INFLUENCE OF PROTEIN SOURCE ON LAMB MUSCLE FATTY ACID PROFILE AND RAPID QUANTIFICATION OF c9,t11-CLA

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Abstract – This study investigated the effects of protein supplements on m. *longissimus dorsi* fatty acid profiles. Crossbred lambs were fed a barley-based diet without (CON) or with peas (PEA), rapeseed cake (RC) or hempseed cake (HC). There was a tendency towards higher total polyunsaturated (P<0.06) and n-6 (P<0.09) fatty acids, largely owing to the 18:2n-6 content with the HC diet. Muscle 22:6n-3 was highest for the HC diet (P<0.01), with the RC diet not different than the HC diet. Muscle *t*11-18:1 content did not differ between diets, however *t*10-18:1 and total *trans*-18:1 was lower for the RC diet. The CON and PEA diets increased muscle *anteiso* fatty acids (P<0.01), indicative of a shift towards a more amylolytic microbial population, which may also have in part been linked to differences in the trans fatty acid profile. Overall, the RC diet resulted in the healthiest muscle fatty acid profile for human consumption, but differences were rather limited, and its practical relevance would need to be further investigated.

Key Words – hempseed cake, rapeseed cake trans-fatty acid

I. INTRODUCTION

Intensive finishing of lambs using cereal-based diets generally requires protein supplementation to meet animal nutritional requirements. In Sweden, shifting economics and environmental concerns support the use of locally grown protein sources in place of imported soybeans. Peas are a suitable substitute for soybean meal in lamb diets, with potential for lowering the n-6/n-3 fatty acid ratio in meat [1]. Rapeseed and hempseed cakes are also high in protein, and can be sources of n-3 polyunsaturated fatty acids (PUFA). Limited amounts of dietary PUFA by-pass the rumen, with most unprotected PUFA undergoing extensive biohydrogenation. Biohydrogenation intermediates, namely *t*11-18:1 and *c*9,*t*11-conjugated linoleic acid (CLA), have received wide attention due to purported health effects [2]. However, high levels of rapidly fermentable starch in cereal-based diets can alter rumen microflora and biohydrogenation pathways, causing a shift from *t*11- to *t*10-18:1 production [3]. Such a shift could have harmful health repercussions as *t*10-18:1 reportedly has negative effects on blood cholesterol profiles in animal studies [4]. Objectives of the present study were to determine the impact of feeding pea, rapeseed or hempseed cake on lamb m. *longissimus dorsi* fatty acids, and to evaluate a new method for measuring major CLA isomers using an ionic gas chromatography (GC) column

II. MATERIALS AND METHODS

Animal diets

Lambs (87 ± 9 days of age) were divided into four groups and finished on a barley-based diet that included 100g hay/kg diet on a dry matter basis. Pea (PEA, n=6), rapeseed cake (RC, n=6) or hempseed cake (HC, n=5) were substituted for barley to increase protein in experimental diets to 16%, and these were compared to a non-supplemented control diet (CON, n=5). Further description of animal diets and growth performance are reported by Karlsson and Martinsson [5]. At slaughter, m. *longissimus dorsi* samples were collected and frozen at -80°C until analyzed.

Fatty acid analysis

Feed samples were ground through a 1mm screen and directly methylated following the method of Sukhija and Palmquist [6]. Feed fatty acid methyl esters (FAME) were analyzed by GC according to Dugan *et al.* [7]. Muscle samples were extracted following the method of

Hara and Radin [8] and methylated using 0.5N sodium methoxide and 3N methanolic HCl as described by Cruz-Hernandez *et al.* [9]. Muscle FAME were analysed by GC and Ag^+-high performance liquid chromatography (Ag^+-HPLC) under conditions described by Cruz-Hernandez *et al.* [3], except *trans*-18:1 isomers, which were analyzed according to Kramer *et al.* [10]. For comparison purposes, major CLA isomers (*t*7,*c*9- and *c*9,*t*11-18:2) were also analyzed using an ionic GC column at 145°C as described by Turner *et al.* [11].

