Fatty acid composition of beef steers as affected by diet and fat depot

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Abstract

Subcutaneous and perirenal fatty acid (FA) profiles were compared in steers fed a control diet (70: 30 red clover silage (RC) : barley concentrate), a diet with sunflower seed (SS) substituted for barley, and diets with 15% or 30% wheat dried distillers' grain with solubles (DDGS-15 and DDGS-30) substituted for RC and SS. Perirenal fat (PRF) versus subcutaneous fat (SCF) had greater proportions of total saturated FA (SFA) and branched chain FA (BCFA), and lower proportions of total and major cis-monounsaturated FA (c-MUFA). Addition of SS to the diet did not change the proportions of total and major c-MUFA and n-6 polyunsaturated FA (PUFA), but led to decreases in the proportions of total and major SFA, BCFA and n-3 PUFA. Progressive substitutions with DDGS led to no further changes in the proportions of total and major SFA and n-3 PUFA, but decreased the proportions of BCFA and c9-16:1, and increased the proportions of c9-18:1 and n-6 PUFA. Feeding SS and DDGS-15 diets yielded the largest proportions of total and major t-18:1 (t11and t13-/t14-18:1) isomers in PRF and conjugated lineolic acid (CLA) isomers (t7, c9- and t9, c11-18:2) in SCF, but responses were diminished when feeding the DDGS-30 diet. Subcutaneous fat versus PRF from steers fed SS and DGGS diets had larger proportions of non-conjugated 18:2 biohydrogenation products (i.e. atypical dienes) than the control diet. Overall, feeding SS and DDGS-15 diets raised the proportions of t11-18:1 in PRF and c9,t11-18:2 in SCF, which have potential human health benefits, but feeding DDGS-30 was less effective.

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Introduction

In the past two decades, links between intake of trans fatty acids (FA) and saturated FA with heart disease have led to recommendations to limit their consumption in foods, including beef (Mapiye *et al.*, 2015; Nantapo *et al.*, 2015; Vahmani *et al.*, 2015). Recently, growing consumer interest in healthy diets has triggered research to enrich beef with polyunsaturated fatty acids (PUFA) and biohydrogenation products (BHP), particularly rumenic acid (*cis* 9, *trans* 11-18:2) and its precursor vaccenic acid (*trans* 11-18:1; Mapiye *et al.*, 2012; 2015) which have potential human health benefits (Field *et al.*, 2009; Dilzer *et al.*, 2012). The proportions of these BHP in beef can be increased by feeding forages in combination with high levels of PUFA (Mapiye *et al.*, 2012; 2015; Vahmani *et al.*, 2015). However, feeding PUFA in high-forage diets results in lower growth rates, smaller carcasses and an undesirable meat appearance compared with high concentrate diets (Webb, 2006; Mapiye *et al.*, 2013a). Recently, it was demonstrated that these problems could in part be alleviated by replacing forage (red clover silage (RC)) with wheat dried distillers' grains plus solubles (DDGS) as a non-forage fibre source (Mapiye *et al.*, 2014a).

The composition of PUFA-BHP in beef is influenced by fat depot, with external depots (e.g. subcutaneous fat (SCF)) and internal depots (e.g. perineal fat (PRF)) with the greatest propensity to accumulate rumenic and vaccenic acids, respectively (Jiang *et al.*, 2013; Mapiye *et al.*, 2013b; 2014b). These differences have important health implications when one examines which depot could be incorporated into meat products such as minced beef. Currently, SCF is used when making minced beef, which is considered by many to be the most popular and most versatile of all beef products (Brewer, 2012). On the other hand, PRF is an underutilized fat depot, but has a higher content of total PUFA-BHP and is more easily accessible during the slaughter process than SCF (Mapiye *et al.*, 2014b). Strategies to enhance the PUFA-

BHP composition of beef products should therefore include feeding designer diets and exploiting fat depot heterogeneity. The current study was an extension of the research by Mapiye *et al.* (2014a). The objective was to compare PUFA-BHP profiles in SCF and PRF fat from the same group of steers fed RC sunflower seed and varying levels of wheat dried distillers' grain with solubles (DDGS).

