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Original article

The Influence of Dietary Lipid Source on Renal Adipose Tissue Fatty Acid Composition in Suffolk Rams

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Abstract
This study investigated the influence of dietary lipid source on the fatty acid (FA) composition of kidney (perirenal) adipose tissue in Suffolk rams. The objective was to evaluate the efficacy of replacing a palm-based protected fat (Megalac, high in palmitic acid) with a protected fish oil supplement, with or without the inclusion of Green Lip Mussel (GLM) extract, to reduce the content of less desirable saturated fatty acids (SFAs) and enhance the deposition of beneficial long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFAs; EPA and DHA). Twelve rams were randomly allocated to four dietary treatments: (1) Megalac (SFA control), (2) Megalac + GLM, (3) Fish Oil, and (4) Fish Oil + GLM. After the feeding period, kidney fat samples were analyzed for FA composition. The principal finding was that dietary lipid sources significantly altered the tissue FA profile. Rams fed fish oil-based diets exhibited a substantial reduction ($P < 0.01$) in the proportion of palmitic acid (C16:0) compared to those fed Megalac-based diets. However, the enrichment of kidney fat with LC n-3 PUFAs was minimal; only docosahexaenoic acid (DHA) showed a small but significant increase ($P < 0.03$) in fish oil groups, while eicosapentaenoic acid (EPA) remained at trace levels. The inclusion of GLM extract did not consistently enhance LC n-3 PUFA deposition. The fraction of "remaining fatty acids" was significantly higher ($P < 0.05$) in the fish oil groups, suggesting the accumulation of minor or unidentified FAs. In conclusion, protected fish oil supplementation effectively reduced the proportion of nutritionally unfavorable C16:0 in rams' kidney fat but was largely ineffective in achieving substantial enrichment with EPA and DHA, highlighting the persistent barrier posed by ruminal biohydrogenation. These results indicate that future strategies to improve the FA profile of ruminant products require more effective methods to bypass rumen metabolism, potentially through combined approaches involving advanced protection technologies and specific rumen modifiers .
Keywords. Kidney Fat, Dietary Lipids, Fish Oil, Ruminal Biohydrogenation, Omega-3 Fatty Acids.

Introduction

The nutritional manipulation of livestock diets is a fundamental practice in animal science, aimed at improving animal health and product quality [1]. Specifically, the fatty acid (FA) composition of kidney fat (perirenal adipose tissue) is a critical determinant of meat quality attributes, including its nutritional value (e.g., omega-3 content) and oxidative stability [2]. While it is well-established that dietary lipids can influence tissue FA profiles, the specific effects of marine-based oils (rich in long-chain omega-3 FAs like EPA and DHA) versus plant-based fats (rich in C18 polyunsaturated FAs like linoleic and α-linolenic acid) in ruminants, such as sheep, require further elucidation due to the complex and extensive biohydrogenation processes in the rumen, which differentially modify these lipid sources before absorption [3].

The fat profile of ruminant meat and adipose tissue is typically characterized by a high proportion of SFAs, such as Palmitic acid (C16:0) and Stearic acid (C18:0), resulting in a low polyunsaturated to saturated (P/S) ratio [4]. High dietary intake of certain SFAs, including C16:0, has been associated with less favorable cardiovascular health outcomes; reducing these specific FAs in animal products is a desirable nutritional goal [5]. Conversely, long-chain omega-3 PUFAs, such as Eicosapentaenoic acid (EPA; C20:5 n-3) and Docosahexaenoic acid (DHA; C22:6 n-3), are indispensable for human health, playing crucial roles in cardiovascular function, cognitive development, and the regulation of inflammatory processes [6]. Consequently, there is a strong incentive to develop nutritional strategies that can effectively increase the deposition of these beneficial FAs in edible animal tissues. The primary biological hurdle in modifying the FA profile of ruminants is the process of ruminal biohydrogenation (BH). In the rumen, microorganisms extensively hydrogenate dietary unsaturated fatty acids, converting them into more saturated forms before they can be

