Branched chain fatty acids frequently occur, although they account for only a small percentage of the total fatty acid.

Williams and Chapman (1970) suggest that chain length, degree of unsaturation, and branching are regulated in phospholipid formation to permit the phospholipids present in the membrane to perform precisely defined roles. Chain length may assist in maintaining the hydrophobic-hydrophilic balance necessary for proper functioning, while optimum structural fluidity may be achieved by adjustments in all three variables. Shorter chain lengths, greater unsaturation and increased branching, would then result, for example, in a reduction of dispersion forces between chains. This would permit increased diffusion, raising the rate of metabolic activity. Although the place of specific fatty acids in

phospholipid organization and function has not been defined, it appears that they each fulfil a particular function and are not interchangeable.

Free fatty acids. Free fatty acids provide 20-50 percent of the fuel for skeletal muscle during prolonged moderate exercise (Masoro, 1968). The constant biosynthesis and degradation of triglycerides are thought to be responsible for the presence of some free fatty acids in adipose tissue, but cannot explain the increased levels observed in aged meat. Such increases in free fatty acids in aging beef have been attributed to the degradation of the more complex lipids (Pearson, 1968; Hood and Allen, 1971).

Factors Affecting Fatty Acid Composition of Bovine Lipids. A comprehensive survey of naturally occurring fats and oils has been compiled by Hilditch (1964). His tables indicate a wide variation in kinds and relative percentages of fatty acids from different animal sources. While those of aquatic origin contain a wide range of mainly unsaturated fatty acids with chain lengths from C16 - C22, the majority of the fatty acids in the depot fats of terrestrial animals consist of fatty acids in the C16 - C18 series, with a much higher degree of saturation.

In a detailed comparison of the composition of beef, pork, lamb and poultry fats, Hubbard and Pocklington (1968) found that the variation in the relative percentages of the major fatty acids within these species was generally greater than the variation between the species. The only exception to this was in the levels of C18:2 in pork and poultry which were higher than those in beef and lamb although even in this case there was some overlapping of the ranges. Thrall and Cramer, (1971b) stressed the importance of determining the normal range of

biological variation within lipid fractions, which their studies showed could be quite considerable. Intramuscular lipid from twenty-six Hereford bulls contained from 13.7 - 29.1 percent C18:0. and from 0.9 - 10.1 percent C18:2. Because of the wide range in the relative percentages of fatty acids normally presented in biological lipids, the numbers of animals represented in a study must be sufficient for valid comparisons to be made.

Age, climate, sex, and the anatomical site of the fat depot, are factors listed by Thrall and Cramer (1971a) as influencing the composition of ruminant fat. Nutrition, which may be responsible for dramatic changes in the fatty acid composition of the fat depots in non-ruminants (Tove, 1960) is of minor importance in ruminants, because rumen microorganisms can hydrogenate ingested fatty acids, converting the polyunsaturates to more saturated forms (Scott, 1971).

Post-mortem changes in the fatty acid composition of the neutral, phospholipid and free fatty acid fractions of bovine lipids were investigated by Hood and Allen (1971). They reported that differences due to aging were much greater in the free fatty acid fraction than in the other fractions. The amount of free fatty acids which can be extracted from fresh meat is so much less than that of neutral or phospholipids, that the consequences of lypolytic activity would naturally be detected first in the free fatty acid fraction.

Effect of Anatomical Location

Skeletal muscles differ in the amount of work they perform as well as in the speed and time required to carry out their specific physiological roles. Some require energy for brief, fast action, while others are involved in more sustained movement. The latter depend on free fatty acids to supply the greater part of their high energy requirement, and their metabolism is predominantly respiratory. Typical "red" muscle contains concentrated capillary and mitochondrial systems which are necessary for fatty acid oxidation. These are less abundant in "white" muscle, which depends more on glycogen to supply its energy needs. Skeletal muscles are generally of mixed red and white muscle types, whose proportions determine the extent to which respiratory or glycolytic metabolism predominates (Marsh, 1970; Masoro, 1968; Havel, 1970)

Neutral lipid fraction. Lipid from the semitendinosus, longissimus dorsi, and triceps brachii of thirteen animals was fractionated into neutral and phospholipid moieties by O'Keefe et al (1968).
When the fatty acid composition of the neutral fraction was analysed statistically, no significant differences were apparent. In a similar investigation, Terrell and Bray (1969) compared the composition of the neutral lipid from the triceps brachii, transversus abdominus and psoas major muscles of thirty-five animals. In this study there were marked differences in the relative percentages of eleven of the thirteen fatty acids reported. The most noticeable contrast was in the higher levels of long chain unsaturates present in the psoas major phospholipids than

in those of the triceps brachii. The discrepancies between the results reported by these two groups may be due in part to the different sample sizes involved. With the smaller sample, normal biological variation might have been sufficient to obscure significant comparisons. The means of the six major fatty acids from the neutral lipid extract of the three muscles studied by Terrell and Bray (1969), which comprised over ninety percent of the total, are tabulated in Table 1. Considerable variation existed in the amounts of individual C18 fatty acids; however a comparison of the total amounts of C18 fatty acids within each muscle reveals variations of only ±0.7 percent.

Subcutaneous fat bordering the transversus abdominus, semitendinosus and triceps brachii exhibited fewer compositional differences than did the corresponding intramuscular neutral lipids (Terrell, 1967). Only C16:1, C18:0 and C18:1 fatty acids differed between depot sites, but changes in the proportions of these three acids were sufficient to make the outer layers of subcutaneous fat more unsaturated than the inner layers at each location. In Table 2 the values for these three major fatty acids in the inner and outer layers have been compared.

Phospholipid fraction. There are profound differences in the composition of the phospholipid and neutral lipid fractions from intramuscular lipid. The former contains a wider range of fatty acids, and a much higher percentage of unsaturates. As much as twenty percent of the phospholipid fatty acids have been reported to be C20 or C22 acids (Hornstein et al.,1961; Hornstein et al., 1967). This contrast between the neutral and phospholipid fatty acid patterns was made

Table 1

Mean Comparison of the Six Major Fatty Acids of the Neutral Fraction from Three Bovine Muscles[1,2]
(Terrell and Bray, 1969)

Fatty Acid	Triceps Brachii	Psoas Major	Transversus Abdominus
C14:0	2.75[a]	3.21[b]	3.05[b]
C16:0	31.98[a]	33.77[b]	33.43[b]
C16:1	3.92[a]	2.98[b]	3.65[a]
C18:0	9.77[a]	14.10[b]	12.26[c]
C18:1	44.24[a]	38.97[b]	41.52[c]
C18:2	2.41[a]	2.08[b]	1.92[b]

[1] Means in the same line with the same superscript are not significantly different ($P < 0.05$)

[2] Means expressed as a relative percentage of the total fatty acids measured

Table 2

Effect of Depot Site on the Fatty Acid Composition of Bovine Subcutaneous Fat Depots[1,2]
(Terrell et al., 1969a)

Fatty Acid	Transversus abdominus		Semitendinosus		Triceps brachii	
	inner layer	outer layer	inner layer	outer layer	inner layer	outer layer
C16:1	5.48[a]	7.01[b]	6.70[b]	8.06[c]	7.85[bc]	9.44
C18:0	10.93[a]	9.71[a]	9.03[a]	7.59[b]	7.48[b]	6.09
C18:1	46.05[ab]	46.70[b]	46.44[b]	47.76[bc]	48.25[bc]	48.77[a]

[1] Means in the same line with the same superscript are not significantly different ($P<0.05$)

[2] Means expressed as a relative percentage of the total fatty acids measured.

evident in data reported by Terrell (1967), (Table 3) in which only the percentage of C18:0 was the same for both fractions.

The fatty acid composition appears to vary less between phospholipid fractions than between neutral lipid fractions from the same muscle. This is illustrated by the fact that only six phospholipid fatty acids in one study, and seven in another, were reported to vary with respect to muscle location (O'Keefe et al., 1968; Terrell and Bray, 1969). These results are summarized in Table 4. Differences in the red and white fiber content of skeletal muscles could be responsible for varying phospholipid fatty acid patterns, and for the somewhat higher phospholipid/cholesterol ratios reported for more active muscles (Terrell et al., 1969b).

Effect of Breed

The objective of animal breeding is to produce animals of superior productivity, capable of transmitting their inherited characteristics to their off-spring. The heritability of one trait may be markedly different from that of another, although in general the traits associated with carcass quality and growth after weaning tend to have high heritabilities. Crossbreeding is a necessary step in developing a new breed but it is also used to produce hybrid vigor. Hybrid animals usually gain more quickly and efficiently than nonhybrids, but the traits of importance to carcass quality depend almost entirely on inherited characteristics, and are not influenced by heterosis.

Simmental and Limousin cattle imported from Europe are of a larger build than the common British breeds, the Angus, Hereford and Shorthorn.

Table 3

Comparisons of Thirteen Fatty Acids from the Neutral and Phospholipid Fractions of Three Bovine Muscles[a,b]
(Terrell 1967)

Fatty Acid	Fraction	
	Neutral	Phospholipid
C8	.41	1.02
C10	.15	.32
C12	.10	.24
C14	3.00	1.87
C14:1	.96	.23
C15	.42	.66
C16	33.06	30.75
C16:1	3.51	1.99
C17	1.09	.75
C18	12.04	11.91
C18:1	41.57	28.07
C18:2	2.14	18.66
C18:3	1.54	3.51

[a] Means not underlined are significantly different ($P < 0.01$)

[b] Means expressed as a relative percentage of the total fatty acids measured

Table 4

Mean Comparison of the Major Fatty Acids in the Phospholipid Fractions of Three Bovine Muscles[1,2]

Fatty Acid	O'Keefe and Wellington (1968)			Terrell & Bray (1969)		
	Semi-tendinosus	Longissimus Dorsi	Triceps Brachii	Triceps Brachii	Psoas Major	Transversus abdominus
C14:0	0.28^{a}	0.36^{a}	0.24^{a}	1.07^{x}	1.58^{x}	1.49^{x}
C16:0	18.16^{a}	22.55^{b}	18.66^{a}	24.32^{x}	28.43^{y}	28.01^{y}
C18:0	9.68^{a}	7.78^{b}	9.97^{a}	10.36^{x}	10.02^{x}	10.72^{x}
C18:1	23.11^{a}	24.34^{a}	25.38^{a}	24.42^{x}	24.03^{x}	24.94^{x}
C18:2	23.85^{a}	23.02^{a}	22.59^{a}	17.52^{x}	15.66^{y}	15.33^{y}
C18:3	1.44^{a}	2.00^{b}	1.61^{a}	3.25^{x}	2.99^{x}	2.84^{x}
C20:4	15.23^{a}	12.54^{a}	14.21^{a}	11.46^{x}	9.43^{y}	9.21^{y}

1 Means in the same line with the same superscript are not significantly different ($P < 0.05$)

2 Means expressed as a relative percentage of the total fatty acids measured

When used as a "Sire" breed, they can be expected to increase the carcass value and growth rate of their progeny. Whether these breeds will remain terminal breeds, used to produce slaughter cattle from purebred or hybrid females, remains to be seen (Seale and Parker, 1971).

Beef animal breeding has been largely directed towards an increase in the efficiency of meat production. Factors used for breed comparison include weight gain per day per pound of feed, percentage dressing loss, and pounds of defatted primal cuts per animal (Rahnefeld et al, unpublished paper). The quality characteristics of tenderness, flavor and juiciness, have not so far played an important role in determining breeding programs. If investigation shows that the quality and composition of beef, like pork, can be made more desirable through controlled breeding, then more consideration will have to be given to the assessment of these characteristics.