Material and Methods

Animal management and the ingredients and nutritional composition of experimental diets were previously described by Mapiye *et al.* (2014a). Briefly, sixty-four 12-month-old British × Continental crossbred steers with an initial mean bodyweight (BW) of 362.7 ± 4.50 kg were randomly allocated to four dietary treatments (control, sunflower seed (SS), DDGS-15 and DDGS-30), with two pens of eight steers per dietary treatment. The control diet was composed of 70% red clover silage, 25.8% barley grain and 4.2% vitaminmineral supplement on a dry matter (DM) basis (Table 1). The SS diet contained 11.4% SS substituted for barley grain, and the DDGS-15 and DDGS-30 diets contained 15% and 30% DDGS, respectively, substituted for red clover silage and SS to maintain a targeted 5% added oil in the diets from SS or DDGS (Table 1).

At slaughter, PRF was collected closest to the geometric centre of the whole PRF, vacuum packed and held in a 2 °C cooler with a wind speed of 0.5 m/sec pending FA analysis. Carcasses were cooled for 24 h under the same conditions prior to collecting and vacuum packing SCF covering the *longissimus thoracis et lumborum.* A 50 g subsample of each fat depot was homogenized using a Robot Coupe Blixir BX3 food processor (Robot Coupe USA Inc., Ridgeland, Miss, USA) and frozen at -80 °C for subsequent FA analysis.

For FA analysis, 50 mg fat was freeze dried and directly methylated with 0.5 M sodium methoxide (Aldai *et al.*, 2009). Fatty acid methyl esters were analysed by gas chromatography (GC) using a CP-Sil88 column (100 m, 25 µm ID, 0.2 µm film thickness) in a CP-3800 gas chromatograph equipped with an 8600-series autosampler (Varian Inc., Walnut Creek, Calif, USA). Two GC analyses were conducted per sample using complementary temperature programmes with 150 °C and 175 °C plateaus according to Kramer *et al.* (2008). Conjugated linoleic acid (CLA) isomers not separated by GC were further analysed using Ag⁺-HPLC as described by Cruz-Hernandez *et al.* (2004). Individual peaks were identified with reference standards (GLC-603, Nu-Chek Prep. Inc., Elysian, Minn, USA; BC-Mix1, Applied Science, State College, Pa, USA) and peak orders and retention times reported in the literature (Cruz-Hernandez *et al.*, 2004; Kramer *et al.*, 2008; Gómez-Cortés *et al.*, 2009).

Statistical analyses were conducted using Proc Mixed of statistical analytical systems (SAS, 2009). All the data were analysed as a two-way ANOVA, including fixed effects of diet, fat depot and diet × fat depot interaction, and slaughter day and animal (diet) as random effects. Treatment means were generated and separated using the LSMEANS and PDIFF options, respectively. The significance threshold was set at P <0.05.

Results and Discussion

Fatty acids of dietary or endogenous origin

Diet and its interaction with fat depot did not (P > 0.05) affect total FA content (Table 2). Overall, the proportions of total saturated FA (SFA), 14:0 and 16:0 decreased (P < 0.05) with SS addition, but further substitutions with DDGS led to no further changes (P > 0.05; Table 2). Conversely, the proportions of 18:0 increased (P < 0.05) with the addition of SS to the control diet, but further substitutions with DDGS led to no (P > 0.05) further changes. These results resemble patterns of dietary proportions of the individual SFA observed in the current study. Current results may also be partly linked to the influences of both rates of complete biohydrogenation of PUFA to 18:0 and high proportions of 18:2*n*-6, which were previously reported by Shingfield *et al.* (2013) to down-regulate Δ -9 desaturase activity in adipose tissues.

The proportions of total *cis*-monounsaturated FA (c-MUFA) were not (P > 0.05) influenced by diet. The proportions of *c*9-14:1 tended to decline (P = 0.06) with the addition of SS to the control diet. The inclusion of DDGS led to further reductions (P = 0.06). Shingfield *et al.* (2013) reported similar results and attributed them to the inhibitory effects of high dietary levels of 18:2*n*-6 on *de novo* synthesis of FA, including conversion of 14:0 to *c*9-14:1. Adding SS to the control diet did not (P > 0.05) change the proportions of *c*9-16:1 (Table 2), but further substitutions with DDGS did reduce (P < 0.05) its proportions. The results could be linked partly to the high proportions of 18:2*n*-6 in adipose tissues that were observed when feeding DDGS diets (Table 2).

