SLIP POINT OF SUBCUTANEOUS ADIPOSE TISSUE LIPIDS AS AN INDICATOR OF BEEF CARCASS QUALITY

A Thesis

by

LINDSAY PAIGE WARD

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2008

Major Subject: Animal Science

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Approved by:

Chair of Committee,	Stephen B. Smith
Committee Members,	Marcos Sanchez-Plata
	Jeffrey W. Savell
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ABSTRACT

Slip Point of Subcutaneous Adipose Tissue Lipids as an Indicator of Beef Carcass Quality. (May 2008)

Lindsay Paige Ward, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Stephen B. Smith

We hypothesized that slip point of subcutaneous (s.c.) adipose tissue lipids would predict beef carcass quality. To address our hypothesis, 79 *M. longissimus dorsi* (LD) steaks from cattle of unknown background were used to provide information on slip points, percentage intramuscular lipid, fatty acid composition, and MUFA:SFA ratios. Overlying s.c. adipose tissue was separated from the muscle lean, which contained intramuscular (i.m.) adipose tissue. Lipids were extracted from s.c. adipose tissue and muscle lean by a modified chloroform:methanol procedure and subjected to various analyses. The hypothesis was tested by developing regression equations to determine which fatty acid variables were most useful in predicting carcass composition. There was a high correlation between s.c. MUFA:SFA ratio and s.c. slip points ($P < 0.001$) with an R^2 of 0.557. Also, the MUFA:SFA fatty acid ratios of s.c. and i.m. adipose tissue were significantly correlated and an R^2 of 0.440 was observed ($P < 0.001$) when regressed against each other. The current data set observed s.c. MUFA:SFA ratios (0.73) lower than previous studies, which suggests a population of young or unfinished cattle. This study demonstrated that it is possible to predict the intramuscular lipid (IML) MUFA:SFA ratio by measuring s.c. slip point (R^2 of 0.097; $P < 0.01$). However,

our hypothesis of predicting amount of marbling, hence quality grade, from the melting temperature of s.c. adipose tissue lipids proved incorrect ($R^2 = 0.001$). Nonetheless, these data indicate that LD fatty acid composition can be estimated by measuring s.c. adipose tissue slip point.

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NOMENCLATURE

AMSA	American Meat Science Association
FAME	Fatty acid methyl ester/esterification
HDL	High density lipoprotein
i.m.	Intramuscular adipose tissue
IML	Intramuscular lipid
LD	*M. Longissimus dorsi*
LDL	Low density lipoprotein
MUFA	Monounsaturated fatty acid
NMR	Nuclear Magnetic Resonance
PUFA	Polyunsaturated fatty acid
s.c.	Subcutaneous adipose tissue
SCD	Stearoyl-CoA desaturase
SFA	Saturated fatty acid
UFA	Unsaturated fatty acids
VLDL	Very low density lipoprotein

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CHAPTER I

INTRODUCTION

Marbling is a major contributor to the determination of carcass quality. Therefore, prediction of this carcass quality trait would be beneficial in allowing cattle producers to finish their cattle at the appropriate time. Both s.c. and i.m. adipose tissues are positively related to nearly all of the organoleptic properties of beef (tenderness, juiciness, and beef flavor intensity) thus, dictates consumer satisfaction of a product (Smith et al., 1984; Wheeler, Cundiff & Koch, 1994; Tatum, Smith & Carpenter, 1982). The type as well as the amount of a specific fatty acid in beef affects consumer preference (May, Sturdivant, Lunt, Miller & Smith, 1993) with unsaturated fatty acids (UFA) identified as the most influential pertaining to flavor (Westerling & Hendrick, 1979). Monounsaturated and saturated fatty acids (MUFA & SFA, respectively) determine the melting temperature (slip point) of adipose tissue aiding in the identification of specific fatty acids in the adipose tissue. Stearoyl-CoA desaturase (SCD) is the enzyme responsible for converting SFA to MUFA in bovine adipose tissue (Martin, Lunt, Britain & Smith, 1999).

This thesis follows the style and format of *Meat Science*.

Subcutaneous fatty acid composition has been shown to be similar to i.m. adipose tissue fatty acid composition (Sturdivant, Lunt, Smith & Smith, 1992). Subcutaneous fatty acid composition determines s.c. slip points (melting temperature) dictated via the specific fatty acids present in the adipose tissue (Smith, Yang, Larsen & Tume, 1998; Smith, Lunt, Chung, Choi, Tume & Zembayashi, 2006). The i.m. lipid percentage in beef muscle is highly correlated with the concentration of specific fatty acids in s.c. adipose tissue (Lunt, Choi, Chung & Smith, 2005; Smith et al., 2006). This information suggests that s.c. slip points should be highly correlated with i.m. marbling scores.

The economic advantages associated with processing cattle at the ideal time while able to assess the relative stage of marbling development aids in maximizing carcasses quality and value. This is not only beneficial for producers but packers as well, thereby reducing the number of carcasses with undesirable yield grades. The primary objective of this study was to predict the amount of i.m. adipose tissue by measuring the MUFA:SFA ratio of the s.c. adipose tissue. This study determined a high correlation between the s.c. MUFA:SFA ratio and the s.c. slip points as well as between the MUFA:SFA ratios in both i.m. and s.c. adipose tissue. This is beneficial as well because by measuring s.c. slip points, the composition of the marbling can be predicted. However, the hypothesis of predicting the amount of marbling from s.c. slip points was proven incorrect.

CHAPTER II

REVIEW OF LITERATURE

2.1 Importance of adipose tissue in meat

The quantity and chemical composition of beef lipids have been shown to influence sensory quality and acceptability of fresh beef. Adipose tissue affects many attributes of beef from tenderness to palatability. Studies have concluded that marbling is positively related to Warner-Bratzler shear force (Smith et al., 1984; Wheeler et al., 1994). Further, s.c. adipose tissue, or external fat, which is often considered waste fat, also positively affects taste panel ratings of juiciness, tenderness, and overall palatability within a certain constraint of fat thickness (~ 0.8 cm) (Tatum et al., 1982).

For beef, the level of lipid in meat necessary to achieve consumer acceptance is between 3% and 7.5% on an uncooked basis (Smith, Smith & Lunt, 2004). Carcasses with moderately abundant marbling scores produced loin, top round, bottom round and eye of round steaks that had higher sensory panel ratings or lower shear force values than did carcasses with lower marbling scores (Smith et al., 1984), indicating that more marbling creates a more palatable steak. As the amount of marbling increases from Practically Devoid (and upwards), fat cells (adipocytes) lodged between the muscle fibers contribute to the lipids in beef (Rule, Smith & Romans, 1995).

