

final report

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Hard Fat Project

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1. BACKGROUND AND OBJECTIVES

MRC Project CS.272 provided support for the visit of Dr Stephen Smith, Texas A&M University (TAMU), College Station, Texas, to work with Dr Ron Tume on several biochemical aspects relating to the Hard Fat problem of some Australian beef. Dr Smith was in Australia from June 6th until October 2nd 1996 on Faculty Development Leave from his University.

Dr Smith had obligations to TAMU for Faculty Development Leave and he set the overall objectives for his visit, namely; to address specific lipid biochemical problems that directly impact U.S. and Australian meat production, and to interact with Australian scientists and producers to develop interdisciplinary collaborative research.

The **specific research objectives** of this project were:

- (i) to document the relative proportions of triglyceride species in beef fat varying widely in melting points (fat hardness); and
- (ii) to quantify the amount of saturated fatty acids located in the outer (sn-1/3) positions of triglycerides.

It was anticipated that such investigations would provide important information that would help explain differences in fat hardness across a variety of fatty acid compositions. This is one of a number of research paths being pursued at DFST, Brisbane Laboratory which aims to minimise hard fat of beef carcasses.

2. SPECIFIC RESEARCH OBJECTIVES

2.1. Rationale

Grain feeding of cattle in Australia usually leads to a substantial increase in fat hardness, particularly where feeding is continued beyond 100 days. For Australian meat processors, this results in a significant increase in production costs due to difficulties in boning carcasses with hard fat. In order to avoid hard fat, carcasses are boned at higher temperatures which has significant implications for meat quality and food safety. The effect of long-term grain feeding is opposite to that observed in the U.S.. Long-term feeding of cattle in the U.S. generally results in an increase in the concentration of unsaturated fatty acids, which would reduce fat hardness.

Our research had two specific objectives: 1) to document the relative proportions of triglyceride species in beef fat varying widely in melting points (fat hardness); and 2) to quantify the amount of saturated fatty acids located in the outer (sn-1/3) positions of triglycerides.

Animal fat is composed primarily of triglycerides. These are molecules that have three fatty acids attached to a glycerol backbone. The fatty acids can be saturated (no double bonds), monounsaturated (one double bond), or polyunsaturated. The most abundant saturated fatty acids are palmitic (16:0) and stearic (18:0) acids. There are three common monounsaturated fatty acids which are, in increasing abundance, myristoleic (14:1), palmitoleic (16:1), and oleic (18:1) acids. These are synthesized in adipose tissue from their saturated counterparts by a single enzyme, stearoyl coenzyme A desaturase. The most abundant polyunsaturated fatty acids are linoleic (18:2) and linolenic (18:3) acids, both of which are derived from plant sources of the diet.

Saturated fatty acids have high melting points (60 and 70°C for palmitic and stearic acids, respectively), whereas polyunsaturated fatty acids have the lowest melting points (-11°C for linoleic and linolenic acids). The melting points of monounsaturated fatty acids are intermediate (0 and 16°C for palmitoleic and oleic acids, respectively). Thus, generally speaking, melting point decreases as number of double bonds increases. However, the double bonds in monounsaturated fatty acids occur in one of two configurations, *cis* and *trans*. The *cis*-double bonds are synthesized by cellular desaturases, whereas *trans*-double bonds are produced by ruminal microflora or chemical hydrogenation and are absorbed and incorporated into cellular lipids. Unlike palmitoleic and oleic acids, which are *cis*-monounsaturated fatty acids, *trans*-monounsaturated fatty acids such as *trans*-vaccenic (18:1,t11) have melting points that are well above room temperature (44°C).

Naturally occurring triglycerides are a mixture of fatty acids, and this mixture will dictate the melting point (or hardness) of the fat. The triglycerides composing animal fat can exist in the following forms: as trisaturates (SSS), typically mixtures of 16:0 and 18:0; mixtures of saturates and monounsaturates (SSM and SMM); and mixtures of saturates, monounsaturates, and polyunsaturates (SMP). These triglyceride species can be separated by high performance liquid chromatography (HPLC). Other molecular species of lesser abundance in beef fat that can be separated by HPLC are SSMt, SMMt, and SMtP, all of which contain some proportion of *trans*-monounsaturated fatty acids.

It has been demonstrated that the presence of 18:0 in the outer, sn-1/3 position of triglycerides causes a greater increase in melting point than if the 18:0 was located in the inner, sn-2 position. R.K. Tume has demonstrated that long-term feeding of cattle in eastern Australia increases the percentage of 18:0 in beef fat. What was unknown was the extent to which this 18:0 migrated from the sn-2 position (where it occurs naturally) to the sn-1/3 positions as its concentration increased. If this migration to the sn-1/3 position occurred to an appreciable extent, then this would exacerbate the problem of fat hardness.

2.2. Approach

2.2.1. Animals

We measured the relative proportions of the triglyceride species in fat from specifically targeted groups of cattle. The groups were classified as: saturated (SFA, enriched with 18:0); a group enriched with *trans*-fatty acids (TFA); three monounsaturated groups (MUFA1, MUFA2, and MUFA3); and a group enriched with polyunsaturated fatty acids (PUFA). The cattle were fed either in Australia (SFA, TFA, MUFA3 and PUFA) or Japan (MUFA1 and MUFA2). Assignment to these groups was based on measurements by R.K. Tume of total fatty acids in adipose tissue samples. Groups (each consisting of fat from 5 individual animals) were deliberately chosen to give as wide a range of fatty acid compositions as possible.

2.2.2. Slip points

Subcutaneous adipose tissue was obtained at slaughter and lipids were extracted into chloroform:methanol. Slip points were measured to verify that fat from each treatment group differed in hardness. Solvents were removed exhaustively by heating the lipid samples to 60°C under a nitrogen stream and the lipids were drawn 1 cm into capillary tubes while still warm. Triplicate capillary tubes were collected for each sample. The samples in the capillary tubes were stored overnight at 4°C, and then placed vertically in a chilled water bath. The temperature was increased gradually in the water bath, and that temperature at which the lipid began to move up the capillary tube (slip point) was recorded.

2.2.3. HPLC

Lipids were fractionated on a silver-nitrate impregnated silica column, which separated the triglycerides based on numbers of double bonds. Components of the split eluant stream were detected using an evaporative light scattering detector. For each animal, the triglyceride fractions were collected from 3 - 5 separate runs to provide enough lipid in each fraction (approximately 20 μ g) to allow for analysis of fatty acid positional distribution.

2.2.4. Lipase digestion

The HPLC fractions were dried and emulsified in 1 mL buffer containing 1 mg/mL Triton X-100, 0.04 M Tris (pH 7.2), and 0.05 M borate. The fractions were sonicated for 1 - 2 minutes to ensure complete emulsification. One-half of each fraction was transferred to a separate tube, to which was added approximately 100 units of lipase from *Rhizopus arrhizus delemar*, which removes only the fatty acids in the sn-1/3 positions. The fractions were lipase digested for 60 minutes at 37°C. The portion of the samples which did not receive lipase was incubated under the same conditions. Reactions were terminated by the addition of 0.5 mL 1 N acetic acid and 3 mL chloroform:methanol (2:1, v/v). The lipids were extracted 3 times with chloroform:methanol.

2.2.5. Trans-methylation

The solvent portion, containing the lipids, was dried exhaustively to eliminate all traces of acetic acid, and the remaining glycerides were trans-methylated by incubating for 30 min at 65°C in 1 mL 0.1 N NaOH in methanol. Lipids were extracted with 3 x 3-mL volumes of hexane. The hexane was evaporated, and the samples were redissolved in 200 μ L hexane. A total of 6 to 8 μ L of each sample was used for gas-liquid chromatography for the analysis of fatty acid methyl esters.

The NaOH:methanol methylation procedure does not methylate nonesterified fatty acids. Therefore, the fatty composition of the lipase-digested and undigested (total) fractions could be used to calculate the average composition of fatty acids in the sn-1/3 positions:

Average sn-1/3 % = (3 X % fatty acid in total lipids) - (2 X % fatty acid in sn-2 position)

2.3. Results

2.3.1. Total fatty acid compositions

The fatty acid composition of the unfractionated samples are indicated in Table 1. This information, combined with nutritional history, provided the basis for the assignment to the various groups. The SFA group exhibited an unusually high percentage of 18:0 (26%), whereas the TFA group was enriched with 18:1,t11 (11%). The MUFA groups contained 49 to 53% 18:1, and the MUFA1 and MUFA3 groups also were especially high in 16:1 (> 5%). The PUFA group was remarkable for its high concentration of 18:2 (over 4%), which is unusual for bovine adipose tissue.

Table 1 also contains results for lipase digestions of the total lipid fractions, as well as the calculated fatty acid composition of the sn-1/3 position. In general, lipase digestion resulted in an enrichment of 18:1 and 18:2 at the sn-2 position, indicating that these fatty acids are located primarily in this position. Conversely, 16:0 was enriched primarily at the sn-1/3 positions. As the percentage of 18:0 was increased by dietary regimen, the proportion of 18:0 observed in the sn-1/3 position increased. Thus, 18:0 was randomly distributed throughout the triglyceride molecule in the MUFA groups, but was disproportionately enriched in the sn-1/3 position in the SFA and TFA groups. The data suggest that, as total 18:0 exceeds 15%, a greater proportion of 18:0 is incorporated into the outer fatty acid ester positions.