According to Nakamura *et al.* (2004), high levels of 18:2*n*-6 can inhibit Δ -9 desaturase activity, consequently reducing *de novo* synthesis of *c*9-16:1 from 16:0. Feeding SS diet had no (*P* >0.05) effect on the proportions of *c*9-18:1, but further substitutions with DDGS increased (*P* <0.05) its proportions (Table 2). In line with current findings, Cruz-Hernandez *et al.* (2007) demonstrated that increasing 18:2*n*-6 in the diet is capable of inhibiting biohydrogenation in the rumen, leading to the accumulation of intermediate products in

adipose tissues. Overall, variations in *c*-MUFA are difficult to interpret, because *c*-MUFA can originate from both diet and endogenous synthesis.

	Dietary treatments				
Variable	Control	SS	DDGS-15	DDGS-30	SD
Ingredients (% DM basis)					
Sunflower-seed	0.0	11.4	9.2	7.0	N/A
Dried distiller' grains with solubles	0.0	0.0	15.0	30.0	N/A
Barley grain	25.8	14.4	14.4	14.4	N/A
Red clover	70.0	70.0	57.2	44.4	N/A
Vitamin/mineral supplement ¹	4.2	4.2	4.2	4.2	N/A
Nutrient composition (g/kg DM)					
Dry matter	426	402	442	501	42
Crude protein	131	134	165	208	36
Crude fat	18.9	64.0	58.0	59.0	20.9
Calcium	8.6	9.2	8.1	6.9	1.0
Phosphorus	3.1	3.2	4.1	5.3	1
Acid detergent fibre	337	370	338	284	36
Neutral detergent fibre	433	487	445	385	42
Digestible energy (MJ/kg)	11.3	10.8	11.4	12.2	0.59
Fatty acids (% of total fatty acids)					
14:0	0.35	0.17	0.15	0.15	0.10
16:0	18.8	10.6	11.9	13.5	3.60
18:0	2.86	4.15	3.67	3.24	0.56
20:0	1.11	0.65	0.49	0.41	0.31
22:0	1.29	1.11	0.85	0.71	0.26
24:0	1.25	0.70	0.52	0.44	0.36
<i>c</i> 9-18:1	9.49	12.4	13.0	13.3	1.75
c11-18:1	0.93	0.73	0.76	0.78	0.09
18:3 <i>n</i> -3	18.9	7.09	6.26	5.59	6.32
18:2 <i>n</i> -6	39.0	59.6	60.3	60.1	10.5

Table 1 Ingredients, nutrients and fatty acid composition of the dietary treatments

SS: sunflower seed; DDGS-15: 15% wheat dried distillers' grain with solubles + sunflower seed; DDGS-30: 30% wheat dried distillers' grain with solubles + sunflower seed; SD: standard deviation; N/A: not applicable. ¹ Vitamin/mineral supplement per kg DM contained 18.6 g calcium; 9.3 g phosphorus; 5.6 g potassium; 2.1 g sulphur; 3.3 g magnesium; 9.2 g sodium; 265 mg iron; 314 mg manganese; 156 mg copper; 517 mg zinc; 10.05 mg iodine; 5.04 mg cobalt; 2.98 mg selenium; 49722 IU vitamin A; 9944 IU vitamin D₃; 3222 IU vitamin E.

Inclusion of SS in the control diet led to reductions (P < 0.05) in the proportions of total and major *n*-3 PUFA, but further substitutions with DDGS led to no (P > 0.05) further changes (Table 2). This is consistent with the dietary proportions of 18:3*n*-3 for the various dietary treatments. Thus, these results could be related to the substitution of barley grain and RC with SS and DDGS, which both have relatively lower concentrations of 18:3*n*-3. There is also a possibility that high proportions of 18:2*n*-6 in the adipose tissues of steers fed SS and DDGS might have reduced the elongation of 18:3*n*-3 to 22:5*n*-3 due to competition for the Δ -6 desaturase (Johnson *et al.*, 2012).