Furthermore, an adequate amount of fat cover is necessary for proper chilling rates which have been shown to reduce the cold-induced myofibrillar toughening (Smith, Dutson, Hostetler & Carpenter, 1976). As an animal matures, there is decreased muscle growth and increased fat deposition. For this reason, it is important for producers to

harvest their animals at the appropriate age to allow for adequate fat cover. If an animal is losing body condition, its body generates energy from fat first, indicating the first depot to disappear would be marbling, as it was the last depot to appear.

2.2 Fatty acid composition

Fat cells are deposited in several body depots; in cattle, these include: 1) perinephric (internal); 2) intermuscluar (seam); 3) intramuscular (i.m., marbling); and 4) subcutaneous (s.c., external). These fat depots have unique fatty acid compositions although, in all depots, the primary fatty acids are palmitic acid (16:0), stearic acid (18:0), and oleic acid (18:1) (Rule et al., 1995). Depending on what location of the carcass is being observed, the fatty acid profile of that area is unique due to the order in which lipid is deposited throughout the carcass. The fatty acid composition of a carcass is analyzed closely because the specific fatty acids present affect the palatability, the healthfulness of meat, and the melting point.

Fatty acid composition of bovine fat plays a significant role in palatability. Westerling and Hedrick (1979) reported sensory flavor panel scores that were positively associated with total UFA content. Dryden and Marchello (1970) determined that the flavor score was positively correlated with the amount of oleic acid concentration. As USDA carcass quality grade increases, the proportion of oleic acid increases and stearic acid decreases, creating an inverse relationship between oleic and stearic acids. The concentration of IML in the *M. longissimus dorsi* ranges anywhere from 3% in cattle fed in the US market to 25% or more in Japanese Black or American Wagyu cattle fed to a Japanese endpoint (Zembayashi, 1994). As the percentage IML accumulates, there is a

direct response in the elevation in the concentration of oleic acid from 30% to 50% (Smith et al., 2006; Chung et al., 2006). Palmitic and stearic acid are SFA, whereas oleic is the most abundant of the MUFA in beef.

Adipose tissue of corn-fed steers contained higher concentrations of palmitoleic acid (16:1), linoleic acid (18:2), oleic acid, more total MUFA and polyunsaturated fatty acids (PUFA), and higher 16:1:18:0 ratios than adipose tissue of hay-fed steers (Chung et al., 2006). The grain-based diets contribute more MUFA because either corn-based diets stimulate Δ9 desaturase gene expression or hay based diets depress desaturase activity/gene expression (Chung et al., 2006). Cattle fed forage based diets are shown to have an increased amount of SFA in their adipose tissue than those fed grain-based diets (Westerling & Hendrick, 1979).

2.3 Subcutaneous adipose tissue as an indicator of marbling

The s.c. adipose tissue of Wagyu (Japanese Black) carcasses have been shown to contain low concentrations of stearic acid (melting point = 70°C) (Sturdivant et al., 1992). These cattle also exhibited unusually high percentages of ribeye IML, suggesting a negative relationship between carcass quality and stearic acid in s.c. adipose tissue. It was later confirmed that, as marbling scores increased, the concentration of stearic acid in s.c. adipose tissue decreased (Chung et al., 2006; Smith et al., 2006). As the amount of marbling increases from Practically Devoid to Moderately Abundant, the amount of extractable fat from the i.m. depots increases as well (Savell & Cross, 1988). Jeremiah (1996) demonstrated that carcasses with traces of marbling had less s.c. adipose tissue

than carcasses with more marbling, and carcasses with modest amounts of marbling had more s.c. adipose tissue than carcasses with less marbling in their study.

Previous studies suggested a high correlation between the fatty acid compositions of s.c. adipose tissue to i.m. adipose tissue (Archibeque, Lunt, Gilbert, Tume & Smith, 2005; Sturdivant et al., 1992). More importantly, it has been demonstrated that a high correlation exists between IML (hence, marbling scores) and the concentration of MUFA in the s.c. adipose tissue (Lunt et al., 2005; Chung et al., 2006). Across Angus and Wagyu steers fed to long and short endpoints showed that as IML increased, the amount of MUFA increased accordingly (Lunt et al., 2005). The fatty acid composition of the s.c. adipose tissue determines the slip point because the more MUFA in the sample, the lower the melting point. In addition, it is thought that because s.c. fatty acids contribute to the slip point, these would be related to marbling.

2.4 Monounsaturated:saturated fatty acid ratio

Saturated fatty acids are shown to predispose man to coronary heart disease and should be limited in the diet, while fat intake should be focused more on UFA (U.S. Department of Health & Human Services, 2005). The higher the MUFA, the healthier the food is considered because the monounsaturates have been shown to decrease low density lipoproteins (LDL) and very low density lipoprotein (VLDL) while maintaining levels of high density lipoproteins (HDL) cholesterol (Etherton, Thompson & Allen, 1977). The MUFA:SFA ratio of adipose tissue lipids differs depending upon the carcass quality grades. The typical MUFA:SFA ratio is approximately 1:1 in cattle raised in the US (Smith et al., 2004). In s.c. adipose tissue from Japanese Black cattle this ratio can

be as high as 2.6:1, whereas in Australian cattle, the MUFA:SFA ratio for s.c. adipose tissue can be as low as 0.8:1 (Smith et al., 2004). Sturdivant et al. (1992) reported that Japanese Black cattle with the highest Japanese fat quality grade exhibited the greatest MUFA:SFA ratio in their s.c. adipose tissue. They further determined that the MUFA:SFA ratio of adipose tissue was influenced by breed, sex, nutritional and environmental differences (Sturdivant et al., 1992).

Previous studies also indicated that the MUFA:SFA ratio of both s.c. and i.m. adipose tissue tended to be similar when grouped within sex, diet, or breed (Gilbert, Lunt, Miller & Smith, 2003; May et al., 1993; Sturdivant et al., 1992; Zembayashi & Nishimura, 1996). Chung et al. (2006) reported that feeding endpoint had the strongest and most consistent effect on fatty acids of s.c. adipose tissue, evidenced in Angus steers raised to the Japanese heavy weight endpoint accumulated adipose tissues lipids that were remarkably unsaturated (softer fat). Further, it has been demonstrated that the amount of marbling and s.c. fat thickness are similarly increased during the growing phase (Schoonmaker, Cecava, Faulkner, Fluharty, Zerby & Loerch, 2003).

MUFA:SFA ratios provide verification for the healthfulness of beef to the human diet. The higher the ratios, the more MUFA present, therefore, the dietary guidelines of increasing MUFA have been accomplished. MUFA and PUFA contribute to the overall decreased risk of heart disease, obesity, and cancer.

