Journal of Animal & Plant Sciences, 35(5): 2025, Page: 1223-1234
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2025.5.0104

IMPACT OF VARYING FIBER INTAKE AND PARTICLE SIZE ON THE PROPORTION OF FAT DEPOTS AND THE FATTY ACID PROFILE IN NAIMI LAMBS

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ABSTRACT

The Naimi sheep is one of the main breeds in Saudi Arabia, characterized by a thick tail and substantial fat deposits around the intestines. This study aimed to evaluate the influence of fiber type (alfalfa hay vs. wheat straw) and feed particle size (FPS) on the rate of fat deposition in in three major internal depots: tail fat (FT), omental fat (OF), and kidney knob channel fat (KKCF). Thirty-six weaned male Naimi lambs were divided into four treatments of 9 lambs each. At the end of the feeding trial, lambs were slaughtered and their internal fat depots removed to measure fat volume and fatty acid (FA) profiles. Lambs fed small sized wheat straw (WS2), had the lowest carcass fat weight (23.65 kg), whereas lambs fed uncrushed wheat straw (WS1) and uncrushed alfalfa hay (AH1) showed the highest fat percentages (WS1: 30.23 %; AH1: 28.92 %). Feeding hay alfalfa as a source of fiber to lambs had a significant effect on the levels of omega-3 (n-3) and α- linoleic acid (ALA) levels in in FT, OF and, KKCF. Conversely, WS1 increased the levels of stearic acid and odd-chain fatty acid (OCFA). In contrast, the feed with small sizes as AH2 and WS2 significantly elevated linolenic acid (LA), total polyunsaturated fatty acids (PUFA) and Omega 6 (n-6) in all three depots. Quality indices revealed that lambs fed alfalfa hay and wheat straw had lower atherogenicity index (AI) and thrombogenicity index (TI) indices in the fat tail compared to OF and KKCF. Moreover, the high ratio of hypercholesterolemic FA (hFA) to hypocholesterolmic FA (HFA) resulted in better values for fat tail (1.50), omental fat (1.01) and KKCF (1.26), which do not indicate any risk for human health. Thus, both the source and size of fiber particles had a significant influence on total fat deposition and FA profiles in lambs. Feeding strategies can be tailored by adjusting fiber source and particle size to modulate ruminal volatile fatty acid (VFA) and internal fat deposition.

Keywords: Naimi lambs; fatty acid profile; Omental fat; kidney knob channel fat; fat tail, fiber type, fiber particles size.

Published first online July 22, 2025 Published final September 30, 2025

INTRODUCTION

Most sheep breeds in Asia, Africa, and a some Middle Eastern countries are characterized by a fat tails and high fat content (Atti *et al.*, 2004). Among these, Naimi sheep are the most numerous and popular breed among consumers in Saudi Arabia. They are characterized by a large tail and accumulate high levels of internal fat in tissues such as the stomach and kidneys. Tis fat particularly tail fat, which accounts up to 25 % of the carcass weight, significantly affects carcass quality (Suliman *et al.*, 2021). Although sheep with fat tails are known to be highly resistant to, harsh environmental, conditions and to seasonal weight loss, there is no experimental evidence supporting this claim (Scanlon *et al.*, 2013). Sheep have an extraordinary capacity to accumulate body fat, particularly in females compared to males (Wallace *et al.*, 2020). Fat deposition is divided into subcutaneous, intermuscular, intramuscular, and cavitary (pelvic and renal), and visceral (pericardial, omental or omental and mesenteric). Adipose tissue content and composition may vary depending on species, age, gender, diet, anatomical location, and environmental conditions (Wallace *et al.*, 2020). From a commercial point of view, subcutaneous and intermuscular fatty deposits are valuable in early growth stages of lambs, when deposits are relatively small; however, their value declines once deposition exceeds optimum levels. Pelvic fat is used as an attribute for commercial classification of the carcass, whereas the intramuscular fat affects meat quality rather than carcass grade. Because pelvic fat weight is strongly correlated with total carcass fat, it serves as an index of carcass fattening (Brand *et al.*, 2018).