The effect of breed on the neutral, phospholipid and fatty acid fractions of bovine lipids has not been reported in the literature. However, Gillis (1972) observed that the total intramuscular lipid from Limousin crossbreeds contained significantly more C14:0 and C16:0 fatty acids than did that of Simmental crossbreeds. More C16:1 fatty acid was present in the lipid from Angus than from Shorthorn or Hereford crossbreeds. There also appeared to be a trend towards lower unsaturated/saturated ratios in Limousin crossbreeds, but this was not sufficiently marked to be significant.

Effect of Sex

Color, flavor and tenderness of beef appear to be affected by sex differences (Field et al., 1966; Martin et al., 1971). However, sex differences are not as pronounced in younger animals as they are in

older ones. Bulls, steers and heifers, 300 to 399 days old, were rated the same for tenderness, juiciness and flavor, at 400 to 499 days palatibility of bulls was rated lower, while after 500 days, meat from bulls was significantly less tender than similarly treated roasts from steers and heifers (Field et al., 1966). Martin et al (1971) found that color and shear value correlations for aging meat from steers and heifers operated in a different direction than correlation values for meat from bulls, which increased in both darkness of color and tenderness with aging. In youthful animals sex did not appear to influence quality.

The rate of growth of different muscle groups within an animal is sex-related, and the growth has been shown to be preferentially stimulated by sex hormones (Lawrie, 1966). The effect of sex hormones, or a lack of them, on the animal's enzyme system may also be responsible for the observed tendency of males to have less intramuscular fat than females, and of castrated animals to have more intramuscular fat than the corresponding whole male or female (Lawrie, 1966; Gillis, 1972)

Neutral lipid fraction. The fatty acid patterns of the neutral lipids from heifers, bulls and steers, generally exhibit only minor variations. Compositional differences were not apparent in the neutral lipids from steers and heifers when animals of the same age were compared (Link et al., 1970c). Small but significant differences were reported by Hood and Allen (1971) in the levels of C16:0, C18:0, and C18:2. Steers had more C16:0 and C18:2 but less C18:0 than bulls. In the subcutaneous lipid, Terrell (1967) reported more C16:0 and C18:0 from steers and more C18:1 from heifers. Fewer differences between

steers and heifers were observed by Hood and Allen (1971), although bulls had more C18:0 than either heifers or steers, while heifers had more C18:1 than either steers or bulls. These results were confirmed by data presented by Thrall and Cramer (1971b). Table 5 summarizes the effects of sex on the six major fatty acids present in the neutral subcutaneous lipid fraction and in the total subcutaneous lipid.

Phospholipid fraction. No significant differences in the fatty acid composition of the phospholipids from heifers and steers were reported by Terrell (1967), but Hood and Allen (1971) in studies with bulls, steers and heifers, found that heifers had more C16:0 and less C18:0 than either bulls or steers. Bulls had faster rates of free fatty acid increase than the other sexes, which was attributed more to phospholipid than triglyceride hydrolysis, because over the aging period the relative amounts of the free fatty acids changed to proportions more like that of the phospholipid fraction. The higher aroma scores given to ribs from heifers by the sensory panel was thought to be related to the lower rate of phospholipid degradation.

Free fatty acid fraction. The amount of free fatty acids present in both intramuscular and subcutaneous lipid after 21 days of aging was lower for heifers than for steers or bulls (Hood and Allen, 1971). No differences in free fatty acid content between inner and outer layers of subcutaneous fat was evident, so that the increasing levels of free fatty acids which developed with time could not be attributed to bacterial lipases, but rather must have been the result of the activity of hormone-sensitive lipase present in the tissues. Compositional differences due to sex when the fatty acid compositions were averaged over four

Table 5

Fatty Acid Composition of Subcutaneous Lipid from Bulls, Steers and Heifers[1,2]

Fatty Acid[1]	Terrell (1970)		Hood and Allen (1971) (Triclycerides only)			Thrall & Cramer (1971)	
	Steers	Heifers	Steers	Heifers	Bulls	Heifers	Bulls
C14:0	4.28[a]	3.87[b]	3.3	2.7	5.2	4.7	3.7
C16:0	28.37[a]	26.31[b]	24.9	22.5	24.5	26.9	26.7
C16:1			6.3[c]	5.5[d]	6.2[c]	7.2	5.0
C18:0	9.61[a]	9.02[b]	10.2	10.3	11.0	12.3	17.4
C18:1	45.39[a]	48.50[b]	45.6[c]	49.7[c]	45.1[c]	42.5	40.4
C18:2			2.4	3.4	3.3	1.6	3.1

[1] Means in the same line with the same superscript are not significantly different ($P < 0.05$)

[2] Means expressed as a relative percentage of the total fatty acids measured.

aging periods, were few. Bulls had more C14:0 than heifers or steers, and heifers more C18:1 and C18:2 than bulls or steers.

Although there are numerous studies on the composition of total lipid extracts from adipose and muscle tissue, information is scarce on the composition of the separate fractions. As was evident in the preceeding review of the literature, the role of anatomical location in determining lipid patterns has been more thoroughly investigated than the roles of sex and breed. Clearly more research is required into the nature of the individual lipid components and the factors which bring about changes in their composition.

METHOD

Samples

Source

Twenty-four animals, two bulls and two steers of each of six cross-breeds produced by crossing Simmental and Limousin sires with Hereford, Angus and Shorthorn cows, were used in this study. Samples of the biceps femoris and longissimus dorsi muscles and adjacent subcutaneous fat, were obtained from the Canada Department of Agriculture Research Station, Brandon, Manitoba.

Spring-born male calves were weaned at 6-6½ months, and half were randomly chosen for castration. Bulls and steers were raised to 1000 pounds weight (approximately 452 kg), on a self-fed ration of 50% barley, 30% oats, 15% beet pulp, 2.5% molasses, 0.5% urea and 2% of a mixture of salt, vitamins and minerals. Carcasses were hung until the fourth day after slaughter, when the section of the longissimus dorsi adjacent to the twelfth vertebra and the entire biceps femoris were excised from the right side, and with subcutaneous fat from the same locations, placed in polyethylene bags and held at $3^{o}C$ overnight. The following day the meat and fat were wrapped in polyethylene coated freezer paper, and placed in a $-40^{o}C$ freezer. The frozen sample material was brought from the Brandon Research Station to this laboratory in styrofoam containers, where it was stored at $-37^{o}C$ to $-40^{o}C$. Prior to being freeze-dried, the meat was thawed at $23.5^{o}C$ for fifteen hours, and then aged at $3.5^{o}C$ for seven days. The total aging

period was ten days, including three days on the carcass and seven days under ordinary refrigeration. Subcutaneous fat was thawed before sampling but was not aged.

Sampling Procedure and Preparation of Samples

Intramuscular fraction. A 2.5 cm slice of meat was taken from the proximal end of the aged longissimus dorsi and biceps femoris muscles. Cores 2.54 cm in diameter were removed from the medial, central and lateral portions of each slice. These cores were combined, and then dried on a Virtis Freeze-Mobile Freeze Dryer (Model 10-140BA)[1]. The freeze-dried samples were stored until extracted in screw-top glass jars, under nitrogen, at -10°C.

Subcutaneous fraction. Fat from the longissimus dorsi area was separable into interior and exterior layers, but there were no clearly defined layers in the fat covering from the biceps femoris area. Samples of approximately 10 g were taken from each of the interior and exterior layers of the longissimus dorsi and from the biceps femoris subcutaneous fat. These were dried and stored as described above for the intramuscular samples.

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[1] Virtis Company, Inc., Gardiner, N.Y. 12525.

Chemical Analysis

Lipid Extraction

Intramuscular Lipid. A sample containing 6-8 g of partially frozen lyophylized muscle was diced and homogenized in 95 ml of chloroform-methanol-water (1:2:0.8 v/v/v) for three minutes at medium speed in a Virtis 23 homogenizer[1]. Lipid extraction and separation was achieved following the method of Bligh and Dyer (1959), by adding 25 ml of chloroform and 25 ml of water during the filtration of the homogenate. The washed residue, including the Whatman No.1 filter paper, was rehomogenized, as described, to ensure complete extraction of fat. Filtrate from the two extractions was shaken for one minute with 0.1 g of sodium chloride in a 250 ml separatory funnel and allowed to stand for 15-18 hours.

After separation, the lower (chloroform) layer was filtered through Whatman No.2 filter paper into a 125 ml round-bottomed flask and evaporated to dryness on a Buchler Portable Flash Evaporator (Model PF-100N)[2]. After evaporation the flask was placed in a desiccator containing concentrated sulfuric acid. Both flasks and desiccator were thoroughly flushed with nitrogen.

Subcutaneous Lipid. Lipid was extracted from partially frozen, lyophilized samples of subcutaneous fat in the same manner as that from

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[1] Virtis Company Inc., Gardiner, N.Y. 12525

[2] Buchler Instruments, Inc., 1327 Sixteenth St., Fort Lee, N.J. 07024

the intramuscular samples, except that the residue was not reextracted.

Lipid Fractionation

Phospholipid separation. Separation of the phospholipid from the neutral and free fatty acid fractions of the intramuscular lipid was accomplished by a modification of the method of Choudhury and Arnold (1960), using the solvent system of Hornstein et al. (1967). Silicic acid powder (100-mesh) was activated by heating at 120°C for one half an hour in an unstoppered flask. When removed from the oven the flask was restoppered and the silicic acid cooled to room temperature before being used. 2.5 g of silicic acid was put into a 50 ml volumetric flask and to this was added approximately 300 mg of extracted lipid dissolved in 2 ml of chloroform, and 10 ml chloroform-hexane-diethyl ether solvent (2:1:1 v/v/v). The flask was stoppered and shaken for ten minutes on a Burrell Wrist-Action Shaker[1]. The contents of the flask were filtered through a sintered glass funnel, and the silicic acid washed with 20-25 ml of chloroform-hexane-diethyl ether to extract the neutral lipids and free fatty acids. The solvent was evaporated by placing the flasks in a warm water bath under nitrogen.

The funnel containing silicic acid was transferred to another Buchner flask and the phospholipids washed off with 25-30 ml of methanol. The phospholipids were evaporated to dryness on the flash evaporator, and with the neutral-free fatty acid fraction were desiccated over

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[1] Burrell Corporation, Pittsburgh, Pa.

concentrated sulfuric acid in a nitrogen atmosphere.

Free fatty acid separation. Free fatty acids were separated from neutral lipids by the method described by Hamilton and McDonald (1971), although in this case 250-275 mg of lipid material was used instead of 100-200 mg and acetone was not added to the flasks for moisture removal. The flasks containing both neutral and free fatty acid fractions were desiccated as described earlier.

Preparation of Methyl Esters

Dried lipid was dissolved in petroleum ether and transferred to a 50 ml volumetric flask. The petroleum ether was evaporated by placing the flask in a warm water bath and flushing with nitrogen. The method of Metcalfe et al. (1966) was used for the preparation of the methyl esters, which were then stored in screw-top glass vials at -10°C prior to GLC analysis.

