THE UNIVERSITY OF MANITOBA

THE EFFECT OF CROSSBREED, SEX AND ANATOMICAL LOCATION ON THE CHOLESTEROL AND PHOSPHOLIPID COMPOSITION OF BOVINE INTRAMUSCULAR LIPIDS

by

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ABSTRACT

Thirty-six animals representing six crossbreeds and two sexes were used to investigate the influence of crossbreed, sex and anatomical location on the composition of cholesterol and phospholipids present. These animals represented the F1 generation produced by crossing Limousin and Simmental bulls with Hereford, Shorthorn and Aberdeen Angus cows. Total intramuscular lipid was extracted from the longissimus dorsi and biceps femoris muscles from six crossbreeds by the chloroform-methanol method of Bligh and Dyer. Analyses of variance of the percent moisture and extractable lipid showed a significantly higher extractable lipid content in steers than bulls. Statistical analyses for cholesterol and phospholipids revealed no significant differences due to anatomical location throughout this investigation. Significant sex differences were observed for total, free and esterified choelsterol while crossbreed differences were evident for total and free cholesterol fractions only. Significant crossbreed x sex and crossbreed x muscle interactions were observed for free and esterified cholesterol as well as crossbreed x sex x muscle interactions for all fractions. A significant sex effect occurred for total phospholipids in addition to crossbreed x muscle, sex x muscle and crossbreed x sex x muscle interactions. Sex x muscle and crossbreed x sex x muscle were the only significant interactions evident for the cholesterol/lipid phosphorous ratio. Analyses of variance for the four phospholipid fractions studied revealed no significant differences due to sex or anatomical location although a significant crossbreed effect occurred for phosphatidylserine + phosphatidylinositol. Crossbreed x sex and crossbreed x muscle interactions were evident for sphingomyelin in addition to crossbreed x muscle interactions for

phosphatidylcholine and phosphatidylserine + phosphatidylinositol. A significant crossbreed x sex x muscle interaction was evident for all the phospholipid components investigated. These results revealed that crossbreed and sex exerted a far greater influence on the lipid components in the animals studied than that of anatomical location.

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GENERAL INTRODUCTION

Lipids have been reported to contribute to many of the organoleptic and biochemical properties of meat. While there is a great deal of information on the fatty acid composition of bovine lipids, relatively little is known with respect to the polar lipids of skeletal beef tissue. Phospholipids have been implicated in the deterioration of flavor in both extremely lean meat and freeze-dried meat during storage as well as exerting a possible role in the flavor of processed beef. The importance of phospholipids to meat flavor, however, is still obscure and can only be elucidated by identification of the individual polar lipids present. The incrimination of animal fat relative to the development of atherosclerosis and other human cardiovascular diseases has also stimulated considerable interest in the biochemical properties of meat animal lipids. Many studies have been published on the cholesterol levels in human and animal tissues while comparatively little has been reported for bovine intramuscular fat. It is hoped that the information reported in this thesis will provide a better understanding of those factors influencing the composition of animal lipids.

In a program to evaluate foreign cattle breeds, the Canada Department of Agriculture Research Station in Brandon introduced into Canada, European breeds, two of which are the Simmental and Limousin. Bulls from these breeds were crossed with Aberdeen Angus, Hereford and Shorthorn cows to determine whether the crossbreeds produced better quality meat more efficiently than the traditional breeds. Sensory evaluation of two muscles from bulls and steers from these crossbreeds as well as chemical analysis of the lipid composition were carried out

previously in this laboratory by McLandress (1972), Gillis (1972) and Watts (1972), respectively.

The following study investigated the effect of crossbreed, sex and anatomical location on the quantity and composition of phospholipids and cholesterol in bovine intramuscular lipids.

REVIEW OF LITERATURE

INTRODUCTION

Lipids are an integral part of muscle structure and are associated with many of the organoleptic and biochemical properties of meat (Hornstein et al., 1961; Terrell et al., 1969; Dryden and Marchello, 1970). In addition to contributing to meat quality they can undergo both oxidative and hydrolytic deteriorative reactions leading to characteristic off-flavors and off-odors. The phospholipid fraction in particular is extremely susceptible to oxidation and is responsible for undesirable flavor changes associated with stored meat (Hood and Allen, 1971). This fraction is also subject to hydrolysis leading to deteriorative changes during meat storage (Olley and Lovern, 1960; Fishwick, 1968). The importance of dietary cholesterol and its influence on serum lipids has been widely reported (Connor et al., 1969; Losier, 1972). While the amount of total cholesterol present in meat has been extensively studied little information is available on the quantitative distribution of free and esterified cholesterol in muscle tissues.

This review will attempt to discuss those factors influencing both the cholesterol and phospholipid content of animal muscles.

CHOLESTEROL

The cholesterol content of foods has been the subject of concern in recent years owing to its influence on serum lipid patterns (Connor et al., 1969; Hegsted et al., 1965; Losier, 1972). While trace amounts of cholesterol have been reported in a few isolated plant tissues, it is foods of animal origin that provide the major sources of this sterol (Feeley et al., 1972). The concentration of cholesterol in animal muscle was reported by Wilcox (1962) to be around 0.06 percent and is present in the free and esterified forms. Kritchevsky and Tepper (1961) reported that fish and dairy products generally contained 55-95% of cholesterol in the free form while that found in meats was more esterified (50-70%). This was refuted by later investigators (Tu et al., 1967; Lyaskovskaya and Kelman, 1967) who found the esterified cholesterol content of animal muscles to be less than 10% of the total cholesterol with the majority being in the free form. Cholesterol is not only located in the lipids of animals but it is also bound with the protein as a lipoprotein. While quantitatively cholesterol in the lean muscle is only a minor component, it nevertheless is an active metabolite within the cells as well as a constituent of cell membranes. The role of cholesterol in the latter is still not fully understood although Williams and Chapman (1970) suggested that it may control in a reversible manner the fluidity of the membrane. This might explain in part the relationship between cholesterol and the ease with which different nutrients enter the cell suggested earlier by Wilcox

et al. (1962).

The majority of studies reported in the literature have been concerned with serum cholesterol levels in experimental animals while comparatively little has been published on mammalian muscles.

Effect of Anatomical Location

Studies carried out almost forty years ago by Bloor (1936) demonstrated a wide range of cholesterol values for different muscles both between species as well as among individual animals within a single species. Individual muscle types were differentiated according to their cholesterol levels with smooth or involuntary muscle being associated with the highest levels (0.75 percent of dry weight) and skeletal muscle the lowest levels (0.30 percent of dry weight). Cardiac muscle, however, was associated with cholesterol levels intermediate to those observed for the other muscle types and accounted for around 0.55 per cent of the dry weight as shown in Table 1.

Vecchio et al. (1955) found no significant difference in the levels of cholesterol in both muscle and adipose tissue of a number of different grades and cuts of meat studied. The concentration of cholesterol in the muscle was 40-50 mg per 100 g of muscle in the adult mammals as well as in the thigh (dark meat) of chicken. In white meat of chickens, however, the amount of cholesterol content was lower at around 30 mg per 100 g of muscle. This study demonstrated that the cholesterol content tended to be higher in the muscles of young, growing animals compared to the mature adults of the same species, as indicated in Table 2.

Allen and co-workers (1967) investigated the cholesterol content

Table 1. Cholesterol Content of Muscles (per cent of dry weight)[a]

Muscle	Cholesterol
Smooth muscle (gastrointestinal tract)	0.70
Ventricle (warm blooded animal)	0.55
Skeletal muscle (vital)	0.34

[a]Adapted from W.R.Bloor (1936).

Table 2. Mean Values for Concentration of Cholesterol in mg per 100 g Muscle[a].

Hen (Light meat)	30.8
Hen (Dark meat)	51.6
Beef	43.3
Veal	71.6
Pork	44.6
Pig	82.4
Mutton	50.7
Lamb	60.3

[a]Adapted from Vecchio et al., 1955.

of three porcine muscles and observed no significant differences between the diaphragm, longissimus dorsi and psoas major muscles. The mean value of cholesterol in the diaphragm, however, did appear higher than that for the longissimus dorsi and psoas major. Terrell et al. (1969) in their studies on six bovine muscles similarly observed no significant differences between the levels of cholesterol among the six anatomical locations investigated. This was attributed in part to the large standard deviation in addition to a 7-10% error in the cholesterol assay used.

Tu et al. (1967) investigated the free and esterified cholesterol levels of animal muscles. The average free cholesterol concentration of bovine and porcine muscles was reported to be 58 and 65 mg %, respectively, while that for the esterified form was 6 mg %. This differed somewhat from that observed by Lyaskovskaya and Kelman (1967) in which the free cholesterol of beef was found to be higher than that of pork. However, the variability between animals could account for these differences as well as the method of analysis.

Differences between the cholesterol levels of bovine intramuscular and subcutaneous lipids from the longissimus dorsi were reported by Stromer et al. (1966). The intramuscular lipid was reported to be considerably higher in cholesterol than the corresponding subcutaneous fat. Significant ($P < 0.05$) differences were also evident between the external and internal layers of the subcutaneous fat although these were no longer apparent after the animal had reached six years of age.

Luddy et al. (1970) investigated the free cholesterol of porcine muscles based on the light and dark portions. They found the mean value for the semimembranosus (light) muscles was 2.3% of the total

lipid compared to 2.7% for the quadriceps femoris (dark) muscle. These values were considerably greater than that observed for the semitendinosus light and dark muscle lipids which were 1.0% and 1.2%, respectively.

While some of these investigators have indicated differences in cholesterol level between muscles, further research is required to establish more fully the nature of these differences.

Effect of Sex

Hood and Allen (1971) in their studies on bovine lipids did report a sex effect for total cholesterol. Bulls appeared significantly ($P < 0.01$) higher in cholesterol than heifers but did not differ from that of steers when determined as percent of muscle or percent of fat-free muscle. When cholesterol was expressed as mg per g of lipid, however, bulls were significantly ($P < 0.01$) higher than both steers and heifers in addition to being inversely related to the intramuscular lipid as illustrated in Table 3.

Terrell et al. (1969) reported a significant ($P < 0.05$) difference for total cholesterol in bovine muscles for weight groups 420 kg and 455 kg. While no statistical differences were evident between the total cholesterol for steers and heifers, there was a tendency for steers to be higher than heifers as shown in Table 4. Further investigations appear to be necessary to establish more definitively, differences in cholesterol between normal and castrated animals.

Effect of Marbling and Other Factors

Feeley et al. (1972) in reviewing previous work stated breed among those factors studied in relation to cholesterol. However, while breed might be expected to exert some influence, there has been no published information relating the effect of this factor. A number of

Table 3. Total Lipid Cholesterol Levels in Bovine Longissimus Dorsi Muscle among Three Sex Groups. (Hood and Allen, 1971)

Sex	Total lipid	Total cholesterol		
	% of muscle	% of muscle	% of fat-free muscle	mg/g lipid
Bull	4.03^{ab}	0.046^{b}	0.048^{b}	11.51^{ab}
Heifer	7.29^{ac}	0.057^{ab}	0.061^{ab}	7.79^{a}
Steer	5.98^{bc}	0.049^{a}	0.052^{a}	8.10^{b}

^{abc}Means in each column having the same superscript are significantly different ($P < 0.01$)

Table 4. Mean Total Cholesterol of Six Bovine Muscles by Sex and Live Weight[a]. (Terrell et al., 1969)

Weight group	Sex	
	Steer	Heifer
386 kg	80.17	75.49
420 kg	72.81	71.49
455 kg	86.62	78.17

[a]Values expressed on mg per 100 g wet tissue.

investigators, however, have attempted to correlate the amounts of cholesterol with fat or degree of marbling. Stromer et al. (1966) observed no significant ($P < 0.05$) difference in cholesterol content when expressed on a tissue weight basis accompanying a corresponding increase in marbling score. However, when expressed on a lipid content basis, a significant ($P < 0.05$) decrease in cholesterol level was found to accompany an increase in marbling score for the three maturity groups of bovine animals studied as shown in Table 5. This indicated that the majority of cholesterol was located in the cell membranes and intracellular structures with no detectable amounts observed in the marbling fat. Conflicting evidence was presented by Tu et al. (1967), however, who suggested that if the intramuscular marbling fat contained negligible amounts of cholesterol then the cholesterol content would decrease with an increase in lipid concentration. These workers plotted the percent lipid values against total cholesterol concentration along with a regression line for bovine and porcine muscles. The total cholesterol content for the bovine muscle appeared to increase slightly with increasing percent lipid as shown in Fig. 1. The regression line was found to be $\hat{Y} = 48.9 + 1.7x$ in which an increase of 1.7 mg % in total cholesterol per 1% increase in lipid content was estimated for bovine muscle. A similar trend was observed for porcine muscles in which an increase of 0.5 mg % in total cholesterol per 1% increase in lipid content was observed. These workers indirectly concluded that marbling fat did in fact contain about the same amount of total cholesterol as the muscle tissue. Further support for this can be found in studies carried out on bovine muscles by Terrell et al. (1969). While the latter workers found no significant differences for the total cholesterol between

Table 5. Cholesterol Content of Intramuscular Lipid from Bovine Longissimus Dorsi in the Fifth to the Eighth Thoracic Vertebrae Region.[a] (Stromer et al., 1966)

	Maturity Group					
	A		B		F	
Marbling Score	Lipid	Tissue	Lipid	Tissue	Lipid	Tissue
Moderately abundant	4.06	0.45	4.20	0.44	11.20	0.44
Slightly abundant	4.47	0.43	3.26	0.39	3.56	0.39
Modest	6.35	0.43	4.59	0.41	5.16	0.44
Small	6.36	0.41	5.36	0.43	4.90	0.36
Traces	11.20	0.46	8.81	0.41	7.06	0.37
Practically devoid	14.52	0.46	12.51	0.42	11.20	0.38

[a]mg per g lipid
A: 15 to 18 months
B: 20 to 24 months
F: Over 6 years

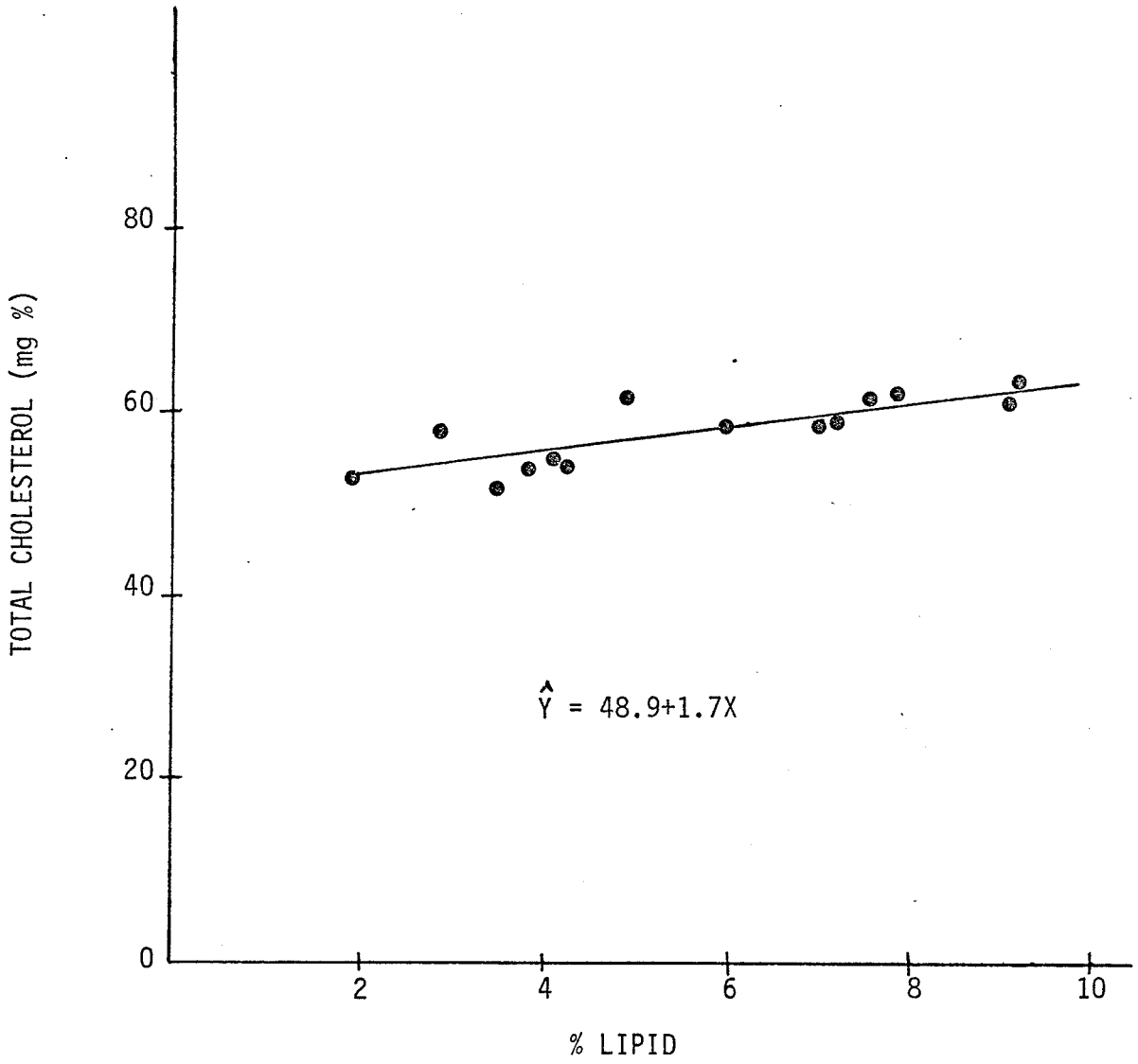

Fig. 1. Regression of total cholesterol concentration on percent lipid for beef muscle.

muscles as discussed earlier, an increase in extractable lipid did appear to accompany a slight increase in total cholesterol.

PHOSPHOLIPID

Phospholipids are essential constituents of all cell membranes and in meat account for about 1% of the total tissue weight. Their highly unsaturated fatty acid content renders them extremely susceptible to oxidative attack. This can result in the production of rancid off-flavors which have a deleterious effect on meat quality as observed in studies on turkey, pork and beef (Acosta et al., 1966; Hornstein et al., 1961; Younathan and Watts, 1960). As essential components of membranes it has been suggested that phospholipids are also involved in energy transfer, triggering mechanisms, nerve impulse mechanisms, protein synthesis and cell adhesiveness in addition to being implicated in cancer and atherosclerosis (Williams and Chapman, 1970).

This section will discuss those factors influencing the quantity and composition of phospholipids in animal muscles with particular reference to anatomical location and sex.