The rumen acts as natural bioreactor for breaking down fibers in feed with rumen microbes playing a key role in this vital process that generate end

products (Ceconi *et al.*, 2015; Wang *et al.*, 2020). including volatile fatty acids (VFA), which are subsequently converted to long-chain fatty acids (LCFA) and deposited in tissues (Brzozowska *et al.*, 2016; Wang *et al.*, 2022). The VFA profile influences types of fatty acids deposited in tissues (Alstrup *et al.*, 2016). VFA proportions depend on feed fiber type, source, and size (Ribeiro *et al.*, 2015; Bharanidharan *et al.*, 2018), which in turn influences rumen bacterial activity (Zhang *et al.*, 2017). As well as the percentage of each fiber content (Neutral detergent fiber (NDF) and acid detergent fiber (ADF)), their nutritional value and fermentation properties (Holt *et al.*, 2010) and rumen activity dynamics (Fernando *et al.*, 2010). Thus, VFAs are major determinants of meat tissue fatty acid composition (Brzozowska *et al.*, 2016). Considering current trends, high fat-to-muscle ratios remain a concern for consumers globally (Garrido *et al.*, 2023). This issue arises because most sheep breeds, particularly in the Middle East have thick tails (Razzaque and Mohammed, 2010) and tend to accumulate fat subcutaneously, intramuscularly, viscerally (Li *et al.*, 2024; Liu *et al.*, 2024), and in the tail (Zeng *et al.*, 2020). The tail is among the largest fat-containing organs, Suliman *et al.* (2021) reported tail fat weights of 11.59 kg, 13.78 kg, and 12.64 kg for the Awassi, Harri, and Najdi breeds, respectively. Differences in FA deposition proportions are dependent on several factors, including genetics, breed, gender, and nutritional factors, as well as individual variation (Wood *et al.*, 2008). However, the most important factor is feed, including fiber type and source, particle size, fiber content, vitamins and minerals, and dietary fat (Matar *et al.*, 2020; Queiroz *et al.*, 2021). The fatty acid composition of Naimi lamb adipose tissues remains largely uncharacterized. Because animals exposed to fiber naturally mobilize their fat depots in the tail, lipolysis increase and deposition decreases. We hypothesized that this mobilization would also alter fatty acid composition. This study aimed to evaluate the influence of fiber source (alfalfa hay vs. wheat straw) and particle size on fatty acid proportions in the major fat depots of growing Naimi lambs specifically tail, omental, and kidney knob channel fat.

MATERIALS AND METHODS

Management and Design: Thirty-six post-weaning Naimi lambs (mean body weight 22.5 ± 0.75 kg), were selected and evaluated for body weight, clinical health status and live weight.

The study lasted for three months from December through February. The lambs were divided into four groups of nine and housed under stable conditions as follows diagram:

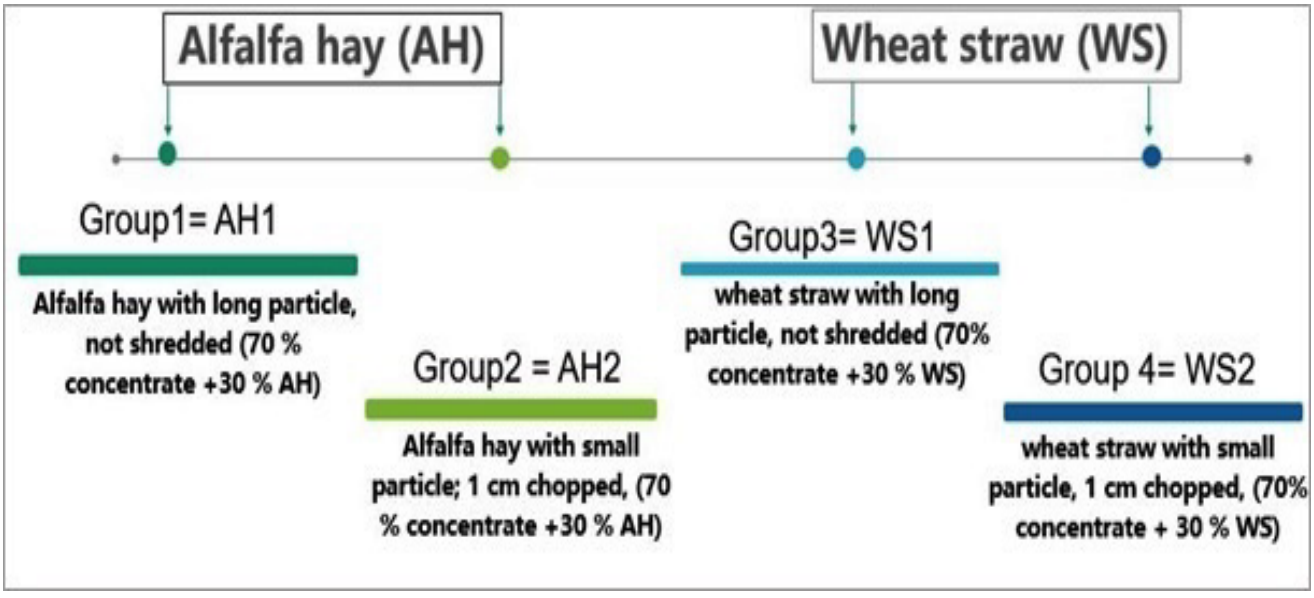

Table 1. list the ingredients of the experimental diets. Animals were fed individually twice a day and given *ad libitum* access to water.

Animal Slaughter and Sampling: At the end of the study, three lambs from each treatment group were fasted for 16 hours before slaughter at the Al-Diriyah State slaughterhouse, located approximately 5 km from the farm. Animals were weighed before and after slaughter and all internal organs and omental fat, KKCF and tail fat of lambs were weighted and stored on ice, and transported to the Animal Production Department, KSU until analysis as previously described by Balevsca *et al.* (1967).

Table 1. Ingredients and chemical composition of the experimental diets.

Item, unit	Dietary treatments[1]	
	WS	AH
Ingredients, % of dietary DM		
Alfalfa hay	-	30.0
wheat straw	30.0	-
concentrate %	70.0	70.0
Nutrient composition, DM basis		
Dry Matter, (%)	90.1	89.9
Ash, %	7.23	7.17
Crude Protein, %	13.52	14.12
Ether Extract, %	1.64	1.89
Neutral Detergent Fiber, % (NDF)	34.67	30.01
Acid Detergent Fiber, % (ADF)	11.78	15.81
Metabolizable energy, Mcal/kg	2.84	2.95
Lipid Profile, %		
C8:0	0.59	0.62
C10:0	0.69	0.77
C12:0	12.01	12.62
C14:0	5.31	5.07
C16:0	12.63	11.42
C16:1 cis 9	0.13	0.11
C17:0	0.07	0.08
C18:0	3.02	2.92
C18:1 cis 9	25.20	25.80
C18:1 cis 11	0.68	0.72
C18:2 cis9 cis12	35.08	36.62
C18:3 cis9 cis12 cis15	2.93	3.68
Saturated fatty acids (SFA)	32.13	29.10
Monounsaturated fatty acids (MUFA)	25.76	33.60
Polyunsaturated fatty acids (PUFA)	43.86	37.50

[1] Alfalfa hay chopped (AH2), while alfalfa hay without chopped (AH1), was added to the concentrate in a ratio of 70:30. In addition, wheat straw chopped (WS2), or wheat straw without chopped (WS1), was added into concentrate in a ratio of 70:30.

Sample Collection and Analysis:

Extraction and methylation of lipids: Fat from internal organs (tail, omental and Kidney renal knob fat) was collected post slaughter and stored at −80 ℃ until analysis. Total lipids were extracted in duplicate each depot. Fatty acid profile was determined by a methylation method, modified from Palmquist and Jenkins (2003) and Jenkins (2010), in which fatty acids are expressed as methyl esters. Briefly, 0.5 g of fat sample were placed into polypropylene tubes, then 3 ml of 0.5 M sodium methoxide in methanol (to protect the unsaturated fatty acids) were added. Tubes were stirred for 1 min and incubated in a 50 ° C water bath for 10 minutes. They were then removed and cooled to 24 ° C for 5 minutes, after which 3 mL of 5% methanolic hydrochloric acid was added and stirred for 1 minute to extract all fat from the samples. Samples were returned to 80 ° C water bath for 10 minutes and were cooled for 7 minutes. Next, 3.5 ml hexane and 5 ml of 6% potassium carbonate were added for fat dissolution and saponification. After vortex for 1 min and centrifuging at 2500 × g for 5 min, the upper hexane layer was transferred to fresh tubes, treated with 0.5 g anhydrous sodium sulfate and 0.1 g activated carbon to remove moisture and color, vortexed, and centrifuged again at 1500 × g for 5 min. The final hexane extract was collected into vials and stored at for chromatographic analysis.

