RELATIONSHIP BETWEEN DIETARY N-6/N-3 AND Δ5-, Δ6-DESATURASE PROTEIN EXPRESSION IN BOVINE MUSCLES

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Abstract – Linseed (L), canola (C) and soybean (S) supplemented diets ranging in n-6/n-3 were fed to steers to investigate the effect of diet on polar fatty acid composition and Δ5-(Δ5d), Δ6-(Δ6d) desaturase protein expression in cheek and diaphragm muscle. Diet did not affect polar lipid saturated fatty acid content, however the C diet did result in higher 18:1c-9 content of the muscles (P<0.001). The S diet resulted in higher 18:2n-6 content in muscles (P<0.001), which accounted for the higher n-6 and polyunsaturated fatty acid (PUFA) content (P<0.001). The L diet resulted in higher 18:3n-3 content in the muscles (P<0.001), as well as the longer chain derivatives. Diet did not affect Δ5d and Δ6d expression; however there was a stronger association between Δ5d expression and n-3 PUFA in cheek and diaphragm, being slightly stronger for 20:5n-3 than 18:3n-3. Δ5d expression had a negative association toward 18:2n-6, but neutral or positive towards 20:4n-6 in cheek and diaphragm respectively. There were no clear associations between Δ6d expression and PUFA. Results show the muscle PUFA profile responds to the dietary n-6/n-3 ratio of processed oilseeds and Δ5d desaturase protein expression has a stronger association to n-3 PUFA, which could affect the muscle LC-PUFA profile.

Key Words – long-chain polyunsaturated fatty acid, processed oilseeds

I. INTRODUCTION

Health recommendations call for increased intake of long-chain polyunsaturated fatty acids (LC-PUFA), particularly those derived from 18:3n-3. Health benefits of n-3 LC-PUFA include reduced inflammatory response and cardiovascular disease risk. Red meat can play an important role towards meeting recommended n-3 LC-PUFA intakes [1]. Ruminant meat LC-PUFA can be altered by diet, including oilseed supplementation [2]. Desaturation of essential fatty acids, namely 18:2n-6 and 18:3n-3, to their LC-PUFA derivatives is through the actions of Δ5- (Δ5d) and Δ6-desaturase (Δ6d). Few studies have investigated how diet can influence the actions of these enzymes in ruminants. Herdmann *et al.*[3] reported high dietary n-3 decreased Δ6d protein expression in muscle, but not in subcutaneous fat, whereas Hiller *et al.* [4] found that Δ6d gene expression was not altered in either tissue.

Objectives were to (i) investigate the effects of feeding oilseeds over a range of n-6/n-3 ratios on the polar lipid fatty acid profile of cheek (m. *masseter*) and diaphragm (*pars costalis diaphragmatis*) muscle (ii) determine the effect of diet on the expression of Δ5d and Δ6d proteins in the muscles and (iii) investigate the associations between the polar lipid profile and Δ5d and Δ6d protein expression. Selected muscles are accessible at slaughter; and diaphragm has previously been used to demonstrate dietary effects on muscle fatty acids [5]. Likewise, desaturase protein expression was reportedly similar between diaphragm and more typical skeletal muscles [6]. Cheek and diaphragm observations are expected to be similar as both consist of predominantly oxidative muscle fibres undergoing continual phasic contraction.

II. MATERIALS AND METHODS

Animal trial

Forty-eight steers were individually fed a basal diet, as a total mixed ration between 100 and 120 days, consisted on a dry matter (DM) basis of 300 g/kg grass silage 685 g/kg barley grain

and 15 g/kg mineral/vitamin premix formulated to provide 129.8 g/kg DM CP, 34.7 g/kg DM fat and 500 IU of vitamin E daily. Roasted or extruded oilseed supplements were top dressed at 2.5 kg/day as-fed, with the dietary n-6/n-3 treatments as linseed (L, 0.7, n=16); canola (C, 1.5, n=16), and soybean (S, 2.2, n=16). One animal was removed from the trial due to factors unrelated to diet. Further feeding and animal performance are reported by McNiven *et al.* [7].