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absorbed by the host animal [7]. This process is highly efficient, leading to the limited transfer of dietary PUFAs, especially the highly desirable long-chain omega-3 FAs found in marine sources, to the animal's tissues. To circumvent this limitation, researchers have explored various dietary interventions, including the use of protected fat supplements and marine-derived oils, which are rich in EPA and DHA. Fish oil is a well-established source of long-chain omega-3 PUFAs, which have been widely investigated as a feed supplement to enrich ruminant products [8]. However, the efficacy of this strategy is often constrained by the extent of ruminal BH. Furthermore, the use of novel feed additives, such as Green Lip Mussel (GLM) extract, which is also a source of marine lipids and has anti-inflammatory properties, presents an alternative or complementary approach to dietary manipulation [9]. The combined effect of these supplements on the specific FA profile of internal adipose depots, such as kidney fat, which serves as a significant metabolic reservoir, warrants detailed investigation.

The present study was designed to evaluate the impact of replacing a conventional protected fat source (Megalac) with fish oil, with or without the inclusion of Green Lip Mussel extract, on the fatty acid composition of kidney fat in Suffolk rams. Based on the known effects of marine oils, we hypothesized that the fish oil-based diets would successfully modify the kidney fat profile by reducing the concentration of less desirable SFAs and facilitating the deposition of beneficial long-chain omega-3 PUFAs, despite the challenge of ruminal biohydrogenation.

Material and Methods

Animal and diets

Twelve Suffolk rams with a mean age of 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. This study was designed to investigate the main effect of different dietary fat sources, Megalac, Megalac + GLM، fish oil, and fish oil with GLM, on the fatty acid composition of kidney fat in Suffolk rams. The central hypothesis was that these distinct lipid supplements would lead to significant and measurable differences in the tissue's FA profile.

The four trial treatments were created by supplementing this basal diet as follows:

1. Megalac Diet: This diet served as a saturated fatty acid (SFA) control, supplemented with 36 g/kg of Megalac (Volac Ltd., UK), a calcium soap of palm acid oil known to be high in palmitic acid (C16:0). This treatment was intended to provide a baseline for comparison against the more unsaturated and novel supplements.
2. Megalac + GLM Diet: This diet was supplemented with 35 g/kg Megalac and 6 g/kg of a novel, glycosaminoglycan-rich powder (Bionovate GLM Powder). The inclusion of GLM aimed to test its potential to confer greater resistance to rumen biohydrogenation due to its unique, cold-extracted composition, high in protein and complex carbohydrates.
3. Fish Oil Diet: This diet was designed to directly supply preformed long-chain n-3 PUFAs by incorporating 65 g/kg of a protected fish oil adsorbed onto a vermiculite matrix (Trouw UK Ltd). This supplement was specified to be rich in eicosapentaenoic acid (C20:5 n-3; 165 g/kg) and docosahexaenoic acid (C22:6 n-3; 110 g/kg).
4. Fish Oil + GLM Diet: This treatment combines 61 g/kg of the protected fish oil with 6 g/kg of the GLM powder. It was formulated to evaluate any potential synergistic or additive effects between the preformed marine n-3 PUFAs and the rumen-protective properties of the GLM supplement on the resulting kidney fatty acid profile.

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

Variables	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)				
C16:0	15.3	14.3	7.3	8.3
C18:2 *n*-6	7.2	7.1	14.9	16.8
C18:3 *n*-3	3.2	2.9	2.2	2.6
C20:5 *n*-3	0.2	0.4	1.8	2.1
C22:6 *n*-3	n.d.	0.2	0.6	0.7

n.s.: not detected

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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. Carcass tissue samples were collected for fatty acid analysis from the kidney fat tissue. Tissue samples were stored at -20 °C until used for lipid extraction and analysis of fatty acid methyl esters.

Gas Chromatographic Analysis of Fatty Acid Methyl Esters (FAMEs)

Fatty acid methyl esters were analyzed using a Hewlett-Packard HP 6890 Plus gas chromatograph equipped with an Agilent 7683 series auto-injector and a Varian CP-Sil 88 fused silica capillary column (100 m × 0.25 mm, film thickness 0.20 µm). Helium served as the carrier gas at a constant flow rate of 0.5 ml/min, with splitless injection. The oven temperature program initiated at 160°C, increased to 220°C at a rate of 1.5°C/min, held for 10 minutes, and then ramped to 230°C at 5.0°C/min.

