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Feeding protected fish oil to increase n-3 PUFA of kidney tissue in rams

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Abstract
In recent years awareness of the importance of the fatty acid composition of ruminants meat and milk products has increased, with a particular emphasis on *n*-3 polyunsaturated fatty acids, for example, eicosapentaenoic acid (EPA; C20:5 *n*-3) and docosahexaenoic acid (DHA; C22:6 *n*-3), as these can have beneficial effects on animal performance and reduce the risk of coronary heart disease in humans. The aim of this study was to investigate the effects of feeding dietary protected fish oil (FO) and Green lipped mussel of (GLM) on lipid composition of kidney tissue. Twelve Suffolk rams with a mean age 7.0 months ± 15 days with an average live weight of 93 (±3.5) kg were divided randomly into four groups of 3 rams per group and housed individually. The rams were randomly allocated to each of foure fatty acid (FA) sources; Megalac (M), Megalac with Green lipped mussel (MGLM), Fish oil (FO) and Fish oil + Green lipped mussel (FOGLM). Rams offered fish oil and fish oil with GLM diets contain higher level of C20:5 *n*-3 and C22:6 *n*-3 (p<0.001) in the kidney tissue compared to rams offered on the Megalac diets. In conclusion, dietary GLM, FO and FOGLM altered the fatty acid composition of lipids of kidney tissue of rams.

Keywords: ram kidney, dietary fish oil, green lipped mussel, kidney tissue fatty acid profile

1. Introduction
It is well known that lipid in red meat are one of the major sources of saturated fatty acids (SFA) which affect human health predisposing consumers various kinds of cardiovascular diseases [1, 2]. Increasing the concentrations of *n*-3 PUFA in ruminant meat products can be achieved by supplementing animal diet with fish meal/fish oil, linseed/linseed oil or forages [3, 4]. Studies in which ruminant animals were fed unprotected sources of eicosapentaenoic acid (EPA; C20:5 *n*-3) and docosahexaenoic acid (DHA; C22:6 *n*-3) have demonstrated high levels of biohydrogenation in the rumen [5, 6, 7] and it was recommended by [8] that some form of protection of PUFA against the effects of microorganisms in the rumen was required. The aim of this study was to investigate the efficacy of dietary fat sources evaluated for their susceptibility to biohydrogenation to improving the lipid composition of kidney tissues of Suffolk rams. Also, to evaluate the susceptibility to rumen biohydrogenation of the *n*-3 PUFA contained in the Bionovate GLM powder®.

2. Material and Methods
2.1. Diets
Four diets were formulated to provide a similar fat level (50 g/kg) from different fat sources as shown in Table 1. The rams were fed a 70:30 basal diet of haylage and concentrate. On a fresh weight basis, the rams consumed 1 kg of concentrate (86% DM) and 3 kg of haylage per day. The four treatment diets were prepared by supplement the basal die as follows:

2.1.1 Megalac (M): Megalac (a calcium soap of palm oil: Volac Ltd.UK) contained 36g/kg which is high in palmatic acid (C16:0), a saturated fatty acid.

2.1.2 Megalac + GLM (MGLM): Megalac with GLM powder contained 35g/kg Megalac with 6g/kg Bionovate GLM Powder®. Bionovate GLM Powder® is produced using a unique, cold extraction process, resulting in a supplement that is high in protein and glycosaminoglycan-rich carbohydrates. These characteristics of the product may confer greater resistance to rumen biohydrogenation compared to other PUFA supplements.

2.1.3 Fish oil (FO): Fish oil adsorbed onto a vermiculite matrix, (Trouw UK Ltd, Nantwich, Cheshire) contained 65 g/kg protected fish oil (Trouw UK Ltd, Nantwich, Cheshire) its specification: 50% oil, C20:5 *n*-3 (165 g/kg, C22:6 *n*-3 (110 g/kg).

2.1.4 Fish oil+GLM (FOMGL): Fish oil with GLM powder a mixture of 61g/kg protected fish oil and 6g/kg GLM. The novel fat supplement (Bionovate GLM Powder®) and protected fish oil supplied preformed long chain *n*-3 PUFA, C20:5 *n*-3 and C22:6 *n*-6.

2.2. Animals and managements
Twelve pedigree Suffolk rams with a mean age 7.0 months ± 15 days with an average live weight of 93 (±3.5) kg were divided randomly into four groups of 6 rams per group and housed individually.

Table 1: Raw material of experimental diets containing different fat sources and the daily intake of fatty acids.

	Megalac	Megalac+GLM	Fish oil	Fish oil+GLM
Ingredients (g/kg DM) Concentrate	273	272	266	264
Haylage	691	687	673	669
Megalac	36	35	0	0
GLM powder	0	6	0	6
Protected fish oil	0	0	61	61
Fatty acid intake (g/d)				
C18:3 *n*-3	3.2	2.9	2.2	2.6
C20:4	2.2	2.2	2.2	2.2
C20:5 *n*-3	0.2	0.4	1.8	2.1
C22:6 *n*-3	n.d.	0.2	0.6	0.7

Ns: not detected

2.3. Samples taken at slaughter

At the end of the experiment, rams (three rams per treatment) were fasted for 18 hours and taken to the abattoir. They were subsequently killed by electric stunning followed by exsanguination. Kidney tissue samples were collected for fatty acid analysis. Kidney tissue samples were stored at -20 °C until used for lipid extraction and analysis of fatty acids methyl esters.