Total Phospholipid

Effect of Anatomical Location

Early investigations on the respiratory muscles of mammals and birds by Bloor and Snider (1934) indicated a positive correlation between physiological activity and the phospholipid content of muscle. More recent studies by Masoro et al. (1964) and Shchesno (1965) on mammalian muscles also showed that those muscles more active physiologically were richer in phospholipids. A similar relationship between phospholipids and the physiological activity has since been reported for chicken and

turkey muscle (Peng and Dugan, 1965; Marion and Miller, 1968; Neudoerffer and Lea, 1968; Wangen et al., 1971). Turkki and Campbell (1967) suggested that the phospholipids of skeletal muscle were an intrinsic property of the muscle and were more closely related to the physiological activity of the individual fiber than to simply muscle exercise. Evidence for this was provided by the fact that red muscles contained more total phospholipid than white muscles due to the larger density of mitochondria present. The major difference between these two fibers is related to their primary energy source for myofibrillar contraction which in the case of the red fiber is aerobic oxidation while that in white fibers is derived from anaerobic glycolysis (Beecher et al., 1965). Turkki and Campbell (1967) also investigated the phospholipid concentration for the extensor carpi radialis and psoas major muscles of beef. When expressed on a dry-weight basis no significant difference was apparent between the levels of phospholipids. However, when expressed as percent of total lipid the phospholipids in the extensor muscle were significantly ($P < 0.01$) higher than that in the psoas major muscles. The variability in the distribution of red and white fibers between these muscles was thought to account for the significant difference in phospholipids observed. O'Keefe et al. (1968) in studies on bovine lipids also reported a location effect in which the semitendinosus was significantly ($P < 0.05$) higher in phospholipids than that of the longissimus dorsi or triceps brachii when expressed as a percent of the total fat. Allen et al. (1967) studied the diaphragm, longissimus dorsi and psoas major muscles of porcine animals and observed the lipid phosphorous to be significantly ($P < 0.01$) higher in the diaphragm. Hornstein et al. (1967) reported that the diaphragm muscle of bovine animals contained 60%

more phospholipids than the transversus abdominis while the psoas major was 20% richer in phospholipids than the corresponding transversus abdominis, longissimus dorsi and semitendinosus.

Luddy and co-workers (1970) determined phospholipid content in the light and dark portions of the semitendinosus muscle, the semimembranosus (light) and the quadriceps femoris (dark) muscles. A significant ($P < 0.05$) difference between muscles was observed due to the quadriceps femoris (dark) muscles being richer in phospholipids than the semimembranosus (light) muscle (Table 6). The dark muscles in this study were approximately 40% higher in total phospholipids than the corresponding light muscles. This was in agreement with earlier studies on chicken and turkey muscles in which the dark meat of the thigh muscle was considerably higher in phospholipids than the corresponding light meat of the breast muscle (Peng and Dugan, 1965; Marion and Miller, 1968; Acosta et al., 1966). The differences observed in phospholipids between the dark and light muscles are a reflection of the differences in physiological activity and fiber content of the individual muscle tissues.

Effect of Sex

Comparatively few studies have reported the effect of sex on the phospholipids of bovine muscles. Link et al. (1970) found steers to be significantly higher ($P < 0.05$) than heifers when phospholipids were based on mg per 100 g muscle. When expressed as mg per g lipid, however, steers were significantly higher in phospholipids than heifers at the 1% level ($P < 0.01$). Hood and Allen (1971) reported a significant difference ($P < 0.01$) in intramuscular lipid levels among bulls, heifers and steers in the order of heifer > steer > bulls. When the phospholipids

Table 6. Mean Values of Phospholipid from Various Porcine Muscles[a]. (Luddy et al., 1970)

Semimembranous (light)	Quadriceps femoris (dark)	Semitendinosus Light	Dark
19.8±4.3	34.3±7.6	16.6±1.8	27.4±3.0

[a]Expressed as per cent of total lipid.

were expressed as mg per g lipid, they were significantly higher ($P<0.01$) in bulls and lower in heifers when compared to steers. Both these studies suggested that the intramuscular fat or marbling fat contained little or no phospholipids (Table 7). These differences were consequently attributed to the variation in the amount of intramuscular lipid between the sexes. This inverse relationship between the amount of intramuscular lipid and phospholipids in beef was reported by several investigators (Callow, 1962; Turkki and Campbell, 1967). Turkki and Campbell (1967) found this relationship to be curvilinear for eight psoas major and eight extensor carpi muscles. O'Keefe et al. (1968) in studies on bovine muscles similarly observed that an increase in phospholipids accompanied a decrease in total muscle fat when expressed as percent of total fat content. This relationship was further clarified by Campbell and Harrill (1971) who plotted the log percent of phospholipid against log percent of total lipid for four bovine muscles. A straight line relationship was observed from which a significant ($P<0.01$) regression coefficient was evident for these two parameters (Fig. 2).

The phospholipid content as related to sex was also reported in chicken and turkey by Marion and Miller (1968) and Acosta et al. (1966), respectively. The female chicken has a higher lipid content in the breast, heart and liver tissues and a lower lipid content in the thigh compared to the male. The phospholipid concentrations, however, were rather inconsistent between the sexes among the four tissues investigated, so that no definite trends were apparent (Marion and Miller, 1968). Acosta et al. (1966) reported the total muscle lipid of turkey to be higher in one-year old females than in sixteen-week old males while the corresponding percent of phospholipids based on the total lipid was

Table 7. Total Lipid and Phospholipid in Bovine Longissimus Dorsi Muscle.

	Hood and Allen (1971)			Link et al. (1970)		
		Phospholipid			Phospholipid	
Sex	Intramuscular lipid %	% of muscle	mg/g lipid	Intramuscular lipid %	% of muscle	mg/g lipid
Bull	4.03^{rs}	0.56^{a}	138.4^{rs}			
Heifer	7.29^{rt}	0.52^{ab}	71.8^{rt}	11.00^{r}	0.52^{a}	75.3^{r}
Steer	5.98^{st}	0.57^{b}	95.5^{st}	8.10^{r}	0.55^{b}	100.6^{r}

^{ab}Means in each column with same superscript are significantly different ($P < 0.05$)

^{rst}Means in each column with same superscript are significantly different ($P < 0.01$)

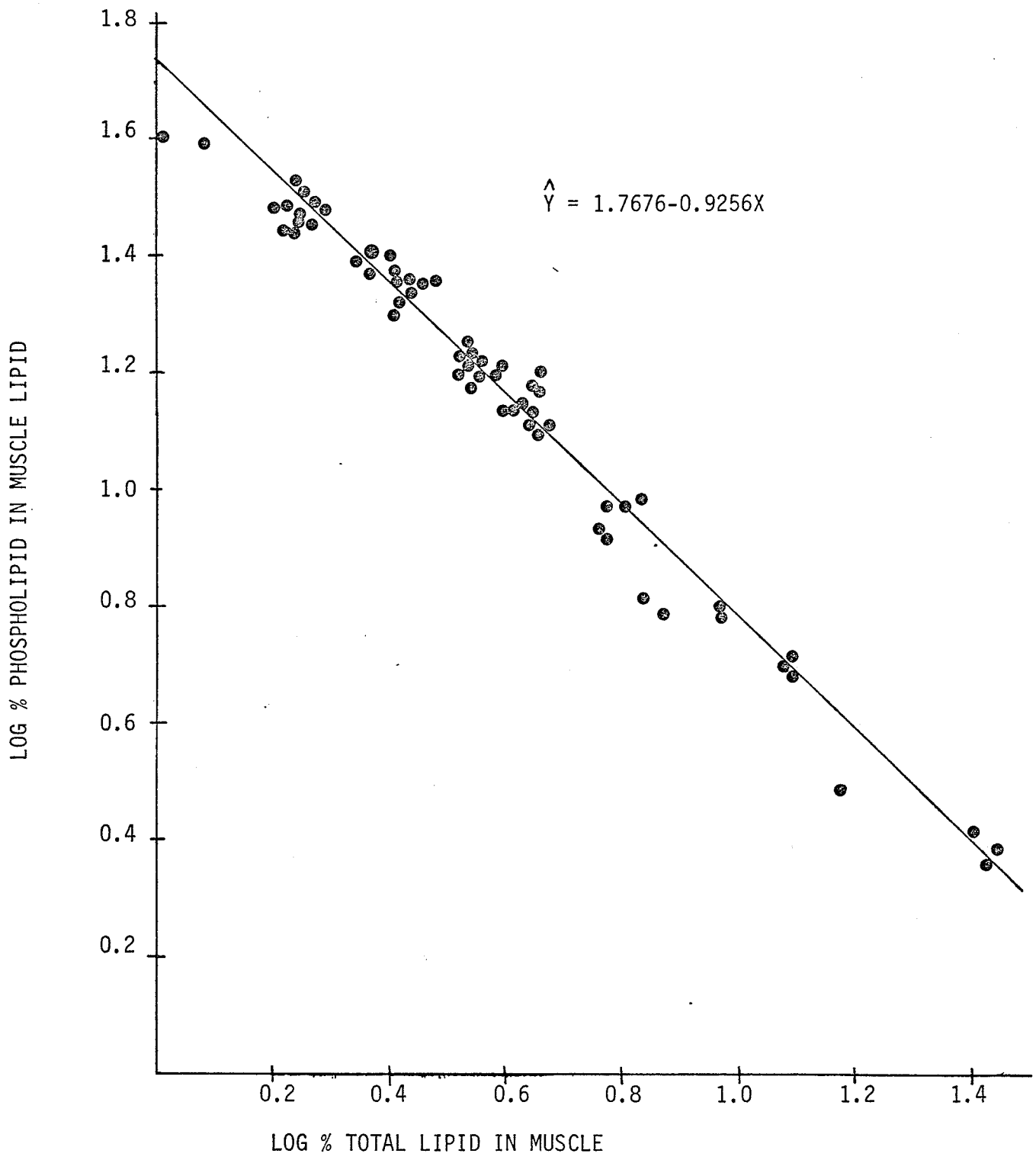

Fig. 2. Relation of phospholipid concentration in lipid to muscle total lipid concentration, wet weight basis.

higher in sixteen-week old males than one-year old females. This agreed with that reported for bovine muscles which was related to the amount of lipid present.

Cholesterol/Phospholipid Ratio

Cholesterol/phospholipid ratio as an indicator of muscular activity was suggested by Bloor and Snider (1934). These workers demonstrated that changes in cholesterol levels also accompanied changes in phospholipids although those involving cholesterol occurred to a much smaller degree. Consequently a lower cholesterol/phospholipid ratio was associated with a more active muscle tissue. This ratio was reported for mammalian muscles by Allen et al. (1967) and Terrell (1967) (Table 8). Allen et al. (1967) in studies on porcine animals reported the ratio of cholesterol/lipid phosphorous to be significantly ($P<0.05$) higher in the longissimus dorsi than in the diaphragm muscle and attributed this to the greater activity associated with the diaphragm. Investigations on bovine animals by Terrell (1967) showed that the psoas major had a higher extractable lipid and a higher cholesterol/lipid phosphorous ratio than triceps brachii. This indicated the psoas major muscle to be a slower developing and less active muscle than the triceps brachii which contained less extractable lipid and a lower cholesterol/lipid phosphorous ratio as indicated in Table 8.

Phospholipid Composition

Information regarding the quantitative distribution of the individual phospholipid fractions in fresh muscle tissues of meat animals is rather limited. Hornstein et al. (1961) fractionated the phospholipids of both beef and pork into phosphatidylcholine, phosphatidylethanolamine and sphingomyelin. Phosphatidylcholine and phosphatidyl-

Table 8. Intramuscular Lipid and Cholesterol to Lipid Phosphorous Ratio in Porcine and Bovine Muscles.

Anatomical Location	Allen et al. (1967)		Terrell (1967)	
	Total lipid %	Ratio	Total lipid %	Ratio
Diaphragm	10.01	1.96		
Psoas major	3.05	2.36	11.44	3.66
Longissimus dorsi	4.64	2.96	10.03	3.43
Transversus abdominis			9.88	3.28
Semimembranosus			6.23	3.58
Triceps brachii			6.05	3.04
Semitendinosus			5.97	3.15

ethanolamine were found to be present in similar amounts of around 40 to 45% while sphingomyelin accounted for 10 to 15% of the total phospholipid. Mabrouk et al. (1969) separated the intramuscular polar lipids of beef into phosphatidylcholine, lysophosphatidylcholine, phosphatidylethanolamine, phospahtidylserine and phosphatidylinositol. This section will attempt to discuss where possible the influence of a number of factors including anatomical location and sex on the phospholipid composition of animal muscle.

Effect of Anatomical Location

Kuchmak and Dugan (1963) found phosphatidylcholine and phosphatidylethanolamine to be the major components present in pork muscle accounting for 61% and 31% of the total phospholipids, respectively. Of the remainder phosphatidylserine accounted for 5% and sphingomyelin 3%. This study indicated small variations in the relative amounts of each phospholipid among belly, ham, loin and rib as illustrated in Table 9. These workers found that phosphatidylethanolamine had a tendency to be present in greater amounts in those tissues containing less total lipid while the reverse situation appeared to be the case for sphingomyelin. No distinct patterns however were evident for phospahtidylcholine and phosphatidylserine throughout this study.

Masoro et al. (1964) compared the phospholipid composition of the soleus and gastrocnemius muscles of monkeys. The predominant phospholipids present were phosphatidylcholine and phosphatidylethanolamine which accounted for 50 and 25% of the total lipid phosphorous, respectively. A comparison of these muscles from the same leg showed the gastrocnemius had 55% of the lipid phosphorous present as phospha-

Table 9. Percentage of each Phospholipid Type in the Phospholipid of Hog Muscle Tissue. (Kuchmak and Dugan, 1963).

	Carcass Location			
Phospholipid Composition	Belly	Ham	Loin	Rib
Phosphatidylethanolamine	32.8	34.2	33.3	28.4
Phosphatidylserine	4.7	7.8	4.7	2.5
Phosphatidylcholine	58.6	54.7	60.8	63.0
Sphingomyelin	3.9	3.3	1.2	6.1

tidylcholine compared to 53% for the soleus, a small but significant ($P < 0.05$) difference. A further significant ($P < 0.01$) difference was also evident for polyglycerophosphatide which accounted for 7% in gastrocnemius and 9% in soleus as illustrated in Table 10.

Turkki and Campbell (1967) in studies on the phospholipids of two bovine muscles found that phosphatidylcholine, phosphatidylethanolamine and sphingomyelin accounted for approximately 62, 30 and less than 10% in both extensor carpi radialis and psoas major muscles as shown in Table 11. While the mean values for the phosphatidylethanolamine component for the extensor and psoas major muscles were very similar at 29.0 and 31.4%, respectively, this difference nevertheless approached significance. These investigators also observed that while the overall average for sphingomyelin in the extensor was higher than that for the psoas, this difference was not statistically significant. The order of magnitude reported for the individual phospholipid fractions by these workers was in general agreement with that observed in earlier studies on porcine and monkey muscles (Kuchmak and Dugan, 1963; Masoro et al., 1964).

Recent investigations on the phospholipid composition of poultry muscles showed phosphatidylcholine to be the major phospholipid present accounting for almost half of the total phospholipids in turkey (Neudoerffer and Lea, 1968; Wangen et al., 1971) and in chicken (Peng and Dugan, 1965). Phosphatidylethanolamine, phosphatidylserine, sphingomyelin, lysolecithin and cardiolipin accounted for the remaining phospholipids present in the skeletal muscle of poultry. From these studies it would appear that there are some variations in phospholipid distribution between muscles although a more comprehensive investigation is

Table 10. Percent Distribution of Lipid Phosphorous in Phospholipid Classes in Monkey Muscles[a]. (Masoro et al., 1964)

Phospholipid Component	Gastrocnemius	Soleus
Phosphatidylcholine*	55	53
Phosphatidylethanolamine	25	26
Sphingomyelin	4	3
Phosphatidylinositol	7	6
Phosphatidylserine	2	3
Polyglycerophosphatide**	7	9

[a]Means expressed as relative percent of six phospholipid fractions.

* $P < 0.05$

** $P < 0.01$

Table 11. Distribution of Lipid Phosphorous among Phosphatidylcholine, Phosphatidylethanolamine and Sphingomyelin in Two Beef Muscles[a]. (Turkki and Campbell, 1967)

Phospholipid class	Muscle		Signif. of diff.
	Extensor	Psoas	
Phosphatidylcholine	62.3±1.47	61.6±0.24	
Phosphatidylethanolamine	29.0±0.98	31.4±0.95	$0.1 > P > 0.05$
Sphingomyelin	8.7±1.12	7.0±0.80	

[a]Percent of the sum of the fractions, each value is an average for 2 determinations for each of 8 animals ± std. deviation.

required to delineate more fully the nature of these differences.

Other factors

Little information is available on the effect of sex and breed on the phospholipids of animal muscles. Wangen et al.(1971) did report some variations in the phospholipid composition of the breast and thigh muscles of turkey during aging. Phosphatidylethanolamine showed an inverse relationship with age in both tissues studied. The main phospholipids were phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine + phosphatidylinositol, lysophosphatidylcholine and sphingomyelin which accounted for 50, 22, 10, 5 and 10% of the total phospholipids, respectively.

As is evident from the preceeding review of the literature, the role of anatomical location in determining cholesterol and phospholipids has been more thoroughly investigated than that of sex and breed. More research is required to provide a greater understanding of these factors influencing lipid composition particularly in relationship to meat quality.

METHOD

Samples

Source

Thirty-six animals representing six crossbreeds and two sexes 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 were obtained from the Canada Department of Agriculture Research Station, Brandon, Manitoba.

Spring-born male calves were weaned at 6-6½ months and half of the males were randomly chosen for castration. Bulls and steers were raised to 1200 pounds weight (approximately 545±20 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, then the section of the longissimus dorsi adjacent to the twelfth vertebra and the entire biceps femoris were excised from the right side, placed in polyethylene bags and held at $3^{o}C$ overnight. The following day the meat was wrapped in polyethylene coated freezer paper, and placed in a $-40^{o}C$ freezer. The frozen samples were transported from the Brandon Research Station to this laboratory in styrofoam containers where they were stored at $-37^{o}C$ to $-40^{o}C$. After thawing for fifteen hours at $23.5^{o}C$, the meat samples were 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.

Sampling Procedure and Preparation of Samples

A 2.5 cm slice of meat was taken from the proximal end of the 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 Dryer (Model 10-140BA)[1]. The freeze-dried samples were stored until extracted in screw-top glass jars, under nitrogen, at -10°C.

Chemical Analysis

Lipid Extraction

Total lipid extraction and separation was carried out by the method of Bligh and Dyer (1959). A sample containing 5-10 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 full speed in a Virtis 23 homogenizer[1]. The extraction mixture was filtered with slight suction through Whatman No. 1 filter paper and the residue washed with 25 ml of chloroform and 25 ml of water. The washed residue was re-homogenized to ensure total fat extraction. The filtrate from the two extractions was shaken with 0.1 g of sodium chloride in a 250 ml graduated cylinder and allowed to stand overnight at room temperature. The lower (chloroform) layer was recorded and the upper (aqueous alcoholic) layer removed by aspiration. The chloroform extracts were sealed in vials with teflon lined screw caps in a nitrogen atmosphere and refrigerated until analyzed.

Total Lipid Determination

The total lipid content was determined by evaporating a 10 ml aliquot of the chloroform layer in a 25 ml beaker on a steam bath.

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

After cooling to room temperature, the beaker containing the oily residue was placed in the dessicator over calcium sulphate ($CaSO_4$) to remove any residual moisture. The lipid content was then determined on the dried sample gravimetrically.

