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Degummed crude canola oil supplementation affects fat depot melting points in purebred and first-cross Merino sheep

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Abstract: The objective of this study was to test the hypothesis that degummed crude canola oil (DCCO) will lower fat melting points (FMP) of both visceral and subcutaneous fats in lambs. Twenty-four lambs comprising purebred and first-cross Merino progeny from Dorset, White Suffolk and Merino sires mated to purebred Merino ewes were supplemented with varying levels of DCCO over a nine-week period. The experimental treatment groups were: Control (1kg plain wheat-based pellets only), Medium (500g plain wheat-based pellets + 500g wheat-based pellets containing DCCO), and High (1kg wheat-based pellets containing DCCO at a concentration of 50ml/kg) supplementation levels. The flock comprised eight wether and ewe lambs per treatment. However, at the end of the trial, four Merino ewes were retained in the flock for breeding purposes, while the remaining twenty lambs were slaughtered in a commercial abattoir. Visceral fat samples were taken from the kidney region and subcutaneous fat samples were taken from the *Longissimus dorsi* muscle. FMP was determined using temperature slip point methodology in the laboratory. DCCO had significant effects on the FMP of both subcutaneous ($p < 0.0002$) and visceral ($p < 0.0001$) fats, with the lowest FMP achieved at high levels of supplementation in both fat depots. Significant sire breed differences ($p < 0.0001$) were also detected in which Dorset-sired progeny had the highest melting points in both fat depots. The results of this study indicate that within fat depots, DCCO supplementation produced softer fats with lower melting points, suggesting potentially healthier fats likely to contain higher levels of unsaturated fatty acids.

Keywords: Degummed Crude Canola Oil, Fat Melting Point, Subcutaneous Fat, Visceral Fat, Sire Breed

1. Introduction

Fat melting point (FMP) is the key determinant of fat hardness or softness. The lower the melting point, the softer the fat and vice-versa. From a production viewpoint, hard fats on animal carcasses present a processing challenge in the boning room of meat processing plants [1, 2]. Softer

fats are preferable as they are easier to trim or remove by hand, thereby improving workplace occupational health and safety conditions. Nutritionally, softer fats contain higher levels of unsaturated fatty acids, whereas harder fats contain higher levels of saturated fatty acids [3-5]. Unsaturated fatty acids are preferred in human diets as they aid in the prevention of rheumatoid inflammation and

cardiovascular diseases [6]. However, from a meat quality perspective, softer fats may be more prone to oxidation compared to harder fats, thereby initiating the onset of rancidity in meat, which can have subsequent adverse impacts on shelf life, colour and flavour [3-5].

Of late, there is increasing interest in the inclusion of lipids as alternative, energy-dense supplementary feed sources in the diet of ruminants [7]. Published literature has established that when lipids are included in sheep diets, they can have sequential effects on fatty acid compositions [8-12] and therefore, the melting points of fats. However, there is currently a knowledge gap on the use of degummed crude canola oil (DCCO) as a high energy, unsaturated fatty acid supplementary feed source in sheep diets and its impact on fat melting point.

In this study, we tested the hypothesis that *supplementation of sheep with degummed crude canola oil lowers the melting points of both visceral and subcutaneous fat depots*. The objective of this study was to assess the impact of dietary DCCO supplementation on the melting points of visceral and subcutaneous fat depots in genetically divergent sheep.

2. Materials and Methods

2.1. Animals and Management

This experiment was conducted at the University of Tasmania Farm, Cambridge, Hobart, Tasmania, Australia. All procedures were approved by the University of Tasmania Animal Ethics Committee and conducted in accordance with the 1993 Tasmanian Animal Welfare Act and the 2004 Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Twenty-four purebred and first-cross lambs, weaned at six months of age, were subjected to a nine-week feeding trial using 3 levels of DCCO supplementation: Control (1kg plain wheat-based pellets only), medium (500g plain wheat-based pellets + 500g wheat-based pellets containing DCCO), and high (1kg wheat-based pellets containing DCCO at a concentration of 50ml/kg). Lambs were sired by Dorset, White Suffolk and Merino rams crossed with pure bred Merino ewes, consisting of eight lambs per genotype comprising four ewes and four wethers. All supplementation levels were replicated within each genotype and sex. During the trial, all experimental animals were given *ad libitum* access to lucerne hay and water. The nutritional composition of the experimental diets is given in Table 1. Four Merino ewes were retained in the flock for breeding purposes, while the remaining twenty lambs were slaughtered at a commercial abattoir. Visceral fat samples from the slaughtered animals were taken from the kidney region and subcutaneous fat from the *Longissimus dorsi* muscle between the 12th-13th rib interface.

