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Breed differences and heterosis in triacylglycerol fatty acid composition of bovine adipose tissue

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Summary

Subcutaneous adipose tissues were biopsied in purebred Jersey (n=17), purebred Limousin (n=17) and reciprocal F_1 Jersey × Limousin crossbred (n=33) calves at the age of 9–10 months. Triacylglycerol fatty acids were extracted and analysed for sex and breed differences. Heterosis, additive and maternal variances were estimated. All calves were pasture-fed in a single management group and biopsied from the same anatomical site. Heifer calves had significantly higher proportions of palmitoleate, total mono-unsaturated fatty acids, desaturation index and lower stearate than steer calves. Significant breed differences were observed in that Limousin calves had the highest proportions of palmitate and total saturated fatty acids, whereas Jersey calves had the most palmitoleate and desaturation index. Dominance effects were evident in the proportions of palmitate, stearate, desaturation and elongation enzyme indices due to the observed highly significant heterosis effect. Myristate, palmitate and total saturated fatty acids were considered heritable due to the observed highly significant additive genetic effect.

Introduction

The mating of closely related animals has long been known to lead to 'inbreeding depression' as a result of loss of fitness in the offspring. On the other hand, crossbreeding animals results in increased hybrid vigour since the offspring generally, perform better than the average of the parental breeds. This is termed heterosis, an important animal breeding tool for the improvement of traits like growth, musculature and survival.

It has not yet been established if crossbreeding cattle that have high proportions of the undesirable saturated fatty acids in their adipose tissue with those with high proportions of desirable mono-unsaturated fatty acids would lead to an improvement in the crossbred animals. Therefore, the hypothesis tested in this study was that there are breed differences between the Jersey and Limousin such that any significant heterosis effect could be exploited for genetic improvement.

The initial step in genetic improvement is the establishment of breed differences for the traits of interest. Next, is the question of how heritable the traits are. Traits that show significant additive genetic effects are considered heritable whereas significant dominance indicates the heterosis effect. The aim of this experiment was to investigate sex and breed differences between purebred and crossbred calves at a single age, and to estimate the heterosis of triacylglycerol fatty acids.

Materials and methods

Subcutaneous adipose tissues were biopsied from the area between the 12th and 13th ribs in 67 Jersey, Limousin and reciprocal F_1 Jersey × Limousin calves at 9–10 months of age. The calves were all pasture-fed and maintained in a single management group at the J.S. Davies Cattle Gene Mapping herd, Martindale, South Australia. Triacylglycerol fatty acids were extracted, methylated and analysed by gas-liquid chromatography as described in our previous work (Malau-Aduli et al. 1997; Siebert et al. 1998; Malau-Aduli 1998).

The normalized values of fatty acid percentages were statistically analysed using PROC GLM (SAS 1989). The first model included the fixed effects of sex and breed, and their interaction. Least squares means were computed to test for significant differences between sexes and breeds. The second model for the estimation of mode of inheritance included the fixed effects of sire and dam breeds and their interaction. Sire breed accounted for additive genetic effect whereas dam breed estimated combined additive and maternal effects. If dam effect was different from sire effect, it was concluded that the difference was due to maternal effect. Heterosis, that is dominance, was estimated by the interaction between sire and dam breeds. Thus, heterosis for a given fatty acid was computed as the mean deviation between the crossbred and parental breeds. Similarly, additive genetic effect was computed as the mean deviation between Jersey and Limousin sire breeds, and the deviation between the dam breeds, respectively, was the combined additive and maternal effects.