Total Cholesterol

Total cholesterol analyses were carried out on 2 ml aliquots of the lipid-containing chloroform extracts by a modification of the method of Mann (1961) described by Tu et al. (1967). The 2 ml aliquots were transferred to pyrex tubes and the solvent in each extract evaporated to dryness at 60°C under a stream of nitrogen in a water bath. To each sample 4 ml of absolute ethanol and 0.5 ml of 50% KOH solution were then added and saponification carried out in a 60°C water bath for 1½ hours during which period the sample was swirled at 20 minute intervals. After cooling, 3 ml of 5% NaCl solution and 10 ml petroleum ether were added to each tube and mixed thoroughly for 1 minute using a vortex mixer. The mixture was then allowed to stand at room temperature to facilitate separation of the petroleum ether layer. A 3 ml aliquot of the ether layer was then pipetted into a dried test tube and the solvent evaporated under nitrogen in a 60°C water bath. To each tube 4 ml of glacial acetic acid and 2 ml of $FeCl_3$-H_2SO_4 reagent were added and mixed thoroughly. The solution was allowed to stand at room temperature for 30 minutes and the optical density recorded at 560 mμ using a Junior Spectrometer[1] standardized with the reagent blank. The μg of cholesterol present in the solution was determined from a standard curve.

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[1] Coleman Instruments Inc., Maywood, Ill., U.S.A.

Free Cholesterol

Free cholesterol was determined according to the method of Sperry and Webb (1950) on 2 ml aliquots of each lipid extract. Free cholesterol was precipitated by the addition of 0.5% digitonin solution and the precipitate separated by centrifugation at 2400 r.p.m. for 20 minutes using International Centrifuge (Model CS)[1]. The amount of cholesterol in the precipitate was determined according to the method of Tu et al. (1967) described previously.

Esterified Cholesterol

The esterified cholesterol content was estimated on the difference between total and free cholesterol measured.

Lipid Phosphorous

Determination of lipid phosphorous was carried out according to the method of Fiske and Subbarow (1925) with the following modifications described by Losier (1972). The samples were evaporated to dryness in 30 ml micro-Kjeldahl flasks on a steam bath. They were then charred with 1 ml 5N sulfuric acid on the Kjeldahl digestion apparatus. 30% hydrogen peroxide was added directly to the hot acid mixture to give quicker oxidation. The relative amount of phospholipid in the total lipid extract was calculated by multiplying the amount of lipid phosphorous by a factor of 25.

Quantitative Analysis of Phospholipids by Thin-Layer Chromatography

Preparation of Plates

Thin-layer plates were prepared as described by Skipsi et al. (1964). 40 g of silica gel H[2] was slurried with 90 ml of 0.01M Na_2CO_3 solution and mixed at medium speed in a Virtis 23 homogenizer[3] for five

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[1]International Equipment Company, Boston, Mass., U.S.A.

[2]E. Merck (Darmstadt), Applied Science Laboratories Inc., Penna 16801, U.S.A.

[3]Virtis Company Inc., Gardiner, N.Y. 12525, U.S.A.

minutes, and then transferred to an adjustable applicator[1]. The amount of slurry was sufficient to prepare three to four plates (200 mm x 200 mm) of 500 mu thickness. The silica gel coated plates were then allowed to dry at room temperature for ½ - 1 hour and then activated at 110°C for 1 hour. The plates were then stored in a dessicator prior to chromatographic analysis.

Sample Application

Samples (35-50 ul) of the lipid-containing chloroform extracts were applied 2.5 cm from the lower edge of the plate and approximately 3 cm apart using a lambda pipette.

Chromatography

A modified solvent system was used for developing the chromatograms from that described by Skipski et al. (1964) consisting of a chloroform:methanol:acetic acid: water (25:12.5:4:1.5; V/V/V/V) mixture.

The chromatoplates were developed in TLC chromatanks[2] lined with Whatman No.3 MM filter paper saturated with the solvent mixture (Skipski et al., 1965). The solvent mixture was allowed to move approximately 19 - 19.5 cm from the bottom of the plate (approximately 2¼ hours).

Detection and Identification of Spots

The thin-layer plates were sprayed with a 3% cupric acetate solution in 8% aqueous phosphoric acid (H_3PO_4) until uniformly transparent (Fewster et al., 1969). The individual spots were developed by placing the plates in a 180°C oven for 25 minutes.

Quantitative Analysis

Quantitative analysis of individual phospholipids was carried out by measuring the optical densities of the charred spots with a TLC

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[1]Colab Laboratories Inc., Chicago Heights, Ill., U.S.A.

[2]Colab Products, Consolidated Laboratories, Weston, Ontario, Canada.

densitometer (Model 530) including a Light Source (Model 52-C) with TLC stage equipment, Multiplier Photometer (Model 501-A) and Varicord (Model 42B) single pen recorder[1].

The amount of each of the phospholipid fractions identified was determined by measuring the individual peak areas using the Polar Planimeter (4236 M)[2].

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]Photovolt Corp., New York 10010, U.S.A.

[2]Keuffel and Esser Co., Germany.

RESULTS AND DISCUSSION

Percent Moisture and Extractable Lipid

The mean comparison of percent moisture and extractable lipid from the six crossbreeds studied is listed in Table 12. No significant ($P < 0.05$) differences were observed among the crossbreeds for these two parameters which is in agreement with that reported for these same crossbreeds at 452 kg liveweight by Gillis (1972). The moisture content for the thirty-six animals investigated ranged from 66 to 74%. While the amount of moisture lost from these muscles on thawing was not determined in this study, in a similar loss of approximately 4-16% observed by McLandress (1972) from these crossbreeds slaughtered at 452 kg liveweight might also be expected. It is generally recognized that moisture accounts for approximately 75% of the raw muscle (Lawrie, 1966; Woolsey and Paul, 1969; Covington et al., 1970). The crossbreeds in this study thus appear to follow the usual pattern for percent moisture on a raw muscle basis.

Variations in the percent of intramuscular lipid have been reported between those breeds reared primarily for meat production compared to those reared for milk production (Lawrie, 1966). The animals reared for beef production were found to contain higher levels of intramuscular lipid in the longissimus dorsi muscle at the level of the 4th, 5th and 6th lumbar vertebrae than the dairy breeds (Callow, 1947; Lawrie, 1961). Differences in marbling scores have also been observed among beef cattle breeds (Kauffman et al., 1968; Bramblett et al., 1971). No significant differences ($P < 0.05$) in moisture content were observed among crossbreeds in this study so that a significant

Table 12. Mean Comparison of Moisture (%) and Extractable Lipid (%) from Six Crossbreeds[a,b].

	Crossbreed					
	S x H	S x SH	S x A	L x H	L x SH	L x A
Moisture %	70.68	70.43	71.20	70.86	70.41	70.85
Extractable lipid %	3.49	4.42	4.19	4.16	4.03	4.77

[a]S = Simmental, L = Limousin, A = Aberdeen Angus, H = Hereford, SH = Shorthorn.

[b]No significant differences ($P<0.05$).

difference in lipid content would not be expected. Table 12 indicates that there was a tendency among Limousin crossbreeds to contain more extractable lipid than Simmental crossbreeds.

A significant ($P < 0.05$) sex effect was observed for the percent extractable lipid. Table 13 shows that steers contained significantly ($p < 0.05$) more extractable lipid than bulls in this study. This is consistent with the sensory panel evaluation reported on these same animals by McLandress and Diamant (1972) in which steers were rated greasier than bulls. No significant ($P < 0.05$) difference was found in moisture content, but the mean value of bulls was higher than that of steers. These observations are in agreement with that reported by other researchers (Turton, 1962; Lawrie, 1966; Hood and Allen, 1971; Field, 1971; Gillis, 1972). It is well recognized that castration alters the amount and distribution of fat in the carcass resulting in bulls having less fat than steers.

No significant ($P < 0.05$) differences between the longissimus dorsi and biceps femoris were observed for percent moisture and extractable lipid. From Table 13, however, it is evident that the biceps femoris tended to have a higher content of extractable lipid which is similar to that observed by Gillis (1972) for these same crossbreeds slaughtered at 452 kg liveweight. Many researchers have reported that red muscle has a greater supply of lipid than white muscle. It has since been demonstrated that the main marbling deposits are associated with a heavy vascular network (Blumer et al., 1962). The longissimus dorsi is recognized as a typical white muscle with approximately 35% of its fibres being of the red fibre type (Moody and Cassens, 1968), while the biceps femoris has a larger proportion of red fibres (Allen et al.,

Table 13. Mean Comparison of Percent Moisture and Percent Extractable Lipid from Two Sexes and Two Anatomical Locations[a].

	Sex		Anatomical Location	
	Bull	Steer	Longissimus Dorsi	Biceps Femoris
Moisture %	71.52	69.96	70.75	70.73
Extractable Lipid %	3.49	4.87	3.83	4.17

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

1967). The slight variation in the lipid content observed between these muscles can thus be attributed to the differences in fibre type and vascular distribution as well as in the enzymatic components of the muscle fibres.

Cholesterol

The ug of cholesterol present in each sample was determined by referring to a standard curve as illustrated in Fig.3. A statistical analysis was carried out for total, free and esterified cholesterol. Mean squares and F values used to test the main effects and interactions are shown in Table 14.

Effect of Anatomical Location

No significant ($P<0.05$) differences between the longissimus dorsi and biceps femoris were observed for total, free or esterified cholesterol when expressed as mg per g of lipid or mg percent of the wet muscle. The mean comparison of total, free and esterified cholesterol from the two anatomical locations studied are shown in Table 15. These results indicate that there was a tendency for the mean value of the biceps femoris to be slightly higher in total cholesterol than the corresponding longissimus dorsi when expressed as mg percent of the wet muscle. This is in agreement with that reported by earlier workers that a more active muscle is associated with a higher content of cholesterol (Bloor and Snider, 1936). Investigations by Terrell et al. (1969) on bovine lipids also indicated no significant differences due to muscle which they attributed in part to the large standard deviation obtained. Previous studies by Allen et al. (1967) on porcine muscles similarly found no significant difference due to anatomical location, although the mean value of cholesterol in the diaphragm did tend to be higher than that in

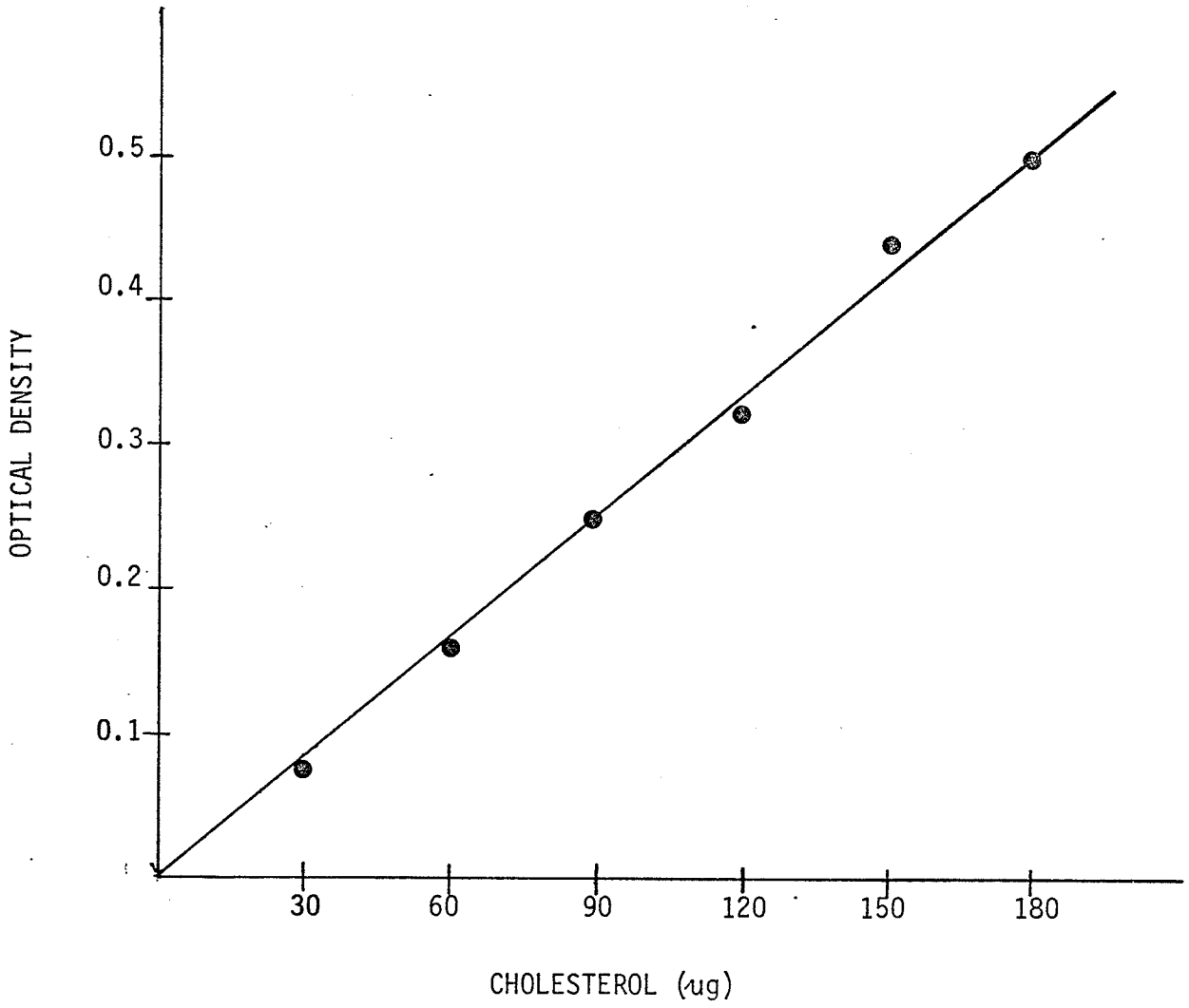

Fig. 3. Standard curve for cholesterol determination.

Table 14. Analysis of Variance for Extractable Lipid, Total, Free and Esterified Cholesterol of Bovine Intramuscular Lipid

Source	df	Extractable Lipid		Total Cholesterol				Free Cholesterol				Esterified Cholesterol			
				mg/g lipid		mg %		mg/g lipid		mg %		mg/g lipid		mg %	
		MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Crossbreed	5	1.42	0.35	26.54	0.89	123.39	3.03*	15.96	0.77	147.13	4.68*	3.82	0.90	12.88	0.34
Sex	1	54.41	13.57*	594.44	19.85*	37.80	0.93	419.34	20.14*	61.53	1.96	9.95	2.33	199.60	5.25*
B x S	5	3.86	0.96	35.44	1.18	10.69	0.26	34.17	1.64	97.62	3.10*	8.33	0.35	149.39	3.93*
Among Animals	24	4.01		29.94		40.71		20.82		31.45		4.27		38.00	
Muscle	1	2.15	1.99	0.27	0.01	103.42	3.24	0.91	0.08	40.32	1.04	0.07	0.02	9.12	0.32
B x M	5	0.91	0.84	36.77	1.88	63.10	1.98	20.73	1.87	205.76	5.30*	13.87	4.42*	159.12	5.56*
S x M	1	0.12	0.11	12.25	0.63	38.65	1.21	6.19	0.56	129.34	3.33	1.49	0.47	3.26	0.11
B x S x M	5	25.64	23.74*	260.36	13.32*	667.53	20.91*	183.73	16.57*	1573.91	40.54*	49.36	15.72*	530.10	18.53*
A x M within crossbreed and sex	24	1.08		19.54		31.93		11.09		38.82		3.14		28.60	

*Significant ($P < 0.05$)

Table 15. Mean Comparison of Total, Free and Esterified Cholesterol from Two Anatomical Locations[a,b].

Cholesterol	Anatomical Location	
	Longissimus Dorsi	Biceps Femoris
Total (mg/g lipid)	14.66±0.74	14.77±0.74
(mg percent of wet muscle)	49.39±0.94	51.76±0.94
Free (mg/g lipid)	10.51±0.56	10.73±0.56
(mg percent of wet muscle)	35.28±1.04	37.05±1.04
Esterified (mg/g lipid)	4.02±0.30	3.96±0.30
(mg percent of wet muscle)	14.03±0.89	14.75±0.89

[a]All values are not significantly different ($P < 0.05$)

[b]Mean values from thirty-six observations.

the longissimus dorsi and psoas major muscles. The biceps femoris, being primarily a locomotive muscle, is more active than the longissimus dorsi and accounts for the variation in the content of total cholesterol observed.

The corresponding mean value for the free cholesterol in the biceps femoris was also found to be slightly higher than in the longissimus dorsi (Table 15). This is to be expected since the free cholesterol accounted for approximately 70% of total cholesterol present in the muscles. Luddy and co-workers (1970) determined the free cholesterol content of porcine muscles based on the light and dark portions. These workers found that the level of free cholesterol was always higher in the dark portion than in the light portion as illustrated by quadriceps femoris (dark) and semimembranosus (light) muscles which contained a mean value of 2.3% and 2.7%, respectively. Luddy et al. (1970) also confirmed the importance of fiber type in relation to muscle activity which probably explains the differences observed in this study.

No significant differences were evident for esterified cholesterol when expressed as mg percent of the wet muscle or mg per g of lipid.

Effect of Sex

A significant ($P < 0.05$) sex effect was observed for total and free cholesterol when expressed as mg per g of lipid (Table 14). Bulls had significantly ($P < 0.05$) more total and free cholesterol than steers, in addition to being inversely related to the extractable lipid as illustrated in Table 16. The data collected for total cholesterol is in agreement with that reported for bovine animals by Hood and Allen (1971). The sex effect observed appeared to be due primarily to the variation in amount of extractable lipid associated with the normal male compared to

Table 16. Mean Comparison of Total, Free and Esterified Cholesterol from Two Sexes[a,b]

Sex	Extractable Lipid	Cholesterol		
	% of Muscle	Total	Free	Esterified
		mg/g lipid	mg/g lipid	mg % of wet muscle
Bull	3.49±0.33	17.58±0.91	13.03±0.76	12.73±1.03
Steer	4.87±0.33	11.83±0.91	8.21±0.76	16.06±1.03

[a]All values show a significant ($P < 0.05$) difference.

[b]Mean values from thirty-six observations.

the castrated animal.

A significant ($P < 0.05$) sex effect was observed for esterified cholesterol when expressed as mg percent of the wet muscle. In this case the mean value of steers appeared to be significantly higher than that of bulls as indicated in Table 16.

Effect of Crossbreed

Significant ($P < 0.05$) crossbreed effects were observed for total and free cholesterol when expressed as mg percent of the wet muscle A division of the crossbreed main effect into sire, cow and sire x cow effects (Table 17) illustrated that sire contributed to the significant ($P < 0.05$) crossbreed effect observed for total cholesterol. A comparison of means presented in Table 18 shows that the Simmental crossbreeds were significantly higher in total cholesterol than the corresponding Limousin crossbreeds. While no significant cow effect was evident, there was a tendency for the Angus crossbreeds to contain more total cholesterol than either the Hereford or Shorthorn crossbreeds. In the case of free cholesterol, both a sire and sire x cow interaction contributed to the significant ($P < 0.05$) crossbreed effect observed. Fig. 4 illustrates the significant sire x cow interaction for free cholesterol when expressed as mg percent of the wet muscle. The high levels in the Simmental x Hereford and Simmental x Angus crossbreeds compared to the low levels in the corresponding Limousin x Hereford and Limousin x Angus crossbreeds together with the reverse situation for the Shorthorn crossbreeds appeared to account for the interaction effect observed.