The increase in 18:0 in the sn-1/3 position was accompanied by a proportionate decrease in 18:1, rather than 16:0. Thus, the total saturates in the outer triglyceride positions exceeded 70% for the SFA and TFA groups, and exceeded 60% in the PUFA group. When *trans*-fatty acids are included, the proportion of high-melting point fatty acids in the sn-1/3 positions was 75, 87, and 68% for the SFA, TFA, and PUFA groups, respectively. As the percentage of saturated fatty acids occupying the sn-1/3 positions increases, there is a disproportionate increase in melting point. The very high percentage of saturated (and high-melting point *trans*-monounsaturated) fatty acids in the outer triglyceride positions indicates that the SFA, TFA, and PUFA samples would have higher melting points than would be predicted based on total fatty acid composition alone.

2.3.2. Slip points

As anticipated, slip point temperatures were highest for the SFA and TFA groups (45.1 and 41.5, respectively; Table 2), reflecting their high concentrations of saturated and/or *trans*-fatty acids. Although the PUFA was enriched with 18:2, it displayed a relatively high slip point (38.5°C), as

predicted by the high percentage of saturated fatty acids in the sn-1/3 positions. The lowest slip point was exhibited by MUFA1 (22.8°C). These samples were liquid at room temperature (approximately 25°C).

2.3.3. Proportions of HPLC fractions

The SFA and TFA groups had the greatest proportion of triglycerides containing three saturated fatty acids (SSS), and the TFA was especially high in those fractions containing *trans*-fatty acids (SSMt, SMMt, and SMtP; Table 3). SSMt eluted prior to the SSM fraction at variable retention times, whereas the more abundant SSMt eluted between the SSM and SMM fractions. Because of its low abundance and inconsistent retention time, the SSMt peak was not analyzed further.

The SMM fraction comprised about 50% of the triglycerides for the three MUFA groups, which also were high in the fraction containing three monounsaturated fatty acids (MMM). The PUFA group exhibited the highest proportions of those fractions containing polyunsaturated fatty acids, SMP and SMtP.

2.3.4. Fatty acid composition of HPLC fractions

It is apparent that the percentage of 18:0 in total lipids can influence its distribution in the triglyceride molecule. What was unknown was which triglyceride fraction would demonstrate the greatest change in positional distribution. To address this, triglyceride species were collected by HPLC and analyzed for fatty acid positional distribution by specific lipase digestion.

The MUFA1 and PUFA groups contained a small percentage of 18:1 in their SSS fraction which should not have been observed (Table 4). The predominant fatty acid in the SSS fraction was 18:0 for the SFA, TFA, and MUFA1 groups, but was 16:0 for the MUFA2 and PUFA groups. The PUFA group had a relatively small percentage of 18:0 in the sn-1/3 position (15%), but a high percentage of 16:0 (68%). For the other treatment groups, the distribution of 16:0 and 18:0 was similar at all three triglyceride positions. In other words, total saturated fatty acid percentage had no effect on positional distribution in all but the PUFA group, in which 18:0 primarily occupied the inner, sn-2 position.

The SSM HPLC fraction represented the predominant triglyceride species for the SFA and TFA groups (Table 3). This fraction also exhibited the greatest percentages of 18:0 in the sn-1/3 position for the SFA and TFA treatment groups (Table 5). Stearic acid comprised 44 to 49% of the fatty acids in the sn-1/3 position for the SFA and TFA groups, but only 18 to

22% for the other treatment groups. The abundance of the SSM fraction and the enrichment of the sn-1/3 position in 18:0 for the SFA and TFA groups indicate that the SSM fraction would be primarily responsible for the melting point characteristics of samples from these treatment groups.

The PUFA samples contained the highest percentage of *trans*-fatty acids in the SMMt fraction, which was unexpected (Table 6). The MUFA1 group had the lowest percentage *trans*-fatty acids in the SMMt fraction in addition to the lowest abundance of the SMMt HPLC peak (Table 3). There was no enrichment of 18:0 in the sn-1/3 positions in the SMMt fraction; rather, 16:0 and the *trans* fatty acid 18:1,t11 were in greatest abundance in the sn-1/3 position of the SMMt triglycerides.

The SMM HPLC fraction was abundant in all the groups, and was the predominant fraction in the MUFA and PUFA groups (Table 3). There was an enrichment of 18:1 in particular in the sn-2 position, whereas 16:0 was enriched at the sn-1/3 position (Table 7). There was less than 14% 18:0 in the SMM fraction, and the 18:0 was evenly distributed throughout the SMM triglycerides. Therefore, the fatty acid composition and positional distribution of the SMM fraction would have had little impact on differences in melting points among treatment groups.

The same was true for the SMtP, MMM, SMP HPLC fractions (Tables 8-10). These were relatively low in abundance, and there was a fairly uniform distribution of all fatty acids except 16:0 throughout the triglyceride molecules. It appears, then, that the positional distribution of fatty acids within the SSM fraction, and the relative abundance of this fraction, were primarily responsible for the observed differences in melting points across treatment groups.

2.4. Discussion

The mechanism by which changes in fatty acid positional distribution are accomplished is unknown. It generally is assumed that, as triglycerides are synthesized in bovine adipose tissue, 16:0 is esterified first to the sn-1 position, 18:1 subsequently esterified to the sn-2 position, and finally a random mixture of fatty acids (including polyunsaturated fatty acids) are esterified to the sn-3 position. Our results modify this pattern somewhat, in that 16:0 and 18:0 comprise up to 20 and 15%, respectively, of the fatty acids in the sn-2 position, and 18:1 can comprise as much as 35% of the sn-1/3 positions. More importantly, it appears that, in bovine adipose tissue, 18:2 is preferentially incorporated into the sn-2 position, which occurs primarily in the SMP fraction. Thus, the acyltransferases responsible for triglyceride biosynthesis in bovine adipose tissue exhibit only partial specificity.

In adipose tissue with normal levels of stearoyl coenzyme desaturase activity, 18:1 is the predominant fatty acid incorporated into the sn-2 position and 18:0 is distributed randomly. In tissues with reduced desaturase activity, in which 18:0 accumulates, the proportion of 18:0 at the sn-2 position increases perhaps merely due to its greater concentration within the cell. There appears to be a fixed limit to the amount of 18:0 that can be esterified at the sn-2 position (approximately 15%). After this limit is reached, the acyltransferases place more 18:0 in the outer positions. Because the concentration of 16:0 in the sn-1/3 position generally is unaffected by the concentration of 18:0 in the samples, and because 16:0 generally is esterified to the sn-1 position prior to esterification of fatty acids in the other positions, we can conclude that enrichment of 18:0 occurs primarily at the sn-3 location.

3. INTERACTION WITH AUSTRALIAN SCIENTISTS

3.1. Rationale

Australia and the U.S. share the common goal of producing high quality beef for export to Japan. The Japanese market demands highly marbled beef with white, soft fat. This is accomplished in the U.S. by feeding corn, which also is the basis for the production of meat for the domestic U.S. market. In Australia, feedlot rations predominantly use grains such as barley, wheat, or sorghum; very little corn is available for animal production. It also is common in Australia to add whole cottonseed to increase the energy and protein content of the diets.

Producing beef for export in Australia is hindered by two production problems, each of which appears to confound the other:

- (i) Low marbling scores, relative to those produced in the U.S. in genetically similar cattle fed for the same period of time.
- (ii) Hard fat in long-term fed cattle.

In order for Dr Smith to learn more of the production methods and biochemical bases for these problems, Dr Tume organised and accompanied Steve Smith on visits to several feedlots, farms, and research centres in Queensland and New South Wales. In addition, a number of seminars were organised in the Brisbane area where Dr Smith had the opportunity to meet with many research personnel having similar interests.

The following is a summary of their itinerary and findings. For those periods not specified, Steve Smith worked in Dr. Tume's laboratory developing techniques and processing samples that formed the basis of the research findings presented here.

3.2. Details of Meetings and Visits

June, 1996

Dr Brian Siebert (University of Adelaide, Waite Agricultural Research Institute) visited Cannon Hill where we discussed our respective research programs on fat quality. Visited Toowoomba to collect experimental fat samples and meet with **Alan Rich** (Australian Agricultural Nutrition Pty Ltd), with whom we discussed the contribution of cottonseed feeding to the evolution of hard fat. Mr. Rich also indicated that Australian cottonseed can only be fed at relatively low levels (< 15%) without decreasing performance. Visited with **Philip Green** of **Australian Meat Standards** and **Dr. Graham Trout** of **Victoria University** about meat production in the U.S..

July, 1996

Dr Smith presented a seminar "Production of Meat from Japanese Style Cattle" to the scientists and staff at CSIRO DFST, Cannon Hill.