Fatty Acid Determination

The fatty acid methyl esters were separated on a dual column Aerograph (Model 1740-1)[1] gas chromatograph equipped with flame ionization detectors and using helium[2] as a carrier gas. Samples were injected onto 2.7 m x 3.2 mm steel columns packed with 10% EGSS-Y on 100/120 mesh Gas CHROMQ.[3] The flow rates were 36 ml/min. for the helium, 25 ml/min. for hydrogen[2], and 250 ml/min. for air[2]. The columns were operated isothermally at a temperature of 200°C with injector and

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[1] Varian Aerograph, 6358 Viscount Rd., Malton, Ontario

[2] Welder's Suppliers, 25 McPhillips St., Winnipeg 3, Manitoba

[3] Applied Science Lab. Inc., P.O.Box 440, State College, Pa. 16801

detector temperatures maintained at 250°C and 230°C, respectively.

The gas chromatograph was equipped with a Varian Aerograph (Model 20)[1] single pen recorder and a Varian Aerograph (Model 477) Digital Integrator[2]. The individual fatty acid methyl esters were identified by comparing retention times with known fatty acid mixtures supplied by the Hormel Institute and by comparing logarithmic plots of retention times.

Determination of the Relative Percentage Recovery of Free Fatty Acids.

To determine the completeness of the separation of free fatty acids from the phospholipids and neutral lipids, palmitic-1-C^{14} acid[5] was added to two samples containing intramuscular lipid. These were carried through the separation described, and the three fractions transferred to liquid scintillation vials[6]. The solvent was evaporated and the residue dissolved in toluene which contained 5.0 g of PPO[7] and 0.3 g POPOP[8] per liter. A blank was prepared in a similar fashion.

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1 Varian Aerograph, 6358 Viscount Rd., Malton, Ontario

2 Varian Aerograph, 6358 Viscount Rd., Malton, Ontario

3 Hormel Institute, University of Minnesota, Austin, Minnesota 55912

4 Freshwater Institute of the Government of Canada, 501 University Crescent, Winnipeg, Manitoba.

5 NEC-075 Palmitic-1-C^{14} acid. New England Nuclear, Boston, Mass.

6 Packard Instrument Inc., Downers Grove, Ill.

7 PPO : 2-5 diphenyl ozazole. Packard Instrument Inc.

8 POPOP : 2,2-p-phenylene bis (5-phenyl ozazole). Packard Instrument Inc.

Total radioactivity in each sample was determined using a Liquid Scintillation spectrometer[1]. The efficency of the counting was ascertained by the Channels Ratio method (Wang and Willis, 1965).

Statistical Analysis

Analysis of Variance was performed and where F values were significant Duncan's multiple range test (1955) was used to compare treatment means.

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[1] Model 8260 Nuclear Chicago Instruments, Des Plaines, Illinois.

RESULTS AND DISCUSSION

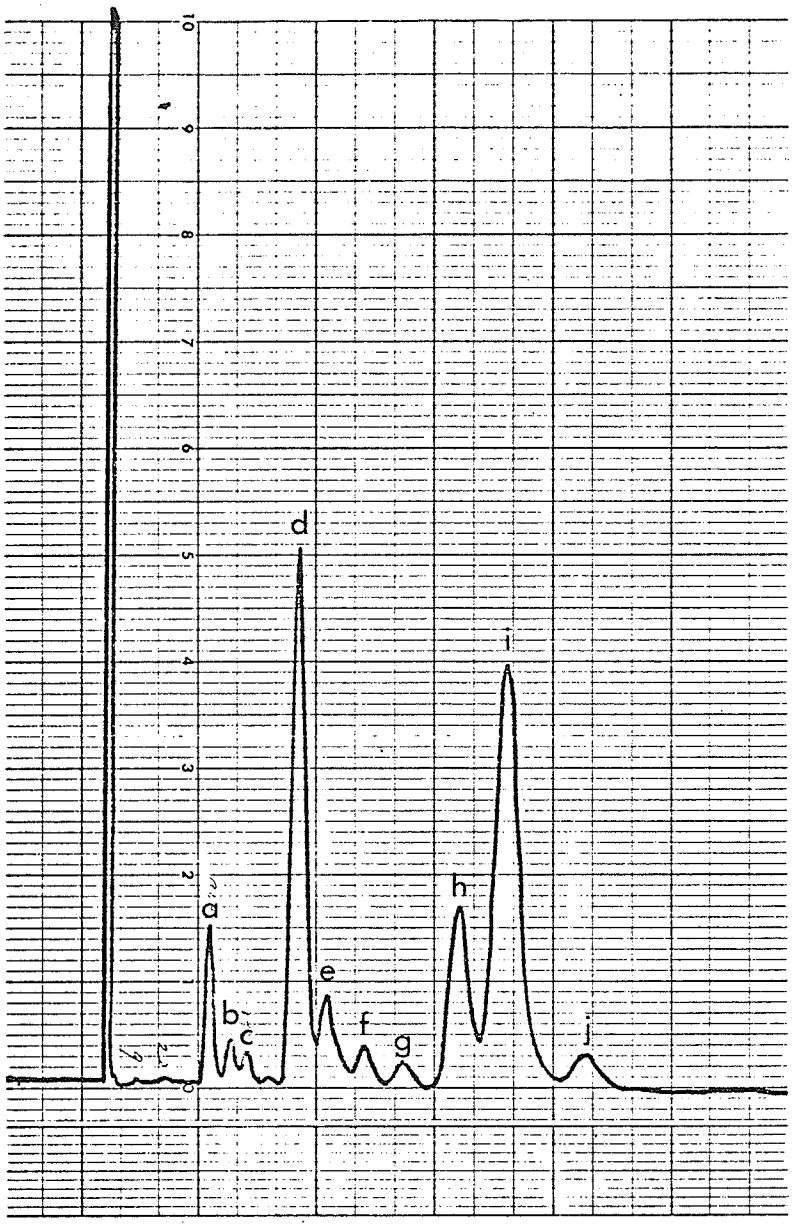
Intramuscular Lipid

Fatty Acid Composition

Typical chromatograms are illustrated in Figures 1, 2 and 3. Peaks C:X and C:Y were initially thought to be C15:1 and C17:1, an agreement with the identification made by Terrell (1967) and Gillis (1972). Hood and Allen (1971), however, concluded that peaks of similar relative retention times were branched fatty acids with 16 and 18 carbon atoms. The relative percentages of these two fatty acids in all the lipid fractions examined by Hood and Allen (1971) agreed closely with the percentages observed in this study for peaks C:X and C:Y. Branched chain fatty acids are of widespread occurrence in phospholipids (Williams and Chapman, 1970) and C:X and C:Y were present in significant amounts only in the phospholipid extracts. Consequently, the most probable identifications of C:X and C:Y are C16:Br and C18:Br, respectively.

The fatty acids C14:0, C16:0, C16:1, C18:0, C18:1, and C18:2 accounted for over ninety percent of the total fatty acids in all but the phospholipid fractions, where these fatty acids amounted to approximately eighty percent of the total. These six major fatty acids were analysed statistically for the main effects of breed, sex and anatomical location, as well as for interaction effects. Tables 6, 7 and 8 summarize the results of the analysis of variance for the neutral, phospholipid, and free fatty acid fractions, respectively. A 0.05 level of significance was used throughout this study.

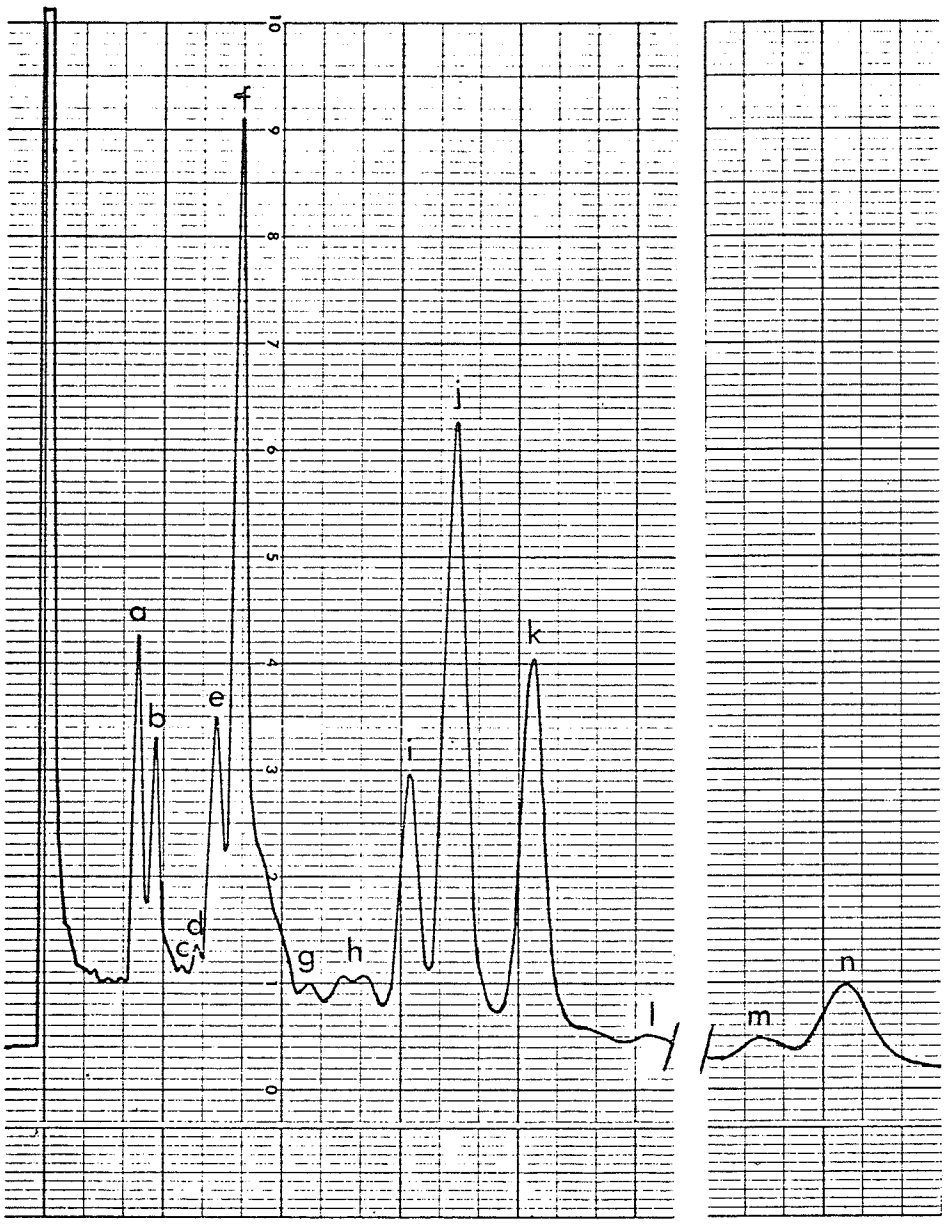
Figure 1. A Typical Chromatogram of the Fatty Acid Methyl Esters from the Neutral Fraction of Bovine Intramuscular Lipid.