2.5 Stearoyl-CoA desaturase activity

The balance between stearic acid and oleic acid (and therefore the MUFA:SFA ratio) is determined by the activity of an enzyme called stearoyl-CoA desaturase (SCD),

also known as Δ9 desaturase (Chung et al., 2006). This enzyme is expressed in virtually all cells, but is especially active in the adipose cells. High activity of Δ9 desaturase is advantageous because the SFA are converted to MUFA, which are deemed nutritionally acceptable by consumers. Chung et al. (2006) reported that feeding endpoint had the greatest effect on fatty acids of s.c. adipose tissue because the longer an animal was fed, the more time the enzyme had to work on the conversion of the adipose tissue. Those cattle fed to the Japanese endpoint (longer), had the greatest amount of oleic acid, MUFA, and higher MUFA:SFA ratios associated with them than did those cattle fed to the US endpoints. The elongated period of time allowed the Δ9 desaturase activity to stimulate conversion resulting in increased unsaturates.

Mitsuhashi, Mitsumoto, Kitamura, Yamashita & Ozawa (1988) previously reported that, in Japanese Black cattle, the melting point of adipose tissue lipids decreased from 35.5°C (96°F) in 14 mo-old steers to 21.2°C (70°F) in 28 mo-old steers, and suggested that melting point may be controlled by Δ9 desaturase. Expression of the SCD gene increases profoundly between weaning and 16 mo of age in the s.c. adipose tissue of Angus steers (Martin et al., 1999). This effect occurs when animals are fed grain-based diets, because the Δ9 desaturase activity is stimulated by the grain to begin converting SFA to MUFA. This stimulation of activity is positively correlated to myristic (14:1), palmitoleic, oleic, and total MUFA (Yang, Larsen, Smith & Tume, 1999). Any elevation of Δ9 desaturase activity conversely reduces the concentration of SFA, especially stearic acid, because of the negative correlation (Chung et al., 2006).

Equally, any depression of Δ9 desaturase activity causes an increase in the deposition of SFA.

2.6 Slip points

Within niche markets in the U.S., soft fat is widely desired because of its positive effect on palatability, which correlates to the amount of marbling within a cut. With the export markets currently open, there is an opportunity to export to major markets in Japan and Korea, creating an economic advantage for the U.S. As indicated by Chung et al. (2006), increasing the MUFA levels in beef decreases the slip point, which produces softer, more desirable fat. The composition of beef adipose tissue is associated with sensory characteristics, with oleic acid being positively correlated with fat softness (Smith et al., 1998). The slip point decreases in relation to an increase in MUFA and alternately increases with more SFA because of the chemical structure of the fatty acid chains.

Slip points are most highly correlated with the concentration of stearic acid; as carcass quality grade increases, the percentage of stearic acid, hence slip point, decreases dramatically (Smith et al., 1998; 2006). The reduction in melting point is due to decreases in stearic acid and concomitant increases in MUFA (Smith et al., 2004). Mitsuhashi et al. (1988) suggested that melting point could be influenced by the Δ9 desaturase activity because of the relationship to MUFA. Slip points are useful to measure because the melting temperature identifies specific fatty acids present in the lipid (saturated versus unsaturated).

Chung et al. (2006) demonstrated that fatty acids in s.c. adipose tissue typically become less saturated between weaning and slaughter in cattle that are fed a grain-based diet. Between weaning and slaughter weight, the ratio of MUFA:SFA increases from 0.66 to 0.86:1. This suggests that as the cattle age, the amount of MUFA increases, lowering the slip point of the lipids in the meat at the same time that marbling scores are increasing dramatically. The objectives of this study were to document the correlation of fatty acid compositions between s.c. and i.m. adipose tissue of different quality grades of beef carcasses, document the relationships among s.c. fatty acid composition, percentage IML, slip points, and carcass quality, and to establish s.c. fatty acid composition and slip point as a prediction tool for edible beef fatty acid composition.

CHAPTER III

MATERIALS AND METHODS

3.1 Sample collection

Subcutaneous adipose tissue samples and 79 facings of the *M. longissimus dorsi* (LD) at the 12^{th}-13^{th} rib were supplied by Merial (Duluth, GA) from finished animals of unknown background. The samples were removed at slaughter, labeled, and immediately frozen. The samples were shipped to Texas A&M University on dry ice and stored at -20°C for up to 3 mo.

3.2 Total lipid extraction

Total lipid was extracted by a modification of the methods of Folch, Lees & Stanley (1957). Approximately 1 g of overlying s.c. adipose tissue or 1 g of the LD facings was homogenized with 5.0 mL of chloroform:methanol (2:1, vol/vol) in a Brinkmann Polytron Homogenizer (Brinkmann Instruments, Westbury, NY). After homogenization, 10 mL of chloroform:methanol was added to the sample for a final volume of 15 mL, which was then left to sit at room temperature (approximately 20°C) for 30 to 60 min for lipid extraction. The homogenate was vacuum filtered through a sintered glass filter funnel fitted with Whatman GF/C filters (Whatman Ltd., Maidstone, England) into a test tube containing 8 mL of 0.74% KCl (wt/vol). The sample was vortexed and allowed to sit for 2 h to allow for phase separation. Once the phases had separated, the aqueous layer was removed and discarded and the lower phase was transferred to 20 mL scintillation vials. The samples were evaporated to dryness by

heating at 60°C under N_2 gas. The remaining liquid was the total extracted lipid used for fatty acid analysis or slip point determination.

3.3 Fatty acid composition

Lipid was extracted according to Folch et al. (1957). Fatty acid methyl esters (FAME) were prepared as described by Morrison and Smith (1964). The total extracted lipid obtained from the procedure described above had 1 mL of 0.5 KOH in MeOH and submerged in a 70°C water bath for 10 min. Then, 1 mL of BF_3 (14%, wt/vol) was added to the sample which was then flushed with N_2, loosely capped, and placed back into the 70°C water bath for 30 min. The samples were removed from the bath and allowed to cool before 2 mL HPLC grade hexane and 2 mL of saturated NaCl were added to the samples and vortexed. This produced two distinct phases of which the upper phase was transferred to a new test tube with 800 mg of Na_2SO_4 to remove any moisture from the sample. Two milliliters of the vortexed solution were added to the tube with the saturated NaCl and vortexed again. The upper layer was transferred into the tube with Na_2SO_4. This final volume was transferred to the scintillation vial to give a final volume of 5 mL of sample in the scintillation vial. The sample was then evaporated to dryness at 60°C under nitrogen and finally reconstituted with HPLC grade hexane and analyzed using a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 autosampler, Varian Inc., Walnut Creek, CA) (Smith et al., 2002). Separation of FAME was accomplished on a fused silica capillary column CP-Sil88 [100 m x 0.25 mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands), with helium as the carrier gas (flow rate= 1.2 mL/min). After 32 min at 180°C, oven temperature increased at

20°C/min to 225°C and held for 13.75 min. Total run time was 48 min. Injector and detector temperatures were at 270°C and 300°C, respectively. Standards from Nu-Check Prep, Inc. (Elysian, MN) were used for identification of individual FAME. Individual FAME were quantified as a percentage of total FAME analyzed. This equipment can accurately measure all fatty acids normally occurring in beef lean and fat trim, including isomers of conjugated linoleic acid and the omega-3 fatty acids.

