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Conservation of conjugated linoleic, *trans*-vaccenic and long chain omega-3 fatty acid content in raw and cooked lamb from two cross-breeds

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Abstract Available literature mainly describes the fatty acid (FA) content of raw meat, with only limited data explaining the effect of different preparation and cooking temperatures on health-promoting FA such as conjugated linoleic acid (CLA), its metabolic precursor *trans*-vaccenic acid (TVA), and long chain omega-3 FA (LC omega-3 FA). We collected *m. longissimus thoracis* (LT) from rib chops of Poll Dorset × East Friesian (EF) and Poll Dorset × Romney (R) lambs raised together. The FA composition of raw LT was compared with LT prepared with bone and all fat trimmed away or bone and fat left intact before cooking under a fan-grill. The LT from EF lambs had less cooking loss, lower lipid and total fatty acid (TFA) contents, and a lower proportion of saturated fatty acids (SATFA) than the LT from R lambs. Raising cooking temperature from medium to well-done increased cooking losses and dry matter (DM) yield but did not affect FA content or composition. Compared with raw LT, cooking of the trimmed LT resulted in more TFA and lipids in the DM, and these were greater still in the intact LT. Similar increases occurred in the DM content of most individual and groups of FA, except for CLA and LC omega-3 FA which increased with cooking of trimmed LT without further significant increase with cooking intact LT. The proportions of SATFA and TVA in the TFA increased after cooking trimmed LT and increased further after cooking intact LT. In contrast, proportion of polyunsaturated fatty acid (PUFA) was reduced at each step, while the proportion of CLA was not affected by cooking. Thus, the FA content of raw lamb underestimates that of cooked meat, especially if the lamb is cooked with bone and fat left intact allowing infiltration of FA from the surrounding intermuscular and subcutaneous fat. This influx of lipids from the surrounding adipose tissue dilutes the proportions of PUFA and LC omega-3 FA in the TFA.

Abbreviations East Friesian (EF); Romney (R); *m. longissimus thoracis* (LT); *trans*-vaccenic acid (TVA); conjugated linoleic acid (CLA); long chain omega-3 fatty acids (LC omega-3 FA); fatty acids (FA); total fatty acids (TFA); saturated fatty acids (SATFA); mono-unsaturated fatty acids (MUFA); polyunsaturated fatty acids (PUFA).

Keywords lamb; breeds; cooking methods; conjugated linoleic acid; omega-3 fatty acids

INTRODUCTION

Lean beef and lamb are wholesome foods which provide a variety of caloric and essential fatty acids (FA). Among the beneficial, health promoting FA are conjugated linoleic acid (CLA), especially the *cis*-9, *trans*-11 isomer, *trans*-vaccenic acid (TVA), and the long chain omega-3 polyunsaturated FA (LC omega-3 FA). CLA reduces the severity of cancer in a number of animal models exposed to a range of acute carcinogenic stimulants (Belury 1995; Kritchevsky 2000). TVA, the major precursor of CLA, is found mainly in meat and milk of ruminants (Corl et al. 2001) and dietary TVA is known to be

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converted to CLA *in situ* in mice (Santora et al. 2000) and humans (Salminen et al. 1998). The LC omega-3 FA include eicosapentaenoic (EPA; C20:5), docosapentaenoic (DPA; C22:5), and docosahexaenoic (DHA; C22:6) acids, which can reduce the potential for coronary heart disease, cancer, and arthritis (Simopoulos 1996). Less beneficial FA include the saturated FA (SATFA), especially the intermediate chain length lauric, myristic, and palmitic acids that can promote the development of atherosclerosis (Ulbricht & Southgate 1991).

Although the literature and food composition tables are rich with detail on the FA composition of raw beef and lamb, there is less information on cooked meat (Badiani et al. 2002) and very limited data describing effects of cooking on the content of CLA, TVA, and LC omega-3 FA. These unsaturated FA are sensitive to heat, which may cause isomerisation leading to net synthesis or degradation. In the original discovery of CLA in cooked meat it was thought that CLA arose as an "isomeric heat-generated derivative of linoleic acid" (Ha et al. 1987). Shantha et al. (1994) measured CLA content in raw and cooked beef. With cooking they observed 5–25% more total CLA including the *cis*-9, *trans*-11 isomer, but those increases might not be due to conversion of precursors because the cooking affected the total fat content and yield of edible portion of meat. Similarly, West & Chrystall (1989) reported a 16% increase in the recovery of polyunsaturated FA (PUFA) from roasted cuts of beef, lamb, and mutton, which they attributed to the "infiltration of fat from adjacent fatty tissue".