Legend

a	C14:0	d	C16:0	h	C18:0
b	C14:1	e	C16:1	i	C18:1
c	C15:0	f	C17:0	j	C18:2
		g	C : Y		

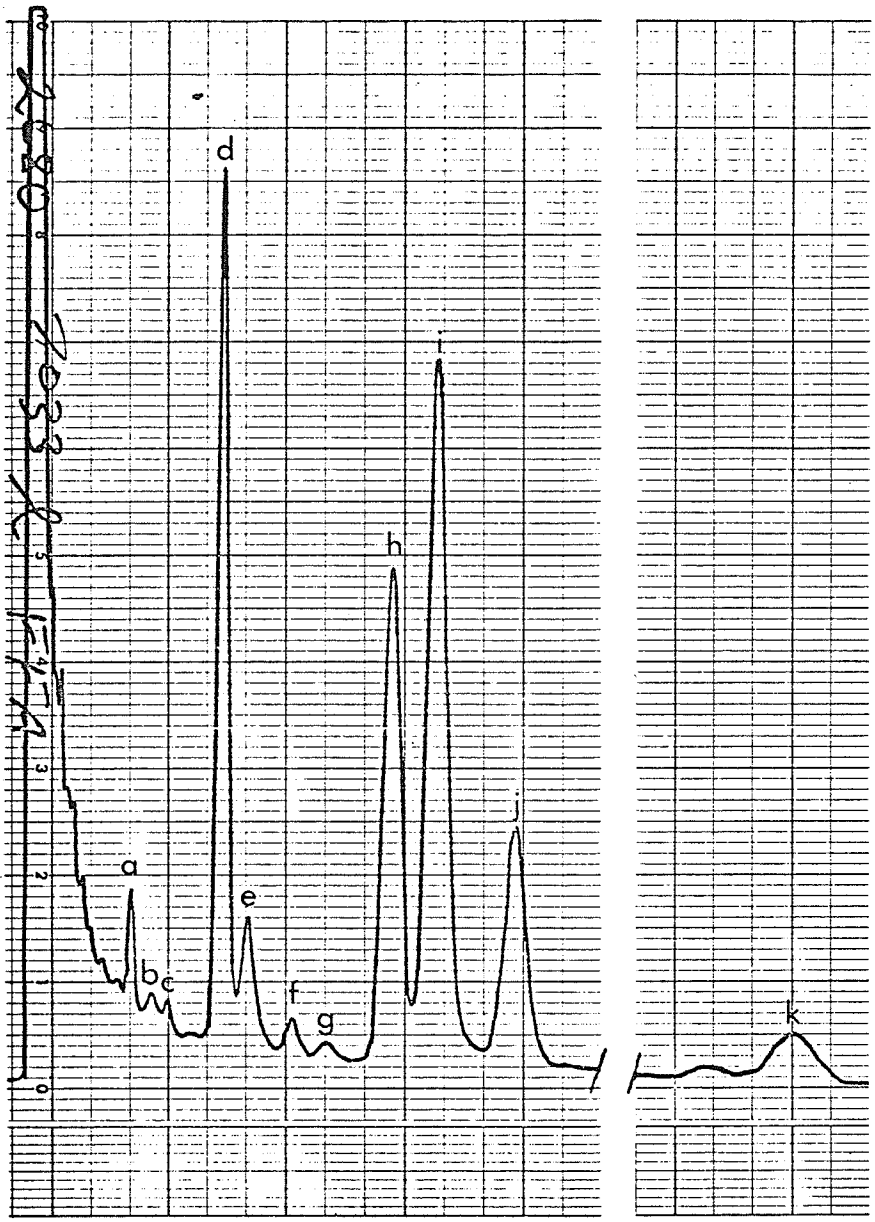
Figure 2. A Typical Chromatogram of the Fatty Acid Methyl Esters from the Phospholipid Fraction of Bovine Intramuscular Lipid.



Legend

a	C13:0	f	C16:0 + C16:1	j	C18:1
b	C14:0			k	C18:2
c	C14:1	g	C17:0	l	C18:3
d	C15:0	h	C : Y	m	C22:0
e	C : X	i	C18:0	n	C20:4

Figure 3. A Typical Chromatogram of the Fatty Acid Methyl Esters from the Free Fatty Acid Fraction of Bovine Intramuscular Lipid.



Legend

a	C14:0	e	C16:1	h	C18:0
b	C14:1	f	C17:0	i	C18:1
c	C15:0	g	C : Y	j	C18:2
d	C16:0			k	C20:4

Table 6

Analysis of variance for Six Fatty Acids of the Neutral Fraction of Bovine Intramuscular Lipid

Source	d.f.	Fatty Acids											
		C14:0		C16:0		C16:1		C18:0		C18:1		C18:2	
		MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Crossbreed	5	0.78	1.83	15.32	2.22	0.96	0.96	3.35	0.62	7.24	0.53	3.08	2.16
Sex	1	0.56	1.33	14.86	2.15	0.01	0.01	3.47	0.65	23.38	1.70	0.08	0.06
BxS	5	1.18	2.80	13.30	1.93	1.68	1.67	5.23	0.97	9.02	0.67	0.93	0.65
Among Aminals	12	0.42		6.90		1.00		5.37		13.73		1.42	
Muscle	1	4.81	5.68*	93.80	14.82*	33.67	40.36*	101.79	36.45*	17.64	0.84	4.32	2.13
BxM	5	0.77	0.91	7.08	1.12	0.19	0.23	0.92	0.33	30.00	1.43	1.95	0.96
SxM	1	0.75	0.89	25.67	4.05	0.70	0.84	0.00	0.00	63.71	3.04	0.30	0.15
BxSxM	5	0.51	0.60	6.51	1.03	0.50	0.60	1.48	0.53	19.89	0.95	1.00	0.49
AxM within Crossbreed and Sex	12	0.85		6.33		0.83		2.79		20.95		2.03	

* Significant ($P<0.05$)

Table 7

Analysis of variance for Six Fatty Acids of the Phospholipid Fraction of Bovine Intramuscular Lipid

Source	d.f.	Fatty Acids C14:0		C16:0+C16:1		C18:0		C18:1		C18:2	
		MS	F	MS	F	MS	F	MS	F	MS	F
Crossbreed	5	7.63	1.62	3.96	0.85	4.67	1.71	66.03	6.01*	19.08	1.32
Sex	1	0.02	0.00	6.31	1.35	15.19	5.55*	0.48	0.04	1.84	0.13
BxS	5	6.56	1.40	7.09	1.52	8.56	3.13*	0.15	0.56	19.45	1.35
Among Animals	12	4.70		4.67		2.73		10.99		14.40	
Muscle	1	134.67	34.96*	4.08	0.73	0.67	0.30	0.01	0.00	103.84	16.76*
BxM	5	9.44	2.45	10.46	1.88	3.51	1.60	12.34	1.85	20.36	3.28*
SxM	1	2.94	0.76	13.96	2.51	20.40	9.27*	7.75	1.16	2.25	0.36
BxSxM	5	4.67	1.21	2.22	0.40	8.08	3.67*	12.37	1.86	9.57	1.54
AxM within Crossbreed and Sex	12	3.85		5.56		2.20		6.66		6.20	

* Significant ($P<0.05$)

Table 8

Analysis of variance for Six Fatty Acids of the Free Fatty Acid Fraction from Bovine Intramuscular Lipid

Source	d.f.	Fatty Acids											
		C14:0		C16:0		C16:1		C18:0		C18:1		C18:2	
		MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Crossbreed	5	5.44	1.53	9.07	0.53	4.93	2.00	10.42	0.82	81.99	1.10	32.28	1.88
Sex	1	0.00	0.00	1.17	0.07	0.00	0.00	0.56	0.04	11.90	0.16	4.56	0.27
BxS	5	5.44	1.53	4.08	0.24	1.59	0.64	16.45	1.30	32.90	0.44	6.51	0.38
Among Animals	12	3.55		17.09		2.46		12.67		74.21		17.15	
Muscle	1	17.52	3.04	10.92	0.43	1.80	0.43	234.97	20.64*	11.70	0.49	22.96	1.90
BxM	5	8.40	1.46	7.93	0.31	0.80	0.19	16.08	1.41	79.89	3.35*	23.91	1.98
SxM	1	3.52	0.61	4.75	0.19	5.68	1.35	1.92	0.17	52.71	2.21	11.02	0.91
BxSxM	5	5.32	0.92	2.82	0.11	0.03	0.01	6.19	0.54	43.96	1.84	9.82	0.81
AxM within Crossbreed and Sex	12	5.76		25.42		4.21		11.38		23.87		12.09	

* Significant ($P<0.05$)

Neutral Fraction

Effect of anatomical location: Significant differences were observed for C14:0, C16:0, C16:1 and C18:0. The biceps femoris had higher levels of C14:0, C16:0, and C16:1, and lower levels of C18:0 than the longissimus dorsi. Table 9 gives the total fatty acid composition of the neutral fraction of the intramuscular lipid at two locations. Mean comparisons of the six major acids are presented in Table 10.

Gillis (1972) studying bulls and steers of the same six crossbreeds, reported similar compositional differences for the total intramuscular lipid from the biceps femoris and longissimus dorsi. Although these differences were not always of the same magnitude as were observed for the neutral fraction, they were consistently in the same direction: more C14:0, C16:0, C16:1 and C18:2 in the biceps femoris, but more C18:0 and C18:1 in the longissimus dorsi (Gillis, 1972). The unsaturated/saturated ratio for the neutral extract from the biceps femoris was 1.12 compared to 1.08 from the longissimus dorsi, indicating a slightly greater degree of unsaturation in the biceps femoris. Comparable ratios for the total intramuscular lipids were reported by Gillis (1972) to be 1.30 and 1.15, respectively.

Terrell and Bray (1969) suggested that the differences in the proportions of neutral fraction fatty acids in the triceps brachii, transversus abdominus, and psoas major were a result of the different metabolic functions of the respective muscles. This could also be true for the biceps femoris and longissimus dorsi, since the former is primarily a locomotor muscle which engages in sustained activity, while the latter is used for faster, less sustained movement.

Table 9

Fatty Acid Composition of the Intramuscular Neutral Fraction from Two Sexes and Two Anatomical Locations[a]

	Sex		Anatomical Location	
Fatty Acid	Bull	Steer	Biceps Femoris	Longissimus Dorsi
C14:0	4.3	4.0	4.5	3.8
C14:1	1.7	1.5	1.9	1.3
C15:0	1.2	1.2	1.5	1.0
C16:0	25.2	24.1	27.7	26.1
C16:1	5.7	5.8	6.6	4.9
C17:0	2.0	2.2	2.2	2.0
C : Y	1.8	2.1	2.4	1.6
C18:0	12.4	11.9	10.7	13.6
C18:1	40.5	41.9	40.6	41.8
C18:2	2.7	2.6	2.9	2.3
Unsaturated/ Saturated	1.12	1.19	1.12	1.08

[a] Means expressed as relative percentages of ten fatty acids.

Table 10

Mean Comparison of the Six Major Fatty Acids of the Intramuscular Neutral Fraction from Two Anatomical Locations[a]

Fatty Acid	Anatomical Location	
	Biceps Femoris	Longissimus Dorsi
C14:0	4.5	3.8
C16:0	27.7	26.1
C16:1	6.6	4.9
C18:0	10.7	13.6
C18:1	40.6	41.8
C18:2	2.9	2.3
Unsaturated/Saturated[b]	1.12	1.08

[a] Means underscored by the same line are not significantly different ($P < 0.05$)

[b] Ratios based on the total fatty acid composition

The biceps femoris has a higher "red" to "white" fiber ratio than the longissimus dorsi, a typical white muscle (Moody and Cassens, 1968). Metabolic and functional differences between muscles are a reflection of their fiber composition. Comparatively dark muscles, such as the biceps femoris, have greater capillary and mitochondrial density, and are richer in oxidative enzymes than lighter muscles which rely more on glycolysis for energy production (Beatty and Bocek, 1970). The differences in fatty acid composition of the neutral lipids from the biceps femoris and longissimus dorsi may be related to their different degrees of dependence on oxidative and glycolylic metabolism.