11-14 July

Steve Smith and Ron Tume travelled from Brisbane through agricultural areas of Toowoomba, Dalby, Condamine, Goondiwindi, North Star, Moree, returning to Brisbane via Warwick. Visited **Teys Feedlot** at Condamine, Qld and met with **Keith Adams** (Feedlot Manager) and **David Evans** (Quality Assurance). Discussed the various grains that are available for feedlot finishing in Australia and the use of whole cottonseed in the rations. Visited the **Myola Feedlot** at North Star, NSW. There we met with **Roger Matthews** (Feedlot Manager) and with visitor **Greg Chappell** (Angus stud producer and MRC coordinator), and then travelled to Greg Chappell's Angus property near Moree and discussed stud production and meat quality issues.

17-19 July

Travelled to Rockhampton by air and drove to **Overflow** station to visit with **Wally and Susan Rea** about the production of Japanese Black (Wagyu) cattle in Australia. We also discussed grain feeding versus grass feeding, and the production of cattle in Northern Queensland. Dr Smith agreed to translate an article from Japanese to English from the Japanese trade journal *Beef Journal*. The purpose of this translation was to determine if the Japanese were using embryo transfer and in vitro fertilization to increase carcass quality. As it turned out, the article focused primarily on the improvement of the Japanese dairy herd. Dr Tume arranged for analysis of fat sample biopsies from Wagyu breeding stock.

Steve Smith and Ron Tume met with **Greg Harper, Graeme McCrabb, and Peter Allingham** of the **Tropical Beef Centre** in Rockhampton. Dr Smith presented a seminar *Production of Meat from Japanese Style Cattle* to scientists of the Tropical Beef Centre. The discussion following the presentation focused heavily on the merits of incorporating Wagyu genetics into Australian beef production systems.

28-30 July

Armidale, **University of New England, Beef Cattle CRC**. Steve Smith and Ron Tume visited Armidale to meet with colleagues involved in the Beef Cattle CRC. Dr Smith presented a seminar *Production of Meat from Japanese Style Cattle; Production, Cellular, and Genetic Aspects* to scientists and students of the University of New England. The discussion after the seminar focused on the limitations of marbling development in Australian cattle. There was also interest in designing experiments to ultimately increase the availability of glucose to marbling adipose tissue, with the express purpose of differentially increasing its hypertrophy. Discussions we held with **Drewe Ferguson** and others about the CRC project. We then met with **Dr John Thompson**, and established a small collaborative project based on research Steve Smith has been doing involving brown adipose tissue ontogenic development in pre- and postnatal calves. Dr. Thompson will CAT scan *Bos indicus* and *Bos taurus* calves at several stages of postnatal development to study the involution of brown adipose tissue to white adipose tissue.

August, 1996

1-2 August

Sydney. Dr Smith visited with **Trevor Scott** and **John Ashes** at **CSIRO Division of Animal Production** at Prospect NSW. Also present was **John MacPhillamy** of **Rumentek Industries**. Discussion centred on Trevor and John's recent publications about the modification of bovine fatty acids via the feeding of protected lipids. Also addressed was the hypothesis that providing the ruminant small intestine with more starch will lead to greater marbling development relative to fat accretion in other depots. This was based primarily on their observation that the U.S. finishes cattle on corn, and on some research that Dr Smith had conducted in the mid 1980s. To test this hypothesis directly, Mr. MacPhillamy offered to provide Dr Smith with sufficient Rumentek protected starch product to perform a small trial investigation in the U.S. Dave Lunt (Supervisor, Texas A&M University Research at McGregor, TX) has identified 40 Brangus steer calves that will be ready to go on feed in March, 1997.

Dr Smith met with **Dr. Ian McCausland**, Managing Director of the **Meat Research Corporation of Australia**. Discussions focussed on the involvement of cottonseed as a contributor to the evolution of hard fat in Australia. Although cottonseed is used to a limited extent in the U.S., the presence of other components such as corn, may minimise the hard fat problem. It was also discussed that the

development of marbling in Australia appeared to be limited by the types of grains used to finish cattle in the feedlot. More specifically, it appears that the U.S. has gained an advantage in producing more highly marbled cattle with softer fat by its extensive use of corn in its feedlot industry. During this meeting, Dr. McCausland asked **Dr David Skerman** (Program Manager Feedlots) to join the discussion.

22 August

Dr. Robin Shorthose arranged for Dr Smith to present a seminar *Production of Meat from Japanese Style Cattle* at the **Department of Primary Industries, Yerongpilly** to the Brisbane chapter of the **Australian Society of Animal Production**. The discussion once again focussed on the genetic and metabolic limitations of marbling development. Steve Smith met with **Dr. John Doyle** (Consulting Nutritionist) of **Nutrition Service Associates (Toowoomba)**. Dr. Doyle provided insight about the differences between the Australian and U.S. feedlot industries.

Dr Smith had informal discussions with **John Gaughan** of the **University of Queensland Gatton Agricultural College**.

September, 1996

Dr Tume arranged a meeting at Cannon Hill with **Dr. Ian Johnsson** (Program Manager) of the **Meat Research Corporation** during his brief return visit from the U.S. The above research work was summarized and we expressed our belief that migration of 18:0 to the outer positions of the triglyceride molecule in long-fed cattle was exacerbating the hard fat problem. Ron Tume discussed recent results (A. Yang) on the inhibition of desaturase activity of fat from cattle fed protected cotton seed oil, which highlighted the impact of sterculic acid on desaturase activity.

Dr Hutton Oddy also visited Cannon Hill, and we had a lively discussion about whether there is preferential use of metabolic substrates in subcutaneous and intramuscular adipose tissues.

16 September

Dr Smith presented seminar *Production of Meat from Japanese Style Cattle: Cellular and Molecular Aspects* at **University of Queensland, St. Lucia** at the invitation of **Dr. Jay Hetzel, CSIRO, Division of Tropical Animal Production, Molecular Animal Genetics Centre** and met with **Dr. Roger Drinkwater** and other scientists. During the last two weeks of September, Dr Smith worked part-time in Dr. Drinkwater's laboratory, extracting RNA from adipose tissues differing in 18:0 content to quantify amounts of stearoyl coenzyme A desaturase mRNA.

19 September

Steve Smith and Ron Tume visited **Griffith University** as a guest of **Dr. Peter Rogers**. Dr Smith presented the seminar *Production of Meat from Japanese Style Cattle* to a small group that consisted primarily of undergraduate students in biology and a few faculty members.

4. OVERALL SUMMARY

Australian beef producers are faced with a serious production dilemma. The export of high quality grainfed beef to Japan represents a major source of revenue, but demands that cattle be fed grain for long periods of time. Biochemical data generated in this laboratory (desaturase activities, MRC CS.258, Project STR003) in addition to anecdotal information gathered in the field, indicate quite clearly that feeding whole cottonseed for long periods of time leads to the production of very hard fat via inhibition of stearoyl coenzyme A desaturase. As the percentage of 18:0 increases above approximately 15% of total fatty acids, the proportion of 18:0 located in the outer positions of triglycerides increases. The occurrence of saturated fatty acids in the sn-1/3 position disproportionately increases the melting point of the fat, which explains why seemingly small increases in 18:0 in fat can have such a profound effect on fat hardness (melting points of 1,2 dioleostearin and 1,3 dioleostearin are 24 and 12°C respectively).

Whole cottonseed is widely used in Australian feedlot rations (eastern regions). In the U.S., it is more cost-effective to feed whole/cracked corn than whole cottonseed, so hard fat is not a production problem. This is in spite of the fact that U.S. carcasses are often chilled for up to 48 hours prior to grading and fabricating. In Australia, carcasses are generally graded after 20 hours chilling, frequently not below 10°C.

This leads to a second production problem faced by the Australian beef industry. For that small percentage of Australian cattle not fed whole cottonseed, grading the carcasses at 10°C would appear to lead to a drastic underestimation of beef marbling score. This would be especially true for carcasses from corn-fed cattle, which would contain a higher percentage of monounsaturated fatty acids (as do cattle raised in Japan). It is likely that corn feeding (as opposed to sorghum or other grains) in the U.S. is largely responsible for the greater quality of carcasses produced there. Yet Australian producers would not be rewarded for corn feeding unless carcasses were graded at a lower temperature (eg. 4°C). It is clear that U.S. carcasses also are fatter, so it remains to be determined if corn feeding causes a differential hypertrophy of marbling, or simply increases hypertrophy of all fat depots.

Obviously, there are no simple solutions. There is no inexpensive alternative to whole cottonseed, and the use of whole cottonseed by beef producers undoubtedly supports cotton producers. It also is unlikely that corn production will increase substantially because growing conditions in most of the arable parts of Australia do not favour its production. Research must continue to find a means of increasing, or at least minimizing the decrease in, desaturase activity in the fat depots of cattle produced in Australia.

4.1 Personal Summary (From Dr Smith)

I clearly benefitted from my time in Australia, and especially through my interaction with Dr. Ron Tume and his staff. I gained a much greater understanding about beef cattle production and the science that addresses beef cattle production in Australia. I also acquired a better understanding of the international beef market. I gained valuable laboratory experience through my collaboration with Dr. Tume and, to a lesser extent, with Dr. Roger Drinkwater. I am grateful to the Meat Research Corporation for funding this project.