Overall, feeding SS had no effect (P > 0.05) on total and major *n*-6 PUFA, but feeding increasing levels of DDGS raised (P < 0.05) the proportions of these FAs (Table 2). The proportions of total PUFA declined (P

<0.05) when feeding SS, and increased (P < 0.05) when feeding increasing levels of DDGS (Table 2). These findings may be explained by a combination of dietary fibre, 18:2*n*-6 and DM intake reported for the various diets in the current study by Mapiye *et al.* (2014a). The *n*-6 : *n*-3 ratio increased (P < 0.05) with the addition of SS in the control diet. These proportions were accentuated by inclusion of DDGS (Table 2). Overall, including *n*-6 PUFA sources in the diet of the animal increases the proportions of *n*-6 PUFA in the tissues, due mostly to concomitant decreases in the proportions of *n*-3 PUFA, and this subsequently increases the *n*-6 : *n*-3 ratio (Raes *et al.*, 2004). For PUFA : SFA, the DDGS-30 diet had a greater (P < 0.05) ratio than other diets (Table 2) which was associated with higher PUFA and lower SFA reported for this diet.

	Diet				OFM	Dualue
	Control	SS	DDGS-15	DGGS-30	SEM	P-value
∑ FA (mg/g)	900	904	910	907.5	6.55	0.52
Σ SFA	54.0 ^a	50.5 ^b	49.9 ^b	49.5 ^b	0.87	<.0001
14:0	4.34 ^a	3.62 ^b	3.28 ^c	3.30 ^c	0.11	<.0001
16:0	28.7 ^a	23.7 ^b	23.3 ^b	23.9 ^b	0.27	<.0001
18:0	18.1 ^b	20.8 ^a	21.2 ^a	20.4 ^a	0.71	0.01
∑ <i>c</i> -MUFA	34.8	34.6	35.2	36.3	0.84	0.23
<i>c</i> 9-14:1	1.03	0.98	0.83	0.87	0.07	0.06
<i>c</i> 9-16:1	3.66 ^a	3.33 ^a	2.78 ^b	2.75 ^b	0.18	<.0001
<i>c</i> 9-18:1	27.6 ^b	27.5 ^b	29.0 ^a	30.2 ^a	0.64	0.001
∑ <i>п</i> -3	0.51 ^ª	0.37 ^b	0.35 ^b	0.34 ^b	0.01	<.0001
18:3 <i>n</i> -3	0.44 ^a	0.32 ^b	0.31 ^b	0.30 ^b	0.01	<.0001
22:5 <i>n</i> -3	0.05 ^a	0.04 ^b	0.04 ^b	0.04 ^b	0.01	0.001
∑ <i>п</i> -6	1.61 ^b	1.59 ^b	1.71 ^b	2.20 ^a	0.05	<.0001
18:2 <i>n</i> -6	1.46 ^c	1.47 ^c	1.59 ^b	2.04 ^a	0.05	<.0001
20:3 <i>n</i> -6	0.08 ^b	0.06 ^c	0.06 ^c	0.09 ^a	0.01	<.0001
Σ PUFA	2.11 ^b	1.96 ^c	2.06 ^{bc}	2.54 ^a	0.06	<.0001
∑ <i>n</i> -6: ∑ <i>n</i> -3	3.33 [°]	4.33 ^b	4.92 ^b	6.44 ^a	0.67	0.04
∑ PUFA : SFA	0.04 ^b	0.04 ^b	0.04 ^b	0.05 ^a	0.01	<.0001

Table 2 Effect of diet on adipose tissue fatty acids (% total FA) of endogenous or dietary origin from beef steers

^{a,b,c} Means with different superscripts for a particular fatty acid profile are significantly different (P < 0.05); SEM: standard error of mean.

SS: sunflower seed; DDGS-15: 15% wheat dried distillers' grain with solubles + sunflower seed; DDGS-30: 30% wheat dried distillers' grain with solubles + sunflower seed; FxD: fat depot by diet interaction; *c: cis; t: trans;* Σ FA: total fatty acids in mg per g of tissue; Σ PUFA: sum of polyunsaturated fatty acids= $\Sigma n-6 + \Sigma n-3$; $\Sigma n-6$: sum of 18:2*n*-6, 20:3*n*-6, 20:4*n*-6; $\Sigma n-3$: sum of 18:3*n*-3, 20:5*n*-3, 22:5*n*-3; Σc -MUFA: sum of *c*9-14:1, *c*7-16:1, *c*9-16:1, *c*11-16:1, *c*9-17:1, *c*9-18:1, *c*11-18:1, *c*13-18:1, *c*14-18:1, *c*15-18:1, *c*9-20:1, *c*11-20:1; Σ SFA: sum of 14:0, 15:0, 16:0, 17:0, 18:0, 19:0, 20:0.