3.4 Fat and moisture content

Lean muscle moisture and fat percentages were determined from ground samples of ribeye facings by use of the CEM SMART system (microwaving drying system manufactured by CEM Corp., Matthews, NC) and SMART Trac (NMR system manufactured by CEM Corp., Matthews, NC) as outlined by Keeton, Hafley, Eddy, Moser, McManus & Leffler (2003). A sample of LD was ground to a fine paste using a commercial food processor. Approximately 4 g of sample was transferred and spread onto a tared CEM sample pad using a Teflon-coated spatula. A second tared CEM sample pad was then placed on top of the sample in a sandwich-like fashion. The sample was then placed inside the microwaving drying system (AOAC, 2007). After this procedure, the dried sample pads were taken out and rolled in SMART Trac Film. The rolled sample was placed into a CEM Trac Tube and compressed. This sleeve with sample was then placed into the NMR chamber for analysis.

3.5 Slip points

Melting points of the s.c. adipose tissue lipids were approximated by determining slip points (Smith et al., 1998). After heating to approximately 45°C, the lipids were drawn 1 cm into glass capillary tubes and frozen at -20°C. After freezing, the capillary tubes were suspended vertically in a chilled water bath with the portion of the tube containing the lipid submerged in the water. The water bath was heated at 2°C/min with constant stirring. Temperature of the water was monitored with a Type K thermocouple (model KTSS-HH, Omega Engineering, Inc., Stamford, CT) attached to a digital thermometer (model 91100-50, Cole-Parmer Instrument Co., Vernon Hills, IL). Slip point is defined as the temperature at which the lipid moves up the capillary tube.

3.6 Statistical analysis

The null hypothesis for this study is that the individual fatty acids will not differ between s.c. and i.m. adipose tissue depots. A portion of the data was analyzed using SuperANOVA in order to calculate predefined regression equations (SuperANOVA, Berkley, CA). Simple correlation coefficients were calculated between dependent and independent variables. When analysis of variance indicated a significant difference ($P \leq 0.05$), means were separated by Fischers least squares differences (SuperANOVA).

CHAPTER IV

RESULTS

4.1 Slip point values, % IML, and % moisture

Means and measures of variation were similar between s.c and LD facing samples (Table 1). The mean slip point values for s.c and LD lipids were 38.80°C and 38.50°C, respectively; the standard deviations for s.c. and LD lipids were 3.53 and 3.12, respectively. The variance for the s.c. and LD lipids varied from 12.45 and 9.72. The minimum values for s.c. and LD were 31.1°C and 30.8°C, and the maximum slip point was 45.3°C for both s.c. and LD lipids.

The mean % IML in the LD was 7.01, and the standard deviation was 1.80. The variance for the LD % IML was 3.25, and the minimum and maximum values were 3.6 to 12.8, respectively. There was a negative relationship between % IML and % moisture, with the mean value for % moisture of 68.65 and a standard deviation of 1.27. The variance for % moisture was 1.61. The minimum and maximum values for % moisture were 64.8 and 71.3, respectively.

The mean value for marbling score in the LD was 419.7, and the standard deviation was 93.9. The minimum value was 260 (Sl60) and the maximum value was 670 (SlAb 70).

4.2 Subcutaneous and M. longissimus dorsi *fatty acid composition*

The mean values for stearic acid were 15.7 and 13.6 g/100 g total fatty acids for s.c adipose tissue and IML, respectively. The standard deviation for stearic acid was 2.23 in s.c. and 1.41 in the IML. In s.c. adipose tissue, stearic acid values ranged from a

minimum of 11.3 to a maximum of 20.1 g/100 g total fatty acids. The IML values varied from a low of 10.6 to a high of 17.4 g/100 g total fatty acids.

The mean values for oleic acid were 33.29 and 35.57 for s.c. adipose tissue and IML, respectively. The standard deviation for oleic acid was 2.92 in s.c. adipose tissue and 2.45 in the IML. In s.c. adipose tissue, oleic acid values ranged from a minimum of 25.3 to a maximum of 38.8 g/100g total fatty acids. The IML values varied from a low of 28.7 to 41.3 g/100 g total fatty acids.

The mean values for palmitoleic acid were 2.83 and 3.30 for s.c. adipose tissue and IML respectively. The standard deviation for palmitoleic acid differed only slightly from 0.59 in s.c. and 0.53 in the IML. In s.c. adipose tissue, palmitoleic acid values ranged from a minimum of 1.9 to a maximum of 4.5 g/100 g total fatty acids. The IML values varied from a low of 2.0 to a high of 4.9 g/100 g total fatty acids.

4.3 Regression parameters

Subcutaneous lipids. Subcutaneous slip point was poorly correlated with % IML ($R^2 = 0.001$; $P = 0.734$) (Figure 3), though the IML MUFA:SFA ($R^2 = 0.097$; $P < 0.01$) (Figure 1), and the IML slip point ($R^2 = 0.185$; $P < 0.001$) (Figure 2) were both highly correlated with s.c. slip point. The s.c. MUFA:SFA ratio was more strongly correlated with IML MUFA:SFA ($R^2 = 0.440$; $P < 0.001$), IML slip point ($R^2 = 0.236$; $P < 0.001$), and s.c. slip point ($R^2 = 0.557$; $P < 0.001$), than to % IML ($R^2 = 0.002$). Subcutaneous stearic acid was highly correlated with both s.c. slip point ($R^2 = 0.651$; $P < 0.001$) and s.c. palmitoleic acid ($R^2 = 0.702$; $P < 0.001$), but % IML was not as highly correlated ($R^2 = 0.008$).

Intramuscular lipids. Intramuscular slip point was strongly correlated to IML MUFA:SFA ($R^2 = 0.252$; $P < 0.001$), and IML UFA:SFA ($R^2 = 0.261$; $P < 0.001$); however, % IML was not associated with IML slip point ($R^2 = 0.002$). Conversely, the i.m. MUFA:SFA ratio was poorly correlated to % IML ($R^2 = 0.007$). Intramuscular stearic acid was strongly correlated to IML slip point ($R^2 = 0.127$; $P < 0.01$) and IML palmitoleic acid ($R^2 = 0.590$; $P < 0.001$), though i.m. stearic acid was not related to % IML ($R^2 = 0.004$).