5. Acknowledgments

We wish to thank Dr Aijun Yang for developing the HPLC-separation method in this laboratory and for her valuable contribution to discussions of this work. The expert technical assistance of Mr Tom Larsen and Mrs Maree Brewster is gratefully acknowledged. We wish to thank the Meat Research Corporation for their support.

Table 1. Total, sn-2 and Average sn-1/3 Fatty Acid Compositions for Each Treatment Group

Fraction/Group	Fatty Acid (% distribution)											
	14:0	14:1	16:0	16:1	17:0	17:1	18:0	18:1,t11	18:1,c9	18:1,c11	18:2	18:3
Total												
SFA	1.5	0.1	24.2	1.6	1.3	0.1	26.1	2.3	39.8	0.95	1.6	0.5
TFA	2.0	0.5	26.3	2.2	1.7	0.5	20.0	11.1	32.4	1.04	2.2	0.1
MUFA1	1.3	1.3	24.2	5.2	0.4	1.1	7.6	0.7	52.9	2.99	2.0	0.2
MUFA2	1.4	0.6	22.3	4.0	1.0	0.7	12.2	3.4	49.4	1.96	2.6	0.5
MUFA3	1.6	0.6	23.5	5.2	1.0	1.0	10.5	2.5	49.3	2.51	1.8	0.4
PUFA	1.8	0.2	23.8	2.8	1.1	0.4	17.7	3.7	42.4	1.26	4.5	0.2
sn 2												
SFA	2.0		20.0	2.2	0.4		21.3	2.0	49.6	0.55	2.1	
TFA	4.0	0.4	20.2	2.8	0.6	0.3	15.7	7.5	43.4	0.53	4.0	0.6
MUFA1	2.0	1.5	15.6	5.7	0.1	0.3	7.7	0.4	61.9	1.43	3.5	0.2
MUFA2	0.7	0.1	17.5	2.1	0.1	0.1	14.4	1.6	59.7	1.01	2.4	0.1
MUFA3	1.5	0.9	18.9	5.7	0.6	0.8	9.3	1.5	56.7	1.92	1.7	0.4
PUFA	0.7		17.3	2.3	0.6		17.2	2.0	53.1	0.43	6.2	
Average sn 1/3												
SFA	0.5	0.2	32.6	0.4	3.3	0.2	35.6	3.1	20.2	1.76	0.6	1.5
TFA	-2.1	0.6	38.7	0.8	4.0	1.1	28.4	18.3	10.5	2.06	-1.3	-0.9
MUFA1	-0.1	0.9	41.5	4.4	1.1	2.6	7.6	1.1	35.1	6.11	-0.5	0.3
MUFA2	2.9	1.5	31.9	7.6	2.7	1.8	7.8	7.0	28.7	3.87	2.9	1.4
MUFA3	1.6	0.1	32.9	4.0	1.8	1.2	13.0	4.6	34.7	3.67	2.0	0.5
PUFA	3.8	0.7	36.7	3.9	2.0	1.2	18.7	7.3	21.0	2.90	1.0	0.7

Table 2 Slip Point Analysis of Lipids Varying in Fatty Acid Composition

Group	Slip Point (°C)
SFA	45.1
TFA	41.5
MUFA1	22.8
MUFA2	28.4
MUFA3	30.7
PUFA	38.5

Table 3 Percentages of HPLC Fractions of Triglycerides from Each Treatment Group

Group	Fraction							
	SSS	SSMt	SSM	SMMt	SMM	SMtP	MMM	SMP
SFA	14.6	1.8	41.3	5.9	31.4	1.8	2.4	0.8
TFA	9.4	5.1	34.1	16.5	26.4	3.9	2.9	1.8
MUFA1	1.2	0.4	18.7	2.2	55.5	1.6	17.8	2.1
MUFA2	1.7	0.4	19.6	6.7	49.0	4.2	13.4	4.5
MUFA3	3.9	0.3	22.5	5.6	47.6	3.3	13.7	3.0
PUFA	5.8	1.0	25.3	8.1	38.1	6.7	6.6	7.9

Table 4 Total, sn-2 and Average sn-1/3 Fatty Acid Compositions of the SSS Fraction

Fraction/Group	Fatty Acid (% distribution)					
	14:0	16:0	17:0	18:0	18:1,c9	18:1,c11
Total						
SFA	6.1	30.7	1.1	61.5		
TFA	1.8	18.4		79.7		
MUFA1	5.9	23.7		63.1	5.5	1.7
MUFA2	4.0	53.9	0.4	41.3		
MUFA3	16.1	49.2		32.2		
PUFA	5.9	48.3	2.4	40.6	2.0	
sn-2						
SFA	3.6	29.5	1.2	64.3		
TFA	3.3	14.5		82.2		
MUFA1	1.9	30.1		63.1	4.9	
MUFA2	1.6	54.3		44.1		
MUFA3	11.7	52.7		31.7		
PUFA	2.8	38.5	1.5	53.3	2.8	
Average sn-1/3						
SFA	11.0	33.1	1.0	54.0		
TFA	-1.2	26.4		74.8		
MUFA1	13.9	10.8		63.3	6.9	5.2
MUFA2	8.8	53.1	1.3	35.9		
MUFA3	10.1	53.4		34.6		
PUFA	12.0	67.9	4.2	15.3	0.4	

Table 5 Total, sn-2 and Average sn-1/3 Fatty Acid Compositions of the SSM Fraction

Fraction/Group	Fatty Acid (% distribution)							
	14:0	16:0	16:1	17:0	18:0	18:1,t11	18:1, c9	18:1, c11
Total								
SFA	3.0	23.3	1.3	1.0	34.6	0.7	35.4	0.5
TFA	2.2	18.1	0.7	1.7	36.8	5.9	33.2	1.0
MUFA1	2.9	28.8	2.2	1.2	24.7		38.2	1.9
MUFA2	3.3	34.9	2.0	1.2	22.6	1.9	32.0	1.0
MUFA3	6.1	37.8	3.6	1.4	18.0	1.4	29.2	1.1
PUFA	2.6	31.0	2.1	1.5	25.2	2.3	33.7	0.8
sn-2								
SFA	1.8	18.2	1.2	0.6	29.9	0.3	47.3	0.4
TFA	2.1	15.3	1.0	0.5	30.5	4.6	45.7	0.1
MUFA1	3.9	18.5	2.0		27.3		47.6	
MUFA2	2.6	31.5	14.5	0.4	24.6	0.9	37.5	0.6
MUFA3	4.4	30.0	4.0		18.5	0.9	38.7	1.0
PUFA	1.5	26.5	1.5	1.2	26.6	3.4	38.4	0.
Average sn-1/3								
SFA	5.3	33.6	1.6	1.7	44.0	1.3	11.6	0.9
TFA	2.4	23.8		4.1	49.5	8.5	8.2	2.8
MUFA1	0.9	49.4	2.6	3.5	19.4		19.4	5.6
MUFA2	4.7	41.7	3.0	2.6	18.6	3.8	21.0	1.8
MUFA3	5.1	54.2	2.8	1.8	18.1	2.6	12.6	1.5
PUFA	4.6	40.1	3.1	2.1	22.2	0.3	24.2	1.4

Table 6 Total, sn-2 and Average sn-1/3 Fatty Acid Compositions of the SMMt Fraction

Fraction/Group	Fatty Acid (% distribution)							
	14:0	16:0	16:1	17:0	18:0	18:1,t11	18:1, c9	18:1, c11
Total								
SFA	1.3	18.2	0.6	0.3	18.4	16.2	43.4	0.4
TFA	3.8	17.8	0.9	0.6	19.0	23.3	32.6	0.6
MUFA1	12.3	20.8	0.5		20.9	9.4	36.0	
MUFA2	0.9	24.5	2.1	0.6	10.7	15.9	40.7	1.3
MUFA3	1.5	20.8	2.1	0.6	13.0	21.6	38.5	1.2
PUFA	0.2	19.4	1.4	0.7	13.5	23.4	36.8	0.8
sn-2								
SFA	0.9	14.9	0.6	0.2	20.1	10.2	51.3	0.7
TFA	3.3	14.2	2.4	0.5	16.3	19.6	41.3	0.8
MUFA1	13.1	16.3			21.3	7.1	42.2	
MUFA2	0.38	21.71	2.6		11.9	11.9	47.7	0.3
MUFA3	1.94	15.60	2.6		12.6	14.5	49.1	1.3
PUFA	1.24	17.44	1.4	0.18	15.9	17.0	44.6	0.3
Average sn-1/3								
SFA	2.3	24.7	0.5	0.4	14.9	28.4	27.8	-0.2
TFA	4.8	24.9	-2.2	0.7	24.4	30.6	15.2	0.4
MUFA1	10.7	29.9	1.6		20.2	14.1	23.6	
MUFA2	1.9	30.1	1.0	1.7	8.3	23.9	26.6	3.4
MUFA3	-1.1	30.1	0.5	1.7	11.9	34.1	20.8	1.5
PUFA	-1.8	23.3	1.5	1.7	8.5	36.2	21.1	1.9

Table 7 Total, sn-2 and Average sn-1/3 Fatty Acid Compositions of the SMM Fraction