Fat depot influenced the total FA content, with PRF having a higher (P < 0.05) content than SCF (Table 3). This could be linked to differences in water content between these fat depots, with SCF having higher water content than PRF (Anderson *et al.*, 1972). Saturated FA (SFA) and *cis*-monounsaturated FA (*c*-MUFA) were the major families of FA of dietary or endogenous origin (Table 3). Fat depot influenced (P < 0.05) proportions of total SFA, with PRF having larger (P < 0.05) proportions than SCF. These dissimilarities could be linked to the proportions of 18:0, which exhibit a similar pattern. Generally, less mature fat depots situated externally, such as SCF, are less saturated than mature fat depots situated internally, such as PRF (Christie, 1978; Lee *et al.*, 2011; Jiang *et al.*, 2013). This is linked to a greater Δ -9 desaturase activity index in external depots as opposed internal ones, and replacement of 18:0 with *c*9-18:1 in external fat depots.

Fat depot had a significant effect on total and major *c*-MUFA, with SCF having larger (P > 0.05) proportions compared with PRF (Table 3). These findings agree with differences in Δ -9 desaturase indices (Jiang *et al.*, 2013) and mRNA abundance (Lee *et al.*, 2011) reported previously. The proportions of 22:5*n*-3 and 20:4*n*-6 were the only *n*-3 and *n*-6 PUFA influenced by fat depot, with SCF having larger (P < 0.05) proportions of these FA compared with PRF (Table 3). However, these changes were small and did not alter the proportions of total *n*-3 and *n*-6 PUFA. Fat depot had no effect on total PUFA and *n*-6 : *n*-3 ratio. For PUFA : SFA, the fat depot effect was significant, with SCF vs. PRF having a greater (P < 0.05) ratio (Table 3). Given that total PUFA was similar between the two fat depots, the higher PUFA : SFA reported for SCF could be related to the lower proportions of SFA reported for the SCF depot.

	Fat depo	SEM	D volue		
	Subcutaneous fat	Perirenal fat	- SEIVI	r-value	
ΣFA (mg/g)	896 ^b	915 ^ª	6.55	0.04	
Σ SFA	42.4 ^b	59.5 ^a	0.75	<.0001	
14:0	3.68	3.56	0.08	0.38	
16:0	24.7	25.1	0.19	0.12	
18:0	12.1 ^b	28.2 ^a	0.59	<.0001	
∑ <i>c</i> -MUFA	44.2 ^a	26.2 ^b	0.70	<.0001	
<i>c</i> 9-14:1	1.53 ^ª	0.32 ^b	0.05	<.0001	
<i>c</i> 9-16:1	4.87 ^a	1.39 ^b	0.15	<.0001	
<i>c</i> 9-18:1	34.7 ^a	22.5 ^b	0.53	<.0001	
∑ <i>n</i> -3	0.39	0.38	0.01	0.22	
18:3 <i>n</i> -3	0.34	0.35	0.01	0.45	
22:5 <i>n</i> -3	0.05 ^a	0.04 ^b	0.01	<.0001	
∑ <i>n</i> -6	1.81	1.75	0.04	0.13	
18:2 <i>n</i> -6	1.65	1.62	0.04	0.41	
20:3 <i>n</i> -6	0.08 ^a	0.06 ^b	0.003	0.001	
∑ PUFA	2.20	2.13	0.05	0.12	
∑ <i>n</i> -6: ∑ <i>n</i> -3	4.59	4.56	0.02	0.25	
Σ PUFA : SFA	0.05 ^a	0.04 ^b	0.01	<.0001	

Table 3 Effect of fat depot on fatty acids (% total fatty acids) of endogenous or dietary origin from steers fed red clover silage with sunflower seed and dried distillers' grains plus solubles

^{a,b,c} Means with different superscripts for a particular fatty acid profile are significantly different (P < 0.05); SEM: standard error of mean.