Effect of crossbreed and sex. The fatty acid composition of the neutral intramuscular lipid from six crossbreeds is shown in Table 11. Similar compositional data for bulls and steers are presented in Table 9. No significant crossbreed or sex effects were observed. Higher levels of C14:0, C16:1 and C18:2 and a lower level of C18:1 in the total intramuscular lipid from bulls were observed by Gillis (1972). However, in this study there appeared to be more C14:0 and less C18:1 in the lipid extracts from bulls, but no difference in C16:1 and C18:2 levels between the two sexes. Fractionation of the intramuscular lipid into neutral and phospholipid fractions may have sufficiently reduced the extent of the variations so that they no longer appeared significant.

The fatty acid patterns for the neutral intramuscular lipid from steers and heifers have been reported by Link et al., (1970c) to be almost identical. Lister (1970) has pointed out that animals

Table 11

Fatty Acid Composition of the Intramuscular Neutral Fraction from Six Bovine Crossbreeds[a]

	Crossbreed					
Fatty Acid	SxH	SxSH	SxA	LxH	LxSH	LxA
C14:0	3.7	4.5	3.9	4.4	4.1	4.3
C14:1	1.5	1.7	1.6	1.9	1.2	1.6
C15:0	1.2	1.5	1.3	1.3	0.8	1.3
C16:0	24.4	24.0	23.2	24.3	27.3	24.7
C16:1	5.8	5.7	5.5	5.7	5.6	6.4
C17:0	2.3	2.1	2.2	2.2	1.7	2.1
C : Y	2.2	2.1	2.1	2.0	1.4	2.0
C18:0	12.5	11.9	11.5	13.0	12.1	11.0
C18:1	41.9	40.3	41.7	39.9	42.4	41.1
C18:2	2.8	3.4	1.9	2.4	2.0	3.2
Unsaturated/ Saturated	1.12	1.37	1.28	1.18	1.24	1.34

[a] Means expressed as relative percentages of ten fatty acids.

raised primarily for their meat are generally slaughtered at a relatively young age, often before attaining full sexual maturity. The effects of hormonal differences may not be as great as they would be in older animals. Data presented by Laurie (1966) on the chemical constituents of the longissimus dorsi muscles from twelve month old bulls and steers of the Ayrshire-Red Poll breed, indicated differences only in the amount of intramuscular lipid present. The steers used in this study were castrated at six and one half months of age, and were slaughtered at twelve to fourteen months (1000 ± 20 lbs.). Although marked developmental and conformational changes result from castration, the chemical composition of muscle and adipose tissue appears to be affected to a lesser degree. Compositional differences may, however, increase with age in a similar way to that observed for sensory related differences (Field et al., 1966).

Phospholipid Fraction.

Effect of anatomical location: Significant differences were observed for C14:0 and C18:2. The longissimus dorsi contained much more C18:2 and slightly larger amounts of the other long chain unsaturated fatty acids than the biceps femoris, which had more C14:0. The composition of the phospholipid fraction for the two locations is given in Table 12. Mean comparisons of the major fatty acids are given in Table 13.

Phospholipids are essential to the structure of cellular and intracellular membranes (Masoro, 1968). The observed variations in phospholipid composition at the two muscle locations may be related to contrasting densities of intracellular membranes. Mitochondria are

Table 12

Fatty Acid Composition of the Intramuscular Phospholipid Fraction from Two Sexes and Two Anatomical Locations[a].

	Sex		Anatomical Location	
Fatty Acid	Bull	Steer	Biceps Femoris	Longissimus Dorsi
C13:0	5.7	5.9	6.3	5.2
C14:0	7.4	7.4	9.1	5.8
C14:1	0.6	0.6	1.0	0.2
C15:0	1.5	2.6	2.2	1.9
C : X	5.2	6.2	4.6	6.8
C16:0 + C16:1	24.1	23.4	24.0	23.4
C17:0	1.7	1.1	1.3	1.5
C : Y	4.4	3.4	3.6	4.2
C18:0	8.8	10.0	9.4	9.4
C18:1	18.8	19.1	18.6	18.9
C18:2	16.9	16.6	15.2	18.2
C18:3	0.6	0.4	0.4	0.7
C22:0	0.2	0.4	0.3	0.3
C20:4	2.9	3.8	3.1	3.6

[a] Means expressed as relative percentages of 15 fatty acids.

Table 13

Mean Comparison of the Six Major Fatty Acids of the Intramuscular Phospholipid Fraction from Two Anatomical Locations and Two Sexes.[a]

Fatty Acid	Anatomical Location		Bull	Steer
	Biceps Femoris	Longissimus Dorsi		
C14:0	9.1	5.8	7.4	7.4
C16:0 + C16:1	24.0	23.4	24.1	23.4
C18:0	1.3	1.5	8.8	10.0
C18:1	18.6	18.9	18.8	19.1
C18:2	15.2	18.2	16.9	16.6

[a] Means underscored by the same line are not significantly different ($P<0.05$)

much more concentrated in red than in white fibers, while white fibers have a more highly developed sacroplasmic reticulum. The color of the muscle fiber actually results from these differences (Peachey, 1970). Phospholipid fatty-acid patterns in mitochondria and sarcoplasmic reticulum could be responsible for the differences observed between the biceps femoris and longissimus dorsi.

The relationship between flavor characteristics and bovine intramuscular lipids was investigated by Hornstein et al., (1961). They reported a more rapid development of rancidity in the phospholipid than the neutral extract, and attributed it to the presence of more long chain unsaturated fatty acids. The longissimus dorsi with its higher percentage of long chain unsaturates may be more susceptible to rancidity development during processing and storing than the biceps femoris.

Effect of crossbreed and sex. The fatty acid composition of the phospholipid fraction for six crossbreeds is presented in Table 14. A significant crossbreed effect was observed for C18:1. Mean comparisons shown in Table 15 indicate that the Limousin x Angus crossbreed had a much lower level of C18:1 than any of the other crossbreeds. No similar or contrary trend was observed in the neutral or free fatty acid fractions. This lower mean value in the Limousin x Angus did not result from one or two abnormally low values, but was rather the result of a lower range of values. Seven of the eight Limousin x Angus samples examined had less C18:1 than the mean values for any of the other crossbreeds.

Table 14

Fatty Acid Composition of the Intramuscular Phospholipid Fraction from Six Bovine Crossbreeds[a]

	Crossbreed					
Fatty Acid	SxH	SxSH	SxA	LxH	LxSH	LxA
C13:0	5.2	6.2	6.4	5.1	5.1	6.8
C14:0	6.7	8.0	7.4	6.9	6.6	9.1
C14:1	0.1	0.8	0.6	0.6	0.1	1.6
C15:0	2.4	1.9	2.8	1.6	1.6	2.0
C : X	4.2	5.6	6.8	5.8	5.5	6.3
C16:0 + C16:1	23.8	23.8	22.8	23.0	24.4	24.5
C17:0	1.6	0.9	1.2	1.4	1.7	1.7
C : Y	3.4	3.5	3.6	4.3	3.5	4.5
C18:0	9.3	9.4	9.3	10.5	9.8	8.2
C18:1	21.9	18.1	25.5	19.0	19.3	13.3
C18:2	16.4	17.6	13.7	17.1	17.3	17.9
C18:3	0.8	0.3	0.5	0.7	0.3	0.6
C22:0	0.5	0.1	0.3	0.3	0.4	0.2
C20:4	3.8	3.4	3.1	3.5	4.2	2.1

[a] Means expressed as relative persentages of 15 fatty acids.

Table 15

Mean Comparison of Oleic Acid in the Phospholipid Fractions from Six Bovine Crossbreeds[1]

	C18:1					
Crossbreed	Limousin x Angus	Simmental x Shorthorn	Limousin x Hereford	Limousin x Shorthorn	Simmental x Angus	Simmental x Hereford
Relative Percentage	13.3	18.1	19.0	19.3	21.5	21.9

[1] Means underlined are not significantly different ($P < 0.05$)

Significant sex differences were observed for C18:0. The fatty acid composition of the phospholipid fraction for bulls and steers is tabulated in Table 12. Mean comparisons for the major fatty acids presented in Table 13 show that bulls had significantly more C18:0 than steers.

Sex-related differences in C18:0 were reported by Hood and Allen (1971), who observed that the phospholipid extract from heifer longissimus dorsi had more C18:0 than that of bulls or steers. These results suggest a possible hormonal influence on this particular fatty acid. Terrell (1967) found a significant sex effect only in the levels of C14:0, which was present in slightly higher amounts in the phospholipid extract of heifers than steers (steers 1.48%, heifers 1.73%).

Interaction effects. Significant sex x muscle, breed x sex, and breed x sex x muscle interactions were observed for C18:0. There appeared to be more C18:0 in the biceps femoris of steers than of bulls, but more C18:0 in the longissimus dorsi of bulls than of steers, which resulted in a sex x muscle interaction. The breed x sex interaction occurred because there were lower levels of C18:0 in bulls of the Limousin x Hereford crossbreed and higher levels of this fatty acid in steers of the same breed, than in the other crossbreeds.

The breed x sex x muscle interaction appeared to occur because in two crossbreeds, the Simmental x Angus and the Limousin x Hereford, the amounts of C18:0 in the biceps femoris of bulls was much less than in that of steers. Levels of C18:0 in the longissimus dorsi were similar for both sexes throughout all six crossbreeds.

Free Fatty Acid Fraction.

The composition of the intramuscular free fatty acid fraction from the biceps femoris and longissimus dorsi are shown in Table 16. The comparison of means shown in Table 17 indicates that the longissimus dorsi had considerably more C18:0 than the biceps femoris. Neutral lipid from the longissimus dorsi was also higher in C18:0, which may indicate that the two fractions are to some extent interdependent.

Several investigators have reported higher levels of free fatty acids in meat after aging (Pearson, 1968; Hood and Allen, 1971). The increases have been attributed to lipolytic enzyme activity still present in postmortem muscles. Both triglyceride lipases and phospholipases are known to be active in living muscle tissue (Masoro, 1968; Jensen, 1971). Changes in free fatty acid composition over an extended aging period have been attributed more to phospholipase activity than to the hydrolysis of triglycerides (Hood and Allen, 1971). However, this would not explain the higher levels of C18:0 in the longissimus dorsi which were observed in this study, since the phospholipid extracts from this muscle were not higher in C18:0 than those from the biceps femoris.

Lipases specifically hydrolyse free fatty acids at the α position prior to those at the β position (Jensen, 1971), and C18:0 has been reported to be preferentially esterified at the α position in bovine lipids (Brockerhoff, 1966). Enzymatic hydrolysis may therefore result in a more rapid release of C18:0 than of other fatty acids.