Results

Individual fatty acids (C14–C18) from subcutaneous adipose tissues biopsied from the area between the 12th and 13th ribs were measured in young Jersey, Limousin and F_1 Jersey × Limousin calves. The factors fitted in the model and tests of significance used to analyse the fatty acid data are shown in Table 1

Sex differences were significant in the proportions of the individual fatty acids palmitoleate (16:1) and stearate (18:0). Summations of the saturated fatty acids (SFA) and mono-unsaturated fatty acids (MUFA) as well as the ratio of unsaturated to saturated fatty acids (USR) differed between the sexes. Furthermore, sex was a significant source of variation in the calculated indices of desaturation (Table1). Heifers had higher percentages of 16 : 1 (6.9 versus 5.0%), MUFA (46.1 versus 41.9%), USR (0.98 versus 0.85) and desaturation indices in C16 (17.7 versus 13.0%) and C18 (72 .8 versus 67.6%) fatty acids than steer calves (Table 2)

Breed differences between purebred Jersey, purebred Limousin and crossbred Jersey × Limousin calves were significant in the proportions of the individual fatt y acids palmitate (16:0) and heptadecanoleate (17:1) (Table 1). Summations of SFA and MUFA, USR, desaturation and elongation indices also differed between the breeds (Table 1). Limousin calves had the highest proportions of 16:0 (37.1%) and SFA (58.8%), whereas Jersey calves contained the most 16:1 (7.1%) and desaturation index in C18 fatty acids (74.1%) (Table 3)

Proportions of stearate (18:0), desaturation index in C16 fatty acids and elongation index in the crossbred animals (F_1) were higher than the average of Jersey and Limousin purebreds (Table 3). On the other hand, proportions of 16:0, 16:1, oleate (18:1n-9), SFA, MUFA, desaturation index in C18 fatty acids and USR were below the mean of the purebred calves. Table 3 also shows that the proportions of 16:0, SFA, MUFA, desaturation index in C16 fatty acids and USR were the same in both F_1 and Jersey calves. Similarly, percentages of 16:1, 18:0 and desaturation index in C18 fatty acids were the same in both F_1 and Limousin calves. Interestingly too, is the observation that the proportion of the most abundant individual fatty acid in the adipose tissue, oleate (18:1n-9), was the same in all the breeds (Table 3).

Estimates of genetic effects (Table 4) portray significant effects of sire breed on 14:0, 16:0, 18:1n-7 (vaccenate) and SFA, an indication of direct additive genetic variance. Dam breed was significant for 14:1, 16:0, 18:0, SFA, MUFA, desaturation indices in C16 and C18 fatty acids (Table 4), an indication of significant combined effects of maternal and additive genetic variance. Specifically, 14:0, 14:1, 18:0 and desaturation index in C18 fatty acids were due to maternal component of dam effect, whereas 16:0, SFA, MUFA and desaturation index in C16 fatty acids were due to additive effect. Significant effect of heterosis was observed in the proportions of 16:0, 18:0, desaturation index in C18 fatty acids and elongation index (Table 4), an indication of dominance.

Discussion

An ideal model to study heterosis (hybrid vigour) in fatty acids is one that utilizes two diverse breeds of cattle to produce reciprocal F_1 crossbreds. Thus Jersey and Limousin breeds noted for their extreme variation in fat deposition were mated to produce the F_1 crossbreds. To eliminate age differences, purebred Jersey and Limousin calves of the same age as the F_1 crossbreds (9–10 months), were produced for making comparisons. Since the progeny comprised of both heifer and steer calves, sex was fitted as a fixed effect in the model in addition to breed. Sex and breed differences as well as estimates of genetic effects were computed.

Sex differences

There were significant differences between the sexes in two individual fatty acids; 16:1 and 18:0 in which heifers contained higher proportions of 16:1 and lower 18:0 than steer calves (Table 2). Palmitoleate (16:1) is a MUFA that is produced from palmitate (16:0) by the introduction of a double bond by the desaturation enzyme. It has a low melting point of 1°C thus contributing to the softness of fat. It would thus appear that the fat from heifer calves is expected to be softer than that from steer calves. Heifers mature earlier than steers and this is in line with the maturity, fattening and age effects in the previous section. A negative relationship exists between the proportion of unsaturated fatty acids and melting point such that one decreases as the other increases. Litchfield (1972) reported that the melting points of long-chain fatty acids and their methyl esters are related to the arrangement of the molecules within the crystal, the number and relative

positions of the double bonds. Therefore, the higher the proportion of unsaturated fatty acids, the lower the melting point and the softer the fat.