Fraction/Group	Fatty Acid (% distribution)							
	14:0	16:0	16:1	17:0	18:0	18:1,t11	18:1, c9	18:1, c11
Total								
SFA	0.5	15.9	2.4	0.4	11.6		67.1	1.2
TFA	1.1	18.2	3.1	0.5	12.7	3.5	58.5	1.6
MUFA1	0.7	18.6	5.1	0.5	5.9		63.5	3.7
MUFA2	1.0	21.1	4.8	0.6	5.8	1.0	60.0	3.2
MUFA3	1.5	22.4	6.5	0.7	6.1		57.5	2.7
PUFA	0.9	20.2	3.8	0.8	8.0	0.3	62.3	1.9
sn-2								
SFA	1.4	8.0	2.6	0.1	10.8		76.1	0.5
TFA	0.3	12.0	3.0	0.3	13.2	2.7	67.0	1.2
MUFA1	0.9	12.9	5.2	0.1	6.92		68.4	3.5
MUFA2	0.7	14.1	4.5	0.2	6.54	0.86	68.8	2.1
MUFA3	2.1	12.4	7.7	0.4	4.43		67.5	2.0
PUFA	0.5	13.6	2.9	0.4	7.56		71.9	1.6
Average sn-1/3								
SFA	-1.3	31.8	2.0	1.1	13.3		49.1	2.8
TFA	2.6	30.6	3.3	1.0	11.6	5.3	41.5	2.5
MUFA1	0.2	30.1	4.9	1.1	4.0		53.7	4.1
MUFA2	1.6	35.2	5.4	1.3	4.4	1.4	42.4	5.3
MUFA3	-0.9	41.9	4.2	1.3	9.0		39.4	4.3
PUFA	1.5	33.3	5.4	1.7	8.8		43.2	2.6

Table 8 Total, sn-2 and Average sn-1/3 Fatty Acid Compositions of the SMtP Fraction

Fraction/Group	Fatty Acid (% distribution)								
	14:0	16:0	16:1	18:0	18:1,t11	18:1, c9	18:1, c11	18:2	18:3
Total									
SFA	0.6	11.4	0.8	33.2	3.7	40.3	0.9	8.7	
TFA	0.7	9.0	0.8	27.1	8.3	48.4	0.6	5.3	
MUFA1	10.3	14.8	2.7	23.8	2.2	41.0	1.4	3.9	
MUFA2	1.2	18.3	3.9	10.0	12.6	44.2	2.5	6.1	0.7
MUFA3	4.3	14.2	4.2	7.3	13.7	42.1	2.0	7.9	
PUFA	0.9	20.3	1.1	20.0	6.7	28.4	0.8	20.8	0.3
sn-2									
SFA	0.8	8.8		46.6		30.6		13.3	
TFA		8.8		28.1	4.3	51.0		7.7	
MUFA1	2.0	24.7	1.2	27.3		41.3		3.5	
MUFA2	0.4	19.6	3.8	12.8	4.3	49.3	0.9	8.6	
MUFA3	5.8	12.6	4.1	17.0	3.2	44.1	0.3	11.5	
PUFA	1.6	15.7	0.7	18.0	5.0	37.1	1.4	20.5	
Average sn-1/3									
SFA	0.3	16.4	2.3	6.4	11.2	59.8	2.7	-0.5	
TFA	2.1	9.3	2.3	25.0	16.1	43.2	1.7	0.4	
MUFA1	26.8	-5.0	5.5	16.7	6.5	40.5	4.1	4.8	
MUFA2	2.8	15.6	4.0	4.5	29.2	34.0	5.7	0.9	2.0
MUFA3	-1.7	20.3	4.1	3.1	30.4	36.8	5.3	1.2	1.3
PUFA	-0.5	29.3	1.8	24.0	10.2	11.1	-0.4	21.5	0.9

Table 9 Total, sn-2 and Average sn-1/3 Fatty Acid Compositions of the MMM Fraction

Fraction/Group	Fatty Acid (% distribution)								
	14:1	16:0	16:1	17:1	18:0	18:1,t11	18:1, c9	18:1, c11	18:2
Total									
SFA		2.9	2.3	0.6	14.0	0.9	76.0	1.5	1.9
TFA		7.9	1.5		31.4	5.5	46.8	1.0	5.8
MUFA1	0.4	2.8	7.9	1.0	3.4		76.3	7.0	0.2
MUFA2	0.9	4.4	7.3	1.3	6.3	0.8	71.4	4.5	1.8
MUFA3	1.5	0.5	10.4	1.5	0.3	0.5	77.2	6.2	0.8
PUFA	0.1	6.1	4.3	1.0	5.0	3.1	70.5	2.7	7.0
sn-2									
SFA		2.4	1.7		14.2	0.6	76.2	0.8	4.2
TFA		9.9			28.6		52.8		8.7
MUFA1	0.2	4.6	8.9	0.2	6.2		73.8	4.1	0.1
MUFA2	0.5	6.7	6.2	0.7	7.4	0.2	72.3	2.9	3.1
MUFA3	2.7	1.4	10.1	2.0	1.7		75.0	3.1	1.4
PUFA		7.7	3.3	0.1	8.0	1.8	67.3	1.6	9.4
Average sn-1/3									
SFA		3.7	3.5	1.7	13.6	1.4	75.7	3.1	
TFA		3.8	4.5		37.0	16.6	34.9	3.1	
MUFA1	0.9	-0.9	5.9	2.5	-2.3		81.1	12.8	
MUFA2	1.8	-0.1	9.6	2.7	4.1	2.2	69.6	7.9	
MUFA3	-0.2		11.4	1.7	-1.2		80.0	11.8	
PUFA	0.3	2.8	6.3	2.6	-1.1	5.7	77.1	4.7	2.2

Table 4 Total, sn-2 and Average sn-1/3 Fatty Acid Compositions of the SSS Fraction

Fatty Acid (% distribution)	Fatty Acid Compositions of the SSS Fraction		
	18:0	18:1,c9	18:1,c11
Total	30.7	61.5	1.1
SFA	6.1	18.4	1.8
TFA	18.4	79.7	1.1
MUFA1	5.9	63.1	1.7
MUFA2	4.0	41.3	0.4
MUFA3	16.1	32.2	0.4
PUFA	5.9	40.6	2.0
sn-2	29.5	64.3	1.2
SFA	3.6	82.2	3.3
TFA	14.5	82.2	3.3
MUFA1	1.9	63.1	4.9
MUFA2	1.6	44.1	1.6
MUFA3	11.7	31.7	1.7
PUFA	2.8	53.3	2.8
Average sn-1/3	33.1	54.0	1.0
SFA	11.0	74.8	1.0
TFA	-1.2	74.8	1.0
MUFA1	13.9	63.3	6.9
MUFA2	8.8	35.9	1.3
MUFA3	10.1	34.6	0.4
PUFA	12.0	15.3	0.4

Table 1. Total, sn-2 and Average sn-1/3 Fatty Acid Compositions for Each Treatment Group

Fraction/Group	Fatty Acid (% distribution)											
	14:0	14:1	16:0	16:1	17:0	17:1	18:0	18:1,t11	18:1,c9	18:1,c11	18:2	18:3
Total												
SFA	1.5	0.1	24.2	1.6	1.3	0.1	26.1	2.3	39.8	0.95	1.6	0.5
TFA	2.0	0.5	26.3	2.2	1.7	0.5	20.0	11.1	32.4	1.04	2.2	0.1
MUFA1	1.3	1.3	24.2	5.2	0.4	1.1	7.6	0.7	52.9	2.99	2.0	0.2
MUFA2	1.4	0.6	22.3	4.0	1.0	0.7	12.2	3.4	49.4	1.96	2.6	0.5
MUFA3	1.6	0.6	23.5	5.2	1.0	1.0	10.5	2.5	49.3	2.51	1.8	0.4
PUFA	1.8	0.2	23.8	2.8	1.1	0.4	17.7	3.7	42.4	1.26	4.5	0.2
sn 2												
SFA	2.0		20.0	2.2	0.4		21.3	2.0	49.6	0.55	2.1	
TFA	4.0	0.4	20.2	2.8	0.6	0.3	15.7	7.5	43.4	0.53	4.0	0.6
MUFA1	2.0	1.5	15.6	5.7	0.1	0.3	7.7	0.4	61.9	1.43	3.5	0.2
MUFA2	0.7	0.1	17.5	2.1	0.1	0.1	14.4	1.6	59.7	1.01	2.4	0.1
MUFA3	1.5	0.9	18.9	5.7	0.6	0.8	9.3	1.5	56.7	1.92	1.7	0.4
PUFA	0.7		17.3	2.3	0.6		17.2	2.0	53.1	0.43	6.2	
Average sn 1/3												
SFA	0.5	0.2	32.6	0.4	3.3	0.2	35.6	3.1	20.2	1.76	0.6	1.5
TFA	-2.1	0.6	38.7	0.8	4.0	1.1	28.4	18.3	10.5	2.06	-1.3	-0.9
MUFA1	-0.1	0.9	41.5	4.4	1.1	2.6	7.6	1.1	35.1	6.11	-0.5	0.3
MUFA2	2.9	1.5	31.9	7.6	2.7	1.8	7.8	7.0	28.7	3.87	2.9	1.4
MUFA3	1.6	0.1	32.9	4.0	1.8	1.2	13.0	4.6	34.7	3.67	2.0	0.5
PUFA	3.8	0.7	36.7	3.9	2.0	1.2	18.7	7.3	21.0	2.90	1.0	0.7