No significant ($P < 0.05$) crossbreed effect was evident for esterified cholesterol whether expressed as mg per g of lipid or mg percent of the wet muscle. The mean comparison of esterified cholesterol

Table 17. Analysis of Variance for Sire, Cow and Sire x Cow for Cholesterol[a] with a Significant Crossbreed Effect.

Source	df	Cholesterol			
		Total		Free	
		MS	F	MS	F
Sire	1	207.30	5.09*	293.63	9.34*
Cow	2	71.16	1.75	94.96	3.02
Sire x Cow	2	133.67	3.28	126.06	4.01*
Among Animals	24	40.71		31.45	

*Significant ($P < 0.05$).

[a]Means expressed as mg % of wet muscle.

Table 18. Mean Comparison of Total and Free Cholesterol from Two Sires and Three Cows[a,b].

Cholesterol	Sire		Cow		
	Simmental	Limousin	Hereford	Shorthorn	Angus
Total	52.29±1.06	48.89±1.06	49.46±1.30	49.73±1.30	52.57±1.30
Free	38.04±0.93	34.00±0.93	34.93±1.14	34.82±1.14	38.32±1.14

[a]Means expressed as mg percent of wet muscle.

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

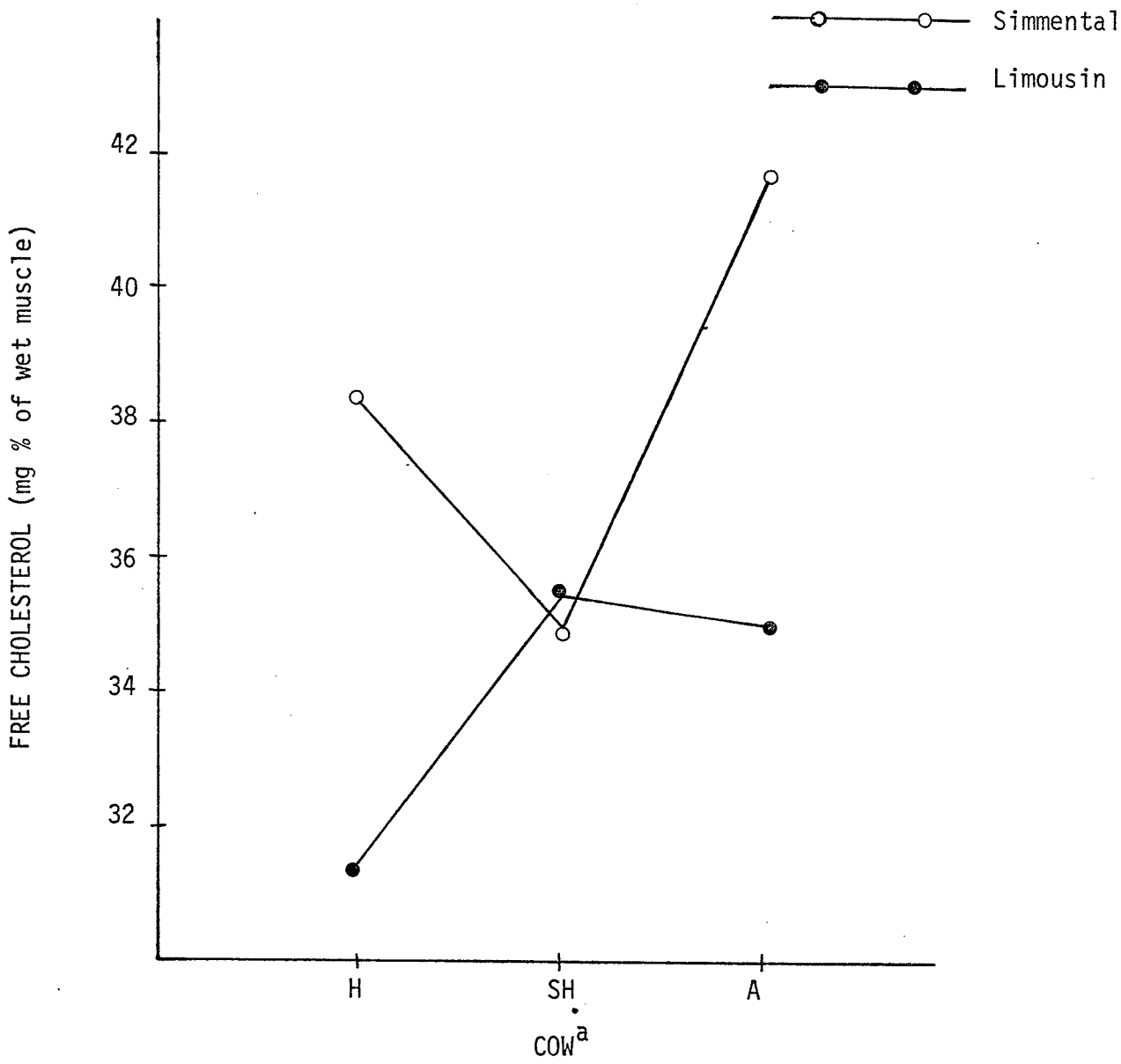

Fig. 4. Significant ($P < 0.05$) Sire x Cow interaction for free cholesterol.

[a]H = Hereford, SH = Shorthorn, A = Aberdeen Angus.

for the six bovine crossbreeds studied is shown in Table 19. There was a tendency for the Simmental crossbreeds to be higher in esterified cholesterol than the corresponding Limousin crossbreeds, with the exception of the Simmental x Shorthorn.

Interaction Effects

A significant crossbreed x sex x muscle interaction was observed for total cholesterol when expressed as mg per g of lipid or mg percent of the wet muscle (Table 14). With the exception of Limousin x Angus, the biceps femoris of bulls was higher in total cholesterol than steers, while with the exception of Simmental x Shorthorn, the longissimus dorsi of bulls was higher in total cholesterol than steers (Table 20). The differences in the levels of total cholesterol between the longissimus dorsi and biceps femoris muscles of bulls and steers within the six crossbreeds appeared to account for the triple interaction effect observed. A significant crossbreed x sex x muscle interaction was also evident when total cholesterol was expressed as mg percent of the wet muscle. There did not appear to be any well defined pattern to explain this interaction which appeared to be rather complex (Table 21).

Significant ($P < 0.05$) crossbreed x sex, crossbreed x muscle, and crossbreed x sex x muscle interactions were observed for free cholesterol when expressed as mg percent of the wet muscle. Fig. 5 illustrates that bulls from the Hereford and Shorthorn crossbreeds were higher in free cholesterol than steers while the reverse situation was found to be the case for Angus crossbreeds. These differences appeared to account for the crossbreed x sex interaction observed. The significant crossbreed x muscle interaction is shown in Fig. 6. The biceps femoris of the Shorthorn and Angus crossbreeds contained higher level of free cholesterol

Table 19. Mean Comparison of Esterified Cholesterol from Six Bovine Crossbreeds[a,b].

	Crossbreeds					
	S x H	S x SH	S x A	L x H	L x SH	L x A
mg/g lipid	4.46±0.60	3.02±0.60	4.41±0.60	3.64±0.60	4.04±0.60	4.35±0.60
mg %	14.55±1.78	12.64±1.78	15.27±1.78	13.72±1.78	15.28±1.78	14.88±1.78

[a]Mean values from twelve observations.

[b]All values are not significantly different ($P < 0.05$).

Table 20. Mean Comparison of Total Cholesterol from Six Crossbreeds, Two Sexes, and Two Anatomical Locations[a,b].

Crossbreed	Anatomical Location			
	Longissimus Dorsi		Biceps Femoris	
	Bull	Steer	Bull	Steer
S x H	20.95	11.86	19.75	14.48
S x SH	11.61	11.97	17.76	8.83
S x A	17.38	13.35	17.51	11.35
L x H	16.22	9.39	17.40	10.93
L x SH	16.88	9.45	24.52	10.31
L x A	19.58	17.06	11.37	12.97

[a]Means expressed as mg per g lipid.

[b]Standard deviation ±2.55.

Table 21. Mean Comparison of Total Cholesterol from Six Crossbreeds, Two Sexes, and Two Anatomical Locations[a,b].

	Anatomical Location			
	Longissimus Dorsi		Biceps Femoris	
Crossbreed	Bull	Steer	Bull	Steer
S x H	54.35	53.27	47.25	56.65
S x SH	36.23	57.53	58.72	42.48
S x A	51.56	58.05	56.72	54.62
L x H	44.58	47.03	46.74	45.83
L x SH	51.09	39.36	50.20	62.27
L x A	49.79	49.85	51.15	48.82

[a]Means expressed as mg percent of wet muscle.

[b]Standard deviation ±3.26.

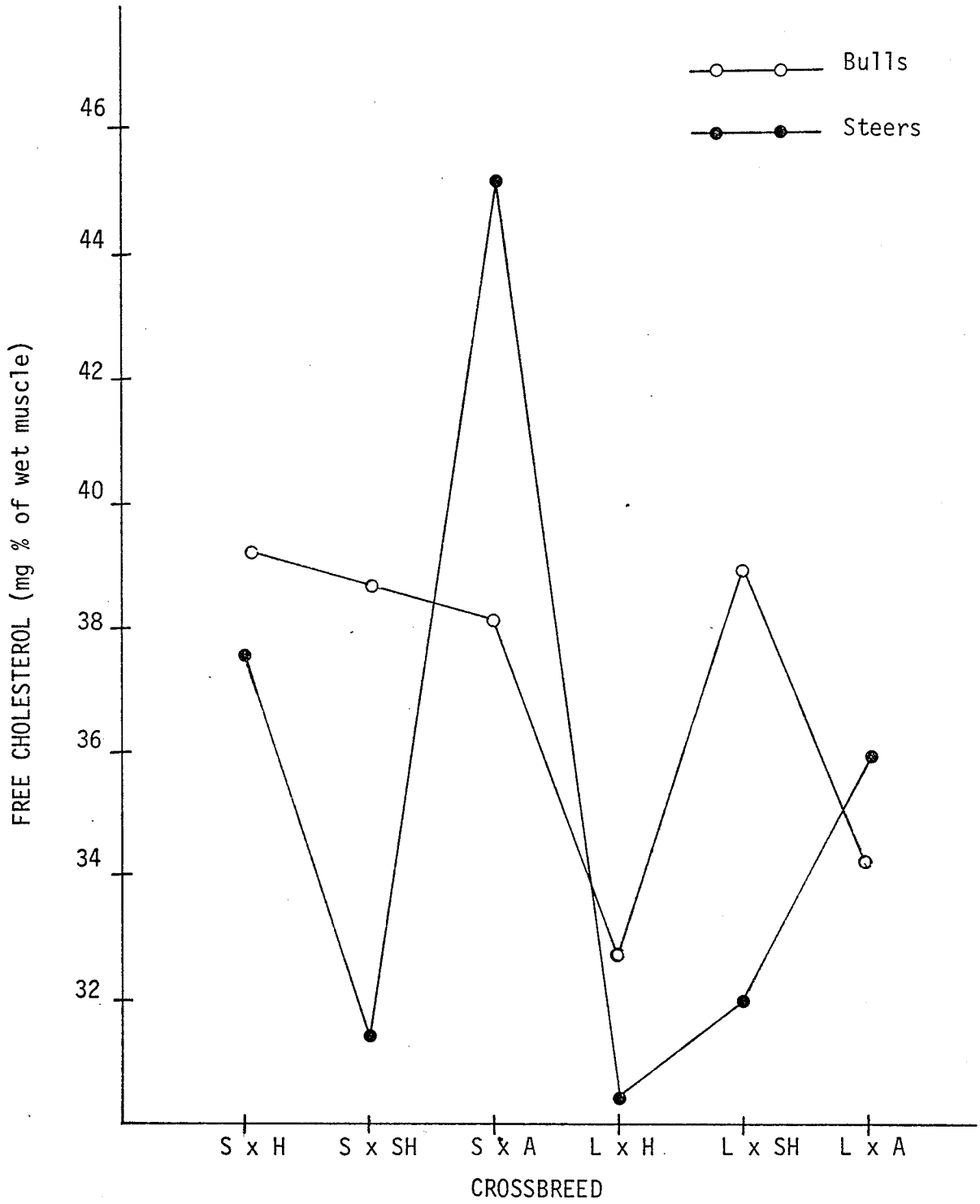

Fig. 5. Significant ($P < 0.05$) crossbreed x sex interaction for free cholesterol.

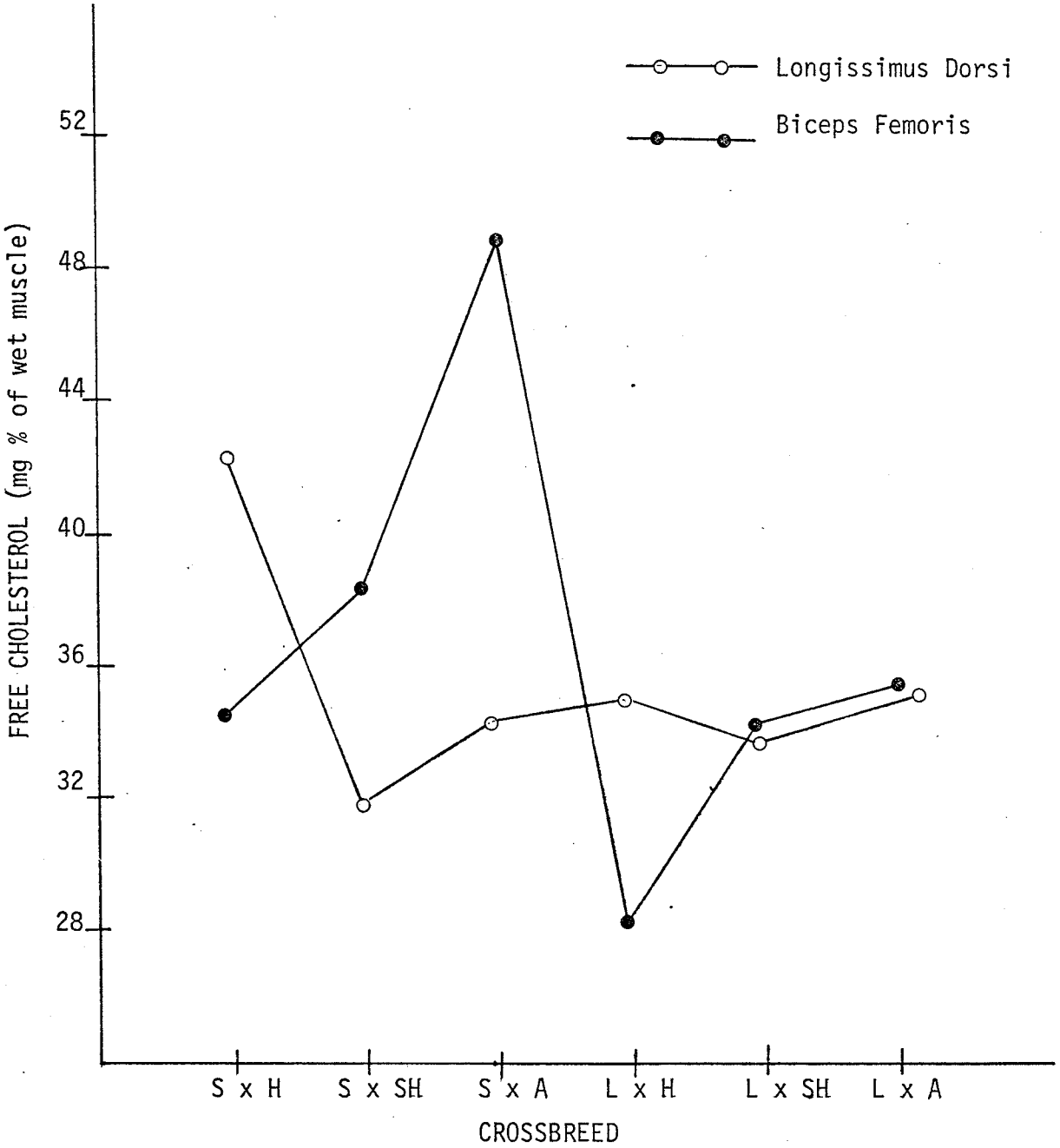

Fig. 6. Significant ($P < 0.05$) crossbreed x muscle interaction for free cholesterol.

than the corresponding longissimus dorsi while the reverse situation was evident for the Hereford crossbreeds. These differences probably account for the crossbreed x muscle interaction effect observed. The crossbreed x sex x muscle interaction was apparently due to the combined effect of the significant crossbreed x sex and crossbreed x muscle interaction discussed earlier. A significant crossbreed x sex x muscle interaction was also observed for free cholesterol when expressed as mg per g of lipid. A comparison of means in Table 22 showed that the amount of free cholesterol was consistently higher in the longissimus dorsi of bulls than steers. A similar pattern was also apparent in the biceps femoris in which bulls contained higher levels of free cholesterol than steers in five of the six crossbreeds studied.

A significant ($P < 0.05$) crossbreed x sex, crossbreed x muscle and crossbreed x sex x muscle interactions were observed for esterified cholesterol when expressed as mg percent of the wet muscle. Fig. 7 illustrates that the crossbreed x sex interaction resulted from the higher levels of esterified cholesterol in the bulls of the Angus crossbreeds whereas steers of the Hereford and the Shorthorn crossbreeds were higher than bulls. The crossbreed x muscle interaction was due to the higher levels of esterified cholesterol in the longissimus dorsi of Simmental x Shorthorn, Simmental x Angus and Limousin x Angus crossbreeds than the biceps femoris, while the reverse situation was found for Simmental x Hereford, Limousin x Hereford, Limousin x Shorthorn crossbreeds (Fig. 8). The combined effect of the significant interactions for crossbreed x muscle and crossbreed x sex discussed earlier was probably responsible for the triple (crossbreed x sex x muscle) interaction observed for the esterified cholesterol.

Table 22. Mean Comparison of Free Cholesterol from Six Crossbreeds, Two Sexes, and Two Anatomical Locations[a,b].

	Anatomical Location			
	Longissimus Dorsi		Biceps Femoris	
Crossbreed	Bull	Steer	Bull	Steer
S x H	15.22	10.15	16.22	7.73
S x SH	8.95	7.66	14.54	6.09
S x A	9.73	9.26	13.08	10.40
L x H	13.25	6.02	11.24	6.93
L x SH	13.01	6.81	18.96	6.07
L x A	15.60	10.43	6.59	10.92

[a]Means expressed as mg per g of lipid.

[b]Standard deviation ±1.92.