As expected, heifer calves had higher proportions of total MUFA than steer calves (Table 2). This indicates that the activity of the desaturation enzyme in converting saturated to unsaturated fatty acids is higher in heifer than steer calves. This is in turn supported by the higher USR values and indices of desaturation in the heifer than steer calves.

Stearate (18:0) is an important saturated fatty acid (the next most abundant fatty acid to 16:0) in the adipose tissue. It is synthesised *de novo* by the elongation of 16:0 through two carbon-unit addition by elongation enzyme. Heifer calves had a lower proportion of 18:0 than steer calves (Table 2). This suggests that the fat from steer calves would be harder than that from heifers since saturated fatty acids have high melting points. Furthermore, this observation might imply that the activity and quantity of elongation enzyme in chain-elongating 16:0–18:0 is greater in steer than heifer calves.

It is important to note that the relative accumulation of 18:0 in the adipose tissue should not be too much of a health concern in relation to cardiovascular diseases as 18:0 is not considered as cholesterol-raising in humans. This is because the bulk of 18:0 synthesised *de novo* is converted by the desaturation enzyme into oleate (18:1n-9), the most abundant MUFA (40%). This is in contrast to 16:0 where only a small proportion (5–7%) of 16:1 results from its conversion, which explains why the accumulation of 16:0 is hypercholesterolaemic in humans.

Sex by breed interaction was not a significant source of variation in fatty acid composition. This suggests that differences observed between heifer and steer calves followed the same pattern in the Limousin and Jersey breeds. Physiological differences between heifer and steer calves exist. For instance, heifer calves reach physiological maturity earlier than steer calves. Also, due to hormonal differences between the sexes (Prior et al. 1983), it is probable that enzyme activities are affected such that females accumulate higher proportions of MUFA than the males.

Breed differences

Breed differences between Jersey and Limousin calves were not detected in most of the fatty acids except 17:1 (heptadecanoleate) (Malau-Aduli et al. 1997; Siebert et al. 1998). A similar trend is evident here as significant breed differences between Jersey, Limousin and F_1 Jersey × Limousin calves were observed in the proportions of 17:1 and 16:0 only (Table 1).

Palmitate (16:0) is the most abundant saturated fatty acid constituting up to 37% of the total fatty acids in the adipose tissue (Table 3). It has been reported to be an undesirable fatty acid in human health studies because it raises cholesterol levels (Mattson and Grundy 1985), even though the levels reported here are lower than the high risk level of 55% of total lipids. Palmitate is also synthesised *de novo* by the elongation of myristate (14:0) via two-carbon unit addition. Limousin calves contained the most 16:0 and total SFA compared with Jersey and F_1 calves (Table 3). This is consistent with the observation of Malau-Aduli et al. (1997) where it was demonstrated that the adipose tissue of Limousin was more saturated than that of Jersey cattle.

It is obvious that crossing the Jersey and Limousin breeds resulted in a decrease in the proportion of 16:0 (Table 3). This is quite significant that a 6% reduction in this deleterious fatty acid could be achieved by crossbreeding the two breeds. It may be possible to even reduce this further if selection is incorporated into the crossbreeding programme. It is very likely that the F_1 crossbreds are exhibiting negative heterosis for 16:0 (percentages less than the average of the parentals), which from the human dietary perspective, is beneficial.

Heterosis

When different breeds of cattle are crossed, the resulting F_1 progeny generally show an increased hybrid vigour in certain characteristics compared with the parental breeds. The amount of heterosis is expressed as the difference between the crossbred and parental breed means (Falconer 1993). From a fat deposition and fatty acid composition perspective, Jersey and Limousin breeds differ, since the Jerseys are early maturing, deposit more intramuscular fat and contain less saturated fatty acids than the Limousins. Furthermore, their desaturation and elongation indices differ (Malau-Aduli et al. 1997; Siebert et al. 1998; Malau-Aduli et al. 1998).

The F_1 progeny resulting from crossbreeding Jersey and Limousin cattle had higher means than the average of the purebreds in the proportions of 18:0, desaturation index in C16 fatty acids and elongation index (Table 3). This is a clear evidence of heterosis which is in turn, a demonstration of the dominance of a gene (or genes) in one breed over the other for these traits. The significant effect of heterosis in the proportions of 16:0, 18:0, desaturation index in C18 fatty acids and elongation index is portrayed in Table 4.

