SUBCUTANEOUS BIOHYDROGENATION PRODUCT PROFILES OF STEERS FED FORAGE-BASED DIETS WITH SUNFLOWER-SEED AND WHEAT DRIED DISTILLERS' GRAINS WITH SOLUBLES

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Abstract – Subcutaneous fat proportions of polyunsaturated fatty acid biohydrogenation products (PUFA-BHP) 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 substituted for RC and SS. Compared to control, the SS, DDGS-15 and DDGS-30 diets had ~4% more crude fat with 20% more 18:2*n*-6. Compared to feeding control, the SS diet did not change but DDGS-15 and DDGS-30 diets progressively increased proportions of total *n*-6 fatty acids in subcutaneous fat. Compared to control, feeding SS and DDGS-15 diets increased *c*9,*t*11-18:2 and *t*11-18:1 (*i.e.*, major BHP of 18:2n-6; *P* < 0.05), but contents were either unchanged (*c*9,*t*11-18:2) or intermediate (*t*11-18:1) when feeding DDGS-30. Compared to control, feeding all other diets increased (*P* < 0.05) total non-conjugated 18:2 BHP (*i.e.*, atypical dienes) and *t*8,*c*12-18:2. Compared to control, feeding SS reduced total and major branched chain fatty acids (*P* < 0.05), and DDGS-15 and DDGS-30 led to further reductions (*P* < 0.05). Overall, feeding SS and DDGS-15 diets raised the proportions of PUFA-BHP with potential human health benefits (*i.e.*, *t*11-18:1 and *c*9,*t*11-18:2), but feeding DDGS-30 was somewhat less effective.**

I. INTRODUCTION

There has been a growing interest in raising proportions of polyunsaturated fatty acid biohydrogenation products (PUFA-BHP), especially rumenic acid (RA, *cis (c)*9, *trans (t)*11-18:2) and its precursor vaccenic acid (VA, *t*11-18:1) in beef (1) owing to their potential benefits to human health (2). This can be accomplished by feeding sources of PUFA in forage based diets, but animal performance and meat quality can suffer compared to feeding high concentrate diets. Recently, we demonstrated these problems can be in part alleviated by replacing forage (red clover silage (RC)) with wheat dried distillers' grains plus solubles (DGGS) as a non-forage fibre source (3). The current study is an extension of Mapiye *et al.* (3) and the objective was to compare PUFA-BHP profiles in subcutaneous fat (SCF) from the same group of steers. We were interested in SCF due to its greater propensity to accumulate RA and VA, and because it is used when making ground beef, the most consumed beef product in North America (4).

II. MATERIAL AND METHODS

Sixty-four 12-month-old British × Continental crossbred steers with an initial body weight (BW) of 362.7 ± 4.5 kg were stratified by weight to four experimental diets (control; SS, DDGS-15 and DDGS-30), with two pens of eight steers per diet. The control diet was composed of 70% RC, 25.8% barley grain and 4.2% vitamin-mineral 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 substituted for RC and SS to maintain ~4% added oil in the diets from either SS or DDGS (DM basis; Table 1).

Table 1 Ingredient, nutrient and fatty acid composition of the dietary treatments

Variable	Dietary treatments				
	Control	SS	DDGS-15	DDGS-30	
Ingredients (% DM basis)					
Sunflower-seed	0.0	11.4	9.2	7.0	
Dried distiller' grains with solubles	0.0	0.0	15.0	30.0	
Barley grain	25.8	14.4	14.4	14.4	
Red Clover	70.0	70.0	57.2	44.4	
Vitamin/mineral supplement[1]	4.2	4.2	4.2	4.2	
Nutrient composition (% DM basis)					SD
Dry matter	42.6	40.2	44.2	50.1	4.2
Crude protein	13.1	13.4	16.5	20.8	3.6
Crude fat	1.89	6.40	5.80	5.90	2.09
Calcium	0.86	0.92	0.81	0.69	0.10
Phosphorus	0.31	0.32	0.41	0.53	0.10
Acid detergent fibre	33.7	37.0	33.8	28.4	3.6
Neutral detergent fibre	43.3	48.7	44.5	38.5	4.2
Digestible Energy (Mcal/kg)	2.71	2.57	2.73	2.91	0.14
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
*c*9-18:1	9.49	12.4	13.0	13.3	1.75
*c*11-18:1	0.93	0.73	0.76	0.78	0.09
18:3*n*-3	18.9	7.09	6.26	5.59	6.32
18:2*n*-6	39.0	59.6	60.3	60.1	10.5

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; [1]Vitamin/mineral supplement per kg DM contained 1.86% calcium, 0.93% phosphorous, 0.56% potassium, 0.21% sulphur, 0.33% magnesium 0.92% sodium, 265 ppm iron, 314 ppm manganese, 156 ppm copper, 517 ppm zinc, 10.05 ppm iodine, 5.04 ppm cobalt, 2.98 ppm selenium, 49722 IU/kg vitamin A, 9944 IU/kg vitamin D3, and 3222 IU/kg vitamin E.