4.4 Correlation coefficients

The correlation central to our hypothesis was the relationship between percentage LD IML and the s.c. slip point. The simple correlation coefficient was -0.038 ($P = 0.73$) (Table 4). The IML slip point was positively correlated with the s.c. slip point ($r = 0.430$; $P < 0.001$). The negative relationship between s.c. stearic acid and i.m. palmitoleic acid, demonstrated previously, was validated with a correlation coefficient of -0.235 and $P = 0.03$. The relationship between i.m. palmitoleic acid and s.c. oleic acid validated our hypothesis that s.c. and i.m. fatty acids are interrelated ($r = 0.229$; $P < 0.05$) (Table 4).

CHAPTER V

DISCUSSION

Chung et al. (2006) and our laboratory (M.A. Brooks & S.B. Smith, unpublished) demonstrated a highly significant correlation between the s.c. MUFA:SFA ratio and % IML in the *M. longissimus dorsi*. The current study, however, did not demonstrate a relationship between % IML (i.e., marbling score) and s.c. slip point in this population of cattle. Thus, we were unable to develop a rapid analytical procedure to predict marbling scores from s.c. adipose tissue. Other important relationships were validated. These included the negative relationship between oleic acid and slip point, the negative relationship between palmitoleic and stearic acid, the positive relationship between i.m. and s.c. slip points, as well as between i.m. and s.c. MUFA:SFA ratios.

Typically, IML fatty acids have been shown to be more saturated than s.c. fatty acids (Chung et al., 2006). Well finished cattle are able to convert more SFA to MUFA (Sturdivant et al., 1992; May et al., 1993; Chung et al., 2006). When cattle are harvested young or brought off pasture and directly harvested without grain-finishing, the ratio reverses where the s.c. lipids are more saturated than the IML (Archibeque et al., 2005).

We previously demonstrated that s.c. adipose tissue, SCD gene expression, and catalytic activity increases with time on a grain-based, finishing diet (reviewed in Smith et al., 2006). Therefore, the MUFA:SFA ratio increases from approximately 0.7 in weaned calves to over 1.1 in finished steers (Chung et al., 2006). The current study analyzed cattle from unknown backgrounds in order to produce a representative population of cattle within the U.S. industry. This data set did not have the range of %

IML that was observed in Chung et al. (2006). Contrary to Smith et al. (2006), the relationship between % IML and s.c. MUFA was not significant. This could be due to the breed type differences (Angus and Wagyu versus cattle of unknown background). In previous studies, cattle were long-fed on pasture or on grain (Sturdivant et al., 1992; Gilbert et al., 2003; Chung et al., 2006). Their % IML ranged from 4 to 30%, whereas in the current study, the % IML ranged from 3.6 to 12.9%. The 3 to 7% IML range is considered the appropriate window of consumer acceptability for marbling and is representative of the average US market (Savell & Cross, 1988). The higher values for % IML reported by Sturdivant et al. (1992) and Chung et al. (2006) are more representative of the Korean and Japanese markets where cattle are known to produce up to 35% extractable fat (Smith et al., 2004).

There were also differences between the slip point data of Chung et al. (2006) and the current data. The s.c. slip point values of Chung et al. (2006) ranged from 20°C to 45°C (25°C difference) whereas the current s.c. slip point values ranged from 31°C to 45°C, a difference of only 14°C (Table 1). Slip point values differ between breed types and adipose tissue depot depending on the size and extent of differentiation of the adipocytes (i.e. larger adipocytes have higher concentrations of MUFA) (Smith et al., 2004, 2006). Differences between breed types, as well as time on feed, could explain why the current slip point values were different from those reported by Chung et al. (2006).

This study and others (Smith et al., 1998; Chung et al., 2006) established a relationship between s.c. stearic acid and s.c. lipid slip point. In the current study, the

positive relationship between s.c. stearic acid and s.c. slip point was significant ($P < 0.001$). As the percentage of stearic acid is increased in adipose tissue, the slip point is also increased due to the high melting point of stearic acid (approximately 70°C). In long-fed cattle, more time is allowed for the conversion of SFA to MUFA via SCD activity (Chung et al., 2006) whereas short-fed cattle rely on the feedlot stage where an intensive grain feeding program is implemented in order to maximize weight gain. When this conversion process of SFA to MUFA is restricted by short term or pasture feeding, a higher concentration of SFA is present, resulting in higher slip point temperatures. The lowest slip point values in this study were 30.8°C, whereas Chung et al. (2006) reported a mean value as low as 20°C. The cattle of Chung et al. (2006) were long-fed a corn-based diet for 16 mo. The cattle with the lowest slip points were of Wagyu genetics, which are associated with higher marbling scores and higher concentrations of MUFA in their lean and adipose tissues.

A high correlation existed between palmitoleic and stearic acids in s.c. lipids, which was significant in this study ($P < 0.001$). This supports data reported by Smith et al. (1998) in that relative SCD activity is best estimated by plotting s.c. palmitoleic acid against s.c. stearic acid, as MUFA increased, SFA decreased. This relationship is influenced by factors such as diet, breed, and time on feed, all of which influence SCD activity in adipose tissue.

This study has confirmed a significant relationship between the i.m. and s.c. slip points as well as between i.m. and s.c MUFA:SFA ratios. The data therefore supports the observations by others (Sturdivant et al., 1992; Gilbert et al., 2003; Archibeque et al.,

2005) that there is a high correlation between fatty acid composition of i.m. and s.c. adipose tissue. This suggests the possibility of using a biopsy of s.c. adipose tissue to determine the composition (hence, stage of development) of i.m. adipose tissue by identifying the fatty acids present in s.c. adipose tissue. The high correlation between i.m. and s.c. slip points also suggests producers may be able to obtain a biopsy of s.c. adipose tissue, determine the melting point, and thereby predict the melting point of the intramuscular adipose tissue.

Muscle or i.m. fatty acids have been shown to be more saturated than s.c. fatty acids (Sturdivant et al., 1992; May et al., 1993; Gilbert et al., 2003). In the early stages of development there is a large bed of preadipocytes that have not yet differentiated, which results in a lower production of MUFA. The enrichment of s.c. adipose tissue with MUFA is the result of extensive differentiation in that depot. Long-fed cattle have more time for the grain-based diet to increase SCD expression and convert SFA to MUFA. This conversion of SFA to MUFA results in a higher s.c. MUFA:SFA ratio. In short-fed cattle, the ratio is reversed and the i.m. MUFA:SFA ratio is higher.