Statistical analysis

Fatty acid data were analysed using a one-way ANOVA with diet as the main effect and individual animal as the experimental unit using the Proc Mixed procedure of SAS v9.2 (Statistical Analysis System (SAS), Cary, NC, USA). The relationship between *t*7,*c*9-CLA as a proportion of combined *t*7,*c*9- and *c*9,*t*11-CLA when analyzed by conventional GC/Ag^+-HPLC, or GC by a 30m SLB IL-111 ionic column was also measured using the Proc Reg and Proc Corr procedures of SAS v9.2.

III. RESULTS AND DISCUSSION

Lamb m. *longissimus dorsi* lipid content for the respective diets was: CON 3.9 g/100 g, PEA 4.2 g/ 100 g, RC 3.6 g/ 100g and HC 3.7 g/ 100g, and did not differ between diets (P=0.67, SEM=0.39). Protein supplements had only minor effects on the fatty acid profile of m. *longissimus dorsi*. Unprotected PUFA in the oilseed cakes were likely extensively biohydrogenated (Table 1), however, there was a tendency for the HC diet to increase the PUFA content of muscle (P<0.06, Table 2). Feeding the HC diet tended (P<0.09) to increase 18:2n-6 and total n-6 in muscle, owing to the higher 18:2n-6 content of hempseed cake. The percentage of muscle 22:6n-3 was higher when feeding the HC diet (P<0.01) compared to the CON and PEA diets, and levels when feeding the RC diet were not different compared to HC and PEA diets. Poor production performance when feeding hempseed cake [5] would likely overshadow any beneficial effects on muscle PUFA profile. The CON and PEA diets increased the muscle *anteiso* fatty acid content (P<0.01), likely as a result of higher starch levels causing shifts towards more amylolytic bacteria in the rumen microflora [12]. Although diets did not affect muscle *t*11-18:1, the *t*10-18:1 content was highest for the PEA diet, and lowest for the RC diet. Lower dietary starch and PUFA in the RC diet likely contributed to the lower total *trans*-18:1 in muscle (P<0.01). Similarly, the percentage of muscle *t*10,*c*12-18:2 was lowest for the RC diet (P<0.05), whereas the HC diet resulted in higher muscle non-conjugated/non-methylene interrupted dienes, due to higher dietary PUFA and possibly relating to the presence of 18:3n-6 and 18:4n-3 in hempseed.

Table 1 Nutrient composition[z] and fatty acid profiles of control (CON) or pea (PEA), rapeseed cake (RC) and hempseed cake (HC) protein supplemented diets.

	CON	PEA	RC	HC
Metabolisable energy (MJ/kg DM)	13	13.4	13.4	12.2
Crude protein (CP, % DM)	11.2	16.1	16.2	16.0
Starch (% DM)	52.9	48.7	38.1	40.2
Fat (% DM)	2.6	2.2	6.3	4.7
Neutral detergent fibre (% DM)	18.5	15.7	20.2	23.8
Acid detergent fibre (% DM)	9.7	10	13.1	15.6
Fatty acid profile (% FAME)[y]				
16:0	23.6	19.9	11.8	14.4
18:0	1.3	2.2	1.6	2.1
*c*9-18:1	12.5	17.8	44.0	11.2
18:2n-6	55.7	52.2	33.6	56.0
18:3n-6	-	-	-	2.2
18:3n-3	6.9	8.0	9.0	13.4
18:4n-3	-	-	-	0.7
18:2n-6/18:3n-3	8.0	6.5	3.7	4.2

[z] Feed table adapted from Karlsson and Martinsson (2011).
[y] FAME- fatty acid methyl esters.

The contents of the major CLA isomers in muscle (*t*7,*c*9-18:2 and *c*9,*t*11-18:2) were not affected by dietary protein source. Methods for their analysis using an ionic GC column versus 100m polar GC/Ag^+- HPLC were in strong agreement (P<0.001, r=0.97) and similar to that found for analysis of beef subcutaneous tissue [13]. Using the ionic GC column for analysis of major CLA isomers in lamb could, therefore, reduce analysis time from over 80 minutes to less than 15, and negate the need for combined GC /Ag^+- HPLC analysis.