Fatty acids were identified by comparing retention times with those of a commercial marine FAME reference standard (Restec, Dorset, UK). Data acquisition and processing were performed using Varian workstation software. Individual fatty acid peaks were quantified based on the percentage of total peak area, and results were expressed as g per kg of total fatty acids.

Statistical analysis

A one-way analysis of variance (ANOVA) was used to compare kidney fat tissue PUFA content among the different treatments (Genstat 9, Lawes Agricultural Trust).

Results

The fatty acid (FA) composition of kidney fat in Suffolk rams was significantly altered by dietary lipid source, while total FA content remained unaffected (Table 1).

Saturated Fatty Acids (SFAs)

Palmitic acid (C16:0) was the most responsive SFA, with its proportion being significantly lower ($P < 0.01$) in rams fed diets containing fish oil (FISH OIL and FISH OIL + GLM) compared to those fed Megalac-based diets. In contrast, the proportions of myristic (C14:0) and stearic (C18:0) acids were not significantly influenced by dietary treatment.

Table 2. Effects of dietary fat source on the fatty acid composition (g/100g total fatty acids) of kidney fat fatty acid of Suffolk rams (n= 3)

FA composition (g/100g TFA)	M	M+GLM	FO	FO + GLM	sed	P
C14:0 (myristic)	1.8	1.9	1.8	1.6	0.16	*NS*
C16:0 (palmitic)	25.0	23.2	20.1	19.6	1.26	*0.007*
C16:1 (palmitoleic)	0.7	0.8	1.0	1.4	0.21	*0.03*
C18:0 (stearic)	30.9	30.3	32.7	32.4	0.94	*NS*
C18:1 *n*-9 (Oleic)	22.6	21.8	19.5	14.3	2.94	*NS*
C18:2 *n*-6 (linoleic)	2.6	3.0	2.0	2.0	0.29	*0.03*
C18:3 *n*-3 (Linolenic)	0.6	0.9	0.9	1.0	0.10	*NS*
C20:5 *n*-3 (Eicosapentaenoic acid)	0.0	0.01	0.01	0.02	0.01	*NS*
C22:6 *n*-3 (Docosahexaenoic acid)	0.0	0.01	0.1	0.02	0.02	*0.03*
Remaining fatty acids	11.0	15.1	21.1	26.5	3.73	*0.05*

NS = not significant, SED = standard error of the difference, GLM = Green Lip Mussel Extract

Monounsaturated Fatty Acids (MUFAs)

Palmitoleic acid (C16:1) showed a significant dietary effect ($P < 0.05$), with the highest value observed in the FO+ GLM group. Oleic acid (C18:1 n-9) exhibited a numerical decline in groups receiving FO+ GLM, although this trend was not statistically significant.

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Polyunsaturated Fatty Acids (PUFAs)

Among the n-6 PUFAs, linoleic acid (C18:2 n-6) was present in a higher proportion in the Megalac + GLM group compared to the fish oil groups ($P < 0.05$). For n-3 PUFAs, α-linolenic acid (C18:3 n-3) was not significantly altered. The long-chain n-3 PUFAs, eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3), were detected only in trace amounts. Nevertheless, DHA showed a small but statistically significant increase ($P < 0.05$) in the fish oil-fed groups.

Remaining and Total Fatty Acids

The fraction of "remaining fatty acids" was significantly greater ($P < 0.05$) in the kidney fat of rams fed fish oil diets, suggesting an accumulation of minor or unidentified FAs from the marine lipid source. Despite these compositional shifts, the total fatty acid concentration (mg/g tissue) did not differ significantly among dietary treatments.