It is pertinent to stress that heterosis is not always positive because it is also possible to have negative heterosis for other traits. The latter situation is evident in that proportions of 16:0, 16:1, SFA, MUFA, desaturation index in C18 fatty acids and USR in the F_1s were below the means of the purebreds (Table 3). The negative heterosis in 16 : 0 and SFA are indeed, beneficial from the human health perspective of fat consumption since it is a direct counter-measure to cholesterol build-up.

Percentages of 16:1, 18:0 and desaturation index in C18 fatty acids were the same in both Limousin and F_1 calves (Table 3), an indication of the dominance of Limousin over the breeds. On the other hand, the proportions of 16:0, SFA, MUFA, desaturation in C16 fatty acids and USR were the same in both Jersey and F_1 calves. Although it is difficult to say the number of genes involved, Table 4 clearly demonstrates that the dominant effect observed in the proportions of 16:0, 18:0, desaturation index in C18 fatty acids and elongation index was highly significant.

Additive genetic effect is of interest in the estimation of breeding value and heritability of traits in selection programmes. It is an important measure of genetic variance because it is the chief cause of resemblance between relatives and therefore, the major heritable component. Significant additive genetic variance was observed in the proportions of 14:0, 16:0, 18:1n-7 and SFA (Table 4). The implication is that the superiority of sires over their contemporaries for these traits, would on the average, be passed on to offspring in the next generation. Therefore, by preventing sires containing high proportions of 14:0, 16:0 and SFA from becoming parents of the next generation, beef cattle breeders can genetically select against these deleterious fatty acids.

The dam contributes half of her genes to the offspring during gamete formation and is therefore of significance too. However, maternal effects confound the heritable component since between and within breeds, some dams have better mothering ability than others. Therefore, the dam breed estimates a combination of both additive and maternal effects, and these were significant in the proportions of 14:1, 16:0, 18:0, SFA, MUFA and desaturation enzyme indices (Table 4). It is difficult to accurately separate the additive from maternal effects in this experimental design of single crossing. A crude option is to compute the difference between sire and dam breeds, since the sire would account for the additive component only, whereas the dam would account for a combination of both additive and maternal components. A backcross experimental design would ensure complete segregation of the genes and hence a better separation of both components.

In conclusion, the hypothesis tested should be accepted because significant breed and sex differences were observed. Also, significant heterosis effect was observed which could be used in selection programmes to reduce the proportions of total saturated fatty acids and increase total unsaturated fatty acids in the crossbred animals. These changes alter the physical and chemical characteristics of the fat and its physiological health effects in humans.

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Table 1. Tests of significance for triacylglycerol fatty acids in the adipose tissue of Jersey, Limousin and F_1 young calves

Fatty acid	Sex	Breed	Sex × breed
14 : 0	0.91	0.17	0.94
14 : 1	0.33	0.07	0.69
16 : 0	0.16	0.01**	0.66
16 : 1	0.01**	0.08	0.97
17 : 0	0.59	0.80	0.19
17 : 1	0.30	0.01**	0.12
18 : 0	0.01**	0.11	0.70
18 : 1n-9	0.10	0.71	0.68
18 : 1n-7	0.06	0.17	0.82
18 : 2	0.71	0.93	0.76
18 : 3n-3	0.71	0.85	0.12
18 : 3n-6	0.23	0.32	0.40
SFA	0.01**	0.01**	0.87
MUFA	0.01**	0.02*	0.58
PUFA	0.82	0.76	0.45
USR	0.01**	0.01**	0.71
PSR	0.99	0.56	0.51
Desat. (C16)	0.01**	0.02*	0.97
Desat. (C18)	0.01**	0.05*	0.69
Elongation index	0.23	0.02*	0.74

* $p < 0.05$; ** $p < 0.01$

SFA, total saturated fatty acids; MUFA, total mono-unsaturated fatty acids; PUFA, total polyunsaturated fatty acids; USR, unsaturated : saturated fatty acid ratio; PSR, polyunsaturated : saturated fatty acid ratio