Feed and muscle fatty acid analysis

Cheek and diaphragm muscle samples were collected at slaughter, snap frozen and stored at -80°C until lipid and microsomal extraction. Lipid extraction of feed and muscle samples, isolation of muscle polar lipids, methylation of lipids by 0.5M sodium methoxiode and 3N methanolic HCl and gas chromatography conditions used for analysis and quantification of fatty acid methyl esters (FAME) were as described by McNiven *et al.* [7].

Isolation of mircosomes and analysis of desaturase protein expression

The Δ5d and Δ6d microsomal enzymes were isolated from cheek and diaphragm muscle by differential centrifugation using Ca^{2+} method following the procedure described by Ward *et al.* [8] with minor modification of using 45,000*g* for 30 min during the second centrifugation step. Analyses of desaturase protein expression were conducted by Western blotting. Microsomal proteins (10 µg) were separated by SDS-PAGE using Bis-Tris 4-12% precast gels at 125V for 90 min, electoblotted onto ImmunoBlot PVDF membrane at 15V for 30 min (Bio-Rad Laboratories Inc., ON, Canada). Membrane was then probed with either Δ5d or Δ6d primary antibodies at 1:500 v/v (custom produced by Sigma, UK). Immunoreactivity to bovine proteins was previously evaluated by Ward et al. [8]. Membrane with transferred proteins was washed with PBST (100 ml 10x PBS and 900 ml H_2O, 1 ml Tween 20, Bio-Rad Laboratories Inc., ON, Canada), then probed with a commercial goat anti-rabbit secondary antibody at 1:10000 v/v (Santa Cruz Biotechnology Inc. CA, USA). Blots were developed using the Immuno-Star WesternC kit (Bio-Rad Laboratories Inc., ON, Canada). Membranes were scanned using a UVP Biospectrum AC Imaging System (UVP, CA, USA) and intensity of signals of protein bands were quantified using Vision Works LS software v6.7.4 (UVP, CA, USA). Membrane was washed and re-probed using rabbit anti-β actin primary 1:000 v/v, then probed with goat anti-rabbit secondary antibody at 1:10000 v/v (Santa Cruz Biotechnology Inc. CA, USA). The molecular weight of β-actin is 40 kDa (Santa Cruz Biotechnology Inc. CA, USA). Expression of a housekeeping protein, β-actin, was analysed and the ratio of Δ5d/ β-actin and Δ6d/ β-actin were determined to normalize Δ5d and Δ6d signals on the blots for cross comparisons.

Statistical analysis

Data was analysed as a complete randomized design using SAS Proc Mixed v9.1 (Statistical Analysis Systems, Cary, NC, USA). Oilseed type and processing method were fixed effects, with individual animal as the experimental unit. Δ5d and Δ6d protein expression were analysed as a log transformation to normalize variance. Results are reported as the differences between least square means and include the standard error of the means (SEM). Multivariate analysis (Unscrambler X 10.1, CAMO, Oslo, Norway) was used to explore the relationships between tissue fatty acids and desaturase protein expression. Log values of the data were transformed by mean-centring and weight-standardised to give all variable the same variance prior to plotting the principal component analysis (PCA) plots.

III. RESULTS AND DISCUSSION

The lipid content did not differ between oilseed supplements, ranging between 5.0 and 5.6 g/100g muscle for cheek and 12.0 to 14.7 g/100 g muscle for diaphragm. Oilseed processing did not affect the polar lipid fatty acid profile and all further dietary effects will be discussed in relation to oilseed supplements. Influence of diet on the polar lipid profile of the cheek and diaphragm are reported in Table 1. Diet did not affect the saturated fatty acid content of the muscles; however the C diet resulted in a higher muscle monounsaturated fatty acid (MUFA) content ($P<0.01$), primarily due to 18:1*c*-9 ($P<0.001$). Present findings relate to the high

18:1*c*-9 content of canola and the stearoyl-CoA suppressing effects of PUFA. The S diet resulted in higher PUFA in the muscles (P<0.05), largely owing to higher 18:2n-6 content (P<0.001), which was reflected by the higher n-6 content (P<0.001). Interestingly, there was no difference between the diets for 20:4n-6, the major n-6 LC-PUFA, however the minor n-6 LC-PUFA, namely 20:3n-6, 22:4n-6 and 22:5n-6 were generally higher for the S and C diets in both muscles (P<0.05). There was no difference between diets for n-6 LC-PUFA content in either muscle. In contrast, the L diet increased 18:3n-3 content, as well as the 20:5n-3, 22:5n-3 and n-3 LC-PUFA content of the muscles (P<0.001). The L diet increased the 22:6n-3 content in diaphragm (P<0.05), with a similar tendency in cheek (P=0.09).