Table 2 Slip Point Analysis of Lipids Varying in Fatty Acid Composition

Group	Slip Point (°C)
SFA	45.1
TFA	41.5
MUFA1	22.8
MUFA2	28.4
MUFA3	30.7
PUFA	38.5

Table 3 Percentages of HPLC Fractions of Triglycerides from Each Treatment Group

Group	SSS	SSMt	SSM	SMMt	SMM	SMtP	MMMM	SMP
SFA	14.6	1.8	41.3	5.9	31.4	1.8	2.4	0.8
TFA	9.4	5.1	34.1	16.5	26.4	3.9	2.9	1.8
MUFA1	1.2	0.4	18.7	2.2	55.5	1.6	17.8	2.1
MUFA2	1.7	0.4	19.6	6.7	49.0	4.2	13.4	4.5
MUFA3	3.9	0.3	22.5	5.6	47.6	3.3	13.7	3.0
PUFA	5.8	1.0	25.3	8.1	38.1	6.7	6.6	7.9

Table 4 Total, sn-2 and Average sn-1/3 Fatty Acid Compositions of the SSS Fraction

Fraction/Group	Fatty Acid (% distribution)					
	14:0	16:0	17:0	18:0	18:1,c9	18:1,c11
Total						
SFA	6.1	30.7	1.1	61.5		
TFA	1.8	18.4		79.7		
MUFA1	5.9	23.7		63.1	5.5	1.7
MUFA2	4.0	53.9	0.4	41.3		
MUFA3	16.1	49.2		32.2		
PUFA	5.9	48.3	2.4	40.6	2.0	
sn-2						
SFA	3.6	29.5	1.2	64.3		
TFA	3.3	14.5		82.2		
MUFA1	1.9	30.1		63.1	4.9	
MUFA2	1.6	54.3		44.1		
MUFA3	11.7	52.7		31.7		
PUFA	2.8	38.5	1.5	53.3	2.8	
Average sn-1/3						
SFA	11.0	33.1	1.0	54.0		
TFA	-1.2	26.4		74.8		
MUFA1	13.9	10.8		63.3	6.9	5.2
MUFA2	8.8	53.1	1.3	35.9		
MUFA3	10.1	53.4		34.6		
PUFA	12.0	67.9	4.2	15.3	0.4	

Table 5 Total, sn-2 and Average sn-1/3 Fatty Acid Compositions of the SSM Fraction

Fraction/Group	Fatty Acid (% distribution)							
	14:0	16:0	16:1	17:0	18:0	18:1,t11	18:1, c9	18:1, c11
Total								
SFA	3.0	23.3	1.3	1.0	34.6	0.7	35.4	0.5
TFA	2.2	18.1	0.7	1.7	36.8	5.9	33.2	1.0
MUFA1	2.9	28.8	2.2	1.2	24.7		38.2	1.9
MUFA2	3.3	34.9	2.0	1.2	22.6	1.9	32.0	1.0
MUFA3	6.1	37.8	3.6	1.4	18.0	1.4	29.2	1.1
PUFA	2.6	31.0	2.1	1.5	25.2	2.3	33.7	0.8
sn-2								
SFA	1.8	18.2	1.2	0.6	29.9	0.3	47.3	0.4
TFA	2.1	15.3	1.0	0.5	30.5	4.6	45.7	0.1
MUFA1	3.9	18.5	2.0		27.3		47.6	
MUFA2	2.6	31.5	14.5	0.4	24.6	0.9	37.5	0.6
MUFA3	4.4	30.0	4.0		18.5	0.9	38.7	1.0
PUFA	1.5	26.5	1.5	1.2	26.6	3.4	38.4	0.
Average sn-1/3								
SFA	5.3	33.6	1.6	1.7	44.0	1.3	11.6	0.9
TFA	2.4	23.8		4.1	49.5	8.5	8.2	2.8
MUFA1	0.9	49.4	2.6	3.5	19.4		19.4	5.6
MUFA2	4.7	41.7	3.0	2.6	18.6	3.8	21.0	1.8
MUFA3	5.1	54.2	2.8	1.8	18.1	2.6	12.6	1.5
PUFA	4.6	40.1	3.1	2.1	22.2	0.3	24.2	1.4

Table 6 Total, sn-2 and Average sn-1/3 Fatty Acid Compositions of the SMMt Fraction

Fraction/Group	Fatty Acid (% distribution)							
	14:0	16:0	16:1	17:0	18:0	18:1,t11	18:1, c9	18:1, c11
Total								
SFA	1.3	18.2	0.6	0.3	18.4	16.2	43.4	0.4
TFA	3.8	17.8	0.9	0.6	19.0	23.3	32.6	0.6
MUFA1	12.3	20.8	0.5		20.9	9.4	36.0	
MUFA2	0.9	24.5	2.1	0.6	10.7	15.9	40.7	1.3
MUFA3	1.5	20.8	2.1	0.6	13.0	21.6	38.5	1.2
PUFA	0.2	19.4	1.4	0.7	13.5	23.4	36.8	0.8
sn-2								
SFA	0.9	14.9	0.6	0.2	20.1	10.2	51.3	0.7
TFA	3.3	14.2	2.4	0.5	16.3	19.6	41.3	0.8
MUFA1	13.1	16.3			21.3	7.1	42.2	
MUFA2	0.38	21.71	2.6		11.9	11.9	47.7	0.3
MUFA3	1.94	15.60	2.6		12.6	14.5	49.1	1.3
PUFA	1.24	17.44	1.4	0.18	15.9	17.0	44.6	0.3
Average sn-1/3								
SFA	2.3	24.7	0.5	0.4	14.9	28.4	27.8	-0.2
TFA	4.8	24.9	-2.2	0.7	24.4	30.6	15.2	0.4
MUFA1	10.7	29.9	1.6		20.2	14.1	23.6	
MUFA2	1.9	30.1	1.0	1.7	8.3	23.9	26.6	3.4
MUFA3	-1.1	30.1	0.5	1.7	11.9	34.1	20.8	1.5
PUFA	-1.8	23.3	1.5	1.7	8.5	36.2	21.1	1.9

Table 7 Total, sn-2 and Average sn-1/3 Fatty Acid Compositions of the SMM Fraction

Fraction/Group	Fatty Acid (% distribution)							
	14:0	16:0	16:1	17:0	18:0	18:1,t11	18:1, c9	18:1, c11
Total								
SFA	0.5	15.9	2.4	0.4	11.6		67.1	1.2
TFA	1.1	18.2	3.1	0.5	12.7	3.5	58.5	1.6
MUFA1	0.7	18.6	5.1	0.5	5.9		63.5	3.7
MUFA2	1.0	21.1	4.8	0.6	5.8	1.0	60.0	3.2
MUFA3	1.5	22.4	6.5	0.7	6.1		57.5	2.7
PUFA	0.9	20.2	3.8	0.8	8.0	0.3	62.3	1.9
sn-2								
SFA	1.4	8.0	2.6	0.1	10.8		76.1	0.5
TFA	0.3	12.0	3.0	0.3	13.2	2.7	67.0	1.2
MUFA1	0.9	12.9	5.2	0.1	6.92		68.4	3.5
MUFA2	0.7	14.1	4.5	0.2	6.54	0.86	68.8	2.1
MUFA3	2.1	12.4	7.7	0.4	4.43		67.5	2.0
PUFA	0.5	13.6	2.9	0.4	7.56		71.9	1.6
Average sn-1/3								
SFA	-1.3	31.8	2.0	1.1	13.3		49.1	2.8
TFA	2.6	30.6	3.3	1.0	11.6	5.3	41.5	2.5
MUFA1	0.2	30.1	4.9	1.1	4.0		53.7	4.1
MUFA2	1.6	35.2	5.4	1.3	4.4	1.4	42.4	5.3
MUFA3	-0.9	41.9	4.2	1.3	9.0		39.4	4.3
PUFA	1.5	33.3	5.4	1.7	8.8		43.2	2.6

Table 8 Total, sn-2 and Average sn-1/3 Fatty Acid Compositions of the SMtP Fraction