Steers were slaughtered at the Lacombe Research Centre abattoir at an average of 190 d on feed. At approximately 20 min post-mortem, during evisceration, a sample of SCF (5 cm × 5 cm × the thickness of SCF) was collected above the loin posterior end of the 12th rib and stored at −80°C for subsequent FA analysis. For analysis, 50 mg of SCF was freeze-dried and directly methylated with 0.5M sodium methoxide (5). Fatty acid methyl esters were analysed by 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, CA, USA). Two GC analyses were conducted per sample using complementary temperature programs with 150°C and 175°C plateaus according to Kramer *et al.* (6). Conjugated linoleic acid isomers not separated by GC were further analysed using Ag^+-HPLC as described by Cruz-Hernandez, *et al.*, (7). Statistical analyses were conducted using Proc Mixed (8). All the data were analysed as a one-way factorial including main effect of diet, and animal (diet) as the random effect. Treatment means were generated and separated using the LSMEANS and PDIFF options respectively. The significance threshold was set at $P<0.05$.

III. RESULTS AND DISCUSSION

No changes in *n*-6 PUFA were found in SCF comparing control versus the SS diet ($P > 0.05$; Table 2). Feeding the DDGS-15 and DDGS-30 diets led to successive increases in *n*-6 PUFA ($P < 0.05$), indicating a greater rate of ruminal bypass. Feeding the SS, DDGS-15 and DDGS-30 diets had no effect ($P < 0.05$) on the proportions of total conjugated linolenic acid (CLNA) and *c*9,*t*11,*c*15-18:3, the major CLNA isomer (Table 2). Compared to control, feeding the SS diet increased ($P < 0.05$) proportions of total non-conjugated 18:2 BHP (*i.e.,* atypical dienes, AD) and the major AD (*t*8,*c*12-18:2) but no further changes were noted when feeding 15% or 30% DDGS ($P > 0.05$). These findings reflect greater proportions of 18:2*n*-6 when feeding SS and DDGS containing diets. Substituting SS into

the control diet had no effect on *t*11,*c*15-18:2, but further substitutions with DDGS led to reductions ($P < 0.05$). This may be related to dietary proportions of *n*-3 PUFA which declined with addition of SS and DDGS to the diet. During biohydrogenation, 18:3*n*-3 is isomerised to CLNA, which is in turn hydrogenated to *t*11,*c*15-18:2 (9).

Compared to control, proportions of total and major CLA (*t*7,*c*9- and *t*9,*c*11-18:2) and *t*-18:1 (*t*11- and *t*13-/*t*14-18:1) isomers 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. This could be a result of a combination of factors including 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 DDGS, which might have reduced ruminal pH and negatively influenced ruminal biohydrogenation (10; 11). This 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.

Feeding SS *vs.* the control diet led to reductions ($P < 0.05$) in the proportions of total and individual BCFA, and proportions of these FA were further reduced ($P < 0.05$) by feeding DDGS-15 and DDGS-30. Since the majority of BCFA in animal tissue are synthesised *de novo* by rumen microbes (12), the high levels of 18:2*n*-6 in SS containing diets might have inhibited the responsible rumen microbes (13) thereby reducing BCFA production. Further reductions observed when adding DDGS to the diet could be a result of decreased ruminal propionate production from readily fermentable starch (14). Propionate is a precursor of methylmalonate, which is used as a primer for BCFA biosynthesis (12).

IV. CONCLUSIONS

Feeding SS and DDGS-15 diets led to remarkable increases in proportions of VA and RA in SCF, but feeding DDGS-30 was not as effective. Feeding DDGS-15 might, therefore, be a way to improve the healthfulness of SCF fatty acid profiles, while improving overall animal performance and meat quality.