The Δ5d and Δ6d protein expression did not differ between the diets in either muscle (P>0.05). The 3-fold difference in dietary n-6/n-3 may have limited full expression potential. A 5-fold difference in diet n-6/n-3 ratio was sufficient for Herdmann *et al.* [3] to conclude high dietary n-3 can decrease Δ6d protein expression in bovine m. *longissimus dorsi*, yet did not affect Δ5d and Δ6d gene expression [4]. The apparent contrast to our study may also be related to tissue specific differences in protein expression. The clear diet effect on the LC-PUFA n-6/n-3 ratio of the muscles (P<0.001), suggests some influence by the Δ5- and Δ6-desaturases on LC-PUFA profile. Interpretations of the PCA plots indicate a stronger association between Δ5d protein expression and n-3 PUFA

Table 1. Polar lipid fatty acid profile (mg/g FAME), Δ5 (Δ5d) and Δ6 (Δ6d) desaturase expression of cheek and diaphragm muscle of steers supplemented with extruded or roasted linseed (L), canola (C), or soybean (S)

	Cheek						Diaphragm					
	L	C	S	SE[z]	P		L	C	S	SE	*P*	
SFA[y]	300.5	302.1	302.7	4.82	0.65		286.2	301.2	291.9	5.27	0.14	
18:0	178.7	179.7	179.6	5.26	0.99		96.9	95.9	98.7	4.36	0.90	
MUFA	170.7 [b]	198.8 [a]	143.5 [c]	8.71	<0.01		284.3 [b]	320.6 [a]	254.4 [c]	9.54	<0.001	
18:1c-9	133.7 [b]	163.4 [a]	108.8 [c]	8.66	<0.001		247.1 [b]	284.7 [a]	219.8 [c]	9.49	<0.001	
PUFA	424.4 [b]	403.1 [b]	465.8 [a]	10.18	<0.05		331.3 [b]	289.6 [c]	371.1 [a]	11.66	<0.001	
n-6	304.9 [b]	325.3 [b]	393.7 [a]	9.27	<0.001		242.1 [b]	238.7 [b]	319.9 [a]	10.16	<0.001	
18:2n-6	172.5 [b]	183.4 [b]	242.9 [a]	7.47	<0.001		166.2 [b]	163.4 [b]	237.6 [a]	7.99	<0.001	
LC n-6	129.7	139.2	147.7	6.35	0.14		74.3	73.9	80.5	3.88	0.43	
18:3n-6	2.67	2.86	2.98	0.11	0.15		2.21 [b]	2.60 [a]	2.61 [a]	0.09	<0.01	
20:3n-6	28.3 [b]	33.3 [ab]	37.0 [a]	1.88	<0.01		18.1 [b]	19.3 [b]	22.6 [a]	1.10	<0.05	
20:4n-6	91.7	93.5	98.0	4.82	0.63		48.6	45.7	48.4	2.91	0.74	
22:4n-6	5.82 [b]	7.80 [a]	7.96 [a]	0.36	<0.001		4.55 [b]	5.31 [a]	5.80 [a]	0.27	<0.01	
22:5n-6	1.27 [b]	1.78 [a]	1.81 [a]	0.10	<0.001		0.81 [b]	0.95 [ab]	1.04 [a]	0.06	<0.05	
n-3	117.7 [a]	76.2 [b]	70.3 [b]	2.70	<0.001		86.9 [a]	48.9 [b]	49.0 [b]	2.43	<0.001	
18:3n-3	43.5 [a]	21.2 [b]	19.5 [b]	1.18	<0.001		40.6 [a]	18.5 [b]	18.6 [b]	1.27	<0.001	
LC n-3	74.2 [a]	55.0 [b]	50.8 [b]	2.53	<0.001		46.3 [a]	30.4 [b]	30.3 [b]	1.75	<0.001	
20:5n-3	25.7 [a]	16.0 [b]	13.6 [b]	1.23	<0.001		14.3 [a]	7.5 [b]	6.8 [b]	0.70	<0.001	
22:5n-3	42.0 [a]	33.3 [b]	31.5 [b]	1.25	<0.001		28.4 [a]	19.9 [b]	20.6 [b]	0.97	<0.001	
22:6n-3	6.51	5.68	5.63	0.31	0.09		3.65 [a]	2.91 [b]	2.88 [b]	0.20	<0.05	
n-6/n-3	2.6 [c]	4.3 [b]	5.7 [a]	0.16	<0.001		2.8 [c]	4.9 [b]	6.6 [a]	1.53	<0.001	
LC n-6/n-3	1.72 [c]	2.54 [b]	2.87 [a]	0.11	<0.001		1.58 [b]	2.34 [a]	2.57 [a]	0.09	<0.001	
Δ5d[x]	2.21	2.10	2.17	0.05	0.38		2.02	1.96	2.04	0.05	0.64	
Δ6d	1.78	1.69	1.82	0.10	0.61		1.03	0.99	1.12	0.07	0.50	