Table 16

Fatty Acid Composition of the Intramuscular Free Fatty Acid Fraction From Two Sexes and Two Anatomical Locations[a]

	Sex		Anatomical Location	
Fatty Acid	Bull	Steer	Biceps Femoris	Longissimus Dorsi
C14:0	5.2	5.1	5.8	4.5
C14:1	2.2	1.4	2.0	1.7
C15:0	1.6	1.9	1.9	1.5
C16:0	21.2	20.8	20.6	21.5
C16:1	4.7	4.7	4.9	4.5
C17:0	1.8	1.8	1.9	1.8
C : Y	1.2	1.4	1.4	1.2
C18:0	15.3	15.6	13.2	17.7
C18:1	31.4	32.4	32.4	31.4
C18:2	11.2	10.5	11.5	10.2
C20:4	1.7	1.8	1.5	2.0
Unsaturated/ Saturated	1.14	1.12	1.20	1.06

[a] Means expressed as relative percentages of eleven fatty acids

Table 17

Mean Comparison of the Six Major Fatty Acids of the Intramuscular Free Fatty Acid Fraction from Two Anatomical Locations[a]

Fatty Acid	Anatomical Location	
	Biceps Femoris	Longissimus Dorsi
C14:0	5.8	4.5
C16:0	20.6	21.5
C16:1	4.9	4.5
C18:0	13.2	17.7
C18:1	32.4	31.4
C18:2	11.5	10.2
Unsaturated/Saturated[b]	1.20	1.06

[a] Means underscored by the same line are not significantly different ($P < 0.05$)

[b] Ratios based on the total fatty acid composition

This would explain the much higher level of C18:0 in the free fatty acid fraction of the longissimus dorsi whose neutral fraction was richer in C18:0 than the neutral lipid of the biceps femoris.

Effect of crossbreed and sex. No significant crossbreed and sex effects were observed for the free fatty acid fraction. The composition of this fraction for six bovine crossbreeds is shown in Table 18. The free fatty acids present in the intramuscular lipid of bulls and steers are presented in Table 16. Limousin crossbreeds tended to have higher levels of the C18 fatty acids than Simmental crossbreeds, although no significant differences were apparent. The lack of variation due to either crossbreed or sex again suggests a relationship with the neutral intramuscular lipid in which no significant crossbreed or sex effects were apparent.

The differences due to sex observed by Hood and Allen (1971) may have occurred because some of the meat samples used were aged for fourteen and twenty-one days. It was reported by Hood and Allen (1971) that free fatty acid levels increased more rapidly in bulls than in steers. Initial differences in fatty acid composition may have been intensified during these long storage periods.

Interaction effects. A small breed x muscle interaction resulted from exceptionally high levels of C18:1 in the Limousin x Shorthorn biceps femoris as well as in the Simmental x Angus longissimus dorsi, although neither breed nor muscle location were significant in themselves.

Table 18

Fatty Acid Composition of the Intramuscular Free Fatty Acid Fraction from Six Bovine Crossbreeds[a]

	Crossbreed					
Fatty Acid	SxH	SxSH	SxA	LxH	LxSH	LxA
C14:0	4.2	4.7	5.8	5.0	4.7	6.4
C14:1	1.8	2.0	2.8	2.1	1.7	2.2
C15:0	1.2	1.5	1.3	1.3	0.8	1.3
C16:0	21.4	20.6	20.4	20.1	19.9	22.7
C16:1	5.7	3.9	5.6	4.4	4.4	4.2
C17:0	2.3	2.0	2.1	2.0	1.4	2.0
C : Y	2.2	2.0	2.1	2.0	1.4	2.0
C18:0	14.2	14.9	14.8	17.3	15.2	16.4
C18:1	28.3	34.8	27.8	34.6	31.6	34.2
C18:2	9.7	13.5	7.8	11.4	10.4	12.2
C20:4	1.8	1.8	0.7	2.6	1.6	2.0
Unsaturated/ Saturated	1.09	1.28	1.01	1.21	1.18	1.12

[a] Means expressed as relative percentages of 11 fatty acids.

Subcutaneous Lipid

Fatty Acid Composition

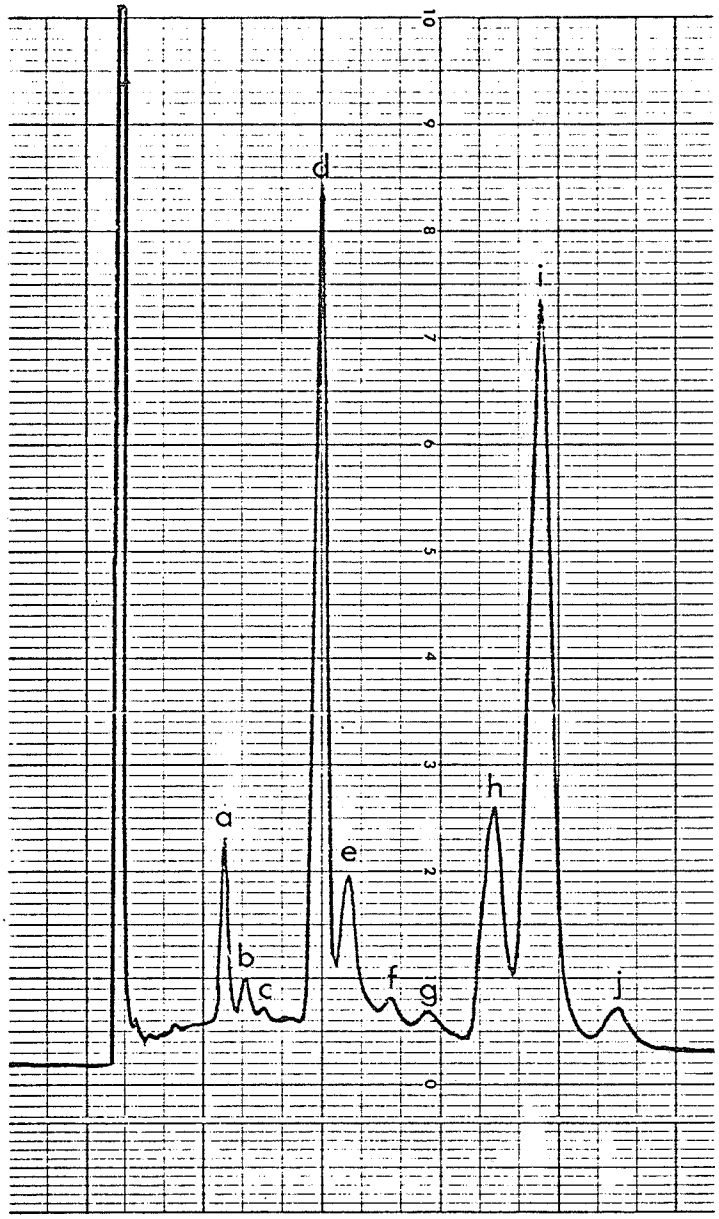
Fatty acids were identified as described in the intramuscular lipid section. Typical chromatograms of the neutral and free fatty acid methyl esters are shown in Figures 4 and 5. Mean squares and F values from the analysis of variance for the neutral fraction are presented in Table 19 and those for the free fatty acid fraction in Table 20.

Neutral Fraction

Effect of anatomical location. Significant differences between anatomical locations were observed for all of the major fatty acids except C18:2. The biceps femoris differed from both the exterior and interior layers of the longissimus dorsi in having less C14:0, C16:0 and C18:0, and more C16:1 and C18:1. Table 21 shows the total fatty acid composition of the neutral fraction from the biceps femoris and the longissimus dorsi, and Table 22 gives the mean comparison of the six major fatty acids.

The unsaturated/saturated ratio was 1.47 for the biceps femoris, compared to the ratios of 1.18 and 1.12 for the exterior and interior layers of the longissimus dorsi. The corresponding unsaturated/saturated ratios reported by Gillis (1972) for the total subcutaneous lipid were almost identical, being 1.47, 1.21, and 1.13, respectively. A comparison of the total amounts of C16 and C18 fatty acids present in these fractions eliminated the variation observed among the depot sites as illustrated in Table 23.

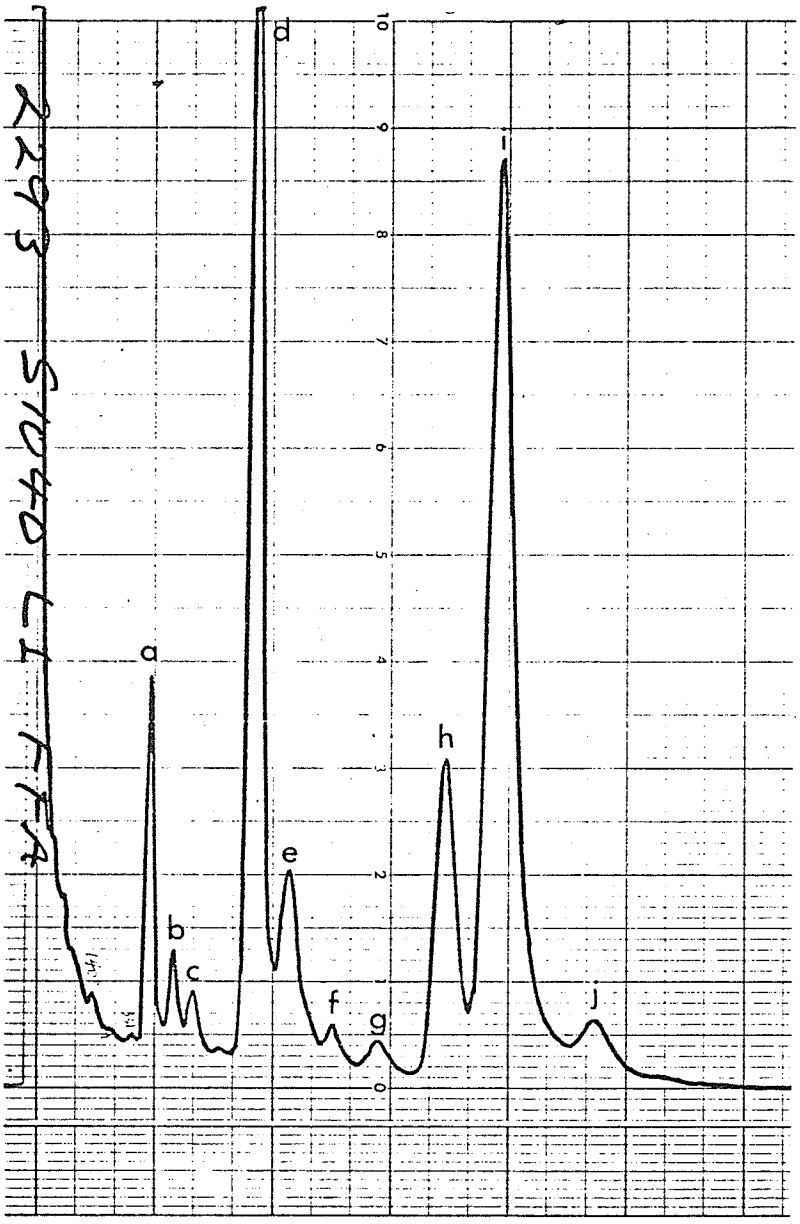
Figure 4. A Typical Chromatogram of the Fatty Acid Methyl Esters from the Neutral Fraction of Bovine Subcutaneous Lipid



Legend

a	C14:0	d	C16:0	h	C18:0
b	C14:1	e	C16:1	i	C18:1
c	C15:0	f	C17:0	j	C18:2
		g	C : Y		

Figure 5. A Typical Chromatogram of the Fatty Acid Methyl Esters from the Free Fatty Acid Fraction of Bovine Subcutaneous Lipid



Legend

a	C14:0	d	C16:0	h	C18:0
b	C14:1	e	C16:1	i	C18:1
c	C15:0	f	C17:0	j	C18:2
		g	C : Y		

Table 19

Analysis of variance for Six Fatty Acids of the Neutral Fraction of Bovine Subcutaneous Lipid