The aim of this study was to assess changes in the beneficial FA content of lamb under different treatments based on preparation and cooking temperature. Uniform rib chops (*m. longissimus thoracis*, LT) from Poll Dorset × East Friesian (EF) and Poll Dorset × Romney (R) lambs received one of two traditional pre-cooking preparations ("trimmed" having bone and fat removed versus "intact" with bone and fat left on) prior to cooking under a consumer fan-grill to one of two endpoint temperatures (medium at 70–75°C versus well-done at 78–84°C).

MATERIALS AND METHODS

Lambs

Muscle samples were collected from lambs raised and slaughtered under conditions typical of New Zealand grazed livestock. Twelve ram lambs, born to six EF and six R ewes and sired by Poll Dorset rams, were slaughtered on the same day at a mean age of 121 ± 2 (SD) days. All lambs were reared as twins on their mothers until slaughter and for the duration of lactation the ewes grazed together on pasture. Carcasses were weighed immediately after slaughter, hung chilled at 4°C for 24 h, and then the rib rack from the 8th to 13th ribs was removed from both sides of each carcass and vacuum packed before freezing at –20°C.

Treatments

The frozen rib racks were cut with a bandsaw into rib chops of similar thickness (approximately 20–25 mm). The 9th, 10th, and 11th rib chops on each side of the 12 carcasses were randomly allocated to one of five treatment groups, with one additional chop used in determining cooking times. The five treatments were (n = 12): raw (uncooked lean LT trimmed free of bone and all external fat); trimmed-medium (the LT without bone or external fat cooked to an endpoint temperature of 70–75°C); trimmed-well-done (78–84°C); intact-medium (the rib chop with its bone and external fat left on then cooked to an endpoint temperature of 70–75°C); or intact-well-done (78–84°C).

The chops to be cooked were thawed overnight at 4°C, prepared with respect to their treatment, and then cooked at 200°C under a fan-grill (Simpson La Scala 3W601W) at 5 cm from the heat source. Cooking times were 8, 10, 12, and 14 min for trimmed-medium, intact-medium, trimmed-well-done, and intact-well-done, respectively. Samples were turned once half way through their cooking time and their final internal temperature was measured by digital thermometer (Comark Foodcheck KM22). Finished samples were allowed to cool at room temperature, blotted dry with paper towels, wrapped individually in aluminium foil, and then vacuum-packed for freezing and storage at –20°C until required for analyses 4–5 days later. All samples were weighed before and after cooking and the difference used to calculate percent cooking loss, however the drip loss material could not be collected.

Sample preparation and analyses

Bone and all subcutaneous and intermuscular fat were removed from the raw and trimmed LT before cooking and from the intact LT after cooking. The LT samples were then weighed, diced, freeze-dried, and ground. Sample weights taken before and after freeze drying were used to calculate proportion of dry matter (DM yield) in the LT. Lipids were extracted from the DM by a modified Folch method (Folch et al. 1957) with part of the extract used to determine total lipid content of the DM gravimetrically and the rest used for the GLC analysis.

Saponification, methylation, and GLC separation of FA in the extracts were by the method described by Knight et al. (2003) with tridecanoic acid (C13:0) used as the internal standard. Total fatty acid (TFA) content of the DM was calculated as the sum of known peaks measured against 39 quantifiable standards, plus all unknown peaks. Chromatograms showed baseline separation for at least 90% of peaks recorded. Positions and quantity (7.4 ± 0.8 (SD) %) of unknowns were not substantially different between treatments. Data are presented as FA content (mg FA g^{-1} DM) and as FA proportion (g FA 100 g^{-1} TFA). The SATFA, mono-unsaturated (MUFA), and PUFA groupings were the same as those described by Knight et al. (2003). SATFA include odd- and even-chain FA, MUFA include *cis* and *trans* FA, PUFA include n-6 and n-3 FA. The CLA, TVA, and LC omega-3 FA are included in their appropriate groups in addition to being presented as individual FA. The predominant (>90 %) CLA isomer in these samples was *cis*-9, *trans*-11 CLA and is the only isomer reported here. The equivalent raw meat values for percent DM and lipid content were calculated by the following equations:

$$\text{Equiv. raw meat \% DM} = \text{DM proportion for cooked LT} \times (100 - \text{\% cooking loss})$$

$$\text{Equiv. raw meat \% lipid} = \text{Equiv. raw meat DM proportion} \times \text{lipid content in g } 100\ \text{g}^{-1}\ \text{DM}$$

Table 1 Mean values and significance of contrasts for the effects of pre-cooking preparation and cooking treatments on *m. longissimus thoracis* (LT) muscle characteristics and the content and proportion of individual and groups of fatty acid (FA) in dry matter (DM) and total fatty acid (TFA). The mono-unsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) groups of FA include *trans*-vaccenic acid (TVA), and conjugated linoleic acid (CLA) and long chain (LC) omega-3 FA, respectively. The contrasts are between raw and trimmed-cooked LT and between trimmed-cooked and intact-cooked LT. As cooking temperature did not affect the lipid or TFA content of the DM or the content and proportions of individual and groups of FA in the DM, values here are averaged over both medium and well-done treatments. SATFA, saturated fatty acids; NS, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

	Preparation				Contrasts	
		Cooked				
	Raw	Trimmed	Intact	Pooled SEM	Raw versus trimmed	Trimmed versus intact
Number of samples (*n*)	12	24	24			
Cooking loss (%)	–	48	37	0.5	–	***
DM yield (%)	25	46	40	0.4	***	***
Equiv. raw DM (%)	25	25	26	0.2	NS	*
Lipid (mg g^{-1} DM)	106	114	126	2.5	**	***
Equiv. raw lipid (%)	2.6	2.8	3.2	0.07	*	***
TFA (mg g^{-1} DM)	72	79	89	1.9	**	***
Fatty acid content (mg FA g^{-1} DM in raw and cooked LT)						
TVA	3.4	3.9	4.5	0.1	**	***
CLA	1.4	1.6	1.7	0.06	**	NS
LC omega-3 FA	2.1	2.3	2.2	0.03	*	NS
SATFA	31	35	40	1.0	**	***
MUFA	27	29	33	0.7	**	***
PUFA	8.5	9.2	9.4	0.1	***	***
Fatty acid proportion (g FA 100 g^{-1} TFA in raw and cooked LT)						
TVA	4.7	4.8	5.0	0.06	(0.07)	*
CLA	1.9	2.0	2.0	0.04	NS	NS
LC omega-3 FA	3.1	2.9	2.6	0.09	NS	***
SATFA	43	44	45	0.2	*	*
MUFA	37	37	37	0.1	NS	NS
PUFA	12.2	11.7	10.9	0.2	(0.07)	**

Statistical analyses

Data were subjected to split-plot analysis of variance (GenStat 2000). Differences between the two breeds were assessed at the whole lamb level, there being six lambs per breed. The five cooking treatments, however, were compared at the sub-plot level, each treatment having been randomly assigned to a rib chop from each lamb. The following contrasts were made among the five treatment groups: raw versus trimmed-cooked; trimmed-cooked versus intact-cooked; medium versus well-done. Breed effects on lamb carcass weight was analysed by ANOVA. Means are presented ± pooled SEM.

RESULTS

There were no interactions between cooking treatment and breed of sheep for any of the variables measured; therefore results for the effects of treatment and breed are presented in separate tables.

Cooking effects

Endpoint temperature

The mean endpoint temperatures measured were 73.4, 81.6, 72.6, and 80.9°C for trimmed-medium, trimmed-well-done, intact-medium, and intact-well-done, respectively. Raising the temperature from medium to well-done increased ($P < 0.001$) percent cooking loss from 39% to 45 ± 0.5% and the resulting yield of DM after freeze drying the cooked sample from 40% to 46 ± 0.4%, and decreased ($P < 0.001$) calculated equivalent raw meat DM from 25% to 24 ± 0.2%. As there were no effects of raising endpoint temperature on the lipid or TFA content of the DM or on the content and proportions of individual and groups of FA in the DM, the results presented in Table 1 have been simplified by averaging cooked treatments across temperatures.