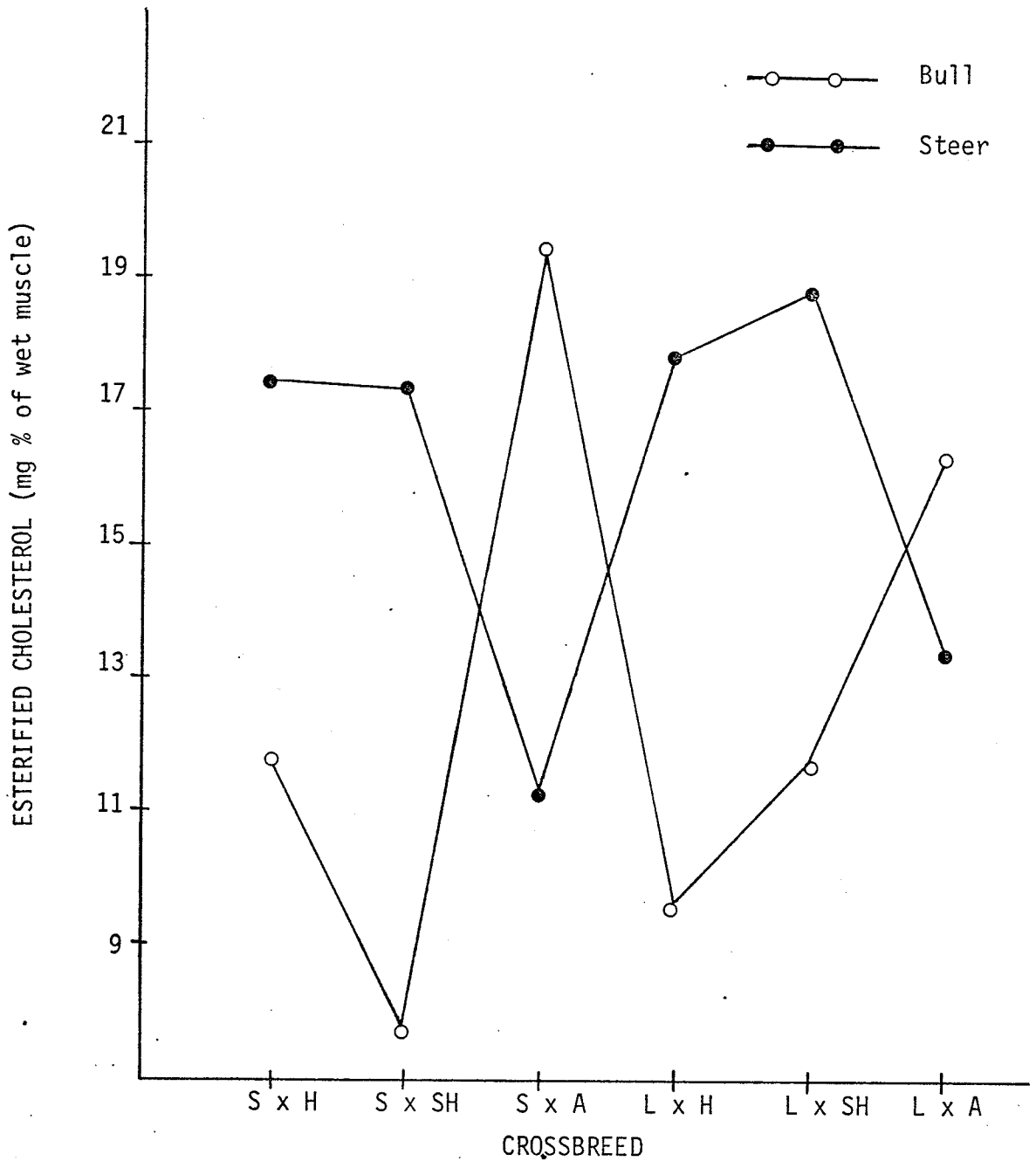

Fig. 7. Significant ($P<0.05$) crossbreed x sex interaction for esterified cholesterol.

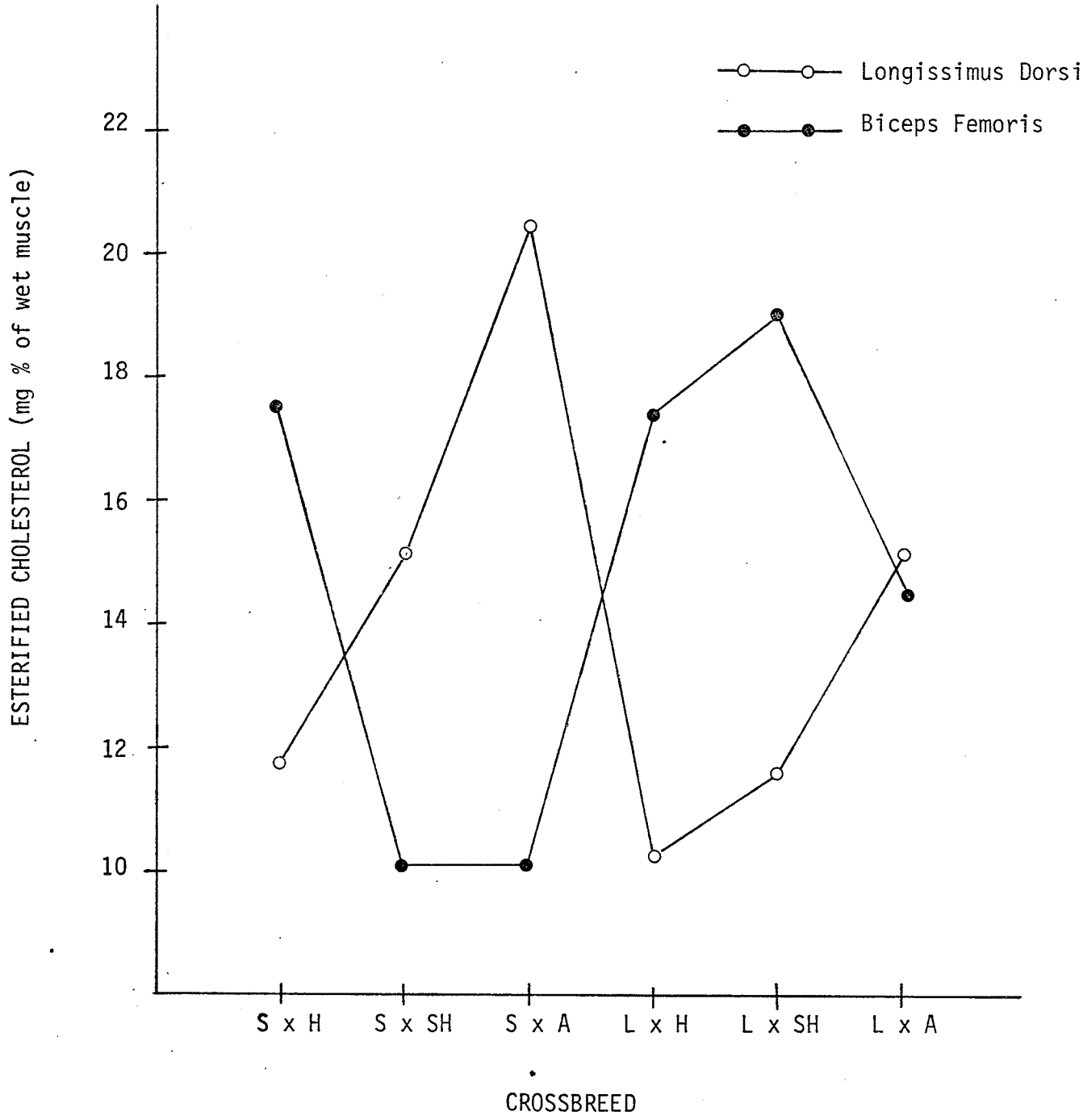

Fig. 8. Significant ($P < 0.05$) crossbreed x muscle location for esterified cholesterol.

Several significant interaction effects were also found when esterified cholesterol was expressed as mg per g of lipid (crossbreed x muscle; crossbreed x sex x muscle). The crossbreed x muscle interaction appeared to follow a similar pattern described earlier for esterified cholesterol based on mg percent of the wet muscle. The longissimus dorsi of Simmental x Shorthorn, Simmental x Angus and Limousin x Angus crossbreeds were higher in esterified cholesterol than the corresponding biceps femoris with the reverse situation occuring in the other crossbreeds (Fig. 9). The crossbreed x sex x muscle interaction observed for esterified cholesterol indicated certain trends responsible for this effect. In longissimus dorsi of bulls from the Simmental x Hereford, Simmental x Angus and Limousin x Shorthorn crossbreeds were higher than the corresponding steers while the reverse situation was evident for the remaining crossbreeds. In the biceps femoris, with the exception of Simmental x Hereford and Simmental x Shorthorn crossbreeds, bulls were all higher in esterified cholesterol than steers as shown in Table 23.

Phospholipids

Total Phospholipids

Statistical analyses were carried out for total phospholipids and cholesterol/lipid phosphorous ratio. Mean squares and F values used to test the main effects and interactions are shown in Table 24.

Effect of Anatomical Location

No significant ($P < 0.05$) difference due to anatomical location was observed for total phospholipids whether expressed as mg per g of lipid, percent of the wet muscle or percent of the fat-free muscle (Table 25).

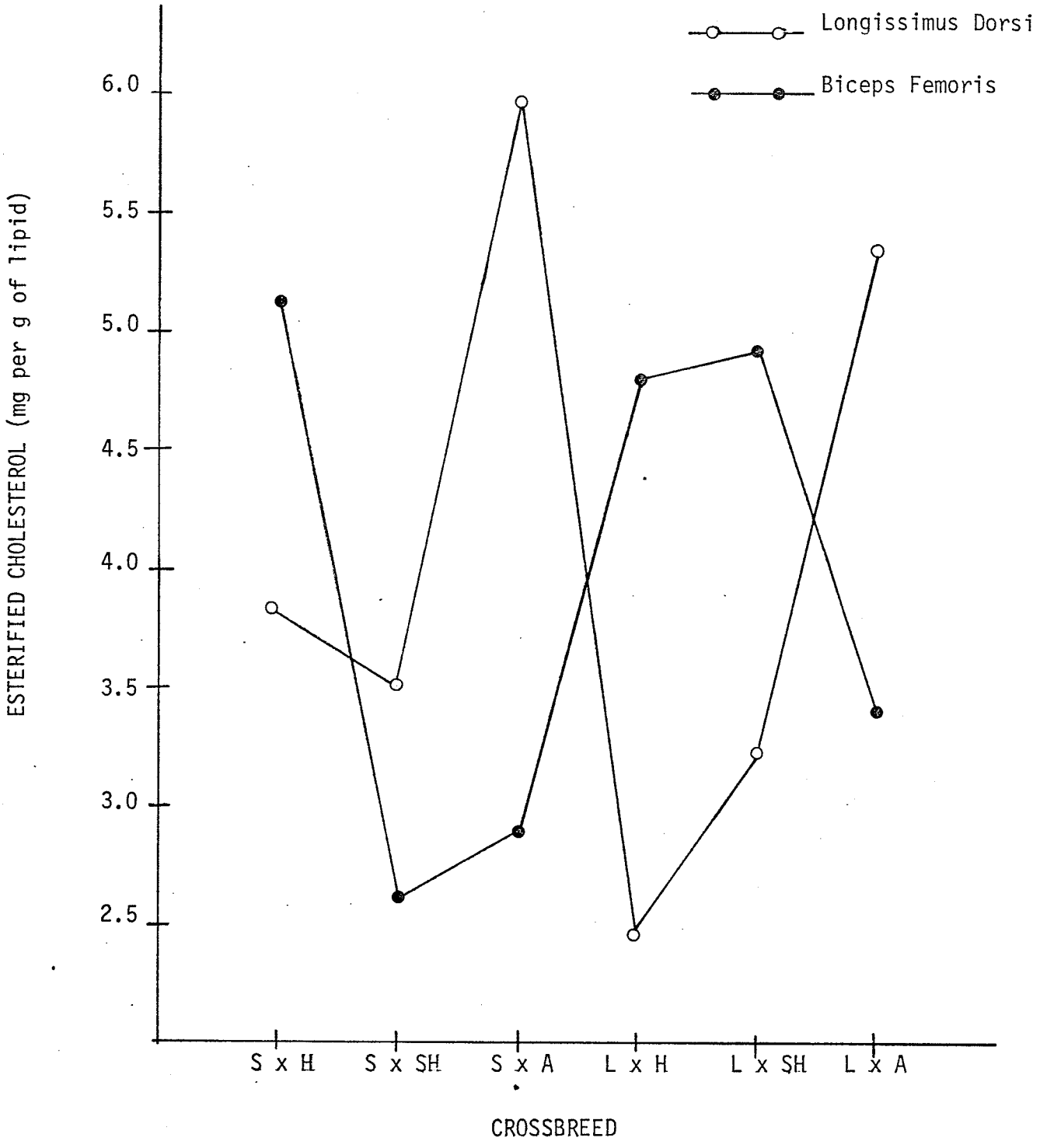

Fig. 9. Significant ($P < 0.05$) crossbreed x muscle location for esterified cholesterol.

Table 23. Mean Comparison of Esterified Cholesterol from Six Crossbreeds, Two Sexes, and Two Anatomical Locations[a,b].

	Anatomical Location			
	Longissimus Dorsi		Biceps Femoris	
Crossbreed	Bull	Steer	Bull	Steer
S x H	5.72	1.84	3.53	6.76
S x SH	2.66	4.31	2.38	2.73
S x A	7.65	4.09	4.94	0.95
L x H	1.61	3.37	5.66	3.92
L x SH	3.87	2.48	5.55	4.24
L x A	3.96	6.63	4.77	2.04

[a]Means expressed as mg per g of lipid.

[b]Standard deviation ±1.02.

Table 24. Analysis of Variance for Total Phospholipid, Cholesterol/Lipid Phosphorous Ratio.

Source	df	% of Muscle		% of Fat-Free Muscle		mg/g lipid		Cholesterol/ Lipid P	
		MS	F	MS	F	MS	F	MS	F
Crossbreed	5	0.019	1.94	0.014	1.13	9686.89	1.14	0.22	1.05
Sex	1	0.086	8.97*	0.157	12.28*	74220.34	8.75*	0.42	2.00
B x S	5	0.008	0.80	0.006	0.480	8646.03	1.02	0.07	0.33
Among Animals	24	0.010		0.013		8478.42		0.21	
Muscle	1	0.011	2.31	0.009	1.35	1389.17	0.34	0.25	2.63
B x M	5	0.019	4.07*	0.021	3.29*	8266.89	2.03	0.21	2.21
S x M	1	0.118	24.71*	0.122	19.30*	15254.81	3.74	0.50	5.26*
B x S x M	5	0.082	17.19*	0.091	14.40*	61736.98	15.15*	2.11	22.21*
A x M within Crossbreed and Sex	24	0.0048		0.006		4073.85		0.095	

* Significant ($P < 0.05$)

Table 25. Mean Comparison of Phospholipids from Two Anatomical Locations[a,b].

	Phospholipids		
Anatomical Location	mg/g lipid	% of muscle	% Fat-Free Muscle
Longissimus Dorsi	203.87±10.64	0.69±0.011	0.72±0.013
Biceps Femoris	195.07±10.64	0.69±0.011	0.72±0.013

[a]Means from thirty-six observations.

[b]All values are not significantly different ($P < 0.05$).

These results indicate little difference between the biceps femoris and longissimus dorsi muscles with respect to the level of phospholipids. Terrell (1967) in studies on bovine lipids reported no significant difference for the levels of lipid phosphorous, expressed as mg per 100 g of the muscle between the longissimus dorsi, semitendinosus, semimembranosus and transversus abdominis muscles although they were significantly lower than the psoas major or triceps brachii muscles. O'Keefe et al. (1968), however, found the levels of phospholipids in semitendinosus and longissimus dorsi to be similar to that of the triceps brachii when expressed as g per 100 g of muscle tissue. Hornstein et al. (1967) also found that the semitendinosus, longissimus dorsi and transversus abdominis muscles were roughly comparable in phospholipid content but considerably less than that for the diaphragm and psoas major muscles. When phospholipids were determined relative to the amount of lipid present , O'Keefe et al. (1968) found the semitendinosus to be significantly higher than the longissimus dorsi and triceps brachii. This resulted primarily from the lower content of intramuscular fat in semitendinosus relative to the longissimus dorsi and triceps brachii muscles. The lack of significance between the longissimus dorsi and biceps femoris observed in this study appeared to be due to the similarity in the levels of fat in these muscles.

Effect of Sex

A significant ($P < 0.05$) sex effect was observed for total phospholipids as indicated in Table 24. The amount of phospholipids present in the bulls and steers from the crossbreeds studied was considerably higher than that for Angus bulls, heifers and steers reported by Hood and Allen (1971). Steers were significantly ($P < 0.05$)

higher in total phospholipids than bulls when expressed as percent of the wet muscle or percent of the fat-free muscle (Table 26). Although Hood and Allen (1971) did not observe a significant difference between the levels of phospholipids between bulls and steers when expressed as percent of the wet muscle or percent of the fat-free muscle, there was a tendency for steers to be slightly higher than bulls. When expressed as mg per g of lipid, however, bulls were significantly higher in phospholipids than steers as observed in studies by Hood and Allen (1971). The latter observation further confirms that the intramuscular fat (marbling) contains little phospholipids since the phospholipid content decreased appreciably with increasing extractable lipid as shown in Table 26.

Effect of Crossbreed

No significant ($P<0.05$) crossbreed effect was observed for total phospholipids. The mean comparison of phospholipids from six crossbreeds is presented in Table 27. There was a tendency for the Limousin crossbreeds, with the exception of Limousin x Hereford, to contain higher levels of phospholipids than the corresponding Simmental crossbreeds.

Interaction Effects

Significant ($P<0.05$) crossbreed x muscle, sex x muscle and crossbreed x sex x muscle interactions were observed for phospholipids when expressed as percent of the wet muscle or percent of the fat-free muscle. Fig. 10 illustrates that the crossbreed x muscle interaction resulted from higher levels of phospholipids in the longissimus dorsi of all crossbreeds with the exception of the Simmental x Shorthorn and Simmental x Angus. The sex x muscle interaction resulted from the

Table 26. Mean Comparison of Extractable Lipid and Phospholipids from Two Sexes[a,b].

Sex	Extractable Lipid	Phospholipids		
	% of wet muscle	mg/g lipid	% of Muscle	% Fat-Free Muscle
Bull	3.49±0.33	231.58±15.92	0.65±0.02	0.66±0.02
Steer	4.87±0.33	167.36±15.92	0.72±0.02	0.76±0.02

[a]Means from thirty-six observations.

[b]All values are significantly different ($P < 0.05$)

Table 27. Mean Comparison of Phospholipids from Six Crossbreeds.