Fraction/Group	Fatty Acid (% distribution)								
	14:0	16:0	16:1	18:0	18:1,t11	18:1, c9	18:1, c11	18:2	18:3
Total									
SFA	0.6	11.4	0.8	33.2	3.7	40.3	0.9	8.7	
TFA	0.7	9.0	0.8	27.1	8.3	48.4	0.6	5.3	
MUFA1	10.3	14.8	2.7	23.8	2.2	41.0	1.4	3.9	
MUFA2	1.2	18.3	3.9	10.0	12.6	44.2	2.5	6.1	0.7
MUFA3	4.3	14.2	4.2	7.3	13.7	42.1	2.0	7.9	
PUFA	0.9	20.3	1.1	20.0	6.7	28.4	0.8	20.8	0.3
sn-2									
SFA	0.8	8.8		46.6		30.6		13.3	
TFA		8.8		28.1	4.3	51.0		7.7	
MUFA1	2.0	24.7	1.2	27.3		41.3		3.5	
MUFA2	0.4	19.6	3.8	12.8	4.3	49.3	0.9	8.6	
MUFA3	5.8	12.6	4.1	17.0	3.2	44.1	0.3	11.5	
PUFA	1.6	15.7	0.7	18.0	5.0	37.1	1.4	20.5	
Average sn-1/3									
SFA	0.3	16.4	2.3	6.4	11.2	59.8	2.7	-0.5	
TFA	2.1	9.3	2.3	25.0	16.1	43.2	1.7	0.4	
MUFA1	26.8	-5.0	5.5	16.7	6.5	40.5	4.1	4.8	
MUFA2	2.8	15.6	4.0	4.5	29.2	34.0	5.7	0.9	2.0
MUFA3	-1.7	20.3	4.1	3.1	30.4	36.8	5.3	1.2	1.3
PUFA	-0.5	29.3	1.8	24.0	10.2	11.1	-0.4	21.5	0.9

Table 9 Total, sn-2 and Average sn-1/3 Fatty Acid Compositions of the MMM Fraction

Fraction/Group	Fatty Acid (% distribution)								
	14:1	16:0	16:1	17:1	18:0	18:1,t11	18:1, c9	18:1, c11	18:2
Total									
SFA		2.9	2.3	0.6	14.0	0.9	76.0	1.5	1.9
TFA		7.9	1.5		31.4	5.5	46.8	1.0	5.8
MUFA1	0.4	2.8	7.9	1.0	3.4		76.3	7.0	0.2
MUFA2	0.9	4.4	7.3	1.3	6.3	0.8	71.4	4.5	1.8
MUFA3	1.5	0.5	10.4	1.5	0.3	0.5	77.2	6.2	0.8
PUFA	0.1	6.1	4.3	1.0	5.0	3.1	70.5	2.7	7.0
sn-2									
SFA		2.4	1.7		14.2	0.6	76.2	0.8	4.2
TFA		9.9			28.6		52.8		8.7
MUFA1	0.2	4.6	8.9	0.2	6.2		73.8	4.1	0.1
MUFA2	0.5	6.7	6.2	0.7	7.4	0.2	72.3	2.9	3.1
MUFA3	2.7	1.4	10.1	2.0	1.7		75.0	3.1	1.4
PUFA		7.7	3.3	0.1	8.0	1.8	67.3	1.6	9.4
Average sn-1/3									
SFA		3.7	3.5	1.7	13.6	1.4	75.7	3.1	
TFA		3.8	4.5		37.0	16.6	34.9	3.1	
MUFA1	0.9	-0.9	5.9	2.5	-2.3		81.1	12.8	
MUFA2	1.8	-0.1	9.6	2.7	4.1	2.2	69.6	7.9	
MUFA3	-0.2		11.4	1.7	-1.2		80.0	11.8	
PUFA	0.3	2.8	6.3	2.6	-1.1	5.7	77.1	4.7	2.2

Table 4 Total, sn-2 and Average sn-1/3 Fatty Acid Compositions of the SSS Fraction

Fatty Acid (% distribution)	Fatty Acid Compositions of the SSS Fraction		
	18:0	18:1,c9	18:1,c11
Total	30.7	1.1	61.5
SFA	6.1	1.8	18.4
TFA	18.4	1.1	79.7
MUFA1	5.9	23.7	63.1
MUFA2	4.0	53.9	41.3
MUFA3	16.1	49.2	32.2
PUFA	5.9	48.3	40.6
sn-2	3.6	29.5	64.3
SFA	3.3	14.5	82.2
TFA	1.9	30.1	63.1
MUFA1	1.6	54.3	44.1
MUFA2	11.7	52.7	31.7
MUFA3	2.8	38.5	53.3
PUFA	11.0	33.1	54.0
Average sn-1/3	11.0	33.1	54.0
SFA	-1.2	26.4	74.8
TFA	13.9	10.8	63.3
MUFA1	8.8	53.1	35.9
MUFA2	10.1	53.4	34.6
MUFA3	12.0	67.9	4.2
PUFA	0.4	15.3	0.4
sn-2	4.9	2.0	1.7
SFA	3.6	29.5	64.3
TFA	1.9	30.1	63.1
MUFA1	1.6	54.3	44.1
MUFA2	11.7	52.7	31.7
MUFA3	2.8	38.5	53.3
PUFA	11.0	33.1	54.0
Average sn-1/3	11.0	33.1	54.0
SFA	-1.2	26.4	74.8
TFA	13.9	10.8	63.3
MUFA1	8.8	53.1	35.9
MUFA2	10.1	53.4	34.6
MUFA3	12.0	67.9	4.2
PUFA	0.4	15.3	0.4
Total	6.1	1.1	61.5
SFA	1.8	1.1	79.7
TFA	5.9	23.7	63.1
MUFA1	4.0	53.9	41.3
MUFA2	16.1	49.2	32.2
MUFA3	5.9	48.3	40.6
PUFA	3.6	29.5	64.3
sn-2	3.6	29.5	64.3
SFA	3.3	14.5	82.2
TFA	1.9	30.1	63.1
MUFA1	1.6	54.3	44.1
MUFA2	11.7	52.7	31.7
MUFA3	2.8	38.5	53.3
PUFA	11.0	33.1	54.0
Average sn-1/3	11.0	33.1	54.0
SFA	-1.2	26.4	74.8
TFA	13.9	10.8	63.3
MUFA1	8.8	53.1	35.9
MUFA2	10.1	53.4	34.6
MUFA3	12.0	67.9	4.2
PUFA	0.4	15.3	0.4

Table 5 Total, sn-2 and Average sn-1/3 Fatty Acid Compositions of the SSM Fraction

Fatty Acid (% distribution)	Total				sn-2				Average sn-1/3					
	14:0	16:0	16:1	17:0	18:0	18:1,t11	18:1,c9	18:1,c11	SFA	TFA	MUFA1	MUFA2	MUFA3	PUFA
	3.0	23.3	1.3	1.0	34.6	0.7	35.4	0.5	18.2	15.3	18.5	31.5	30.0	26.5
	1.8	18.2	1.2	0.6	29.9	0.3	47.3	0.4	3.9	2.1	3.9	2.6	4.4	1.5
	2.1	15.3	1.0	0.5	30.5	4.6	45.7	0.1	MUFA1	MUFA2	MUFA1	MUFA2	MUFA3	PUFA
	2.9	28.8	2.2	1.2	24.7		47.6	0.6	2.6	3.1	2.0	14.5	4.0	1.2
	2.2	18.1	0.7	1.7	36.8	5.9	33.2	1.0	3.3	3.3	3.3	3.3	3.3	3.3
	2.9	28.8	2.2	1.2	24.7		47.6	0.6	3.3	3.3	3.3	3.3	3.3	3.3
	3.3	34.9	2.0	1.2	22.6	1.9	32.0	1.0	6.1	6.1	6.1	6.1	6.1	6.1
	6.1	37.8	3.6	1.4	18.0	1.4	29.2	1.1	2.6	2.6	2.6	2.6	2.6	2.6
	2.6	31.0	2.1	1.5	25.2	2.3	33.7	0.8	1.8	1.8	1.8	1.8	1.8	1.8
	1.8	18.2	1.2	0.6	29.9	0.3	47.3	0.4	3.9	2.1	3.9	2.6	4.4	1.5
	1.8	15.3	1.0	0.5	30.5	4.6	45.7	0.1	3.9	2.1	3.9	2.6	4.4	1.5
	3.9	18.5	2.0	1.2	27.3		47.6	0.6	3.9	2.1	3.9	2.6	4.4	1.5
	2.6	31.5	14.5	0.4	24.6	0.9	37.5	0.6	2.6	3.1	2.0	14.5	4.0	1.2
	4.4	30.0	4.0		18.5	0.9	38.7	1.0	1.5	1.5	1.5	1.5	1.5	1.5
	1.5	26.5	1.5	1.2	26.6	3.4	38.4	0.	5.3	5.3	5.3	5.3	5.3	5.3
	5.3	33.6	1.6	1.7	44.0	1.3	11.6	0.9	4.7	4.7	4.7	4.7	4.7	4.7
	2.4	23.8	4.1	4.1	49.5	8.5	8.2	2.8	0.9	0.9	0.9	0.9	0.9	0.9
	0.9	49.4	2.6	3.5	19.4		19.4	5.6	0.9	0.9	0.9	0.9	0.9	0.9
	4.7	41.7	3.0	2.6	18.6	3.8	21.0	1.8	4.7	4.7	4.7	4.7	4.7	4.7
	5.1	54.2	2.8	1.8	18.1	2.6	12.6	1.5	5.1	5.1	5.1	5.1	5.1	5.1
	4.6	40.1	3.1	2.1	22.2	0.3	24.2	1.4	4.6	4.6	4.6	4.6	4.6	4.6

Table 6 Total, sn-2 and Average sn-1/3 Fatty Acid Compositions of the SMMT Fraction