Glibert et al. (2003) observed higher MUFA:SFA values in s.c. adipose tissue, 1.03, and reported an i.m. value of 0.95. May et al. (1993) compared i.m. and s.c. fatty acid composition in long-fed Angus and Wagyu steers. There was a higher MUFA:SFA value in s.c. adipose tissue (1.17 and 1.50, Angus and Wagyu, respectively) than in the i.m. adipose tissue (1.10 and 1.38, Angus and Wagyu, respectively). Sturdivant et al. (1992) compared s.c. and i.m. adipose tissue and *M. longissimus dorsi* in Wagyu cattle. The s.c. MUFA:SFA was significantly higher (1.98), the i.m. MUFA:SFA was 1.78, and

the muscle MUFA:SFA was 1.66. The current study differed from Sturdivant et al. (1992) because total lipid i.m. adipose tissue and the muscle were combined, yielding a MUFA:SFA ratio of 0.80. Sturdivant et al. (1992) MUFA:SFA ratios were higher than the current study, but they reported a higher s.c. MUFA:SFA ratio, following the trend.

Archibeque et al. (2005) demonstrated MUFA:SFA values that differed from previous studies, i.m MUFA:SFA was 0.73 and s.c. MUFA:SFA was 0.60. The current data also supports this reversal in the trend with a mean i.m. MUFA:SFA fatty acid ratio value of 0.80 and a mean s.c. MUFA:SFA fatty acid ratio of 0.73. The short-fed cattle are typically younger and have less time to convert the SFA to MUFA in the s.c. adipose tissue. When compared to previous studies, the current data set reported the lowest MUFA:SFA values seen to date. These low values could be attributed to a youthful stage of maturity at sampling. When cattle are about 8 mo old, preadipocytes exist and are able to differentiate into adipocytes (i.e., fill with lipid). As soon as this lipid filling occurs, SCD expression increases and SFA are converted to MUFA intramuscularly (Reviewed in Smith et al., 2006). After the cattle reach about 16 mo of age, there is a plateau of the maximum size and number of adipocytes. The SCD may not increase further in i.m. adipose tissue, whereas in s.c. adipose tissue, even at 16 mo of age there are preadipocytes that still have the ability to differentiate, fill with lipid, and be converted to MUFA by the SCD activity (reviewed in Smith et al., 2004; 2006).

CHAPTER VI

CONCLUSIONS

The results obtained in the current study indicate that we were able to determine the fatty acid composition of the intramuscular lipid by measuring the s.c. slip point. Producers will be able to determine how long animals have been fed and whether or not the animal is finished. If the fatty acid composition is around 1.0 then the animal is finished and is ready for harvest. We were able to predict what the composition of the meat, but not the amount of marbling present in a steak.

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APPENDIX

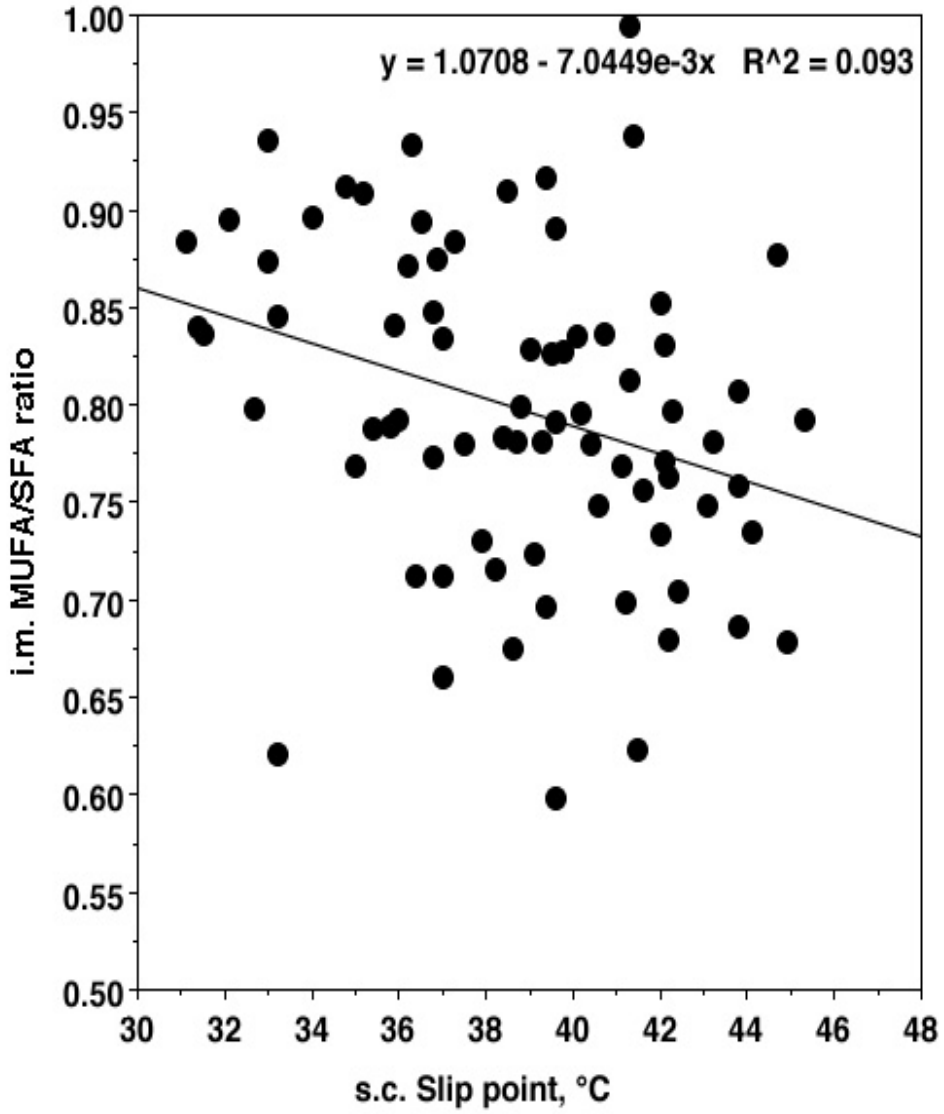

Figure 1. Intramuscular lipid MUFA:SFA ratio as a function of subcutaneous slip point.

