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.