2.2. Determination of Fat Melting Points

Fat melting points (FMP) were determined in triplicates

using five grams of fat from each depot from each animal. These fat samples were melted in an oven set at 105°C for 1 hour and drawn up into open-ended capillary tubes using air suction. Samples were placed in a fridge to enable the fats to solidify. Prior to analysis, sub-samples were removed from refrigeration, the fat level marked with an indelible pen on the capillary tube and attached to a 100°C thermometer, measuring at 1°C unit intervals. This was suspended vertically, submerged in a beaker filled with 80mL of distilled water and placed on a hot plate set at a medium heating level. The submerged capillary tube was then observed, with the slip point of the fat defined as the temperature at which the lipid was observed to have melted and ‘slipped’ within the capillary tube. The temperature at which this occurred was then recorded.

Table 1. Composition of experimental diets as % of dry matter

Component	Crude degummed canola oil- enriched pellets (50ml/kg)	Basal (Plain) wheat-based pellets	Lucerne hay
Dry Matter	91.8	90.9	85.6
Crude Protein	12.7	10.4	17.0
Acid Detergent Fiber	8.0	9.0	44.9
Neutral Detergent Fiber	20.0	21.1	55.2
Total Digestible Nutrients	75.7	72.0	55.4
Ash	9.7	8.9	6.8
Crude Fat	6.2	2.1	

2.3. Statistical Analysis

Statistical analyses of data were performed using the Statistical Analysis System (SAS 2009). Summary statistics of fat melting points by sire breed, sex and supplementation level were computed within each fat depot as part of the data editing procedure to identify any possible outliers using PROC MEANS. The General linear model procedure (PROC GLM) in SAS was then utilized for multi-trait analysis of variance fitting the fixed effects of sire breed, sex, supplementation level and their second-order interactions on subcutaneous and visceral depot fat melting points. Non-significant interactions were dropped from the final model. Pairwise comparisons and mean separations were carried out using Tukey’s test at a minimum threshold of $p < 0.05$ level of significance.

3. Results

3.1. Effect of DCCO Level, Sire Breed and Sex on Subcutaneous and Visceral Fat Depot Melting Points

Within fat depots, level of supplementation with DCCO had significant effects on the melting points of both subcutaneous ($p < 0.0002$) and visceral ($p < 0.0001$) fats (Table 2). As shown in Table 2, the lowest melting point for subcutaneous fat was observed in the *High* level of supplementation ($41.6 \pm 0.3^\circ\text{C}$)

compared to the *Control* ($42.0 \pm 0.2^\circ\text{C}$) and *Medium* ($42.0 \pm 0.4^\circ\text{C}$) supplementation levels. Similarly, visceral FMP was lower at *High* supplementation ($45.3 \pm 0.4^\circ\text{C}$) compared to *Control* ($46.0 \pm 0.2^\circ\text{C}$) and *Medium* ($46.4 \pm 0.4^\circ\text{C}$) supplementation levels.

Fat melting points were also significantly influenced by sire breed ($p < 0.0001$) in both fat depots as depicted in Table 2. Fats from lambs sired by Dorset recorded the highest melting points for both subcutaneous fat ($42.8 \pm 0.2^\circ\text{C}$) compared to White Suffolk ($41.0 \pm 0.3^\circ\text{C}$) and Merino ($41.8 \pm 0.4^\circ\text{C}$), and visceral fat ($46.9 \pm 0.2^\circ\text{C}$) compared to White Suffolk ($45.2 \pm 0.2^\circ\text{C}$) and Merino ($45.7 \pm 0.5^\circ\text{C}$). There were no significant differences ($p > 0.05$) in melting points between ewes and wethers for both subcutaneous and visceral fat depot sites.