[a-c] different letters within a row indicate statistical difference (*P*<0.05) within the muscle.

[z] standard error of the means (SEM)

[y] abbreviations: FAME, fatty acid methyl ester; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; n-6, sum of all n-6 FA; LC n-6, sum of n-6 FA ≥20:3n-6; n-3, sum of all n-3 FA; LC n-3, sum of n-3 FA ≥20:5n-3.

[x] desaturase protein expressed as log value.

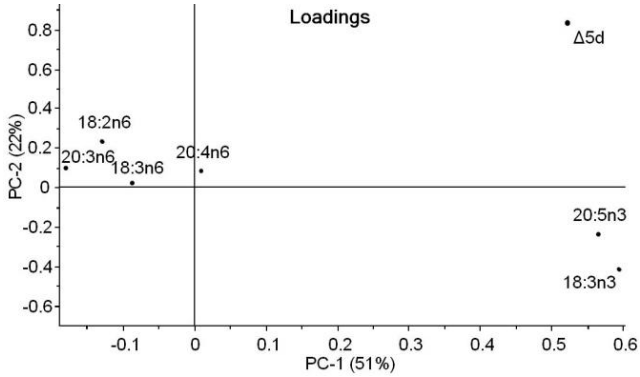

Figure 1. PCA loading plot of associations between Δ5d protein expression and LC-PUFA in cheek.

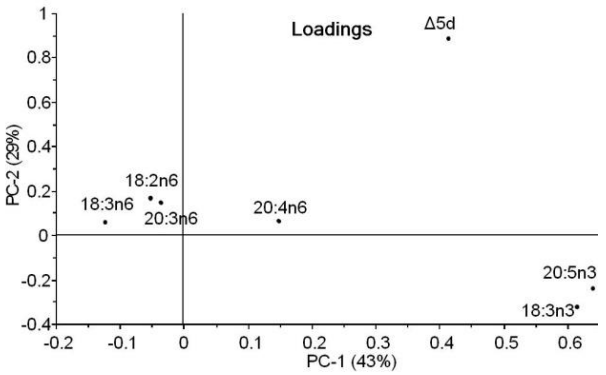

Figure 2. PCA loading plot of associations between Δ5d protein expression and LC-PUFA in diaphragm.

than n-6 PUFA accounting for most of the variance in cheek (Fig. 1) and diaphragm (Fig. 2), being slightly stronger for 20:5n-3 over 18:3n-3 in both muscles. This suggests Δ5d protein expression is increased by n-3 PUFA and influences the polar lipid profile beyond what can be attributed to essential fatty acid concentration alone. No clear associations between Δ6d protein expression and PUFA were apparent from the PCA plots (*figures not shown*).

IV. CONCLUSION

Oilseeds effectively altered the polar lipid profile of the muscles, with linseed significantly increasing the n-3 LC-PUFA content. Both Δ5d and Δ6d proteins are present in cheek and diaphragm; however the present dietary n-6/n-3 range was insufficient to elicit a clear effect on expression. The stronger association between enzyme expression and muscle n-3 LC-PUFA suggests some effect of diet and influence of desaturases on the LC-PUFA profile of cheek and diaphragm.

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