Fatty acid methyl esters (FAME) were extracted using n-hexane and analyzed by mass gas chromatography (GC-MASS) (HP 6890 chromatograph; Hewlett-Packard, Avondale, PA, USA) with a flame ionization detector (FID). Separation of FAME was done on a fused-silica capillary column (100 m, 0.25 mm inner diameter, 0.20 µm film thickness; Chrompack, Varian Inc., Walnut Creek, CA, USA) following Alves and Bessa (2009). Fatty acids identification was carried out by 37-component FAME standard (Supelco, Inc., Bellefonte, PA) and verified by electron impact mass spectrometry with a Shimadzu GC-MS QP2010 Plus (Shimadzu). Individual fatty acids were quantified as grams per 100 grams of total fatty acids.

Nutritional quality was assessing by Atherogenicity index (C12:0 + (4 x C14:0) + C16:0)/ (MUFA + PUFA (n-3: n-6)). Thrombogenicity index (C14:0 + C16:0 + C18:0)/ ((0.5 x MUFA) +(0.5 x PUFA(n-6)) +(3 x PUFA(n-3)) +(n-3/n-6))(Ulbricht and Southgate, 1991). Additionally, the content of hypercholesterolemic FA (HFA) = sum of C12:0, C14:0, and C16:0; Hypocholesterolmic FA (HFA) = C18:1 cis9 + C18:2 n6 + 20:4n6 + C22:5n3 and h/H = (C18:1 cis9 + C18:2 n6 + 20:4n6 + C22:5n3)/ (C14:0 + C16:0) in internal organs fat were measurements according to (Bessa, 1999) and (Santos-Silva *et al.*, 2002)

Statistical analysis: Statistical analysis was performed using SAS (version 9.4, SAS Institute, Cary, NC, USA). Data were analyzed as a 2 × 2 factorial design to test the effects of fiber source (Alfalfa Hay vs. Wheat Straw) and particle size (Fine vs. Coarse particle size), as following

$y_{ijk} = \mu + FT_i + FPS_j + (FT*FPS)_{ij} + \varepsilon_{ijk}$ i = 1,…,*a*; *j* = 1,…,*b*; *k* = 1,…,*n*

where: *yijk* = observation FA in level *i* of fiber type and level *j* of fiber form; μ = the overall mean; *FT* = the effect of fiber type; *FPSj* = the effect of fiber particle size; (*FPS*)*ij* = the effect of the interaction of level *FT* with level *FPS*; εijk = random error with mean 0 and variance σ2; a = number of levels of factor FT; b = number of levels of factor FPS; n = number of observations for each FT x FPS combination.

Analysis of variance was performed using PROC GLM procedures with a 95% confidence interval

($P \leq 0.05$). Treatment means were compared by Duncan's multiple-range test. Results are presented as mean ± SEM, with significance declared at $P \leq 0.05$.

RESULTS

Fat percentage and fatty acids composition of internally deposited fat (omental, kidney knob channel, and tail fat) in Naimi lambs fed two fiber sources (alfalfa hay and wheat straw) and two particle size (non-chopped and chopped) are summarized in Table 2. Overall fat content of Naimi lambs ranges from 23% to 30%. Lambs fed non-chopped alfalfa (AH1) had significantly higher carcass weight and omental fat. ($P \leq 0.05$). In addition, lambs fed WS1 had a higher fat percentage (30.23%), than lambs fed WS2 (23.65) in comparison with the other treatments.

In tail fat, fiber source significantly affected palmitoleic acid (C16:1 cis-7) and α-linolenic acid (C18:3; $P \leq 0.03$), with higher levels in alfalfa-fed lambs (Table 3). Conversely, nonadecanoic acid (C19:0) was higher in lambs fed wheat straw than in lambs fed alfalfa hay ($P \leq 0.01$). A significant effect on LA (C18:2; $P \leq 0.03$), was observed with chopped alfalfa hay. In addition, the fiber source and particle size interaction effect on trans-9.12 linoleic acid (C18:2; $P \leq 0.02$).