Phospholipid	Crossbreed					
	S x H	S x SH	S x A	L x H	L x SH	L x A
mg/g lipid[a]	235.85	158.63	179.0	193.60	222.52	207.24
% of Muscle[b]	0.74	0.62	0.68	0.68	0.71	0.67
% of Fat-free muscle[c]	0.76	0.65	0.71	0.71	0.73	0.69

[a]Standard deviation ±26.58

[b]Standard deviation ±0.028

[c]Standard deviation ±0.032

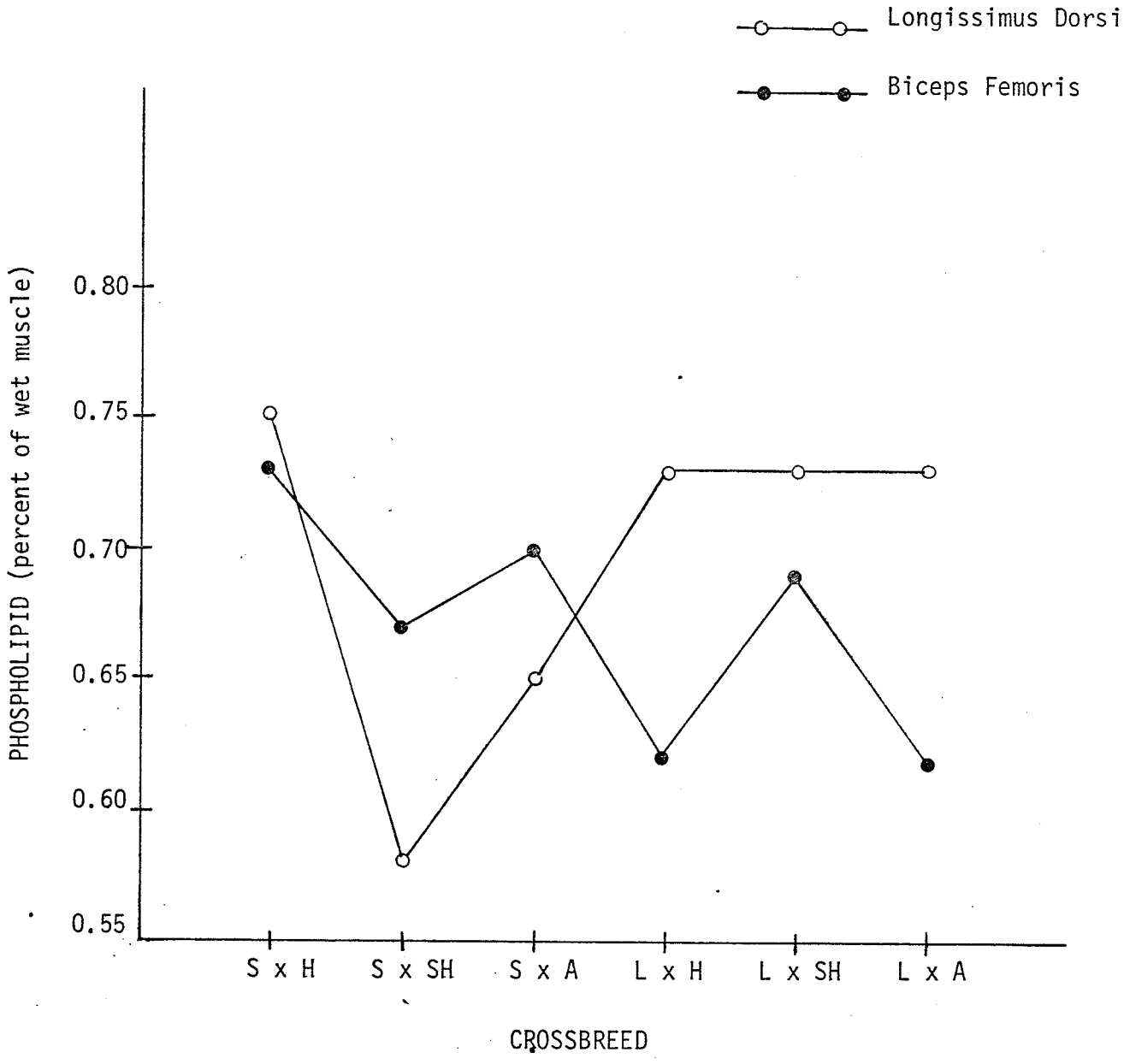

Fig. 10. Significant ($P < 0.05$) crossbreed x muscle location for phospholipid.

higher levels of phospholipids in the longissimus dorsi of steers while in the biceps femoris the reverse situation was observed as shown in Fig. 11. A comparison of means in Table 28 did not indicate any one particular trend responsible for the crossbreed x sex x muscle interaction although certain trends are apparent. In all of the crossbreeds the longissimus dorsi of steers was consistently higher in phospholipids than bulls while in the biceps femoris, with the exception of Simmental x Hereford and Simmental x Shorthorn, bulls tended to be higher than steers. Differences in the levels of phospholipids between the longissimus dorsi and biceps femoris muscles of bulls and steers within the six crossbreeds probably account for the interaction effect observed. When phospholipids were expressed as percent of the fat-free muscle, a similar pattern was evident for crossbreed x muscle, sex x muscle and crossbreed x sex x muscle interactions described above.

A significant ($P<0.05$) crossbreed x sex x muscle interaction was evident for phospholipids when expressed as mg per g of lipid. A comparison of means are shown in Table 29 in which certain trends are evident. In both muscle locations bulls were higher in phospholipids than the corresponding steers with the exception of Simmental x Angus in longissimus dorsi. Differences in the levels of phospholipids between the two anatomical locations of bulls and steers within the six crossbreeds account for the interaction effect observed.

Cholesterol/Lipid Phosphorous Ratio

Effect of Anatomical Location, Sex and Crossbreed

No significant ($P<0.05$) differences due to the anatomical location or sex were observed for cholesterol/lipid phosphorous ratio among the six crossbreeds studied. A mean comparison of cholesterol/

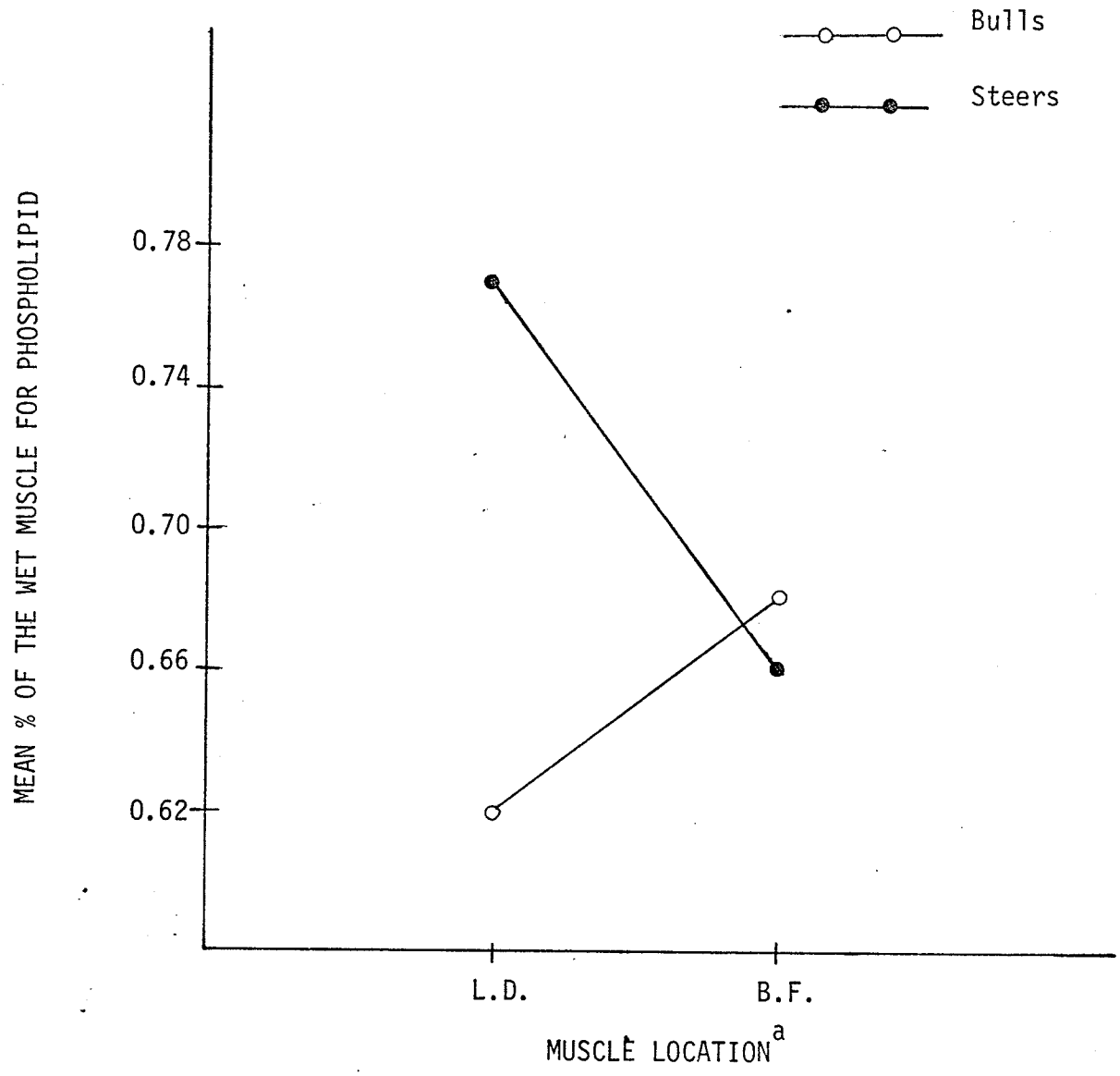

Fig. 11. Significant ($P<0.05$) sex x muscle location for phospholipid.

[a]L.D. = Longissimus Dorsi; BF = Biceps Femoris

Table 28. Mean Comparison of Phospholipids from Six Crossbreeds, Two Sexes, and Two Anatomical Locations[a,b].

	Anatomical Location			
	Longissimus Dorsi		Biceps Femoris	
Crossbreed	Bull	Steer	Bull	Steer
S x H	0.70	0.80	0.72	0.74
S x SH	0.50	0.65	0.63	0.70
S x A	0.51	0.80	0.74	0.65
L x H	0.62	0.85	0.63	0.62
L x SH	0.71	0.74	0.72	0.66
L x A	0.68	0.78	0.63	0.61

[a]Means expressed as percent of muscle.

[b]Standard deviation ±0.040.

Table 29. Mean Comparison of Phospholipids from Six Crossbreeds, Two Sexes, and Two Anatomical Locations[a,b].

	Anatomical Location			
	Longissimus Dorsi		Biceps Femoris	
Crossbreed	Bull	Steer	Bull	Steer
S x H	268.11	188.62	300.63	186.04
S x SH	157.05	134.91	200.64	141.91
S x A	168.65	190.54	223.53	133.27
L x H	228.67	159.96	232.97	152.78
L x SH	238.83	183.25	352.33	115.66
L x A	267.25	260.58	160.81	140.33

[a]Means expressed as mg per g of lipid.

[b]Standard deviation ±36.85.

lipid phosphorous ratios for longissimus dorsi and biceps femoris muscles showed the former muscle to be slightly lower than that for the biceps femoris. This is attributed to the slightly higher phospholipid level in longissimus dorsi reported earlier. Terrell (1967) reported cholesterol/lipid phosphorous ratio for Angus breeds and also found no significant differences among the six anatomical locations studied although the triceps brachii tended to have the smaller ratio. A comparison of means for bulls and steers in Table 30 indicated that steers tended to contain lower ratios compared to bulls. Although there was no significant crossbreed effect observed, there was a tendency for the Simmental crossbreeds to contain higher ratios than the corresponding Limousin crossbreeds (Table 31).

Interaction Effects

Significant sex x muscle and crossbreed x sex x muscle interactions were observed. It is evident from Fig. 12 that sex x muscle interaction resulted from higher cholesterol/lipid phosphorous ratio in the longissimus dorsi of bulls compared to steers; while the opposite situation was evident in the biceps femoris. A comparison of the means for cholesterol/lipid phosphorous ratio in Table 32 did not indicate any one particular trend responsible for crossbreed x sex x muscle interaction observed. In all of the crossbreeds studied, with the exception of Simmental x Shorthorn, the longissimus dorsi of bulls had consistently higher ratios than steers. In the biceps femoris, however, only bulls from the Simmental x Shorthorn, Limousin x Hereford and Limousin x Angus had higher ratios than the corresponding steers. Differences in the ratios between longissimus dorsi and biceps femoris muscles of bulls and steers within the six crossbreeds account for the interaction effect observed.

Table 30. Mean Comparison of Cholesterol/Lipid Phosphorous Ratios from Two Sexes, and Two Anatomical Locations[a].

Sex		Anatomical Location	
Bull	Steer	Longissimus Dorsi	Biceps Femoris
2.00	1.85	1.86	1.98

[a]All values are not significantly different ($P < 0.05$)

Table 31. Mean Comparison of Cholesterol/Lipid Phosphorous Ratios from Six Crossbreeds[a].

Crossbreed					
S x H	S x SH	S x A	L x H	L x SH	L x A
1.86	2.06	2.11	1.77	1.83	1.88

[a]All values are not significantly different ($P<0.05$)

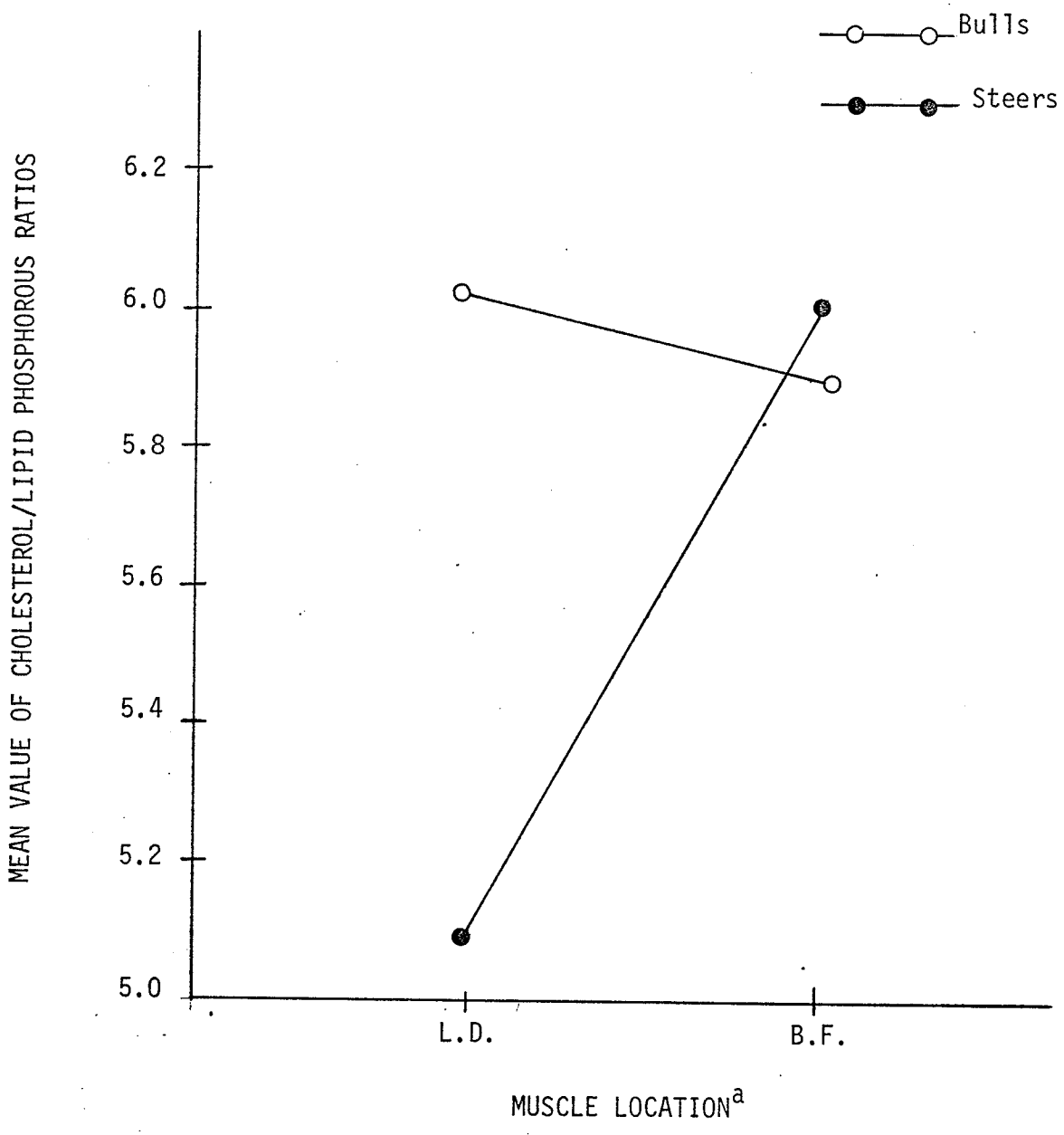

Fig. 12. Significant ($P < 0.05$) sex x muscle interaction for cholesterol/lipid phosphorous ratio.

[a]L.D. = Longissimus Dorsi; BF = Biceps Femoris

Table 32. Mean Comparison of Cholesterol/Lipid Phosphorous Ratio from Six Crossbreeds, Two Sexes, and Two Anatomical Locations[a].

	Anatomical Location			
	Longissimus Dorsi		Biceps Femoris	
Crossbreed	Bull	Steer	Bull	Steer
S x H	2.20	1.67	1.65	1.93
S x SH	1.90	2.22	2.60	1.56
S x A	2.56	1.85	1.92	2.13
L x H	1.80	1.51	1.90	1.87
L x SH	1.84	1.35	1.74	2.41
L x A	1.83	1.63	2.04	2.02

[a]Standard deviation ±0.18.

Phospholipid Composition

Statistical analyses were carried out for phospholipid composition. Mean squares and F values used to test the main effects and interactions are shown in Table 33.

A typical separation obtained by thin-layer chromatography for the extracted lipid samples and standard phospholipid mixtures is shown in Fig.13. The amount of each individual phospholipid fraction was determined by direct photodensitometry of the thin-layer plates and the following chromatogram obtained (Fig.14). Phosphatidylinositol and phosphatidylserine are reported as a single unit owing to the incomplete separation of these phospholipids, although phosphatidylinositol appeared to account for the major portion of this fraction.

The mean comparison of the individual phospholipids are presented in Table 34. It is evident that phosphatidylcholine and phosphatidylethanolamine are the major components present accounting for 41 and 31% of the total phospholipids, respectively. Phosphatidylserine + phosphatisylinositol and sphingomyelin were found to be present in approximately similar amounts of around 13% of the total phospholipids. Since lysophosphatidylcholine accounted for less than 1% of the total phospholipids present, it was not included in this investigation. The amount of phosphatidylcholine observed in the crossbreeds studied compared favourably with that reported by Hornstein et al. (1961) for bovine muscles but was lower than that observed by Turkki and Campbell (1967) for bovine extensor carpi radialis and psoas major muscles. The levels determined for phosphatidylethanolamine were similar to that observed by Turkki and Campbell (1967) although lower than that observed by Hornstein et al. (1961).

Table 33. Analysis of Variance for Four Phospholipid Fractions of Bovine Intramuscular Lipid.