c: *cis*; *t*: *trans*; \sum FA: total fatty acids in mg per g of tissue; \sum PUFA: sum of polyunsaturated fatty acids: $\sum n-6 + \sum n-3$; $\sum n-6$: sum of 18:2*n*-6, 20:3*n*-6, 20:4*n*-6; $\sum n-3$: sum of 18:3*n*-3, 20:5*n*-3, 22:5*n*-3; $\sum c$ -MUFA: sum of c9-14:1, c7-16:1, c9-16:1, c11-16:1, c9-17:1, c9-18:1, c11-18:1, c12-18:1, c13-18:1, c14-18:1, c15-18:1, c9-20:1, c11-20:1; \sum SFA: sum of 14:0, 15:0, 16:0, 17:0, 18:0, 19:0, 20:0.

Fatty acids of microbial origin

Branched-chain FA (BCFA), *trans*-monounsaturated FA (*t*-MUFA), CLA and non-conjugated 18:2 BHP (i.e. atypical dienes (AD)) were the major FA groups of microbial origin (Table 4; Fig 1A). Inclusion of SS in the diet led to reductions (*P* <0.05) in the proportions of total and major BCFA. Proportions of these FA were further reduced (*P* <0.05) by substitution with increasing levels of DDGS. Given that several BCFA in animal tissue are synthesised *de novo* by rumen microbes (Vlaeminck *et al.*, 2006), it may be speculated that high levels of 18:2*n*-6 in SS and DDGS diets might have inhibited rumen microbes (Liu *et al.*, 2011) leading to a decline in BCFA production. Differences in dietary protein content and interaction between dietary nutrients on rumen ecology and fermentation might also have played a role (Vlaeminck *et al.*, 2006). Subsequent decreases when feeding DDGS could be due to reduced ruminal propionate (a precursor for BCFA biosynthesis) production from readily fermentable starch (Pethick *et al.*, 2004). Total and major BCFA were

affected (P < 0.05; Table 4) by fat depot and diet, but their interaction was not significant (P > 0.05). Perirenal fat as opposed to SCF had greater (P < 0.05) proportions of total BCFA and slightly lower (P < 0.05) proportions of *anteiso*-17:0. These findings could be linked to depot-specific differences in incorporation of individual FA (Hood *et al.*, 1976).

Variable -	Diet				0514	
	Control	SS	DDGS-15	DDGS-30	SEM	P-value
Σ BCFA	3.15 ^ª	2.36 ^b	2.23 ^c	1.95 ^d	0.03	<.0001
<i>iso</i> -17:0	0.49 ^a	0.40 ^b	0.37 ^c	0.33 ^d	0.01	<.0001
anteiso-17:0	0.81 ^a	0.57 ^b	0.57 ^b	0.52 ^c	0.01	<.0001
	Fat depot					
	Subcutan	eous fat	Perir	enal fat		
∑ BCFA	2.30	6 ^b	2	.48 ^a	0.02	0.001
<i>i</i> so-17:0	0.4	0	C	.40	0.01	0.57
anteiso-17:0	0.63	3 ^a	0	.61 ^b	0.01	0.02

Table 4 Effect of fat depot and diet on branched-chain fatty acid profiles (% total FA) of beef steers

^{a,b,c} Means with different superscripts for a particular fatty acid profile are significantly different (P < 0.05); SEM: standard error of mean; SS: sunflower seed; DDGS-15: 15% wheat dried distillers' grain with solubles + sunflower seed; DDGS-30: 30% wheat dried distillers' grain with solubles + sunflower seed; \sum BCFA: branched chain fatty acids: sum of *iso*-15:0, *iso*-16:0, *iso*-17:0, *anteiso*-17:0, *iso*-18:0.

The interaction between fat depot and diet was significant for total and major *t*-18:1 FA (Figure 1A). Perirenal fat as opposed to SCF had greater (P < 0.05) proportions of total and major *t*-18:1 FA including vaccenic acid (*t*11-18:1). These proportions were accentuated (P < 0.05) by feeding SS, maintained (P > 0.05) by feeding DDGS-15, and attenuated (P < 0.05) by feeding the DDGS-30 diet. This could be the result of a combination of factors including lower Δ -9 desaturase activity indices in PRF vs. SCF (Lee *et al.*, 2011; Jiang *et al.*, 2013), higher dietary 18:2*n*-6 observed for the diets containing SS, greater bypass of 18:2*n*-6 when feeding DDGS diets, and a decline in dietary fibre with increasing levels of DDGS, which might have reduced ruminal pH and negatively influenced biohydrogenation in the rumen (Hristov *et al.*, 2005; Felix *et al.*, 2012). It may also be due to greater *de novo* synthesis of FA in SCF and dilution of PUFA-BHP when DDGS were added to the diet.