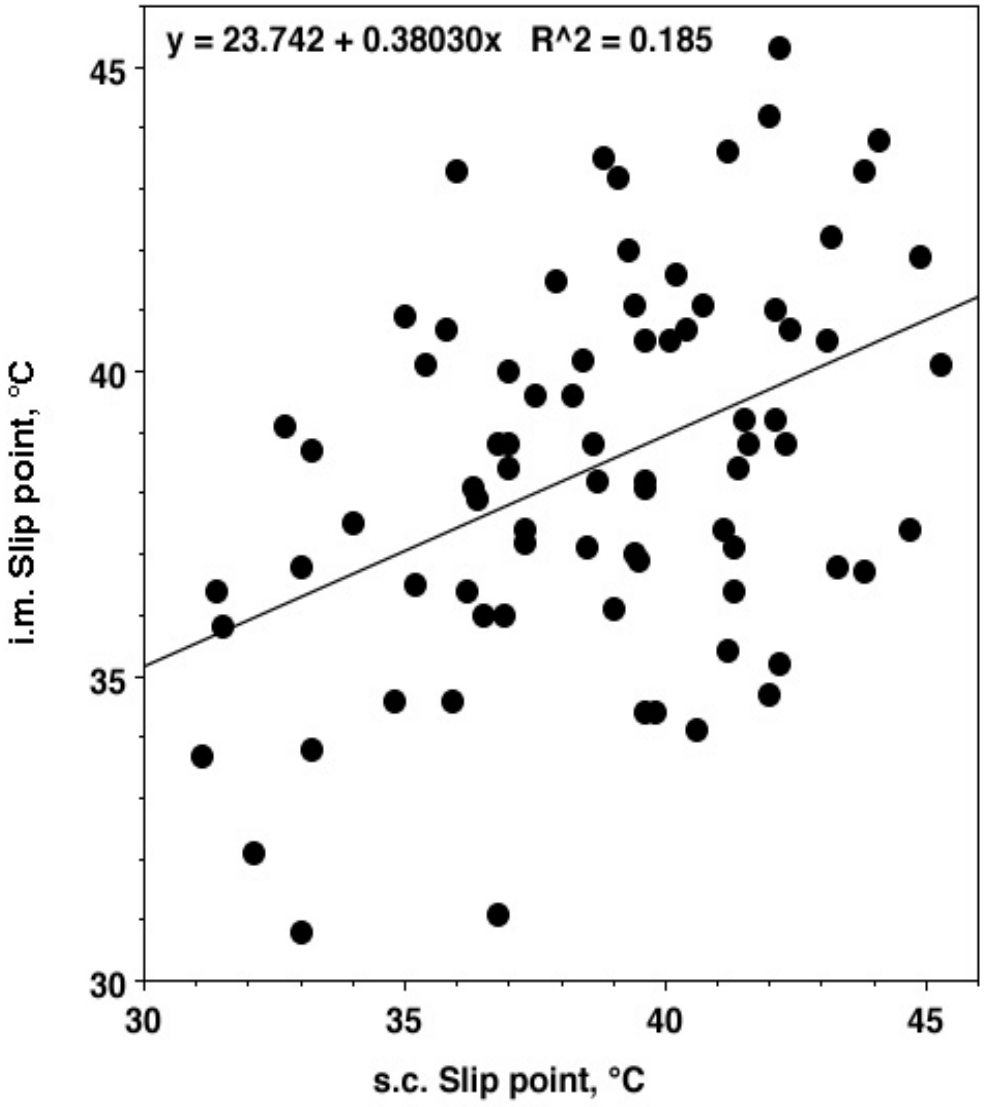

Figure 2. Intramuscular slip point as a function of subcutaneous slip point.

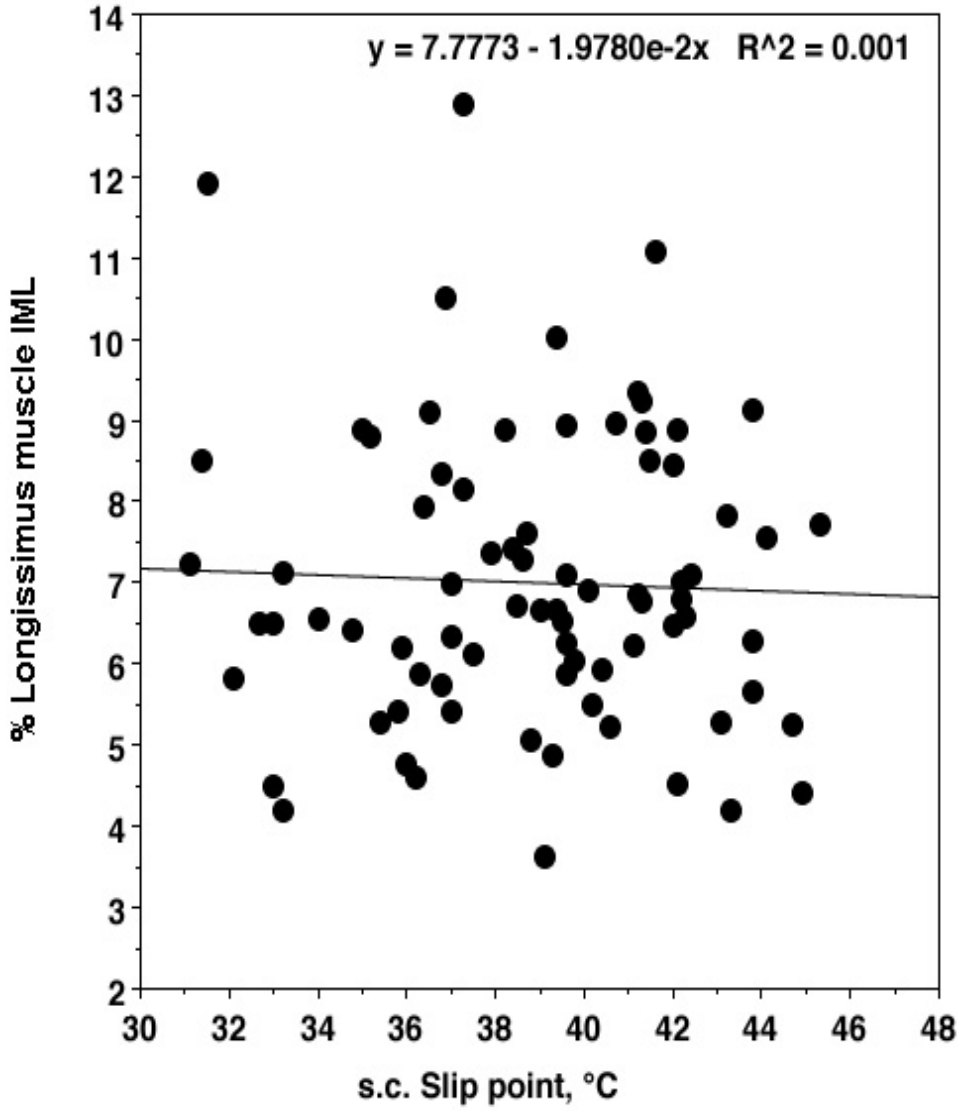

Figure 3. LD intramuscular lipids as a function of subcutaneous slip point.

Table 1. Mean values of s.c slip point temperatures (°C), %IML, % moisture, and marbling score.

Effect	Slip point (°C)		IML (%)		Moisture (%)		Marbling Score[b]	
	s.c.	LD	s.c.	LD	s.c.	LD	s.c.	LD
Mean[a]	38.80	38.50	---	7.01	---	68.65	---	419.7
Std Dev	3.53	3.12	---	1.80	---	1.27	---	93.9
Std Error	0.40	0.35	---	0.20	---	0.14	---	10.6
Variance	12.45	9.72	---	3.25	---	1.61	---	8817.9
Covariance	9.09	8.10	---	25.73	---	1.85	---	---
Min	31.10	30.80	---	3.62	---	64.80	---	260.0
Max	45.30	45.30	---	12.89	---	71.36	---	670.0

[a]Data are for samples from 79 animals.