Discussion

The principal finding of this study is that the fatty acid (FA) composition of renal adipose tissue in Suffolk rams can be significantly modified by the dietary lipid source, corroborating the central hypothesis. Specifically, the replacement of a palm-based protected fat (Megalac) with a protected fish oil supplement led to a substantial reduction in the proportion of palmitic acid (C16:0) and a concurrent, though modest, increase in the deposition of docosahexaenoic acid (DHA). However, the pronounced biohydrogenation (BH) activity of the rumen remained a significant barrier, as evidenced by the only trace-level incorporation of the target long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFAs), eicosapentaenoic acid (EPA), and DHA.

The significant reduction in the proportion of C16:0 in the kidney fat of rams fed fish oil diets (Table 1) is a nutritionally favorable outcome. High dietary intake of C16:0 is consistently associated with adverse cardiovascular health outcomes in humans [10]. This reduction likely reflects the lower dietary intake of C16:0 from the fish oil compared to the palm-acid-oil-based Megalac (Table 2) and suggests a degree of successful dilution or alteration of de novo lipogenesis in the adipose tissue. This aligns with previous work demonstrating that dietary FA profiles can directly influence tissue saturation levels in ruminants, even when ruminal BH is active [11]. The lack of significant change in other major saturated fatty acids (SFAs) like C18:0 further indicates that the dietary effect was specific and not a blanket alteration of all SFA depositions.

The most critical, yet challenging, objective of supplementing ruminant diets with marine oils is to enhance the tissue content of beneficial LC n-3 PUFAs. In the present study, while DHA showed a statistically significant increase in fish oil groups, its absolute concentration remained exceedingly low (<0.1 g/100g total FA). EPA was virtually undetectable. This starkly contrasts with the substantial dietary intake of these FAs provided via the protected fish oil supplement. This outcome underscores the enduring efficiency of the ruminal BH process in saturating dietary unsaturated FAs before they reach peripheral tissues [12]. Recent reviews emphasize that even with protected lipid technologies, the escape of LC PUFAs from complete BH is often partial and variable, leading to inconsistent tissue enrichment [13]. Our results support this view, demonstrating that while protection strategies can facilitate some bypass, the rumen ecosystem remains a formidable filter.

The inclusion of Green Lip Mussel (GLM) extract, hypothesized to confer greater rumen protection, did not produce a consistent synergistic effect with fish oil on LC n-3 PUFA deposition. The "Remaining fatty acids" fraction, however, was significantly higher in both fish oil groups, particularly in the FO+GLM group. This intriguing result suggests an accumulation of minor or branched-chain FAs, possibly of microbial origin or specific marine-derived FAs not quantified in the standard profile (e.g., odd-chain FAs, C20:1, C22:1). Recent metabolomic studies have highlighted that marine lipid supplements can introduce a complex array of minor FAs into the ruminant system, which may have biological significance but are often overlooked in conventional GC analysis [14]. The higher "remaining FA" fraction warrants further investigation with more comprehensive analytical techniques.

The significant increase in palmitoleic acid (C16:1) and the numerical decrease in oleic acid (C18:1 n-9) in fish oil-fed rams may reflect shifts in stearoyl-CoA desaturase (SCD) activity or altered substrate availability for Δ9-desaturation. SCD is a key enzyme converting SFAs to MUFAs. Dietary marine oils, rich in PUFAs, have been shown to modulate SCD gene expression and activity in ruminant tissues, potentially suppressing the conversion of C18:0 to C18:1 [15]. This could explain the observed trends and merits of exploration at the molecular level in future studies.

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Conclusion

The study confirms that dietary lipid source is a potent modulator of kidney fat composition in Suffolk rams. While protected fish oil successfully reduced the proportion of less-desirable palmitic acid, its efficacy in substantially reducing tissue with EPA and DHA was limited, highlighting the persistent challenge of ruminal BH. The novel GLM supplement did not markedly enhance this enrichment under the conditions of this trial but may have influenced the deposition of minor fatty acids. For future strategies aiming to create ruminant products with genuinely enhanced LC n-3 PUFA content, a multi-faceted approach combining advanced lipid protection technologies, perhaps with targeted dietary modifiers of rumen pH (e.g., specific tannins, enzyme inhibitors [16], appears necessary. Furthermore, assessing the impact of these FA profile changes on the oxidative stability and sensory attributes of the derived meat is an essential next step for practical application.

Conflict of interest. Nil

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