Source	d.f.	Fatty Acids											
		C14:0		C16:0		C16:1		C18:0		C18:1		C18:2	
		MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Crossbreed	5	0.94	1.83	10.76	2.86	1.95	2.71	12.86	3.71*	14.12	1.97	5.93	4.64*
Sex	1	0.52	1.12	7.27	1.93	1.68	2.33	4.65	1.34	6.30	0.88	0.46	0.36
BxS	5	0.78	1.69	0.26	0.07	0.85	1.18	3.05	0.88	19.45	2.71	2.92	2.29
Among Animals	12	0.46		3.76		0.72		3.46		7.18		1.28	
Muscle	2	1.64	14.80*	28.10	24.87*	37.64	44.81*	131.72	107.90*	78.93	27.98*	0.18	0.21
BxM	10	0.08	0.75	0.42	0.37	0.64	0.76	1.33	1.09	1.33	0.47	0.33	0.40
SxM	2	0.04	0.41	0.00	0.00	0.42	0.50	1.88	1.54	1.58	0.56	0.18	0.22
BxSxM	10	0.20	1.83	1.38	1.22	0.65	0.77	2.18	1.79	3.46	1.23	0.81	0.97
AxM within Crossbreed and Sex	24	0.11		1.13		0.84		1.22		2.82		0.84	

* Significant ($P<0.05$)

Table 20

Analysis of variance for Six Fatty Acids of the Free Fatty Acid Fraction of Bovine Subcutaneous Lipid

Source	d.f.	Fatty Acids											
		C14:0		C16:0		C16:1		C18:0		C18:1		C18:2	
		MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Crossbreed	5	1.00	0.42	7.58	0.72	3.81	0.76	1.92	0.33	15.36	0.37	3.30	4.21*
Sex	1	1.36	0.57	5.89	0.56	4.30	0.86	0.59	0.10	1.50	0.04	1.23	1.56
BxS	5	2.55	1.06	7.91	0.75	4.67	0.93	4.85	0.83	36.24	0.88	3.13	3.99*
Among Animals	12	2.39		10.59		5.00		5.87		41.12		0.78	
Muscle	2	52.38	17.55*	137.52	14.84*	71.25	47.38*	113.20	35.58*	550.05	20.07*	26.01	17.22*
BxM	10	1.50	0.50	3.90	0.42	1.56	1.04	1.43	0.45	21.52	0.78	1.66	1.10
SxM	2	1.44	0.48	7.32	0.79	2.46	1.64	2.14	0.67	115.66	4.22*	1.42	0.94
BxSxM	10	1.29	0.43	8.02	0.87	1.53	1.02	2.20	0.69	30.40	1.10	1.15	0.76
AxM within Crossbreed and Sex	24	2.98		9.26		1.50		3.18		27.41		1.51	

* Significant ($P<0.05$)

Table 21

Fatty Acid Composition of the Subcutaneous Neutral Fraction from Two Sexes and Two Anatomical Locations[a]

	Sex		Anatomical Location		
Fatty Acid	Bull	Steer	Biceps Femoris	Longissimus Dorsi Exterior	Longissimus Dorsi Interior
C14:0	4.2	4.1	3.8	4.2	4.3
C14:1	1.6	1.6	1.8	1.6	1.5
C15:0	0.8	0.8	0.8	0.8	0.8
C16:0	24.8	24.5	23.5	25.2	25.3
C16:1	6.4	6.7	8.0	5.9	5.7
C17:0	2.0	2.1	2.0	2.0	2.0
C : Y	1.8	2.0	2.3	1.7	1.7
C18:0	12.1	11.6	9.2	12.7	13.6
C18:1	42.1	42.7	44.4	42.0	40.8
C18:2	3.8	3.3	3.6	3.5	3.6
Unsaturated/ Saturated	1.22	1.27	1.47	1.18	1.12

[a] Means expressed as relative percentages of 10 fatty acids.

Table 22

Mean Comparison of the Six Major Fatty Acids of the Subcutaneous Neutral Fraction from Two Anatomical Locations[a]

Fatty Acid	Anatomical Location		
	Biceps Femoris	Longissimus Dorsi Exterior	Longissimus Dorsi Interior
C14:0	3.8	4.2	4.3
C16:0	23.5	25.2	25.3
C16:1	8.0	5.9	5.7
C18:0	9.2	12.7	13.6
C18:1	44.4	42.0	40.8
C18:2	3.6	3.5	3.6
Unsaturated/Saturated[b]	1.47	1.18	1.12

[a] Means underscored by the same line are not significantly different ($P<0.05$)

[b] Ratios based on the total fatty acid composition

Table 23

Mean Comparisons of the Total C16 and C18 Fatty Acids of the Subcutaneous Neutral Lipid from the Biceps Femoris and the Exterior and Interior Layers of the Longissimus Dorsi[1]

Total	Biceps Femoris	Longissimus Dorsi	
		Exterior	Interior
C16	31.5	31.1	31.0
C18	57.2	58.2	58.0

[1] Means expressed as a relative percentage of the total fatty acids measured

The ratio of unsaturated to saturated fatty acids in adipose tissue appears to be maintained by conversion of saturates to unsaturates of the same chain length. Desaturase enzymes may be responsible for the conversion of saturated fatty acids to the more unsaturated forms. This reaction is mediated in animal tissue by enzyme systems of the microsomal fractions of the cell. Monoenoic acids may be desaturated to dienoic, although not to linoleic acid (Wakil, 1970).

Depot site has been shown to influence the composition of both pork and beef adipose tissues (Ostrander and Duggan, 1962; Chacko and Perkins, 1965; Terrell et al., 1967, 1969b). Many reasons have been suggested for the variations encountered at different depot sites. The importance of the particular site as an energy source for adjacent muscle, or in providing insulation, may influence its composition. In addition, the physiological maturity of the animal influences the rate of fat deposition and of metabolic exchange at each location (Thrall and Cramer, 1971b). The fat covering the biceps femoris was much less developed than that over the longissimus dorsi for the animals studied, probably reflecting a different degree of depot site maturity and of metabolic interchange.

Significant differences were observed for C18:0 and C18:1 between the exterior and interior layers of the longissimus dorsi. The exterior layer was higher in C18:1 but lower in C18:0 and therefore less saturated than the interior layer. These results were in agreement with the higher unsaturated/saturated ratio of the total subcutaneous lipid from the exterior layer observed by Gillis

(1972). Other researchers have also reported a higher degree of unsaturation in the exterior fat layer (Terrell et al., 1967; Thrall and Cramer, 1971a). Terrell (1967) suggested that the more unsaturated external layer may be more readily mobilized and more metabolically active than the internal layer of the subcutaneous fat.

Effect of crossbreed and sex. Significant crossbreed effects were observed for C18:0 and C18:2. Limousin x Hereford and Simmental x Hereford crossbreeds had significantly more C18:0 than the Limousin x Angus; while the Simmental x Shorthorn crossbreed had significantly more C18:2 than the other crossbreeds with the exception of the Limousin x Angus. The total fatty acid composition of the neutral lipid from six crossbreeds is presented in Table 24. Mean comparisons for the two fatty acids which differed significantly are shown in Table 25.

The three Simmental crossbreeds had more C18:0 than the corresponding Limousin crossbreeds. In addition to this sire effect, it was apparent that there was an important cow effect, since both crossbreeds with significantly higher levels of C18:0 were Hereford crosses. A similar sire effect was reported by Gillis (1972) for the total subcutaneous lipid extracts.

The fatty acid composition of the subcutaneous neutral fractions of bulls and steers is given in Table 21. No significant sex differences were evident, although the slight variations present correspond with those found by Gillis (1972) for five of the major fatty acids of the subcutaneous lipid of bulls and steers. Other studies reporting the effect of sex on bovine subcutaneous lipid have

Table 24

Fatty Acid Composition of the Subcutaneous Neutral Fraction from Six Bovine Crossbreeds[a]

	Crossbreed					
Fatty Acid	SxH	SxSH	SxA	LxH	LxSH	LxA
C14:0	4.4	3.8	3.8	4.5	4.2	4.2
C14:1	1.7	1.5	1.6	1.7	1.5	1.7
C15:0	0.9	0.7	0.7	0.5	0.7	0.8
C16:0	25.8	23.5	24.2	24.7	25.5	24.2
C16:1	6.1	6.6	6.4	6.3	6.5	7.2
C17:0	2.1	2.0	2.1	2.1	1.9	2.0
C : Y	1.7	2.0	2.0	1.9	1.9	2.8
C18:0	13.0	11.6	12.1	12.9	11.2	10.3
C18:1	40.9	42.8	43.8	41.3	42.8	42.8
C18:2	2.9	4.8	3.0	3.5	3.3	3.9
Unsaturated/ Saturated	1.18	1.16	1.20	1.10	1.11	1.27

[a] Means expressed as relative percentages of ten fatty acids.

Table 25

Mean Comparison of Stearic and Linoleic Acids in the Subcutaneous Neutral Fractions from Six Bovine Crossbreeds[1]

C18:0

Crossbreed	Limousin x Angus	Limousin x Shorthorn	Simmental x Shorthorn	Simmental x Angus	Limousin x Hereford	Simmental x Hereford
Relative Percentage	10.3	11.2	11.6	12.1	12.9	13.0

C18:2

Crossbreed	Simmental x Hereford	Simmental x Angus	Limousin x Shorthorn	Limousin x Hereford	Limousin x Angus	Simmental x Shorthorn
Relative Percentage	2.9	3.0	3.3	3.5	3.9	4.8

[1] Means underlined are not significantly different ($P<0.05$)

usually compared bulls or steers with heifers (Link et al., 1970b; Terrell et al., 1969a; Thrall and Cramer,(1971a). In the one reported comparison of the neutral subcutaneous lipids of bulls and steers, none of the fatty acids differed significantly (Hood and Allen, 1971)

Free Fatty Acid Fraction

Effect of anatomical location: Significant differences due to anatomical location were observed for the six major fatty acids present. Table 26 gives the fatty acid composition of the subcutaneous free fatty acid fraction from the biceps femoris and longissimus dorsi muscles. Mean comparisons of the major fatty acids are presented in Table 27.

The biceps femoris had significantly less C16:0 and C18:0 and more C16:1 than either the exterior or interior layers of subcutaneous fat from the longissimus dorsi. The levels of C14:0, C18:1 and C18:2 in the biceps femoris did not differ from those in the longissimus dorsi exterior fat, but did differ from those of the interior layer. There were pronounced differences in the unsaturated/saturated ratios of the three lipid extracts. The highest ratio, 2.43 was observed for the biceps femoris; and the lowest, 1.35 for the interior layer of the longissimus dorsi. The exterior longissimus dorsi fat had an intermediate ratio of 2.03. The most striking difference observed was in the levels of C18:1. The interior lipid contained 43.7%, compared with approximately 52% in the other two extracts.

The higher unsaturated/saturated ratio in the exterior layer of the longissimus dorsi as compared to that of the interior layer, together with the much higher ratio for the biceps femoris, correspond

Table 26

Fatty Acid Composition of the Subcutaneous Free Fatty Acid Fraction from Two Sexes and Two Anatomical Locations[a]

	Sex		Anatomical Location		
Fatty Acid	Bull	Steer	Biceps Femoris	Longissimus Dorsi	
C14:0	4.4	4.1	3.4	3.4	6.0
C14:1	2.0	2.2	2.2	1.6	2.6
C15:0	1.3	1.6	1.0	1.5	1.9
C16:0	19.7	19.1	17.3	18.9	22.0
C16:1	7.7	8.2	9.8	7.6	6.4
C17:0	1.0	1.2	1.0	1.0	1.2
C : Y	1.4	1.8	2.0	1.5	1.3
C18:0	7.6	7.4	5.4	7.4	9.7
C18:1	49.4	49.1	52.0	52.1	43.7
C18:2	3.7	3.4	4.3	4.1	2.4
Unsaturated/ Saturated			2.43	2.03	1.35

[a] Means expressed as relative percentages of ten fatty acids.