Fat depot x diet interaction influenced (P < 0.05) the proportions of total CLA, t7,c9- and t9,c11-18:2 (Figure 1B). Overall, SCF had greater (P < 0.05) proportions of total CLA, t7,c9-18:2, and t9,c11-18:2 (rumenic acid), the major CLA isomer, and the proportions of these FA were increased (P < 0.05) by feeding SS and DDGS-15 diets (P > 0.05), but responses were somewhat diminished when feeding the DDGS-30 diet. Again, these findings could be ascribed largely to differences in Δ -9 desaturase activity indices between PRF and SCF (Jiang *et al.*, 2013), dissimilarities in dietary 18:2*n*-6 and ruminal pH related to the observed decline in dietary fibre with increasing DDGS.

Total AD and t8,c12-18:2, one of the major AD isomers that is probably derived from 18:2n-6, were affected by fat depot × diet interaction, with SCF having greater proportions than PRF. These AD proportions were increased (P < 0.05) by inclusion of SS, but further substitutions with DDGS led to no further changes (P > 0.05; Figure 1C). These findings could reflect selective uptake or lower rate of metabolism of these AD in SCF (Kramer *et al.*, 1998) and greater proportions of 18:2n-6 observed for the SS containing diets.

Fat depot and its interaction with diet had no (P > 0.05) effect on t11,c15-18:2, one of the major AD isomers probably derived from 18:3n-3 (Figure 1C). Adding SS to the control diet had no (P > 0.05) effect on t11,c15-18:2 either, but further substitutions with DDGS led to reductions (P < 0.05; Figure 1C). This may be related to dietary proportions of n-3 PUFA, which declined with the addition of SS and DDGS to the diet. During biohydrogenation, 18:3n-3 is isomerized to CLA, which is hydrogenated to t11,c15-18:2 (Harfoot et al., 2007).



Fig 1 Effects of fat depot and diet on trans-18:1 isomers (A), CLAs (B) and atypical dienes (C) from beef steers.

Each bar represents mean ± SEM.

^{a,b,c} Means with different superscripts for a particular fatty acid profile are significantly different (P < 0.05); SEM: standard error of mean; SCF: subcutaneous fat; PRF: perirenal fat; SS: sunflower seed; DDGS-15: 15% wheat dried distillers' grain with solubles + sunflower seed; DDGS-30: 30% wheat dried distillers' grain with solubles + sunflower seed; DDGS-30: 30% wheat dried distillers' grain with solubles + sunflower seed; DLGS-30: 30% wheat dried distillers' grain with solubles + sunflower seed; t11,c15-, c9,c15-, t8,c13-, c9,t12-/c16-18:1, t9,c12-, t11,c15-, c9,c15-, c12,c15-18:2; Σ CLA: conjugated linoleic acid = sum of t12,t14-, t11,t13-, t10,t12-, t8,t10-, t7,t9- t6,t8-, c9,t11-, t7,c9-, t11,c13-, t12,c14-, c11,t13-, t10,c12-, t8,c10-, t9,c11-; Σ t-MUFA: sum of trans-monounsaturated fatty acids = t9-16:1, t6,t7, t8-, t9-, t10-, t11-, t12-, t13/t14-, t15-, t16-18:1.

Conclusions

The remarkable differences between fat depots were related to the greater proportions of SFA and lower proportions of *c*-MUFA in PRF, as opposed to SCF. Feeding SS and DDGS-15 diets led to increases in proportions of vaccenic acid in PRF and rumenic acid in SCF, but feeding DDGS-30 was not so effective. Future strategies to enhance the composition of *t*-18:1 and CLA in beef fat without compromising animal performance and meat quality may therefore include feeding SS and 15% DDGS in forage-based diets and exploiting fat depot heterogeneity.

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