Source	df	Phosphatidyl-choline		Phosphatidyl-ethanolamine		Phosphatidylserine + Phosphatidylinositol		Sphingo-myelin	
		MS	F	MS	F	MS	F	MS	F
Crossbreed	5	29.39	2.31	5.42	0.71	32.72	7.14*	1.32	0.80
Sex	1	2.68	0.21	9.32	1.22	0.014	0.003	0.98	0.59
B x S	5	15.26	1.20	8.00	1.05	4.26	0.93	6.91	4.16*
Among Animals	24	12.70		7.63		4.58		1.66	
Muscle	1	27.26	3.79	28.00	3.57	0.09	0.02	1.62	1.26
B x M	5	33.39	4.64*	15.94	2.03	17.41	3.32*	3.62	2.81*
S x M	1	9.17	1.28	0.01	0.001	9.97	1.90	0.06	0.05
B x S x M	5	95.76	13.32*	87.72	11.19*	51.52	9.81*	20.13	15.60*
A x M within crossbreed and sex	24	7.19		7.84		5.25		1.29	

* Significant ($P < 0.05$)

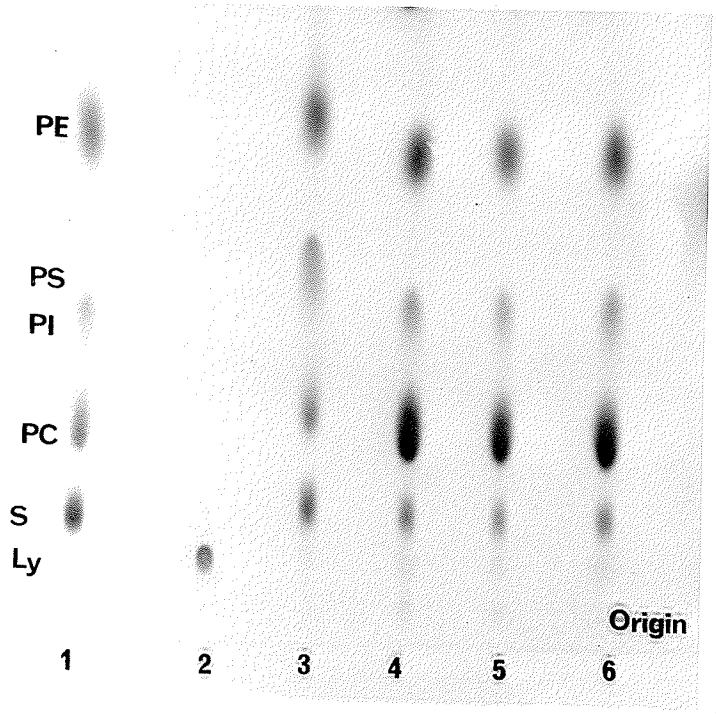

Fig. 13. Thin-layer chromatogram of extracted lipid samples and standard phospholipid mixtures.
1,3, standard phospholipid mixtures; 2, standard phospholipid; 4,5,6, extracted lipid samples. Standard mixtures contain phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylcholine (PC), and sphingomyelin (S); lysophosphatidylcholine (Ly).

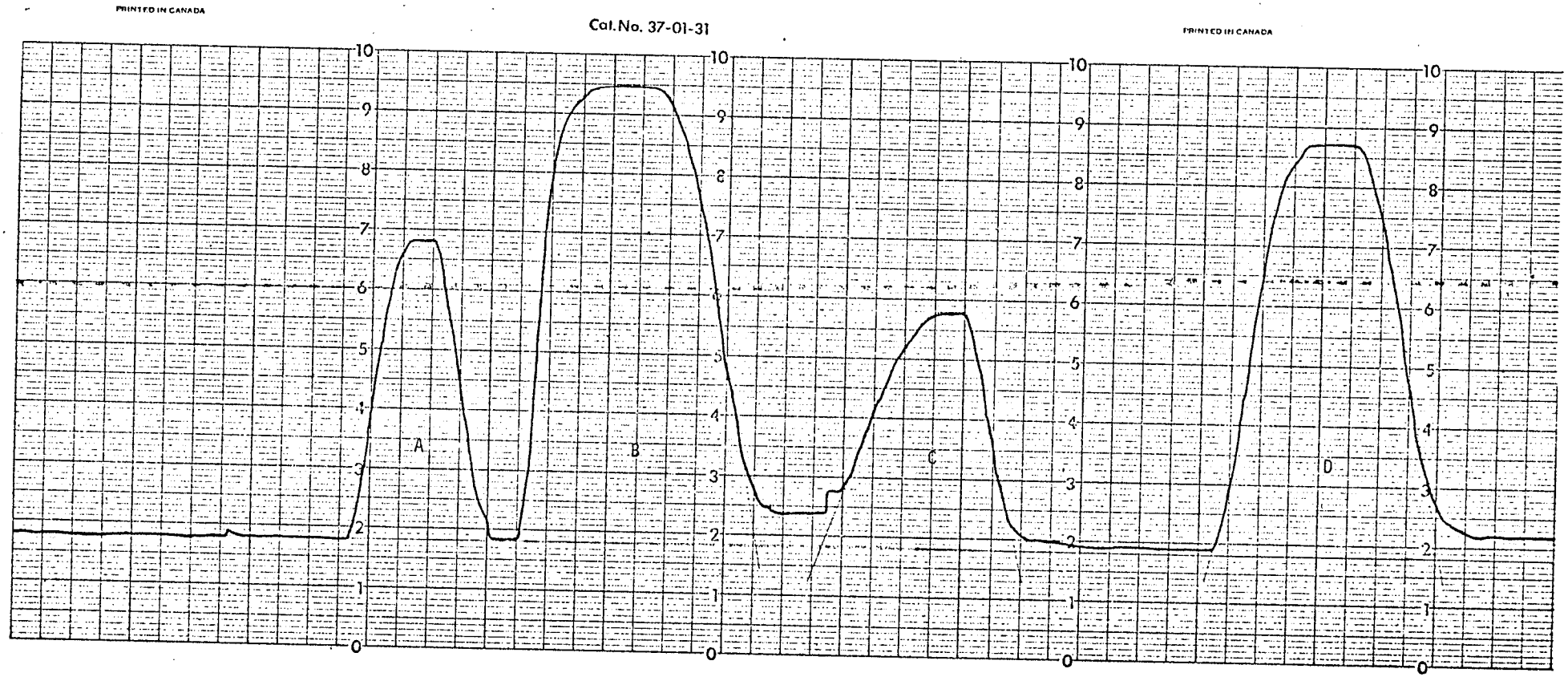

Fig. 14. A typical chromatogram of the phospholipid composition of bovine intramuscular lipid.

A : Sphingomyelin; B : Phosphatidylcholine; C : Phosphatidylserine + Phosphatidylinositol; D : Phosphatidylethanolamine.

Table 34. Mean Comparison of Phospholipid among Phosphatidylcholine, Phosphatidylethanolamine, Phosphatidylserine + Phosphatidylinositol and Sphingomyelin[ab].

Phospholipid Fraction	Relative percentage
Phosphatidylcholine	41.30±0.42
Phosphatidylethanolamine	31.99±0.32
Phosphatidylserine + Phosphatidylinositol	13.62±0.25
Sphingomyelin	13.06±0.15

[a]Means expressed as relative percentage of four phospholipid fractions.

[b]Means from seventy-two observations.

Little information is available regarding the levels of phosphatidylserine + phosphatidylinositol in bovine lipids although the amounts found in the crossbreeds studied were higher than that reported in porcine and poultry muscles (Kuchmak and Dugan, 1963; Wangen et al., 1971). The amount of sphingomyelin determined in this study appeared to come within the range reported by Hornstein et al. (1961) for bovine animals.

The variations in phospholipid composition observed are probably related to the differences in methodology in addition to other factors such as species, breed and age.

Effect of Anatomical Location and Sex

No significant ($P < 0.05$) effects due to anatomical location or sex were observed in this study. A mean comparison of the four phospholipid fractions showed little variation between longissimus dorsi and biceps femoris (Table 35). This is in agreement with that reported by Kuchmak and Dugan (1963) and Turkki and Campbell (1967) for porcine and bovine muscle, respectively. These workers found that the phospholipid composition remained relatively constant in muscles and was independent of carcass location. Turkki and Campbell (1967) reported the means for the cephalin fraction to be the only one approaching a significant difference ($0.1 > P > 0.05$) between the extensor carpi radialis and psoas major muscles. The lack of significance in this study further indicates that there is little variation in individual phospholipid fractions between muscle locations. A similar pattern was obtained with respect to the effect of sex in which there appeared to be no significant differences between the castrated and uncastrated animals.

Table 35. Mean Comparison of Each Phospholipid Fraction from Two Anatomical Locations and Two Sexes[a,b,c].

	Anatomical Location		Sex	
Phospholipid Class	Longissimus Dorsi	Biceps Femoris	Bull	Steer
Phosphatidylcholine	41.9±0.45	40.7±0.45	41.1±0.59	41.5±0.59
Phosphatidylethanolamine	31.4±0.47	32.6±0.47	32.3±0.46	31.6±0.46
Phosphatidylserine + Phosphatidylinositol	13.5±0.38	13.6±0.38	13.6±0.36	13.5±0.36
Sphingomyelin	13.2±0.19	12.9±0.19	12.9±0.21	13.2±0.21

[a]All values are not significantly different ($P < 0.05$)

[b]Means expressed as relative percentage of four phospholipid fractions.

[c]Means from thirty-six observations.

Effect of Crossbreed

A significant ($P<0.05$) crossbreed effect was found for the phosphatidylserine + phosphatidylinositol fraction as shown in Table 33. A division of the crossbreed main effect into sire, cow, and sire x cow effects (Table 36) revealed that cow and sire x cow contributed to the significant ($P<0.05$) crossbreed effect observed. A comparison of means in Table 37 indicates that Angus crossbreeds were significantly higher in this fraction than the other crossbreeds with Hereford crossbreeds being significantly lower than Shorthorn crossbreeds.

Interaction Effects

A significant ($P<0.05$) crossbreed x sex interaction was observed for sphingomyelin. This interaction is a result of the lower levels of sphingomyelin in bulls from the Simmental x Hereford, Simmental x Angus and Limousin x Hereford crossbreeds together with the higher levels in bulls from Simmental x Shorthorn, Limousin x Shorthorn and Limousin x Angus crossbreeds (Fig. 15).

Significant ($P<0.05$) crossbreed x muscle interactions were also evident for sphingomyelin, phosphatidylcholine and phosphatidylserine + phosphatidylinositol fractions. The significant crossbreed x muscle interaction for sphingomyelin resulted from the higher levels in the longissimus dorsi from the Simmental x Shorthorn, Simmental x Angus and Limousin x Hereford crossbreeds and the lower levels in the corresponding Simmental x Hereford, Limousin x Shorthorn and Limousin x Angus crossbreeds as illustrated in Fig. 16. The crossbreed x muscle interaction for phosphatidylcholine as shown in Fig. 17 appears to be due to the higher levels in the longissimus dorsi from the Simmental x Shorthorn, Simmental x Angus and Limousin x Angus crossbreeds together with the reverse situation for the other crossbreeds. The consistently higher

Table 36. Analysis of Variance for Sire, Cow and Sire x Cow for Phosphatidylserine + Phosphatidylinositol with a Significant Crossbreed Effect.

Source	df	MS	F
Sire	1	3.92	0.86
Cow	2	45.79	10.00*
Sire x Cow	2	34.04	7.43*
Among Animals	24	4.58	

*Significant ($P < 0.05$)

Table 37. Mean Comparison of Phosphatidylserine + Phosphatidylinositol of the Intramuscular Lipid from Two Sires and Three Cows[a,b]

Sire[c]		Cow[d]		
Simmental	Limousin	Hereford	Shorthorn	Angus
13.39	13.86	12.23	13.64	15.00

[a]Means expressed as relative percentage of four phospholipid fractions.

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

[c]Standard deviation ±0.97.

[d]Standard deviation ±0.44

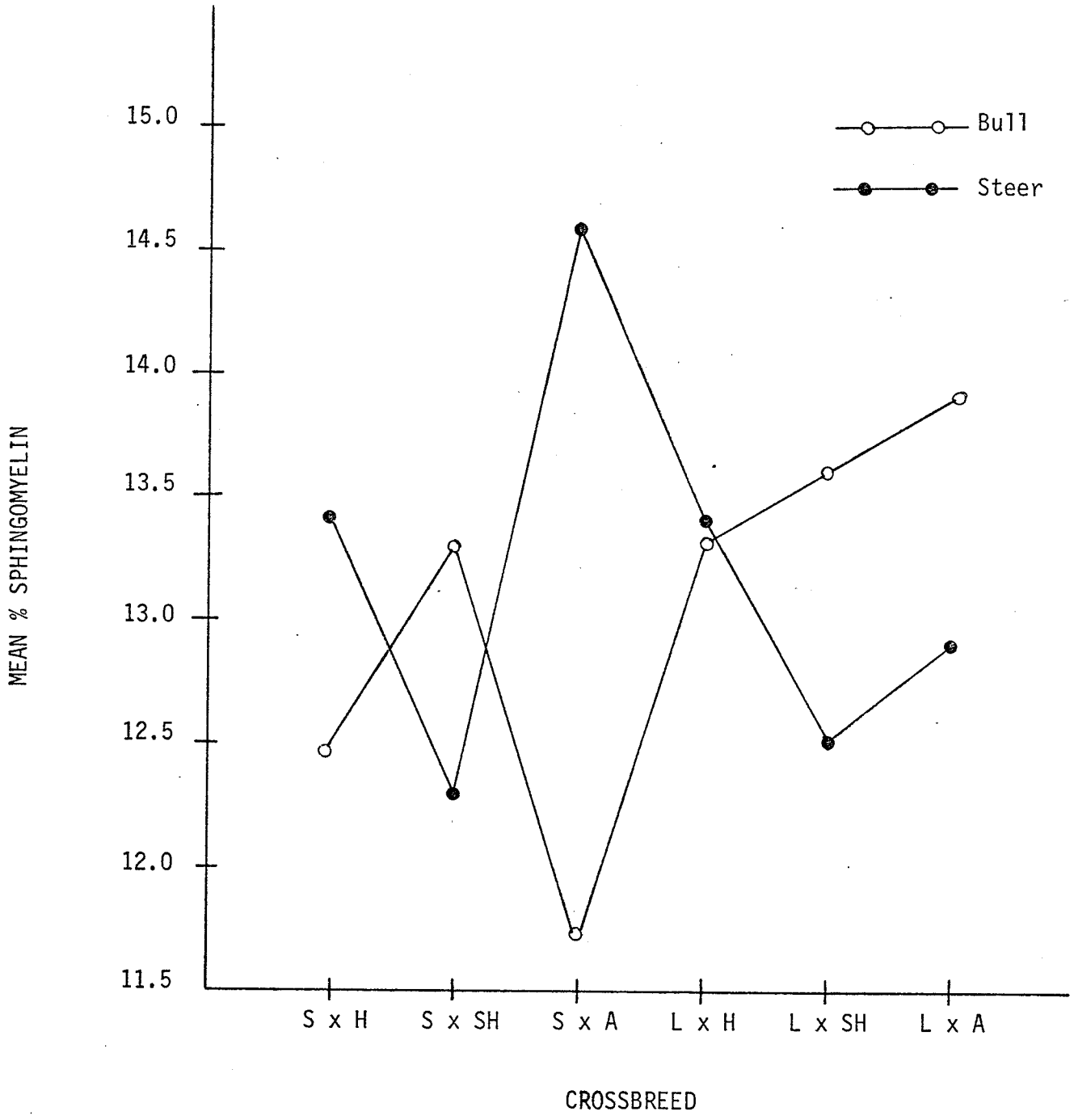

Fig. 15. Significant ($P < 0.05$) crossbreed x sex interaction for sphingomyelin.

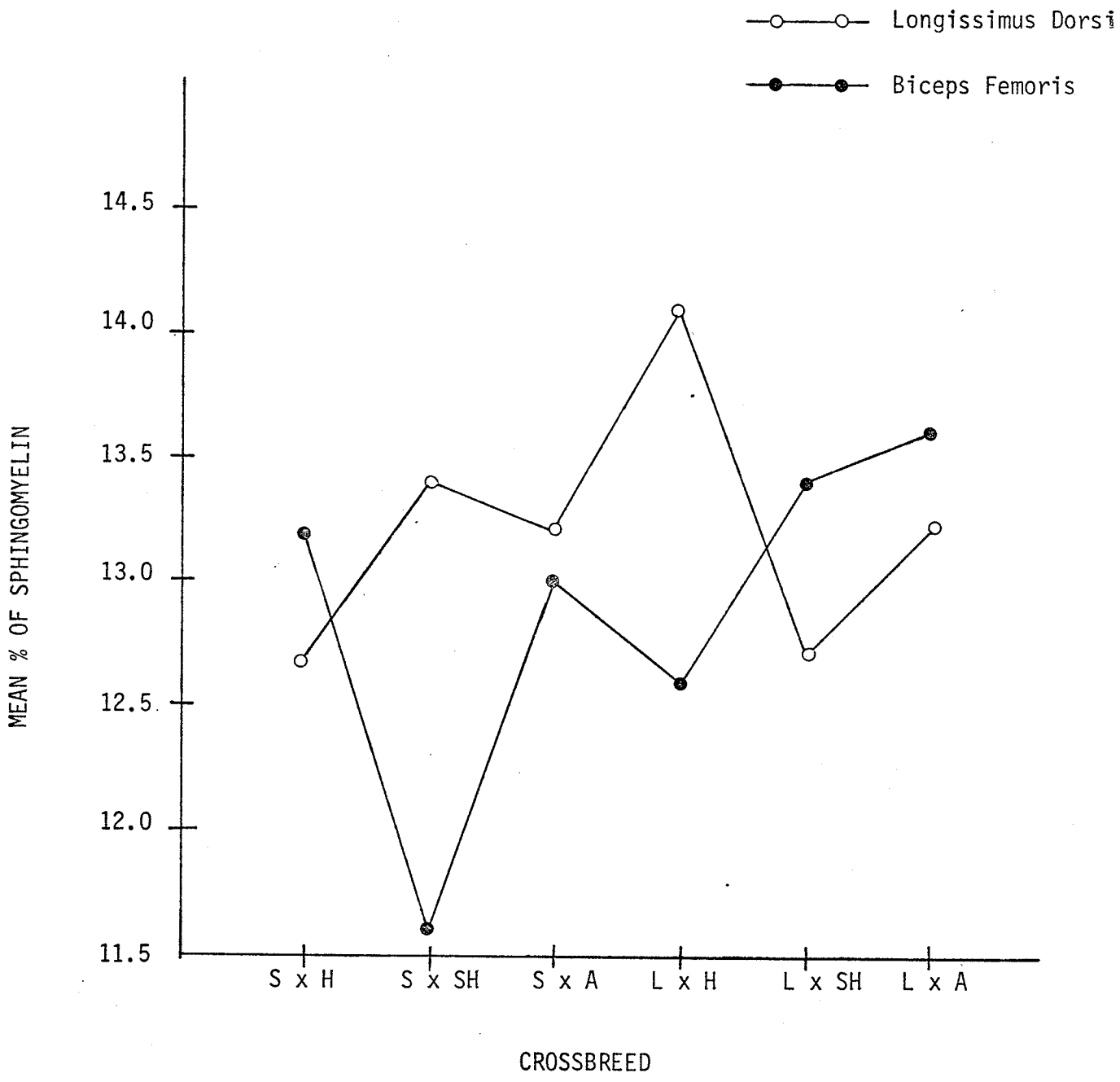

Fig. 16. Significant ($P < 0.05$) crossbreed x muscle location for sphingomyelin.

