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Conjugated linoleic and *trans*-vaccenic acids in grass-fed beef and lamb

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ABSTRACT

Cis-9 *trans*-11 conjugated linoleic acid (CLA) found in ruminant milk and meat has several human health benefits of which the anti-cancer effect is the most prominent. *Trans*-vaccenic acid (TVA) is a precursor of CLA and can be considered as another dietary source of CLA. This paper uses data from three published papers to compare the CLA and TVA concentrations in the *longissimus lumborum* muscle (LL) from cattle and lambs, to determine the within-group variation, and to identify factors influencing this variation. The paper discusses the contribution lean beef and lamb can make to the estimated human daily required intake (DRI) for CLA. The CLA and TVA content was measured in the LL from two groups of cattle (n = 15 and n = 100) and two groups of lambs (n = 30 and n = 12). CLA and TVA concentrations in the total fatty acids (TFA) was higher ($P<0.001$) in lamb than in beef. The coefficients of variation within each group of animals ranged from 23-41% for CLA and 25-68% for TVA, indicating a large variation in the concentration of these fatty acids among animals. A number of factors contributed to these differences but the largest difference was found in lambs, where there was a 41% higher ($P<0.01$) CLA concentration in the TFA from lambs born to ewes with high CLA concentrations than from lambs born to ewes with low CLA concentrations in their milk fat. A 100g portion of lamb containing 2-4% lipid could contribute 2-5% of the estimated 750mg DRI of CLA for humans. However, if dietary TVA was assumed to be equivalent to CLA, then lamb could contribute 13-15% of the DRI. We conclude that lamb can provide a significant proportion of the DRI for CLA for humans and there is scope for farmers to further increasing the CLA and TVA content of beef and lamb.

Keywords: conjugated linoleic acid; *trans*-vaccenic acid; beef; lamb.

INTRODUCTION

The c*is*-9, *trans*-11 isomer of conjugated linoleic acid (CLA), found in milk and meat from ruminants, has several human health benefits with its anti-cancer effects being the most prominent (Kritchevsky, 2000). Most of the CLA in ruminant tissue arises from desaturation of *trans*-vaccenic acid (TVA) that escapes the rumen where it forms during biohydrogenation of linoleic and linolenic acids (Kay *et al*., 2002). Dietary TVA is a precursor of CLA in humans (Turpeinen *et al*., 2002) and can be considered an additional dietary source of CLA.

In order for New Zealand beef and lamb to be promoted in New Zealand for its CLA content, a 100g portion of raw lean meat would need to contain >20% of the daily required intake (DRI) of CLA (Beef and Lamb Marketing Bureau pers. com.). Unfortunately the DRI for CLA to reduce cancer in humans is unknown. Estimated DRI for CLA to reduce the incidence of cancer was 3 g/d (Ip *et al*., 1994). This estimate was based on a daily diet containing 0.1% of a mixture of CLA isomers reducing the number of mammary tumours in rats by 36%. However, this mixture of CLA isomers contained only 43% of the *c*-9 *t*-11 CLA isomer, which is reported to be the active anti-carcinogenic isomer (LavillonniËre & Bougnoux, 1999), and a daily intake of 0.05% of the CLA mixture reduced the number of tumours by 22% (Ip *et al*., 1994). Based on these data, and given the aggressive carcinogens used to induce mammary tumours in the rats relative to the slow development of mammary tumours in humans, a DRI of 750 mg *c*-9 *t*-11 CLA seems reasonable. There would be further reductions in the number of tumours up to a daily intake of about 3750 mg CLA.

The ease with which New Zealand farmers could increase the CLA and TVA content of beef and lamb to provide >20% of the DRI in 100g lean meat depends on the size of the increase needed, the variation in the content of these fatty acids (FA) among animals, and the factors causing this variation. This paper uses data from three published papers to compare the CLA and TVA concentration in the *longissimus lumborum* muscle (LL) from cattle and lambs, to determine the within-group variation, and to identify factors influencing this variation. The paper also discusses the contribution beef and lamb makes to the estimated human DRI for CLA and the importance of TVA as a dietary source of CLA.

MATERIALS AND METHODS

Beef-A (n = 15) was from five 30-month-old Hereford-Friesian bulls, five culled Friesian dairy cows older than 4 years, and five 20-month-old Simmental-Hereford heifers that were selected in the yards of a local processing plant in July 2000. Beef-B (n = 100) was from 50 $^{3}/_{4}$-Jersey $^{1}/_{4}$ Limousin ($^{3}/_{4}$J) and 50 $^{3}/_{4}$ Limousin $^{1}/_{4}$ Jersey ($^{3}/_{4}$L) cattle born over two years (1996, n = 66 and 1997, n = 34) and sired by one of two bulls (sire A, n = 48; sire B, n = 52). Bull calves were castrated at birth. After weaning, heifer (n = 54) and steer (n = 46) calves from each breed were grazed on similar pastures at the Tokanui Research Station near Hamilton. Heifers and steers were slaughtered in randomly selected groups from August to November at 20-24 months of age. Lamb-A (n = 30) was from lambs born to 15 Romney ewes with high CLA (2.6 g/100g TFA) and 15 ewes with low CLA (1.5 g/100g TFA) concentrations in their milk fat. Seventeen of the lambs were single- and 13 were twin-born, and 18 were ram- and 12 were ewe-lambs. All ewes and lambs were grazed together at Flock House Research Station in the Manawatu

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and lambs were weaned at 16 weeks of age and slaughtered at 28 weeks of age. Lamb-B (n = 12) was from ram-lambs sired by Poll Dorset rams and born to six East Friesian and six Romney ewes. All lambs were reared as twins on their mothers until slaughter at 17 weeks of age.