Trimming and cooking treatments

Leaving the LT with fat and bone intact before cooking resulted in less cooking loss, less DM yield, and

Table 2 Mean values and significance for the effect of breed (East Friesian versus Romney) for meat composition. The values here are averaged over all raw and cooked samples within a breed. The MUFA and PUFA groups of FA include TVA and, CLA and LC omega-3, respectively. NS, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Other abbreviations as for Table 1.

	Breed			
	East Friesian	Romney	SEM	Sig. diff.
Number of lambs (n)	6	6		
Cooking loss (%)	40	44	1.0	*
DM yield (%)	39	41	0.4	*
Equiv. raw DM (%)	25	25	0.2	NS
Lipid (mg g^{-1} DM)	107	127	5.5	*
Equiv. raw lipid (%)	2.7	3.2	0.2	(0.05)
TFA (mg g^{-1} DM)	73	90	4.7	*
Fatty acid content (mg FA g^{-1} DM in raw and cooked LT)				
TVA	3.3	4.8	0.7	NS
CLA	1.4	1.8	0.2	NS
LC omega-3 FA	2.2	2.2	0.1	NS
SATFA	31	41	2.0	**
MUFA	27	33	2.4	NS
PUFA	8.7	9.4	0.3	NS
Fatty acid proportion (g FA 100 g^{-1} TFA in raw and cooked LT)				
TVA	4.5	5.1	0.6	NS
CLA	1.9	2.0	0.2	NS
LC omega-3 FA	3.1	2.6	0.2	NS
SATFA	43	46	0.4	***
MUFA	37	36	0.1	NS
PUFA	12.2	10.7	0.6	NS

slightly more calculated equivalent raw meat DM than when the LT were trimmed of fat and bone before cooking (Table 1). Compared to raw LT, many variables were significantly increased in the trimmed-cooked LT and further increased in intact-cooked LT. These include content of lipid and TFA in the DM and equivalent raw meat lipid percent. This greater total lipid was reflected in the content of most individual and groups of FA in the DM. The exceptions were the content of CLA and LC omega-3 FA in the DM, for which there were no differences between trimmed- and intact-cooked LT.

Compared with raw LT, cooking the trimmed LT increased the proportions of the SATFA group and TVA ($P = 0.07$) in the TFA, and there were further increases when cooking the intact LT. In contrast there was a trend ($P = 0.07$) for the proportion of PUFA to be lower in the cooked trimmed LT and significantly lower still for both the PUFA and LC omega-3 FA in the intact LT. There were no effects of either cooking treatment on the proportions of CLA or MUFA in the TFA.

Breed effects

Carcass weights of EF and R lambs were not significantly different (19 versus 18 ± 0.07 kg for EF and R, respectively), but percent cooking loss, DM yield, and the lipid and TFA content in the LT were all lower in EF than in R lambs when averaged across raw and all cooking treatments (Table 2). This lesser total fat content in EF lambs was reflected in markedly lower concentration and proportion of SATFA in the LT. There were no other significant differences in the content or proportion of FA in the LT from EF and R lambs.

DISCUSSION

Although cooking caused significant changes in the content of *cis*-9, *trans*-11 CLA, TVA, and LC omega-3 FA in the DM of the cooked LT from lambs, the changes were small. The treatment differences were likely the result of loss of non-lipid solids in addition to water from the edible portion when the trimmed LT was cooked, together with an influx of lipid from subcutaneous and intermuscular fat when the LT was cooked with bone and fat intact. This interpretation is supported by progressive increase in the content of SATFA, MUFA, and PUFA in the LT from raw to cooked trimmed LT and then to cooked intact LT. Failure to find an increase in the proportion of CLA in TFA when cooking lamb contradicts the observations of Ha et al. (1987) and Shantha et al. (1994) using beef, and does not corroborate their conclusion that cooking generates CLA from precursor FA. Although their proportions of CLA in TFA from beef were markedly lower (four–six-fold) than in the current grass-fed lamb, we have no explanation for the disparity in response to cooking.