Fatty Acid Group	Fatty Acid (% distribution)				Fatty Acid (% distribution)			
	14:0	16:0	16:1	17:0	18:0	18:1,t11	18:1,c9	18:1,c11
Total	1.3	18.2	0.6	0.3	18.4	16.2	43.4	0.4
SFA	3.8	17.8	0.9	0.6	19.0	23.3	32.6	0.6
TFA	12.3	20.8	0.5		20.9	9.4	36.0	
MUFAl	0.9	24.5	2.1	0.6	10.7	15.9	40.7	1.3
MUFAl2	1.5	20.8	2.1	0.6	13.0	21.6	38.5	1.2
MUFAl3	0.2	19.4	1.4	0.7	13.5	23.4	36.8	0.8
PUFA	0.9	14.9	0.6	0.2	20.1	10.2	51.3	0.7
TFA	3.3	14.2	2.4	0.5	16.3	19.6	41.3	0.8
MUFAl	13.1	16.3		21.3	7.1	42.2		
MUFAl2	0.38	21.71	2.6	11.9	11.9	47.7	0.3	
MUFAl3	1.94	15.60	2.6	12.6	14.5	49.1	1.3	
PUFA	1.24	17.44	1.4	0.18	15.9	17.0	44.6	0.3
Average sn-1/3	2.3	24.7	0.5	0.4	14.9	28.4	27.8	-0.2
SFA	4.8	24.9	-2.2	0.7	24.4	30.6	15.2	0.4
TFA	10.7	29.9	1.6	20.2	14.1	23.6		
MUFAl	1.9	30.1	1.0	1.7	8.3	23.9	26.6	3.4
MUFAl2	-1.1	30.1	0.5	1.7	11.9	34.1	20.8	1.5
MUFAl3	-1.8	23.3	1.5	1.7	8.5	36.2	21.1	1.9

Table 7 Total, sn-2 and Average sn-1/3 Fatty Acid Compositions of the SMM Fraction

Fatty Acid (% distribution)	Fraction/Group				Total			
	14:0	16:0	16:1	17:0	18:0	18:1,t11	18:1,c9	18:1,c11
SFA	0.5	15.9	2.4	0.4	11.6	67.1	1.2	1.2
TFA	1.1	18.2	3.1	0.5	12.7	58.5	1.6	1.6
MUFA1	0.7	18.6	5.1	0.5	5.9	63.5	3.7	3.7
MUFA2	1.0	21.1	4.8	0.6	5.8	60.0	3.2	3.2
MUFA3	1.5	22.4	6.5	0.7	6.1	57.5	2.7	2.7
PUFA	0.9	20.2	3.8	0.8	8.0	62.3	1.9	1.9
sn-2								
SFA	1.4	8.0	2.6	0.1	10.8	76.1	0.5	0.5
TFA	0.3	12.0	3.0	0.3	13.2	67.0	1.2	1.2
MUFA1	0.9	12.9	5.2	0.1	6.92	68.4	3.5	3.5
MUFA2	0.7	14.1	4.5	0.2	6.54	68.8	2.1	2.1
MUFA3	2.1	12.4	7.7	0.4	4.43	67.5	2.0	2.0
PUFA	0.5	13.6	2.9	0.4	7.56	71.9	1.6	1.6
Average sn-1/3								
SFA	-1.3	31.8	2.0	1.1	13.3	49.1	2.8	2.8
TFA	2.6	30.6	3.3	1.0	11.6	41.5	2.5	2.5
MUFA1	0.2	30.1	4.9	1.1	4.0	53.7	4.1	4.1
MUFA2	1.6	35.2	5.4	1.3	4.4	42.4	5.3	5.3
MUFA3	-0.9	41.9	4.2	1.3	9.0	39.4	4.3	4.3
PUFA	1.5	33.3	5.4	1.7	8.8	43.2	2.6	2.6

Table 8 Total, sn-2 and Average sn-1/3 Fatty Acid Compositions of the SMTF Fraction

Fatty Acid (% distribution)	Fraction/Group									
	14:0	16:0	16:1	18:0	18:1,t11	18:1,c9	18:1,c11	18:2	18:3	Total
SFA	0.6	11.4	0.8	33.2	3.7	40.3	0.9	8.7	0.7	0.3
TFA	0.7	9.0	0.8	27.1	8.3	48.4	0.6	5.3	0.7	0.3
MUFA1	10.3	14.8	2.7	23.8	2.2	41.0	1.4	3.9	0.7	0.3
MUFA2	1.2	18.3	3.9	10.0	12.6	44.2	2.5	6.1	0.7	0.3
MUFA3	4.3	14.2	4.2	7.3	13.7	42.1	2.0	7.9	0.7	0.3
PUFA	0.9	20.3	1.1	20.0	6.7	28.4	0.8	20.8	0.3	0.3
sn-2										
SFA	0.8	8.8		46.6		30.6		13.3		
TFA	8.8	28.1		28.1	4.3	51.0		7.7		
MUFA1	2.0	24.7	1.2	27.3		41.3		3.5		
MUFA2	0.4	19.6	3.8	12.8	4.3	49.3	0.9	8.6		
MUFA3	5.8	12.6	4.1	17.0	3.2	44.1	0.3	11.5		
PUFA	1.6	15.7	0.7	18.0	5.0	37.1	1.4	20.5		
Average sn-1/3										
SFA	0.3	16.4	2.3	6.4	11.2	59.8	2.7	-0.5		
TFA	2.1	9.3	2.3	25.0	16.1	43.2	1.7	0.4		
MUFA1	26.8	-5.0	5.5	16.7	6.5	40.5	4.1	4.8		
MUFA2	2.8	15.6	4.0	4.5	29.2	34.0	5.7	0.9		
MUFA3	-1.7	20.3	4.1	3.1	30.4	36.8	5.3	1.2		
PUFA	-0.5	29.3	1.8	24.0	10.2	11.1	-0.4	21.5		

Table 9 Total, sn-2 and Average sn-1/3 Fatty Acid Compositions of the MMM Fraction

Fatty Acid Group	Fatty Acid (% distribution)									
	14:1	16:0	16:1	17:1	18:0	18:1,t11	18:1,c9	18:1,c11	18:2	Total
SFA	2.9	2.3	0.6	14.0	0.9	76.0	1.5	1.9	5.8	1.9
TFA	7.9	1.5		31.4	5.5	46.8	1.0	5.8	8.7	4.2
MUFA1	0.4	2.8	1.0	3.4		76.3	7.0	0.2	0.1	8.7
MUFA2	0.9	4.4	1.3	6.3	0.8	71.4	4.5	1.8	0.1	8.7
MUFA3	1.5	10.4	1.5	0.3	0.5	77.2	6.2	0.8	0.1	8.7
PUFA	0.1	6.1	1.0	5.0	3.1	70.5	2.7	7.0	0.1	8.7
SFA	2.4	1.7		14.2	0.6	76.2	0.8	4.2	0.1	8.7
TFA	9.9			28.6		52.8		8.7	0.1	8.7
MUFA1	0.2	4.6	0.2	6.2		73.8	4.1	0.1	0.1	8.7
MUFA2	0.5	6.7	0.7	7.4	0.2	72.3	2.9	3.1	0.1	8.7
MUFA3	2.7	1.4	2.0	1.7		75.0	3.1	1.4	0.1	8.7
PUFA	7.7	3.3	0.1	8.0	1.8	67.3	1.6	9.4	0.1	8.7
Average sn-1/3										
SFA	3.7	3.5	1.7	13.6	1.4	75.7	3.1	3.1	3.1	3.1
TFA	3.8	4.5		37.0	16.6	34.9	3.1	3.1	3.1	3.1
MUFA1	0.9	5.9	2.5	-2.3	2.2	81.1	12.8	12.8	12.8	12.8
MUFA2	1.8	9.6	2.7	4.1		69.6	7.9	7.9	7.9	7.9
MUFA3	-0.2	11.4	1.7	-1.2		80.0	11.8	11.8	11.8	11.8
PUFA	0.3	6.3	2.6	-1.1	5.7	77.1	4.7	2.2	2.2	2.2

Table 10 Total, sn-2 and Average sn-1/3 Fatty Acid Compositions of the SMP Fraction for the SFA, MUFA3 and PUFA Treatment Groups

Fraction/Group	Fatty Acid (% distribution)							
	14:0	16:0	16:1	17:0	18:0	18:1, c9	18:1, c11	18:2
Total								
SFA		22.7	1.2	0.9	9.8	33.9	1.4	30.2
MUFA3	4.3	20.4	2.4		10.4	33.5	1.3	27.2
PUFA	0.5	20.3	1.2	0.5	10.4	33.7	0.9	32.2
sn-2								
SFA		12.3			12.7	34.1		41.0
MUFA3	4.3	11.6	2.9		13.1	29.7		37.3
PUFA		14.5	0.32		11.65	35.3		38.0
Average sn-1/3								
SFA		43.6	3.5	2.6	4.0	33.8	4.2	8.7
MUFA3	-0.5	35.5	0.7		9.0	42.2	2.6	11.4
PUFA	1.6	31.9	2.9	1.6	8.0	30.3	2.4	20.7