Further details on the animals, management, and selection and preparation of samples can be found in Knight *et al*. (2003b) for Beef-A and Beef-B, in Knight *et al*. (2003c) for Lamb-A, and in Knight *et al*. (2003a) for Lamb-B.

Samples of the LL were taken from the carcasses after they had been chilled for 24-72 hr and vacuum-packed before being stored at -20ºC until analysis. The preparation, extraction, saponification, methylation, and analyses of FA in the lean LL were described by Knight *et al*. (2003b). *Cis*-9, *trans*-11 CLA and TVA were the only FA reported in this paper. Lipid content in the meat was measured gravimetrically by evaporating to dryness a sub-sample of the crude chloroform-methanol (2:1; v:v) extract of the meat.

Statistical analyses

All data were analysed by analysis of variance (GenStat, 2000). Details of the analyses for each group are presented in the respective publications. Coefficients of variation (CV) were adjusted for TFA concentration in the LL by calculating residual values for CLA and TVA generated from linear regressions of CLA and TVA with TFA.

TABLE 1: Mean lipid and TFA concentrations in the LL (g/100g), CLA and TVA concentrations in TFA (g/100g), adjusted coefficient of variation (CV), and the correlation between CLA and TVA in the four groups. Comparisons within a row with different superscripts are significantly different at P<0.05.

	Beef-A	Beef-B	Lamb-A	Lamb-B
Number of animals	15	100	30	12
Lipid (g/100g raw LL)	2.1^{b}	3.6^{a}	2.9^{b}	2.6^{b}
TFA (g/100g raw LL)	1.6^{b}	3.2^{a}	2.3^{b}	1.8^{b}
CLA (g/100g TFA)	0.18^{d}	0.34^{c}	0.69^{b}	1.92^{a}
TVA (g/100g TFA)	1.24^{c}	1.43^{c}	3.54^{b}	4.66^{a}
CV for CLA (%)	42	27	41	23
CV for TVA (%)	68	25	27	29
Correlation CLA vs. TVA	0.87***	0.54***	0.48**	0.91***

RESULTS

Comparisons among experiments

There were significant (P<0.01) differences among groups in the concentration of lipid and TFA in the LL, and of CLA and TVA in the TFA (Table 1). Beef-B had higher (P<0.05) lipid concentrations than meat from the other groups. CLA and TVA concentrations were both higher (P<0.05) in Lamb-B than in Lamb-A, and lamb from both groups had at least a 2-fold higher (P<0.05) concentration of these FA in the TFA than the beef. There were significant (P<0.01) correlations between CLA and TVA concentrations in all groups.

Factors influencing within-experiment variation

The CVs, adjusted for TFA concentration in the LL, were high for CLA and TVA in all groups and indicated large variation in the concentrations of these FA among animals (Table 1). Table 2 presents some of the factors that contribute to this variation. CLA concentration was higher in $^{3}/_{4}$J (P<0.001) and progeny born to sire A (P<0.05) than in $^{3}/_{4}$L and progeny born to sire B. TVA concentration was higher for steers (P<0.05), for progeny born to sire A (P<0.05), and for progeny born in 1996 (P<0.001) than for heifers, for progeny born to sire B, and for progeny born in 1997. However, the differences were small. In contrast, in Lamb-A, single-born and lambs born to ewes with high CLA concentrations in their milk fat had 38-41% higher (P<0.01) concentrations of CLA in their TFA than twin-born lambs and lambs born to ewes with low CLA concentrations in their milk fat.

Contribution to daily required intake

Table 3 presents the mean content of CLA and TVA in a 100g portion of LL after removal of all external fat, and the proportion of the DRI of 750 mg CLA that a 100g portion of meat would provide. There were no groups that could provide more than 5% of the DRI for CLA from 100g of meat when considering CLA content alone, and no individual animal provided more than 8% of the DRI. However, the content of TVA was 2-7 times higher than the content of CLA in 100g of meat. The combined dietary intake of CLA and TVA from 100g beef would still provide <10% of the DRI even if it was assumed that the dietary TVA was equivalent to CLA. In contrast, a 100g of lamb would provide 13-15% of the DRI from CLA and TVA. All groups now had individual animals that provided >20% of DRI.

TABLE 2: Factors contributing to the within-group variation in CLA and TVA concentrations are presented. Values in parenthesis are the percentage increase of the larger from the smaller value. n.s. indicates the factor was not significant whereas – indicates the factor was not evaluated.