Table 2 Effect of control diet (CON) or pea (PEA), rapeseed cake (RC) and hempseed cake (HC) protein supplemented diets on the fatty acid profile (% FAME) of lamb m. *longissimus dorsi.*

	CON		PEA		RC		HC		SEM	P value
ΣSFA	45.5		44.4		41.3		42.4		2.80	0.72
16:0	23.4		23.7		22.1		23.0		1.48	0.88
18:0	16.3		15.2		13.8		14.7		1.24	0.56
ΣMUFA	44.5		46.2		48.2		44.9		2.29	0.66
*c*9-18:1	37.6		39.0		41.2		37.4		2.06	0.55
*t*10-	0.86	ab	1.10	a	0.64	b	0.94	ab	0.11	<0.05
*t*11-	0.72		0.65		0.52		0.71		0.06	0.13
Σ *trans*-	2.27	a	2.45	a	1.71	b	2.51	a	0.16	<0.01
*c*9,*t*11	0.31		0.29		0.27		0.33		0.04	0.72
*t*7,*c*9	0.032		0.035		0.025		0.038		0.005	0.31
*t*10,*c*12	0.004	ab	0.002	bc	0.002	c	0.004	a	0.001	<0.05
Σ non-conjugated	0.93	b	0.88	b	0.91	b	1.09	a	0.05	<0.05
Σ*iso*	0.76		0.71		0.65		0.77		0.04	0.16
Σ*anteiso*	0.74	a	0.68	a	0.61	b	0.58	b	0.03	<0.01
PUFA/SFA	0.18		0.17		0.20		0.26		0.03	0.11
ΣPUFA	7.81		7.14		8.33		10.59		0.85	0.06
18:2n-6	4.19		3.77		4.49		5.84		0.55	0.09
18:3n-3	0.69		0.55		0.61		0.78		0.09	0.37
20:4n-6	0.83		0.83		1.06		1.33		0.16	0.12
20:5n-3	0.14		0.14		0.19		0.19		0.02	0.25
22:5n-3	0.32		0.29		0.35		0.43		0.04	0.19
22:6n-3	0.06	c	0.07	bc	0.11	ab	0.14	a	0.01	<0.01
Σn-6	5.23		4.79		5.79		7.48		0.72	0.09
Σn-3	1.20		1.05		1.26		1.54		0.15	0.17
n-6/n-3	4.43		4.85		4.73		4.82		0.52	0.94

Difference letters (^{a,b,c}) within row indicate statistical difference (P<0.05).

Table adapted from Turner *et al.* (2012).

ΣSFA = 14:0+ 16:0+ 17:0+ 18:0+ 19:0+ 20:0+ 21:0+ 22:0+ 24:0; Σiso=15:0iso+ 16:0iso+ 17:0iso+ 18:0iso; Σanteiso= 15:0ante iso+ 17:0 ante iso; ΣMUFA= t9-+ c9-14:1+t10-+ c9-15:1+ t6/t8-+ t9-+ t11/12-+ c7-+ c9-+ c11-+ c13-16:1 + c5-+ c9-17:1+ t6/t8-+ t9-+ t10-+ t11-+ t12-+ t13/t14/c6/c8-+ t15-+c9-+ c12-+ c13- + c14-+ t16-+ c15-+ c16-18:1+ c7-19:1+ c11-20:1+ c13-22:1; Σ trans-t6/8-+ t9 +t10-+ t11-+ t12-+ t13/14-+ t13/t14/c6/c8-+ t15+ t16-18:1; ΣPUFA = 18:2n-6+ 18:3n-3+ conjugated and non-conjugated isomers+ 18:3n-6+ 20:2n-6+ 20:3n-6+ 20:4n-6+ 20:5n-3+ 22:4n-6+ 22:5n-3+ 22:6n3; Σn-6 = 18:2n-6+ 18:3n-6+ 20:3n-6+ 20:4n-6+ 22:4n-6. Σn-3 = 18:3n-3+ 20:5n-3+ 22:5n-3+ 22:6n-3.

IV. CONCLUSION

The lower starch in combination with lower 18:2n-6 content of the RC diet reduced the content of *t*10-18:1 and total *trans*-18:1 in muscle, without affecting the *t*11-18:1 or *c*9,*t*11 CLA content. There were some indications feeding the RC or HC diets could increase muscle 22:6n-3 and lower *anteiso* content. Overall, the RC diet resulted in the healthiest muscle fatty acid profile for human consumption, but differences were rather limited, and the practical relevance would need to be further investigated.

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