[b]100-199= Practically Devoid, 200-299= Traces, 300-399= Slight, 400-499= Small.

Table 2. Least squares mean values and error terms for fatty acid composition of s.c. and IML of the *M. longissimus dorsi.*

Fatty Acids[a]	Mean		Std.Dev.		Min		Max	
	s.c.	IML	s.c.	IML	s.c.	IML	s.c.	IML
14:0	4.03	3.86	0.93	0.81	2.53	2.16	7.97	6.42
14:1	0.99	0.89	0.28	0.24	0.57	0.33	1.79	1.53
16:0	27.54	30.24	2.82	2.84	22.03	25.75	36.40	41.15
16:1	2.83	3.30	0.59	0.53	1.90	2.08	4.50	4.97
17:0	1.23	1.08	0.16	0.25	0.74	0.00	1.74	1.76
17:1	0.65	0.62	0.16	0.21	0.00	0.00	0.99	0.96
18:0	15.74	13.66	2.23	1.41	11.35	10.67	20.15	17.45
18:1t11	5.53	3.69	1.75	1.46	1.36	1.18	11.46	8.89
18:1	33.29	35.57	2.92	2.45	25.37	28.77	38.89	41.35
18:1c11	1.19	1.25	0.20	0.19	0.49	0.00	1.65	1.52
18:2	2.52	3.48	0.48	0.77	1.69	2.01	4.10	6.07
MUFA:SFA[b]	0.73	0.80	0.11	0.08	0.48	0.60	1.00	0.99

[a]Data are for samples from 79 animals.

[b]MUFA= total monounsaturated fatty acids (14:1n-5 + 16:1n-7 + 18:1n-9 + 18:2c9,t11)
SFA= total saturated fatty acids (14:0 + 16:0 + 17:0 + 18:0 + 18:1t1)

Table 3. Statistical relationships between s.c. and i.m. fatty acid and lipid measurements of samples from *M. longissimus dorsi* facings.

Independent/ dependent[a]	R^2	MSE	β_0	SE β_0	β_1	SE β_1
Subcutaneous						
Slip point						
1. % IML	0.001	3.291	7.777	2.268	-0.020	0.058
2. IML MUFA:SFA[b]**	0.097	0.006	1.083	0.100	-0.007	0.003
3. Intramuscular slip point***	0.185	8.019	23.742	3.540	0.380	0.091
MUFA:SFA[b]						
4. % IML	0.002	3.291	6.536	1.375	0.651	1.869
5. IML MUFA:SFA[b]***	0.440	0.004	0.430	0.048	0.504	0.065
6. IML slip point***	0.236	7.058	48.266	2.017	-13.288	2.739
7. Subcutaneous slip point***	0.557	5.593	56.227	1.793	-23.952	2.436
Stearic acid						
8. % IML	0.008	3.271	5.897	1.458	0.071	0.092
9. Subcutaneous slip point***	0.651	4.397	18.720	1.690	1.276	0.106
10. Subcutaneous palmitoleic***	0.702	0.103	6.288	0.259	-0.220	0.016
Intramuscular						
Slip point						
11. % IML	0.002	3.290	7.917	2.545	-0.024	0.066
12. IML MUFA:SFA[b]***	0.252	7.367	53.407	2.946	-18.729	3.681
13. IML UFA:SFA[b]***	0.261	7.278	54.655	3.116	-18.734	3.596
MUFA:SFA[b]						
14. % IML	0.007	3.273	5.586	1.964	1.789	2.454
Stearic acid						
15. % IML	0.004	3.282	8.168	2.005	-0.085	0.146
16. IML slip point**	0.127	8.5902	7.684	3.244	0.791	0.236
17. IML palmitoleic acid***	0.590	0.114	7.220	0.375	-0.287	0.027

[a]Dependent variables for 79 animals.
**P*-value < 0.05; ***P*-value < 0.01; ****P*-value < 0.001.
[b]MUFA= total monounsaturated fatty acids (14:1n-5 + 16:1n-7 + 18:1n-9 + 18:2c9,t11). SFA= total saturated fatty acids (14:0 + 16:0 + 17:0 + 18:0 + 18:1t11). UFA= total monounsaturated plus polyunsaturated fatty acids (PUFA: 18:2n-6 + 18:3n-3 + 20:4n-6).

Table 4. Simple correlation coefficients and the *P*-values for 79 samples of s.c. and *M. longissimus dorsi.*

Independent	s.c. Slip	s.c.14:0	s.c.14:1	s.c.16:0	s.c.16:1	s.c.18:0	s.c.18:1t11	s.c.18:1	s.c.18:1c11
% IML	-0.038	0.033	-0.066	0.053	0.018	-0.066	-0.021	0.121	0.084
	0.734	0.771	0.563	0.642	0.872	0.563	0.849	0.284	0.459
IML slip point	0.430	0.027	-0.250	0.015	-0.374	0.351	0.219	-0.476	-0.240
	<0.001	0.808	0.026	0.888	0.007	0.001	0.052	<0.001	0.033
IML14:0	0.042	0.310	0.120	0.215	0.089	0.020	-0.041	-0.221	-0.140
	0.710	0.005	0.291	0.056	0.433	0.856	0.718	0.049	0.217
IML14:1	-0.208	0.162	0.486	0.066	0.294	-0.290	-0.172	0.123	0.028
	0.065	0.151	<0.001	0.560	0.008	0.009	0.127	0.277	0.801
IML16:0	0.129	0.200	0.099	0.256	0.014	0.076	-0.146	-0.183	-0.234
	0.257	0.075	0.384	0.022	0.897	0.501	0.196	0.105	0.037
IML16:1	-0.218	0.140	0.236	0.061	0.378	-0.235	-0.260	0.229	0.148
	0.052	0.216	0.035	0.590	0.0006	0.036	0.020	0.042	0.190
IML18:0	0.369	-0.039	-0.304	-0.006	0.328	0.457	0.043	-0.265	-0.185
	0.008	0.730	0.006	0.952	0.003	<0.001	0.702	0.018	0.102
IML18:1t11	-0.067	0.131	-0.576	-0.017	0.045	-0.155	0.634	-0.326	0.135
	0.557	0.246	0.613	0.879	0.693	0.171	<0.001	0.003	0.232
IML18:1	-0.198	-0.417	-0.059	-0.260	0.084	-0.070	-0.396	0.681	0.244
	0.080	<0.001	0.605	0.020	0.457	0.538	0.0003	<0.001	0.029

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