3.2. Effect of Level of DCCO Supplementation, Sire Breed and Sex Interactions on Subcutaneous and Visceral Fat Depot Melting Points

Sire breed and supplementation interactions were observed to significantly impact the FMP of both subcutaneous ($p < 0.0001$) and visceral ($p < 0.0001$) fat depots (Table 3).

Table 2. Least square means and standard errors (LSM \pm SE) of subcutaneous and visceral fat depot melting points ($^\circ\text{C}$) of degummed crude canola oil supplemented prime lambs.

Fixed effect	Fat depot	
	Subcutaneous	Visceral
<i>Level of supplementation</i>		
Control	42.0 ± 0.2^a	46.0 ± 0.2^a
Medium	42.0 ± 0.4^a	46.4 ± 0.4^a
High	41.6 ± 0.3^b	45.3 ± 0.4^b
p-value	0.0002***	0.0001***
<i>Sire breed</i>		
Dorset	42.8 ± 0.2^a	46.9 ± 0.2^a
White Suffolk	41.0 ± 0.3^b	45.2 ± 0.2^b
Merino	41.8 ± 0.4^c	45.7 ± 0.5^b
p-value	0.0001***	0.0001***
<i>Sex</i>		
Ewe	41.9 ± 0.3	46.0 ± 0.2
Wether	41.8 ± 0.2	45.9 ± 0.3
p-value	0.9752 ^{ns}	0.7653 ^{ns}

Column means within a fixed effect bearing different superscripts significantly differ ($p < 0.05$). Level of significance: ns not significant ($p > 0.05$) and *** Very highly significant ($p < 0.001$).

There were no significant differences in subcutaneous FMP between treatments within both Dorset and White Suffolk sire breeds. Purebred Merinos exhibited significant differences between treatments - *Control* ($41.17 \pm 0.17^\circ\text{C}$) and *Medium* ($43.63 \pm 0.33^\circ\text{C}$) levels of supplementation, as well as between *Medium* and *High* ($41.00 \pm 0.00^\circ\text{C}$) levels of supplementation. There were no differences between *Control* and *High* supplementation levels for the Merino genotype. Within visceral fat depots, Dorset displayed significant differences between *Control* ($46.22 \pm 0.22^\circ\text{C}$) and *Medium* ($47.56 \pm 0.38^\circ\text{C}$) levels of supplementation,

with no significant differences between *Control* and *High* supplementation or between *Medium* and *High* supplementation levels. White Suffolk sired progeny displayed results comparable to those of Dorset with significant differences between *Control* ($46.00 \pm 0.45^\circ\text{C}$) and *Medium* ($44.67 \pm 0.29^\circ\text{C}$) levels of supplementation, with no differences between other interactions. Purebred Merinos exhibited the greatest degree of differences between FMP in all the three supplementation levels; *Medium* supplementation displayed the highest FMP ($48.00 \pm 0.00^\circ\text{C}$), followed by *Control* ($45.83 \pm 0.17^\circ\text{C}$) and *High* ($43.00 \pm 0.00^\circ\text{C}$).

Supplementation level significantly interacted with sex in both subcutaneous ($p < 0.0001$) and visceral ($p < 0.0171$) fat depot melting points (Table 3). Within ewes, subcutaneous FMP decreased significantly between supplementation levels from $42.44 \pm 0.29^\circ\text{C}$ in the *Control* diet, to $42.00 \pm 0.37^\circ\text{C}$ and $40.83 \pm 0.29^\circ\text{C}$ under *Medium* and *High* supplementation levels, respectively. For visceral fat, there were no significant differences between *Control* ($46.22 \pm 0.22^\circ\text{C}$) and *Medium* supplementation in ewes. However, FMP significantly declined under *High* supplementation ($45.50 \pm 0.67^\circ\text{C}$). There were no significant differences observed in subcutaneous FMP between treatments within wethers. There were no significant differences in visceral FMP between *Controls* ($45.92 \pm 0.23^\circ\text{C}$) compared to either *High* ($46.67 \pm 0.59^\circ\text{C}$) or *Medium* treatments ($45.25 \pm 0.45^\circ\text{C}$), FMP between *Medium* and *High* supplementation were observed. There were no significant differences ($p > 0.05$) in melting points for sex and sire breed interactions for either fat depot (Table 3).