The increase in the proportion of SATFA, no change in the proportion of MUFA, and decrease in the proportion of PUFA when trimmed lamb was cooked was similar to results for beef obtained by Duckett & Wagner (1998), although the magnitude of changes in cooked beef were greater than for lamb. In the present experiment, the decrease in PUFA (9.5%) was larger than the increase in SATFA (2.3%). This supports their suggestion that cooking trimmed meat causes greater losses among the more water-soluble phospholipids found in membranes and comprising PUFA than among the (primarily triglyceride) neutral lipids comprising SATFA.

The increase in neutral lipid associated SATFA when bone and fat are left intact before cooking has been attributed to an influx of lipid from the surrounding subcutaneous and intermuscular fat (West & Chrystall 1989; Badiani et al. 1998). In the current trial, infiltration with additional SATFA and TVA diluted the proportions of PUFA, including the LC omega-3 FA in TFA. However, this influx of lipids from the surrounding adipose tissue did not change the proportions of MUFA in TFA when intact LT was cooked. This was possibly because the proportions of MUFA, but not SATFA and PUFA, in the TFA from intramuscular fat deposits and from adipose tissue surrounding the LT were similar, as observed in milk-fed lambs (Bas & Morand-Fehr 2000). In the present experiment, the proportion of TVA in the TFA behaved like SATFA and the proportion of CLA behaved like MUFA when the trimmed and the intact LT were cooked.

In meat cooked with bone and fat removed, the percent apparent and true retentions (Murphy et al. 1975; Badiani et al. 2002) calculated for grouped and individual FA were consistently greater than 100% (data not shown), although there was no external source of FA. Both measures of retention are sensitive to the loss of meat components other than water and lipid during cooking (e.g., protein, carbohydrate, ash; Murphy et al. 1975; Badiani et al. 2002). Unfortunately, cooking drip loss could not be collected in the present experiment and so the quantity and composition of those solids could not be determined. Higher cooking temperatures increased cooking loss and DM yield, but did not increase the lipid content of the DM, suggesting that mainly water was being lost with increased cooking temperature. If there was additional loss of solid material then the rate of loss must have been similar for lipids and non-lipids to their proportions in the LT (Renk et al. 1985; Luchak et al. 1998).

Leaving bone and fat on during cooking retains moisture, reduces cooking loss, and increases lipid and TFA content of DM through greater retention of lipids (Coleman et al. 1988) and through movement of lipids into the meat from surrounding fat (Coleman et al. 1988; Jones et al. 1992). In this trial, leaving bone and fat on during cooking increased SATFA, MUFA, TVA, CLA, PUFA content, and to a lesser extent LC omega-3 FA when compared with the raw LT. Since most consumers cook lamb loins with bone and some external fat left on the chops, the content of these FA measured in raw lean meat will underestimate their intake even if consumers remove all the external fat after cooking.

At the same age and similar carcass weights, R lambs had more intramuscular fat in the LT than did EF lambs. These deposits largely contained SATFA. Other differences in content and proportions of individual and groups of FA were observed, and these may have reached significance in a larger study with more than six lambs per cross-breed. The EF is a later maturing and larger sheep than R and appears to have a higher proportion of fat in the mesenteric fat depot rather than in subcutaneous, inter- and intramuscular depots (Purchas et al. 2002; Paul Muir pers. comm.).

CONCLUSIONS

Consumer-style cooking caused small increases in the content of health promoting FA *cis*-9, *trans*-11 CLA, TVA, and to a lesser extent LC omega-3 FA in the LT of rib chops from lambs. The results do not support earlier suggestions that cooking causes isomerisation, synthesis or degradation of CLA. The content of SATFA, MUFA, and PUFA in raw lamb underestimates their content in cooked meat, especially if the lamb is cooked with bone and fat left intact allowing infiltration of FA from the surrounding intermuscular and subcutaneous fat. This influx of lipids from the surrounding adipose tissue dilutes the proportions of PUFA and LC omega-3 FA in the TFA.

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