Desat. (C16) = index of desaturation in C16 fatty acids = 100 [(16 : 1)/(16 : 0 + 16 : 1)]

Desat. (C18) = index of desaturation in C18 fatty acids = 100 [(18 : 1n-9)/(18 : 0 + 18 : 1n-9)]

Elongation index = index of elongation in the chain lengthening of C16 to C18 fatty acids = 100[(18 : 0 + 18 : 1n-9)/(16 : 0 + 16 : 1 + 18 : 0 + 18 : 1n-9)]

Table 2. Sex differences in triacylglycerol fatty acids (LSM ± SE) in young calves (% total fatty acids)

Fatty acid	Heifer calves (n = 34)	Steer calves (n = 33)	Significance
16 : 1	6.9 ± 0.4	5.0 ± 0.4	**
18 : 0	13.0 ± 0.7	15.9 ± 0.7	**
SFA	51.3 ± 1.0	55.0 ± 1.0	**
MUFA	46.1 ± 0.9	41.9 ± 0.9	**
USR	0.98 ± 0.04	0.85 ± 0.04	**
Desat. (C16)	17.7 ± 1.0	13.0 ± 1.0	**
Desat. (C18)	72.8 ± 1.0	67.6 ± 1.1	**
Elongation index	54.7 ± 0.8	56.2 ± 0.8	NS

* $p < 0.05$; ** $p < 0.01$

Table 3. Breed differences in triacylglycerol fatty acids (LSM ±ΣE) in young calves (% total fatty acids)[A]

Fatty acid	Jersey (n = 17)	Limousin (n = 17)	F$_1$J × L (n = 33)
16:0	32.8 ± 1.0^a	37.1 ± 1.0^b	31.2 ± 0.7^a
16:1	7.1 ± 0.6^a	5.3 ± 0.6^b	5.9 ± 0.4^b
18:0	12.3 ± 1.0^a	14.5 ± 1.0^b	14.6 ± 0.6^b
18:1n-9	34.5 ± 1.0^a	33.5 ± 0.9^a	33.5 ± 0.6^a
SFA	50.3 ± 1.4^a	58.8 ± 1.3^b	51.9 ± 0.9^a
MUFA	46.9 ± 1.2^a	41.7 ± 1.2^b	44.0 ± 0.8^a
Desat. (C16)	17.7 ± 1.4^a	12.3 ± 1.3^b	15.7 ± 0.9^a
Desat. (C18)	74.1 ± 1.5^a	69.8 ± 1.5^b	69.9 ± 1.0^b
Elongation index	54.0 ± 1.2^a	53.1 ± 1.1^a	56.4 ± 0.8^b
USR	1.0 ± 0.05^a	0.8 ± 0.05^b	0.9 ± 0.03^a

[A] Row means with different superscripts significantly differ ($p < 0.05$)

Table 4. Estimates of genetic effects (additive, maternal and dominance) in triacylglycerol fatty acids expressed as deviations between means (%)

Fatty acid	Sire breed[A] (Additive)	Dam breed[B] (Maternal × additive)	Heterosis[C] (Dominance)
14 : 0	−1.9*	−0.1	−0.6
14 : 1	0.0	0.8**	0.3
16 : 0	−3.3*	−1.3**	−4.3**
18 : 0	0.6	−2.7*	2.3*
18 : 1n-7	0.8*	−0.0	0.4
18 : 1n-9	0.2	0.7	−0.6
SFA	−4.4**	−4.1**	−2.7
MUFA	1.9	3.0*	−0.6
Desat. (C16)	2.4	2.9*	0.6
Desat. (C18)	−0.5	4.7*	−3.7*
Elongation index	2.1	−1.2	3.9**

* $p < 0.05$; ** $p < 0.01$
Only traits that significantly differed are tabulated
[A] (Jersey sire – Limousin sire)/(Limousin sire) × 100
[B] (Jersey dam – Limousin dam)/(Limousin dam) × 100
[C] (Crossbred – Parental)/(Parental) × 100