Table 3. Subcutaneous and visceral fat depot melting points ($^\circ\text{C}$) interactions between sire breed, DCCO supplementation level and sex in prime lambs supplemented with DCCO.

		Fat depot	
Interaction		Subcutaneous	Visceral
Sire	<i>Supplementation Level</i>		
	Control	43.0 ± 0.0	46.2 ± 0.2^a
	Medium	42.9 ± 0.4	47.6 ± 0.4^b
	High	42.3 ± 0.2	46.8 ± 0.2^{ab}
White Suffolk	Control	41.2 ± 0.2	46.0 ± 0.5^a
	Medium	40.6 ± 0.4	44.7 ± 0.3^b
	High	41.2 ± 0.2	45.1 ± 0.4^{ab}
	Control	41.2 ± 0.2^a	45.8 ± 0.2^a
Merino	Medium	43.7 ± 0.3^b	48.0 ± 0.0^b
	High	41.0 ± 0.0^a	43.0 ± 0.0^c
	p-value	<0.0001***	<0.0001***
Sex	<i>Supplementation Level</i>		
	Control	42.4 ± 0.3^a	46.2 ± 0.2^a
	Medium	42.0 ± 0.4^b	46.0 ± 0.4^a
	High	40.8 ± 0.8^c	45.5 ± 0.7^b
Wether	Control	41.6 ± 0.3	45.9 ± 0.2^a
	Medium	42.0 ± 0.6	46.7 ± 0.6^b
	High	41.9 ± 0.2	45.3 ± 0.5^{ac}
	p-value	<0.0001***	0.0171*
Sire breed		Sex	

Interaction		Fat depot	
		Subcutaneous	Visceral
Dorset	Ewe	43.0±0.1	46.6±0.3
	Wether	42.6±0.3	47.2±0.3
White Suffolk	Ewe	40.8±0.3	45.3±0.3
	Wether	41.2±0.4	45.0±0.3
Merino	Wether	41.8±0.4	45.7±0.5
p-value		0.2924 ^{ns}	0.0784 ^{ns}

Column means within a second-order interaction bearing different superscripts significantly differ ($p < 0.05$). Level of significance: ns not significant ($p > 0.05$), * significant ($p < 0.05$) and *** Very highly significant ($p < 0.001$).

4. Discussion

The results of this study demonstrate that within fat depots, DCCO supplementation has the potential to produce softer fats with lower melting points, particularly at high levels of supplementation. This supports our hypothesis that *DCCO supplementation will lower the melting points of both visceral and subcutaneous fat depots in sheep*. The resulting fat softness induced by increased supplementation, indicates that fats from lambs supplemented with DCCO would be easier for meat processors to work with, thereby reducing the incidence of work-related injuries. Furthermore, our findings suggest that fats from sheep supplemented with DCCO have the potential to produce higher concentrations of unsaturated fatty acids in edible fats which are beneficial for human health. However, softer fats containing higher concentrations of unsaturated fatty acids may induce meat oxidation [5]. Therefore, it is reasonable to infer that the observed lower melting points with increasing supplementation levels could have subsequent impacts on meat shelf-life, colour and flavour which could adversely affect meat eating quality and tallow-based products.

Previous studies have recognised that dietary unsaturated fats can extensively impact on the fatty acid profiles of ruminants with resultant increases in unsaturated fatty acid content in the adipose tissue [12, 14-16]. The lowering of melting points with increased supplementation levels observed in the present study reflects the aforementioned annotations. However, Bas and Morand-Fehr [17], Busboom *et al.* [18] and Miller *et al.* [19] propose that changes in FMP are allied with variances in percentages of the fatty acid C18:0, rather than increases in unsaturated fatty acids *per se*. This may account for the observed lower FMP associated with increased supplementation levels herein. Therefore, given that varying compositions of fatty acids can occur, further research assessing the fatty acid composition of adipose tissues from lambs supplemented with degummed crude canola oil is recommended.