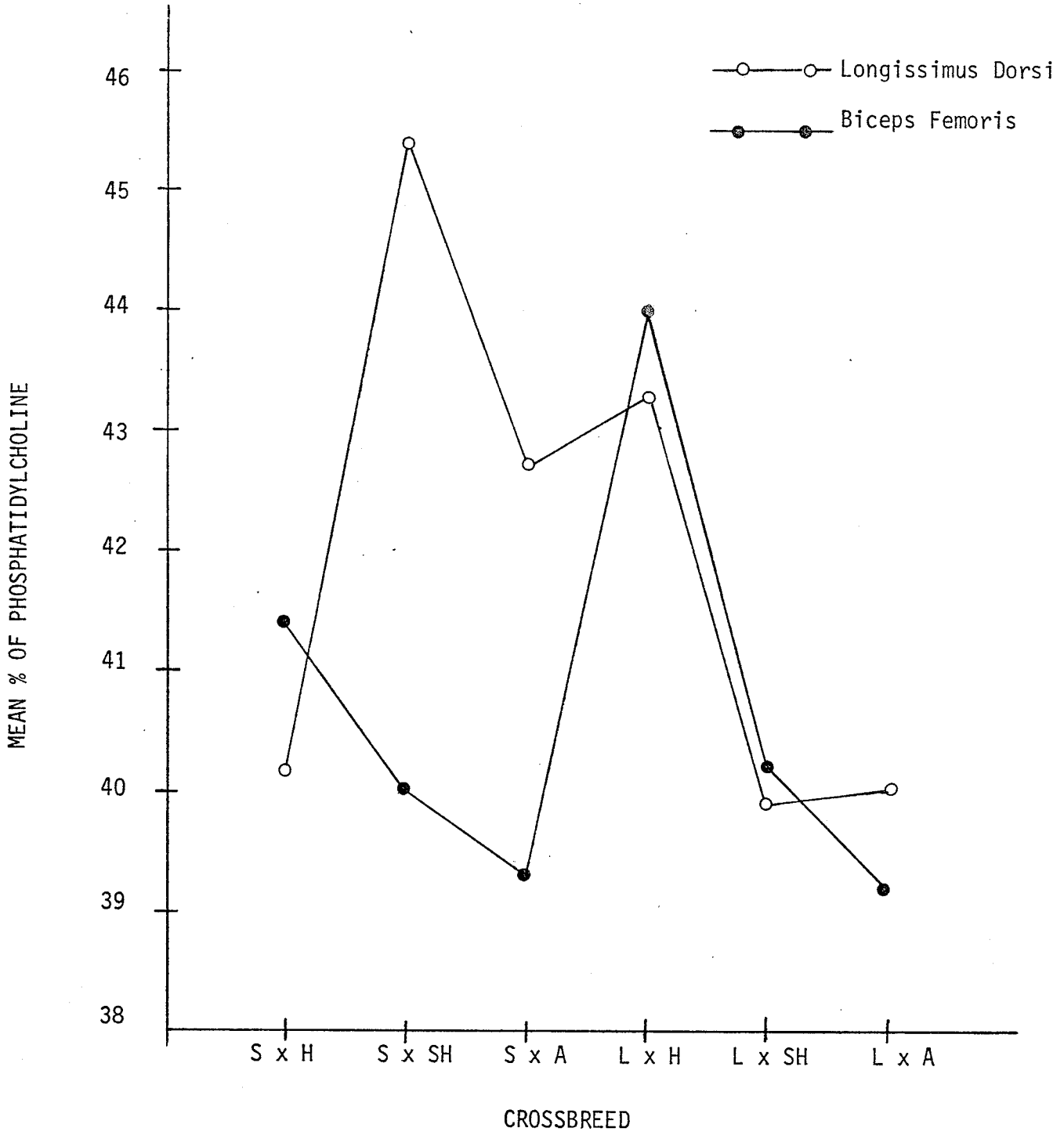

Fig. 17. Significant ($P < 0.05$) crossbreed x muscle location for phosphatidylcholine.

levels of phosphatidylserine + phosphatidylinositol in the longissimus dorsi of all of the crossbreeds, with the exception of the Simmental x Shorthorn, probably contributed to the crossbreed x muscle location observed (Fig. 18).

Significant ($P < 0.05$) crossbreed x sex x muscle interactions were observed for sphingomyelin, phosphatidylcholine, phosphatidylserine + phosphatidylinositol and phosphatidylethanolamine. These triple interactions are somewhat complex although certain trends are obvious. With the excpetion of Simmental x Hereford and Simmental x Angus the longissimus dorsi of bulls were higher in sphingomyelin than steers whereas in the biceps femoris, with the exception of Limousin x Shorthorn and Limousin x Angus, steers were higher than bulls as shown in Table 38.

A comparison of means for phosphatidylcholine is shown in Table 39. In all the Simmental crossbreeds the longissimus dorsi of bulls were higher than steers while the reverse situation was evident in the corresponding biceps femoris. In the Limousin crossbreeds steers from the Limousin x Shorthorn were higher than bulls in both the longissimis dorsi and biceps femoris muscles while bulls were higher than steers in both muscles from the Limousin x Angus. In the Limousin x Hereford crossbreeds, however, steers were higher than bulls in the longissimus dorsi while the reverse situation was observed in the biceps femoris muscle. The differences in levels of phosphatidylcholine between the two anatomical locations of bulls and steers within the six crossbreeds studied probably contributed to the triple interaction observed.

Certain trends were also apparent for the crossbreed x sex x muscle interaction reported for phosphatidylserine + phosphatidylinositol as shown in Table 40. In all the Hereford crossbreeds with the exception

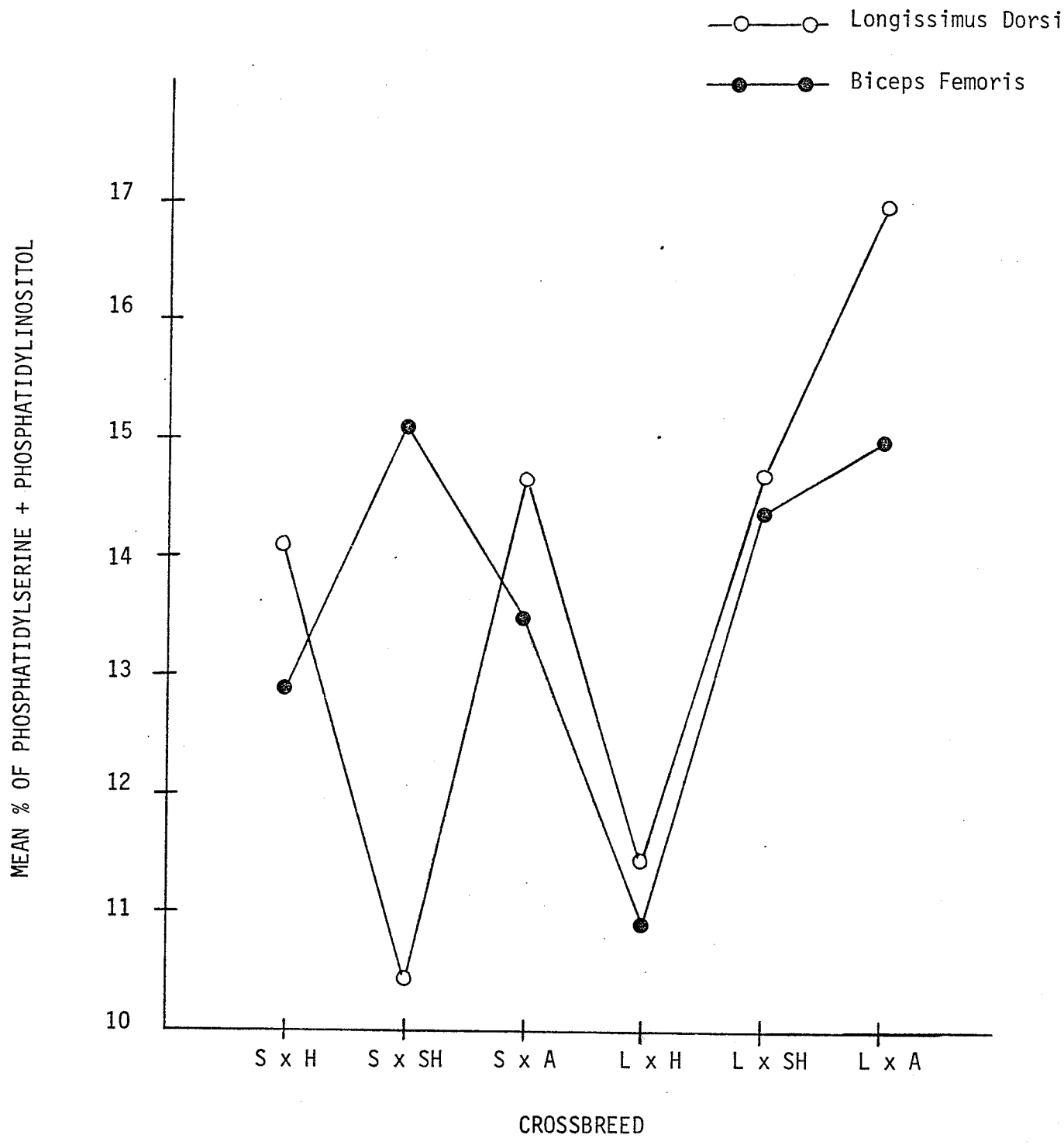

Fig. 18. Significant ($P < 0.05$) crossbreed x muscle location for phosphatidylserine + phosphatisylinositol.

Table 38. Mean Comparison of Sphingomyelin from Six Crossbreeds, Two Sexes and Two Anatomical Locations[a,b].

	Anatomical Location			
	Longissimus Dorsi		Biceps Femoris	
Crossbreed	Bull	Steer	Bull	Steer
S x H	12.4	12.9	12.6	13.9
S x SH	14.1	12.7	11.3	11.8
S x A	10.4	16.0	12.9	13.2
L x H	14.4	13.8	12.2	12.9
L x SH	13.7	11.6	13.5	13.3
L x A	13.3	13.1	14.5	12.7

[a]Means expressed as relative percentage of four phospholipid fractions.

[b]Standard deviation ±0.66.

Table 39. Mean Comparison of Phosphatidylcholine from Six Crossbreeds, Two Sexes and Two Anatomical Locations[a,b].

	Anatomical Location			
	Longissimus Dorsi		Biceps Femoris	
Crossbreed	Bull	Steer	Bull	Steer
S x H	40.6	39.8	40.97	41.87
S x SH	46.2	44.6	36.7	43.20
S x A	43.5	42.0	38.6	40.0
L x H	43.1	43.5	45.9	42.1
L x SH	37.7	42.0	38.7	41.7
L x A	41.3	38.7	40.0	38.5

[a]Means expressed as relative percentage of four phospholipid fractions.

[b]Standard deviation ±1.55.

Table 40. Mean Comparison of Phosphatidylserine + Phosphatidylinositol from Six Crossbreeds, Two Sexes, and Two Anatomical Locations[a,b].

	Anatomical Location			
	Longissimus Dorsi		Biceps Femoris	
Crossbreed	Bull	Steer	Bull	Steer
S x H	14.1	13.8	14.0	11.6
S x SH	9.9	10.8	16.4	13.7
S x A	13.8	15.6	14.1	12.8
L x H	12.2	10.6	9.5	12.1
L x SH	14.7	14.6	14.7	14.2
L x A	14.9	18.8	14.9	15.1

[a]Means expressed as relative percentage of four phospholipids.

[b]Standard deviation ±1.32.

of the biceps femoris of the Limousin x Hereford, bulls were higher in phosphatidylserine + phosphatidylinositol than steers. A similar pattern was also found for the Shorthorn crossbreeds in which bulls were higher than steers in all crossbreeds with the exception of the longissimus dorsi from the Simmental x Shorthorn. In the case of the Angus crossbreeds, however, steers were found to be higher than bulls in all the crossbreeds with the exception of biceps femoris from the Simmental x Angus.

A comparison of means for phosphatidylethanolamine in Table 41 does not indicate any one particular trend responsible for the crossbreed x sex x muscle interaction, although certain trends are apparent. In all the crossbreeds with the exception of Limousin x Angus the biceps femoris of bulls were higher than steers while in the longissimus dorsi with the exception of Simmental x Shorthorn and Limousin x Hereford crossbreeds, a similar trend was found. The differences in levels of phosphatidylethanolamine between the two anatomical locations of bulls and steers within the six crossbreeds probably account for the triple effect observed.

Table 41. Mean Comparison of Phosphatidylethanolamine from Six Crossbreeds, Two Sexes and Two Anatomical Locations[a,b].

	Anatomical Location			
	Longissimus Dorsi		Biceps Femoris	
Crossbreed	Bull	Steer	Bull	Steer
S x H	34.0	33.7	32.5	32.4
S x SH	29.5	31.9	34.9	30.7
S x A	32.2	26.6	34.4	33.8
L x H	30.4	32.5	32.5	32.1
L x SH	33.8	31.8	32.8	30.6
L x A	30.4	29.5	30.9	33.7

[a]Means expressed as relative percentage of four phospholipid fractions.

[b]Standard deviation ±1.62.

SUMMARY AND CONCLUSIONS

The recent introduction of European breeds of cattle into Canada was carried out in order to improve the carcass quality and productivity of the existing breeds. These changes have been shown to have a significant effect on many of the body components which affect the quality of the meat. This program was undertaken to assess the influence of crossbreed, sex and anatomical location on the quantity and composition of phospholipids and cholesterol. The animals used in this study were randomly selected to represent six crossbreeds from the Canada Department of Agriculture Research Station, Brandon. Analysis of the data indicated that crossbreed and sex had a greater influence on the differences observed for the phospholipid and cholesterol components than anatomical location. No significant differences in percent moisture were observed although the muscles of bulls tended to have a higher moisture content than those of steers. Steers had a significantly higher percent extractable lipid on a wet muscle basis than bulls.

A comparison of the levels of total, free and esterified cholesterol determined in this study showed no significant difference between the longissimus dorsi and biceps femoris muscles. A significant crossbreed x muscle interaction was evident for free cholesterol (mg percent of the wet muscle) and esterified cholesterol (mg per g of lipid or mg percent of the wet muscle). The former resulted from biceps femoris of the Shorthorn and the Angus crossbreeds being higher in free cholesterol than the corresponding longissimus dorsi with the reverse situation being the case

for the Hereford crossbreeds. The latter resulted from the higher levels of esterified cholesterol in the longissimus dorsi of Simmental x Shorthorn, Simmental x Angus and Limousin x Angus crossbreeds than the biceps femoris with opposite situation occuring in the Simmental x Hereford, Limousin x Hereford and Limousin x Shorthorn crossbreeds. Definite sex effects were found for total, free (mg per g of lipid) and esterified cholesterol (mg percent of the wet muscle). Bulls were significantly higher in total and free cholesterol than steers while the opposite relationship was apparent for esterified cholesterol. Significant crossbreed x sex interactions were observed for free and esterified cholesterol (mg percent of the wet muscle). The former resulted from the higher levels of free cholesterol in bulls from the Hereford and Shorthorn crossbreeds than steers with the reverse situation occuring in Angus crossbreeds. The significant crossbreed x sex interaction for esterified cholesterol resulted from the higher levels present in steers from the Hereford and Shorthorn crossbreeds and the lower levels in steers from the Angus crossbreeds compared to bulls. The significant crossbreed differences observed for total and free cholesterol (mg percent of the wet muscle) resulted from higher levels in the Simmental crossbreeds compared to Limousin crossbreeds. In addition the Angus crossbreeds were found to be significantly higher in free cholesterol than either the Hereford or Shorthorn crossbreeds. The sire x cow interaction for free cholesterol resulted from the higher levels in Simmental x Hereford and Simmental x Angus crossbreeds compared to corresponding Limousin x Hereford and Limousin x Angus crossbreeds, while the Limousin x Shorthorn were higher than the corresponding Simmental x Shorthorn crossbreeds. Three term interactions (crossbreed

x sex x muscle) were also found for total, free and esterified cholesterol.

No differences due to crossbreed or anatomical location were observed for total phospholipids. The significant crossbreed x muscle interactions for total phospholipids expressed as percent of the wet muscle or percent of the fat-free muscle were attributed mainly to the higher levels present in the longissimus dorsi in four of the six crossbreeds studied. Definite sex effects were evident for total phospholipids when expressed as percent of the wet muscle, percent of the fat-free muscle or mg per g of lipid. Bulls were significantly higher in phospholipids than steers when expressed as mg per g of lipid while the reverse situation occured when expressed as percent of the wet muscle or percent of the fat-free muscle. Significant sex x muscle interactions were apparent for total phospholipids expressed as percent of the wet muscle or percent of the fat-free muscle. This resulted from the higher levels of phospholipids in the longissimus dorsi of steers together with the reverse situation in the biceps femoris. The crossbreed x sex x muscle interactions for total phospholipids were attributed in part to the differences in levels between bulls and steers from the longissimus dorsi and biceps femoris muscles. The sex x muscle interaction for cholesterol/lipid phosphorous ratio resulted from the higher ratio in the longissimus dorsi of bulls than steers with the reverse situation in the biceps femoris.

The phospholipid composition did not vary between muscles and appeared to be independent of anatomical location. A crossbreed effect was observed for the phosphatidylserine + phosphatidylinositol fraction only. This resulted from the higher levels of this fraction in Angus crossbreeds and lower levels in Hereford crossbreeds compared to the

Shorthorn crossbreeds. Significant crossbreed x muscle interactions were observed for phosphatidylcholine, phosphatidylserine + phosphatidylinositol and sphingomyelin. The interaction effect for sphingomyelin resulted from the higher levels in the longissimus dorsi from the Simmental x Shorthorn, Simmental x Angus and Limousin x Hereford crossbreeds and the lower levels in the longissimus dorsi from the Simmental x Hereford, Limousin x Shorthorn and Limousin x Angus crossbreeds. The higher levels of phosphatidylcholine in the longissimus dorsi from the Simmental x Shorthorn, Simmental x Angus and Limousin x Angus crossbreeds and the reverse situation for the other crossbreeds was responsible for the crossbreed x muscle interaction observed for this phospholipid. The higher levels of phosphatidylserine + phosphatidylinositol in the longissimus dorsi in all of the crossbreeds studied,with the exception of Simmental x Shorthorn, were responsible for the crossbreed x muscle interaction. The significant crossbreed x sex interaction for sphingomyelin resulted from the lower levels of this phospholipid in bulls from the Simmental x Hereford, Simmental x Angus and Limousin x Hereford crossbreeds and the higher levels in bulls from the remaining crossbreeds. Significant crossbreed x sex x muscle interactions were observed for the four phospholipid fractions studied. These resulted from differences between bulls and steers from the two anatomical locations among the six crossbreeds studied.

An investigation of the positional distribution of fatty acid within the individual phospholipid fractions should be carried out in order to further clarify the importance of polar lipids in relation to meat quality.

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