		Beef-A	Beef-B	Lamb-A	Lamb-B
Gender	CLA	n.s.	n.s.	n.s.	-
	TVA	n.s.	1.5 vs. 1.3 (15%)	n.s.	-
Breed	CLA	-	0.38 vs. 0.31 (23%)	-	n.s.
	TVA	-	n.s.	-	n.s.
Sire	CLA	-	0.36 vs. 0.32 (13%)	-	-
	TVA	-	1.5 vs. 1.3 (15%)	-	-
Year-born	CLA	-	n.s.	-	-
	TVA	-	1.5 vs. 1.3 (15%)	-	-
Birth rank	CLA	-	-	0.80 vs. 0.58 (38%)	-
	TVA	-	-	n.s.	-
Milk CLA	CLA	-	-	0.81 vs. 0.57 (41%)	-
	TVA	-	-	n.s.	-

TABLE 3: Mean content (mg/100g raw LL) of CLA and TVA and percentage of daily required intake (DRI) supplied by 100g of LL from beef and lamb. The parentheses contain the values for percentage of DRI when individual animals with maximum content of CLA or CLA + TVA are used in the calculations.

	Beef-A	Beef-B	Lamb-A	Lamb-B
Mean CLA (mg/100g LL)	4	11	16	35
Mean TVA (mg/100g LL)	29	46	81	85
% of DRI from:				
CLA in meat	<1 (4)	2 (5)	2 (6)	5 (8)
CLA + TVA in meat	4 (34)	8 (27)	13 (28)	15 (31)

DISCUSSION

The CLA and TVA concentrations in the TFA and their contents in 100g of lean meat were higher for lamb than for beef although there were large variations among animals. This large variation among animals suggests there is scope for farmers to increase the content of these FA in beef and lamb if factors influencing their concentrations could be identified. Factors such as breed, sire, gender, and year of birth had some effects on CLA and TVA concentrations (Table 2). Two factors in lambs that contributed to large increases in CLA concentrations were being born and reared as a single lamb as compared to a twin, and the CLA concentrations in the ewe's milk fat. A possible explanation for these differences was that CLA from milk consumed by the pre-ruminant lamb was absorbed and accumulated in its fat depots. Potentially, higher CLA in the ewe's milk would increase the accumulation of CLA in its lambs, and single-born lambs were likely to consume more milk than twin-born lambs and, thus, accumulate more CLA.

A 100g portion of raw lean (2-4% lipid) lamb or beef supplied <10% of the DRI even when animals with the highest CLA content were considered. The CLA content would need to increase 10-20 fold in beef and 4-10 fold in lamb in order for 100g of meat to supply >20% of the DRI for CLA. This could be difficult for farmers to achieve. However, the TVA content in the LL was 2-7 times higher than CLA and was positively correlated with CLA, indicating that increases in CLA content would be accompanied by increases in TVA. Assuming the absorption of dietary TVA in humans was as effective as CLA and there was 100% conversion to CLA, then 100g of lamb would on average contain >10% of the DRI for CLA. The CLA plus TVA content would now only have to increase 25-35% in lamb and 3-5 fold in beef in order for 100g meat to supply >20% of the DRI for CLA. This magnitude of increase in these FA could possibly be achieved by farmers, especially for lamb.

There are limited data, especially for humans, on the comparative absorption of dietary CLA and TVA, and the proportion of the TVA that is converted to CLA. Dietary TVA increases serum CLA concentrations in humans (Turpeinen *et al*., 2002). Santora *et al*. (2000) fed mice on diets containing 1% of either CLA or TVA. After 2 weeks, 9.5% of the dietary intake of CLA had accumulated in the bodies of the mice fed CLA and 11.4% of the dietary intake of TVA had accumulated as CLA in the mice fed TVA. This suggests dietary TVA may be equivalent to CLA as a source of CLA but more research is required. There may also be a direct anti-carcinogenic affect for TVA since the growth of human cancer cells in tissue culture was reduced by TVA (Awad *et al*., 1995). However, the conversion of TVA to CLA could not be discounted.

Measurements of CLA and TVA content were made on the LL with all external fat removed. Some external fat is usually left on the meat during cooking and could increase CLA and TVA intake. To put this into perspective, the 1.9% of CLA in the TFA in Lamb-B meat was at the upper range of the 1.2-2.2% of CLA found in milk fat from New Zealand dairy cows (Kay *et al*., 2002). Therefore, an extra 2-3g of lamb fat consumed with a lamb chop could provide a similar increase in CLA intake as eating 2-3g of butter or cheese.

Providing there is a high absorption and bioconversion of TVA to CLA, farmer should be able to increase the content of these FA in beef, and especially lamb, to provide >20% of the estimated DRI of CLA needed to reduce the incidence of cancer in humans. Selecting ewes with high CLA concentrations in their milk fat or manipulating the diet of lactating ewes to increase the CLA in milk fat may result in further increases in the CLA and TVA in the meat from their lambs.

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