The research findings of Malau-Aduli *et al.* [20]; Pitchford *et al.* [21] and Siebert *et al.* [22] demonstrated that ruminant FMP can be affected by sire breed. The findings herein, support such established observations. The differences displayed between sire breeds in the current research

indicates that first-cross progeny from Dorset sires are prone to developing fats with high FMP compared to other genotypes, regardless of anatomical site of deposition. This suggests that potentially higher levels of saturated fatty acids which are less susceptible to oxidation and more favourable to maintaining shelf-life stability and meat quality attributes in tallow-based products for longer periods, are in abundance in Dorset-sired progeny than in the other genotypes studied. Conversely, the relatively lower melting points displayed in Merino and White Suffolk genotypes indicates higher proportions of unsaturated fatty acids compared to Dorset. These observations were unanticipated, particularly in Merinos, given previously published literature by Fogarty *et al.* [23] and Wiese *et al.* [24] to the effect that the Merino is a comparatively late-maturing breed than others, with a predisposition for leanness, suggesting indirectly that higher FMP would be observed amongst the purebred Merino studied. Nevertheless, given the fact that fat deposition increases with animal age [25], it can be argued that purebred Merino may well have exhibited its propensity for leanness, and hence, displayed higher melting points than the Dorset meat breed if the animals were older.

Variations in FMP between treatments within genotypes in the present study indicate that the deposition of fatty acid types in subcutaneous and visceral fat depots varies between genotypes. The observed variations in subcutaneous and visceral fat depot melting points in Merinos under varying levels of DCCO supplementation suggests that this genotype is likely more susceptible to changes in dietary fatty acid composition compared to other genotypes. However, the lack variation in subcutaneous FMP between treatments within Dorset and White Suffolk lambs indicates that subcutaneous fat depots are either less susceptible to influxes of unsaturated fatty acids in their diet, or that subcutaneous fat depots are later maturing in these genotypes compared to the Merino. This points to the likelihood that differing levels of dietary fatty acids are more readily diverted into other metabolic processes between genotypes, such as incorporation into the phospholipid component of muscles [26], or intramuscular fat content [27]. However, this requires further investigation.

It is understood that the fats of uncastrated male lambs (rams) are softer than those of castrated lambs (wethers) [28] and females (ewes) [29, 30], thereby exhibiting lower melting points with higher concentrations of unsaturated fatty acids. In the present study, a lack of significant difference between the sexes in terms of fixed effects or sire breed interactions indicates negligible differences between the sexes regarding fat stability and meat quality. The observed lack of sex differentiation supports Warriss [31] who reported that castration eliminates the anabolic effects of male sex hormones in wethers, which in turn, results in wethers producing a carcass composition similar to those of ewes. However, the results herein demonstrated that within sexes, FMP can be influenced by the inclusion of degummed crude canola oil in the diet, thereby affecting meat quality parameters.

5. Conclusion

This study demonstrates that within fat depots, degummed crude canola oil supplementation has the potential to produce softer fats with lower melting points, particularly at high levels of supplementation. These fats would be easier for meat works employees to process, thereby reducing the potential for work-related injuries to occur. Furthermore, the observed lower melting points indicate that there are possibly higher proportions of unsaturated fatty acids beneficial for human health. This study also demonstrates clear and identifiable differences in FMP between sire breeds, and that supplementation with DCCO can affect FMP within sire breed. This will enable prime lamb producers to make informed decisions regarding the use of DCCO in their flock. However, given the potential for variation in fatty acid composition, future work should involve a fatty acid analysis, assessing the nutritional content of fats and meat cuts from sheep fed degummed crude canola oil. This is the next step we are taking in our laboratory to unravel the fatty acid composition of adipose and muscle tissues of lambs supplemented with varying levels of DCCO.

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Competing Interests

The authors declare that they have no competing interests.

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