Table 2 Least square means of polyunsaturated fatty acids and their biohydrogenation products from subcutaneous fat of steers fed sunflower-seed and dried distiller's grains with solubles

Variable	Control	SS	DDGS-15	DGGS-30	s.e.m	P-value
∑ FA (mg/g)	893	895	896	900	907	0.52
∑ *n*-6 PUFA	1.61^{c}	1.61^{c}	1.76^{b}	2.25^{a}	0.06	<0.001
∑ *n*-3 PUFA	0.49^{a}	0.38^{b}	0.36^{b}	0.35^{b}	0.02	<0.001
∑ CLNA	0.07	0.07	0.07	0.07	0.004	0.45
*c*9,*t*11,*c*15-18:3	0.04	0.05	0.05	0.05	0.003	0.20
∑ AD	0.81^{b}	1.20^{a}	1.22^{a}	1.22^{a}	0.04	<0.001
*t*8,*c*12-18:2	0.15^{b}	0.29^{a}	0.31^{a}	0.31^{a}	0.02	<0.001
*t*11,*c*15-18:2	0.21^{a}	0.21^{a}	0.16^{b}	0.14^{c}	0.01	<0.001
∑ CLA	0.82^{c}	1.40^{a}	1.41^{a}	1.32^{b}	0.03	<0.001
*t*7,*c*9-18:2	0.04^{c}	0.07^{b}	0.08^{a}	0.08^{a}	0.003	<0.001
*c*9,*t*11-18:2	0.72^{b}	1.26^{a}	1.25^{a}	1.18^{b}	0.03	<0.001
∑ *c*-MUFA	43.1	44.0	44.2	45.3	1.03	0.50
∑ *t*-MUFA	3.06^{c}	6.09^{ab}	6.49^{a}	5.82^{b}	0.21	<0.001
*t*11-18:1	1.45^{c}	2.80^{ab}	2.88^{a}	2.54^{b}	0.11	<0.001
*t*13/*t*14-18:1	0.37^{c}	0.75^{ab}	0.83^{a}	0.69^{b}	0.04	<0.001
∑ BCFA	3.03^{a}	2.34^{b}	2.20^{c}	1.89^{d}	0.051	<0.001
i*so*-17:0	0.48^{a}	0.40^{b}	0.37^{c}	0.33^{d}	0.01	<0.001
ai-17:0	0.80^{a}	0.59^{b}	0.58^{b}	0.53^{c}	0.01	<0.001
∑ SFA	45.9^{a}	41.7^{b}	41.2^{b}	40.8^{b}	1.04	0.001

^{a,b,c} Means with different superscripts for a particular fatty acid profile are significantly different ($P < 0.05$); s.e.m, standard error of mean; *c*, *cis*; *t*, *trans*; ∑ FA, total fatty acids in mg per g of fat; ∑ PUFA, sum of polyunsaturated fatty acids = ∑ *n*-6 + ∑ *n*-3; ∑ *n*-6 = sum of 18:2*n*-6, 20:3*n*-6, 20:4*n*-6; ∑ *n*-3 sum of 18:3*n*-3, 20:5*n*-3, 22:5*n*-3; ∑CLNA, sum of conjugated linolenic acid = *c*9,*t*11,*t*15-, *c*9,*t*11,*c*15-; ∑ AD, atypical dienes = sum of *t*11,*t*15-, *c*9,*t*13-/*t*8,*c*12-, *t*8,*c*13-, *c*9,*t*12-/*c*16-18:1, *t*9,*c*12-, *t*11,*c*15-, *c*9,*c*15-, *c*12,*c*15-18:2; ∑ CLA, conjugated linoleic acid = sum of *t*12,*t*14-, *t*11,*t*13-, *t*10,*t*12-, *t*9,*t*11-, *t*8,*t*10-, *t*7,*t*9- *t*6,*t*8-, *c*9,*t*11-, *t*7,*c*9-, *t*11,*c*13-, *t*12,*c*14-, *c*11,*t*13-, *t*10,*c*12-, *t*8,*c*10-, *t*9,*c*11-18:2; ∑ *t*-MUFA, sum of *trans*-monounsaturated fatty acids = *t*9-16:1, *t*6,*t*7,*t*8-, *t*9-, *t*10-, *t*11-, *t*12-, *t*13/*t*14-, *t*15-, *t*16-18:1; ∑ *c*-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*12-18:1, *c*13-18:1, *c*14-18:1, *c*15-18:1, *c*9-20:1, *c*11-20:1; ∑ BCFA, branched chain fatty acids = sum of *iso*-15:0, *anteiso*-15:0, *iso*-16:0, *iso*-17:0, *anteiso*-17:0, *iso*-18:0; ∑ SFA= sum of 14:0, 15:0, 16:0, 17:0, 18:0, 19:0, 20:0.

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