Table 4 shows that fiber source significantly affected the classification of fatty acids in tail fat for n-3 and ratio n-3/n-6 ($P \leq 0.03$), but, had no impact on other fatty-acid classes or subdivisions. The use of alfalfa in feed resulted in a 10.11% increase in fat tail of n-3 in comparison with wheat straw. Conversely, lambs fed wheat straw exhibited an n-3:n-6 ratio of 10.67 in tail fat, exceeding recommended levels.

The effect of fiber source and practical size on fatty acid content of the omental fat as shown in Tables 5 and 6. Results showed a significant effect ($P \leq 0.05$) of alfalfa hay as a fiber source on the following fatty acids: C16:0 iso, C19:0 iso, ALA and C20:3, whereas wheat straw had a significant effect ($P \leq 0.05$) C14:1 cis9, C18:1 trans9, C19:0 and odd-chain fatty acid (OCFA). In addition, the proportion of fatty acids in the omental fat was significantly affected by the size of the particle feed which had an AH1 and WS1 significant effect on C10:0 and C16:1 cis9 content, whereas, AH2 and WS2 significant effect on stearic acid (C18:0), PUFA and n6. While, LA, was higher in omental fat in lamb's fad by WS2 ($P \leq 0.05$).

In the same context, the KKCF fatty acid content showed a significant difference in size of the lambs feed by WS2 at C18:1 trans9, C18:2 cis9,12 (LA), PUFA and n6. In contrast, AH1 had a significant effect ($P \leq 0.05$) on OA and MUFA, while AH2 had a significant effect ($P \leq 0.05$) on C18:1 cis 13 and C18:3 cis 6,9,15 (n3- ALA) as shown in Tables 7 and 8. Furthermore, indicate that alfalfa hay had a significant effect on the fatty acid profile ($P \leq 0.05$) such as C20:2 cis11.14, C18:3 cis 6,9,15 (ALA- n3) and n3, whereas wheat straw (WS) had a significant effect ($P \leq 0.05$), on the ratio of n3/n6.

In generally the results focus on the main total FAs and nutrient quality indicators for lambs deposited lipids that are relevant for human health as shown in Table 4, 6 and 8, where source of fiber and practical size feed did not have significant effect on nutrient quality indicators ($P > 0.05$). As observed AI and TI were lower value in tail fat than omental fat and KKCF. In contrast, the levels of hFA and h/H in fat tail were high compared to the omental fat and KKCF in the lambs fed alfalfa hay AH1.

Table 2. Internally deposited fat content and carcass fat percentage of Naimi lambs fed two fiber sources and two levels of particle size feed.

Component kg	Fiber Types				SEM	P value		
	Alfalfa hay		Wheat Straw					
	AH1	AH2	WS1	WS2		Fiber	Size	F*S
Carcass wt.	25.32[a]	22.93[b]	21.02[c]	22.53[b]	0.92	0.03	0.64	0.07
[1]KKC fat	0.25	0.28	0.18	0.27	0.04	0.32	0.19	0.49
Omental fat	0.81[a]	0.63[b]	0.40[c]	0.45[c]	0.12	0.04	0.62	0.39
Mesenteric fat	0.46	0.28	0.31	0.26	0.09	0.38	0.25	0.54
Tail	2.9	2.64	2.85	2.05	0.52	0.55	0.33	0.62
Total Fat %	28.92	28.21	30.23	23.65	2.73	0.56	0.22	0.31

[1]: kidney knob and channel fat; SEM=Standard error of means for treatments effect; AH1: long Alfalfa hay; AH2: chopped alfalfa hay; WS1: long Wheat Straw; WS2: chopped Wheat Straw. Fiber; effect as Alfalfa hay and Wheat Straw - Size; effect of particle size - F*S The combined effect of fiber particle size and fiber source. The superscripts small letters (a-c), the difference in mean of the two fiber types in the rows if considered significant if ($P \leq 0.05$).