Table 27

Mean Comparison of the Six Major Fatty Acids of the Subcutaneous Free Fatty Acid Fraction from Two Anatomical Locations[a]

Fatty Acid	Anatomical Location		
	Biceps Femoris	Longissimus Dorsi Exterior	Longissimus Dorsi Interior
C14:0	3.4	3.4	6.0
C16:0	17.3	18.9	22.0
C16:1	9.8	7.6	6.4
C18:0	5.4	7.4	9.7
C18:1	52.0	52.1	43.7
C18:2	4.3	4.1	2.4
Unsaturated/Saturated[b]	2.43	2.03	1.35

[a] Means underscored by the same line are not significantly different ($P < 0.05$)

[b] Ratios based on the total fatty acid composition.

to ratios reported for the neutral fraction. The relative percentage composition of the two fractions was not identical, however. There was more C18:1 but less C18:0 and C16:0 in the free fatty acid extracts than in the neutral lipid from all the depot sites.

If, as has been suggested by Hood and Allen (1971), free fatty acids in the subcutaneous fat are derived primarily through the hydrolysis of fatty acids esterified at the αposition, then differences between the neutral and free fatty acid fractions would be a consequence of enzyme specificity. Although enzymes specific for particular fatty acids have not yet been identified in animal tissues, a microbial lipase specific for C18:1 has been isolated (Jensen, 1971). Microbial activity on the surface of the longissimus dorsi and biceps femoris subcutaneous fat could account for the differences in the levels of C18:1 observed in this study.

Effect of crossbreed and sex: The free fatty acid composition of the subcutaneous lipid within six bovine crossbreeds is presented in Table 28. Only C18:2 appeared to vary among the crossbreeds. Mean comparisons for this fatty acid are shown in Table 29. A significantly lower amount of C18:2 was observed in the Simmental x Hereford crossbreed when compared with the Simmental x Shorthorn and Limousin x Angus crossbreeds. Since the only significant breed difference in this fraction occurred for a fatty acid which accounted for four percent or less of the total fatty acids, this result cannot be considered of great significance.

No significant sex effects were observed for this fraction. The free fatty acid composition of the subcutaneous fat

Table 28

Fatty Acid Composition of the Subcutaneous Free Fatty Acid Fraction from Six Bovine Crossbreeds[a]

	Crossbreed					
Fatty Acid	SxH	SxSH	SxA	LxH	LxSH	LxA
C14:0	4.3	3.8	4.2	4.4	4.6	4.2
C14:1	2.1	1.9	2.2	2.5	2.2	2.2
C15:0	1.5	1.2	1.5	1.0	1.3	1.4
C16:0	20.7	19.0	18.7	18.7	19.4	19.9
C16:1	7.5	7.8	7.2	7.9	8.2	8.8
C17:0	1.0	1.0	1.2	0.7	1.3	1.3
C : Y	1.2	1.6	1.1	1.4	1.8	1.9
C18:0	7.6	7.4	8.0	7.7	7.3	6.9
C18:1	49.2	50.2	50.4	49.9	48.2	47.7
C18:2	2.9	4.2	3.1	3.6	3.6	4.1
Unsaturated/ Saturated	1.76	1.98	1.87	1.97	1.83	1.86

[a] Means expressed as relative percentages of ten fatty acids.

Table 29

Mean Comparison of Linoleic Acid in the Subcutaneous Free Fatty Acid Fractions from Six Bovine Crossbreeds[1]

	C18:2					
Crossbreed	Simmental x Hereford	Simmental x Angus	Limousin x Hereford	Limousin x Shorthorn	Limousin x Angus	Simmental x Shorthorn
Relative Percentage	2.9	3.1	3.6	3.6	4.1	4.2

[1] Means underlined are not significantly different ($P < 0.05$)

from bulls and steers is given in Table 26. Higher levels of C16:0 and C18:0 in the free fatty acid extract of bulls than of steers was observed by Hood and Allen (1971). A similar trend was evident in this study, but was not large enough to be significant.

Interaction effects: A sex x muscle interaction was observed for C18:1, because levels of this fatty acid in the longissimus dorsi were lower for bulls than for steers, while no sex differences were evident in the biceps femoris.

The Relative Percentage Recovery of the Free Fatty Acid Fraction as Determined by Liquid Scintillation Counting

The palmitic-1-C^{14} acid which was added to two samples of intramuscular lipid and a solvent blank before fractionation, remained almost exclusively in the free fatty acid fraction. A tabulation of the number of counts in each fraction of the three samples, and their corresponding percentages is given in Table 30.

Approximately 98.0 percent of the palmitic-1-C^{14} acid was recovered in the free fatty acid fraction of the intramuscular lipid samples while 96.4 percent was present in the corresponding blank. These results indicate that the method of separation used throughout this study extracted free fatty acids from the other lipid fractions with a high degree of accuracy.

Several researchers have reported difficulties in achieving clean separations of phospholipids by silicic acid adsorption from a chloroform solution (Kuchmak and Dugan, 1963; Hood and Allen, 1971). By increasing the polarity of the solvent system, replacing chloroform with a chloroform-hexane-diethyl ether (2:1:1 V/V/V) mixture, Hornstein *et al* (1967) reported a complete separation of the neutral fraction from the phospholipids. The free fatty acids were further removed using an anion exchange resin, and no contamination by this fraction was found in the phospholipids. Hood and Allen (1971) used acid-washed florisil to separate their lipid fractions, claiming that the silicic acid could not completely remove the free fatty acids from

Table 30

The Relative Percentage Recovery of Palmitic-1-C^{14} Acid from the Neutral, Phospholipid, and Free Fatty Acid Fractions of Bovine Intramuscular Lipid Samples

Sample	Total Counts Recovered	Neutral Lipid		Phospholipid		Free Fatty Acid	
		Counts	%	Counts	%	Counts	%
1	54,473	590	1.0	762	1.3	56,121	97.6
2	61,394	555	0.9	621	1.0	60,219	98.0
B	57,748	885	1.5	1,142	1.9	55,721	96.4

the phospholipids.

Elution with the more polar solvent mixture in this study to separate the phospholipids on activated silicic acid resulted in a clean separation of this fraction with only negligible amounts of free fatty acids remaining. Since the free fatty acid fraction accounted for less than one percent of the total lipid extracted, compared to ten percent or more for the phospholipid fraction, the contamination by the free fatty acids remaining is of little consequence in the overall analysis of the phospholipids. The method of Hamilton and McDonald (1971) which was used to partition the free fatty acids and the neutral lipids proved to be extremely sensitive, in spite of the small amounts of free fatty acids present in the sample. The combination of the methods of Hornstein et al (1967) for phospholipid extraction, and Hamilton and McDonald (1971) for the partitioning of free fatty acids and neutral lipids provides a rapid, convenient and inexpensive method of separation, eliminating the use of acid-treated florasil or anion exchange resins. The latter sometimes require laborious preparation prior to use.

SUMMARY AND CONCLUSIONS

European breeds of cattle are being introduced into Canada to improve the carcass quality and productivity of existing breeds. Factors which influence growth rates and the deposition of lean tissue can also produce compositional changes which affect the quality of the meat. This study was undertaken to examine the effect of anatomical location, crossbreed and sex on the composition of the neutral, phospholipid and free fatty acid fractions of bovine lipids. The animals used in this study were randomly selected to represent six crossbreeds, by the Canada Department of Agriculture Research Station, Brandon. Determinations of lipid composition were carried out on two muscles and the covering subcutaneous fat, from two bulls and steers of each crossbreed.

Intramuscular lipid from the biceps femoris and longissimus dorsi muscles of twenty-four bovine crossbreeds was partitioned into neutral, phospholipid and free fatty acid fractions. A comparison of the neutral lipid from the two muscles revealed that the biceps femoris had a higher unsaturated/saturated ratio and contained more C14:0, C16:0 and C16:1 but much less C18:0 than the longissimus dorsi. The free fatty acids extracted from the biceps femoris were also less saturated and contained less C18:0 than those from the longissimus dorsi. For the phospholipid fractions, however, this relationship appeared to be reversed. The longissimus dorsi contained considerably more C18:2 and less C14:0 than the biceps femoris, and although unsaturated/saturated ratios could not be calculated for this fraction, because C16:0 and C16:1

were reported as a single value, the proportions of the remaining fatty acids indicated a higher degree of unsaturation in the longissimus dorsi.

Differences between the two locations were more pronounced in the subcutaneous lipid. The neutral and free fatty acid fractions from the biceps femoris were much less saturated than the corresponding fractions from the longissimus dorsi, and the exterior layer of the longissimus dorsi was less saturated than the interior layer. Five of the six major fatty acids in the neutral lipids differed between the two muscles, with the biceps femoris having higher percentages of C16:1 and C18:1 but lower percentages of the three unsaturated fatty acids. A comparison of the longissimus dorsi neutral lipids showed that the exterior layer had less C18:0, more C18:1, and a slightly higher unsaturated/saturated ratio than the interior layer. All six major fatty acids of the subcutaneous free fatty acid fraction varied with respect to depot site. The biceps femoris, as was the case for the neutral lipid, had the highest unsaturated/saturated ratio, more C16:1 and C18:1, and less C18:0 than the longissimus dorsi fractions. Unsaturation was much greater in the exterior than in the interior layer of the longissimus dorsi. The three unsaturated fatty acids, C16:1, C18:1 and C18:2 were present in larger amounts in the exterior layer while the interior layer had more of the saturated fatty acids, C14:0, C16:0 and C18:0.

Breed differences were responsible for fewer variations in the lipid fraction composition than were expected, since crossbreed has been shown to influence the composition of the total intramuscular and subcutaneous lipid. The only significant effect of breed on the intramuscular lipids occurred in the phospholipid fraction. Lower levels of

C18:1 were observed for the Limousin x Angus than for the other crossbreeds.

More differences due to crossbreed were evident in the subcutaneous lipids. The neutral fractions from the Limousin x Hereford and Simmental x Hereford had more C18:0 than Limousin x Angus crossbreeds, and the three Simmental crossbreeds each had higher percentages of C18:0 than the corresponding Limousin animals. More C18:2 was present in the Simmental x Shorthorn subcutaneous neutral lipids than in those of other crossbreeds with the exception of the Limousin x Angus. Simmental x Shorthorn and Limousin x Angus subcutaneous free fatty acids also had higher levels of C18:2 than the other crossbreeds, but these levels were only significantly greater than those of the Simmental x Hereford.

Sex differences were responsible for only one compositional difference throughout this study. Phospholipids from bulls had more C18:0 than those from steers. The sex x muscle, breed x sex, and breed x sex x muscle interactions which occurred for C18:0 in the phospholipid fraction were primarily the result of an unusually high level of this fatty acid in the biceps femoris of steers of the Limousin x Hereford crossbreed. Low levels of C18:0 in the biceps femoris of bulls of the Limousin x Hereford and Simmental x Angus crossbreeds also contributed to these interactions. A sex x muscle interaction, observed for C18:1 of the subcutaneous free fatty acid fraction, occurred because of higher levels of C18:1 in the longissimus dorsi of bulls than of steers.

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