2.4 Gas Liquid Chromatography of Fatty Acid Methyl Esters (FAME)

Fatty acid methyl esters were analyzed by gas chromatography using a Hewlett Packard HP 6890 plus GC, an Agilent 7683 series auto injector and equipped with a Varian CPSl88 fused silica capillary column (100 x 0.25mm film thickness). Helium was used as a carrier gas at a constant flow rate of 0.5/min and injection was used. The oven temperature was at 160 ºC then programmed to increase gradually from 160 °C to 220 °C at a rate of 1.5 °C/min, hold for 10 min then increase from 220 °C to 230 °C at a rate of 5.0 °C/min. The fatty acids were identified by comparison with a marine FAME reference mixture (Restec, Dorset, UK). Fatty acids were identified on the basis of their retention time within the capillary column. Data was collected on a Varian workstation and the % area below each of the peaks was calculated and expressed as a % of total peak area. Quantities of individual fatty acids were calculated as g/kg of total fatty acids using the peak areas.

2.5 Statistical analysis

A simple one way Analysis of Variance Comparison of kidney tissue PUFA content between treatments was by one-way ANOVAs using (Genstat 9, Lawes Agricultural Trust).

3. Results

3.1. Diet fatty acid composition

The composition of experimental diets and the intakes of fatty acids are presented in Table 1.

Fatty acid composition for supplement diets containing different fat sources are presented in Table 2. The Megalac diets contained the highest concentrations of palmitic acid C16: 0). The fatty acid composition of C16:0 in rams offered the Megalac diet was 35.0 g/100g TFA; this was approximately two times greater than values for the fish oil and fish oil with GLM powder diets. The mean of C20:5 *n*-3 were 0.4, 0.9, 3.2 and 3.2 g/100gTFA for the Megalac, Megalac with GLM powder, fish oil and fish oil with GLM powder diets respectively. The fatty acid composition of the C22: 6 *n*-3 by rams receiving the Megalac with GLM, fish oil and fish oil with GLM powder diets were on average 0.4, 1.1, and 0.1 g/100gTFA respectively, whilst their intakes were negligible for the Megalac supplemented diets.

Table 2: Fatty acid composition for supplement diets containing different fat sources.

Fatty Acid Concentrations (g/100g TFA)	Megalac	Megalac + GLM	Fish oil	Fish oil + GLM
C16:0	35.0	31.4	13.1	12.5
C20:5 *n*-3	0.4	0.9	3.2	3.2
C22:6 *n*-3	ns	0.4	1.1	1.0
Total fatty acids (mg/g)	38	39	48	57

Ns: not detected

3.2. Fatty acid composition of kidney

The data showing lkidney fatty acid profiles in rams offered *n*-3 PUFA are displayed in Table 3. Rams offered the Megalac and Megalac with GLM diets contained higher levels of C16:0 (p=0.001) compared to rams offered on either the fish oil or fish oil with GLM diets. Rams offered fish oil and fish oil with GLM diets contain higher level of C20:5 *n*-3 and C22:6 *n*-3, (p<0.001) in the kidney compared to rams offered on either the Megalac or Megalac with GLM diets.

Table 3: Effects of dietary fat source on the fatty acid composition (g/100g total fatty acids) content of kidney fatty acid of Suffolk rams (n = 3)

	M	MGLM	FO	FOGLM	SED	P
FA composition (g/100g TFA)						
C16:0 (palmitic)	16.7[a]	15.3[a]	11.6[b]	11.1[b]	0.52	0.001
C18:1 *n*-9 (Oleic)	18.25 [a]	12.7 [a]	5.4[b]	7.5[b]	2.24	0.002
C20:5 *n*-3 (Eicosapentaenoic acid)	1.0[a]	4.0[a]	16.2[b]	16.7[b]	1.86	< 0.001
C22:6 *n*-3 (Docosahexaenoic acid)	1.3[a]	2,8[b]	4.9[c]	5,6[c]	0.46	< 0.001

SED = standard error of the difference

4. Discussion

Fish oils are a rich source of *n*-3 PUFA, C20:5 n-3 and C22:6 n-3. Strong evidences suggest that *n*-3 PUFA reduces cardiovascular diseases risk and this is partly mediated by its potent triglyceride-lowering effects [9, 10], despite a wealth information regarding the effect of *n*-3 PUFA on different organ in mammalian species, there are relatively no data regarding levels of fatty acids in kidney tissues [11].

Reported that increases in longer chain *n*-3 PUFA in muscle phospholipid occur at the expense of *n*-9 monounsaturated fatty acids. This may explain observations in our study where a significant decrease in C18:1 *n*-9 fatty acids were observed in the phospholipid fraction of kidney tissue of rams fed diet containing any *n*-3 PUFA supplements compare to those offered Megalac diet. In our study, compared to Megalac, GLM, FO and FOGLM supplemented rams had higher concentration of C20:5 *n*-3 and C22:6 *n*-3 level. Most human studies [12, 13, 14, 15] and animal studies [16, 17, 18] show similar results.

5. Conclusion

The dietary Green lipped musszle and Fish Oil increase significantly the C20:5 *n*-3 and C22:6 *n*-3 in kidney tissue.

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