Table 3. Influence of fiber source and particle size on fatty acid profile content (g/100g FA) in tail fat of Naimi lambs

FA	Fiber Types				SEM	P value		
	Alfalfa hay		Wheat Straw					
	AH1	AH2	WS1	WS2		Fiber	Size	F*S
C10:0	0.21	0.20	0.23	0.19	0.05	0.98	0.61	0.85
C11:0	0.23	0.15	0.15	0.21	0.06	0.82	0.88	0.36
C12:0	0.48	0.28	0.28	0.34	0.08	0.41	0.38	0.14
C13:0	0.23	0.20	0.16	0.17	0.06	0.52	0.87	0.76
C14:0	6.05	4.37	4.59	4.71	0.56	0.35	0.20	0.15
C15:0 iso	0.15	0.13	0.17	0.11	0.06	0.43	0.85	0.80
C15:0 antiso	0.41	0.38	0.33	0.34	0.04	0.90	0.31	0.59
C14:1 cis9	0.40	0.44	0.36	0.21	0.12	0.30	0.67	0.47
C15:0	1.07	1.48	1.13	1.15	0.22	0.56	0.36	0.41
C16:0 iso	0.16	0.14	0.18	0.24	0.09	0.51	0.83	0.69
C16:1 cis7	0.27^{a}	0.23^{b}	0.19^{c}	0.19^{c}	0.03	0.05	0.51	0.50
C17:0	0.19	0.24	0.18	0.18	0.05	0.53	0.64	0.66
C16:0	27.22	25.09	25.13	24.74	1.25	0.36	0.34	0.51
C17:0 antiso	0.41	0.60	0.61	0.46	0.14	0.85	0.90	0.27
C17:0 iso	0.43	0.38	0.39	0.43	0.04	0.81	0.92	0.26
C16:1 cis9	2.81	3.24	2.96	1.88	0.60	0.34	0.60	0.24
C17:0	2.89	3.51	3.43	3.91	0.36	0.23	0.16	0.85
C18:0 iso	0.13	0.14	0.17	0.13	0.01	0.49	0.23	0.11
C17:1 cis10	1.51	2.07	1.91	1.48	0.45	0.84	0.89	0.30
C18:0	10.08	8.49	9.54	12.60	1.42	0.24	0.61	0.14
C18:1 trans9	0.45	0.42	0.34	0.40	0.12	0.64	0.92	0.74
C19:0	1.74^{c}	2.00^{b}	4.66^{a}	2.83^{b}	0.51	**0.01**	0.19	0.10
C18:1 cis 9 (OA)	36.21	38.15	37.97	37.03	1.62	0.84	0.76	0.40
C18:1 cis 11	1.08	1.37	1.56	1.10	0.19	0.61	0.66	0.09
C18:1 cis 13	0.37	0.47	0.35	0.36	0.08	0.46	0.54	0.64
C19:0 iso	0.10	0.13	0.10	0.09	0.06	0.70	0.89	0.76
C18:2 trans9,12	0.31	0.47	0.39	0.23	0.05	0.18	0.99	0.02
C18:2 trans12,15	0.22	0.21	0.18	0.17	0.04	0.37	0.82	0.99
C18:2 LA	2.82^{B}	3.31^{A}	2.66^{B}	3.29^{A}	0.33	0.79	**0.03**	0.83
C20:2 cis11 cis14	0.25	0.18	0.14	0.13	0.07	0.28	0.54	0.66
C20:0	0.12	0.08	0.11	0.08	0.03	0.85	0.39	0.84
C18:3 cis6,9,15 (ALA)	0.44^{a}	0.49^{a}	0.37^{b}	0.27^{c}	0.05	**0.03**	0.66	0.21
C18:2 cis9, trans11(CLA)	0.84	0.90	0.68	0.67	0.11	0.13	0.83	0.80
C20:4 cis5,8,11,14	0.05	0.07	0.04	0.06	0.02	0.72	0.47	0.95

[1]AH1: long Alfalfa hay; [2]AH2: chopped alfalfa hay; [1]WS1: long Wheat Straw; [2]WS2: chopped Wheat Straw. **Fiber**; effect as Alfalfa hay and Wheat Straw - **Size**; effect of particle size - **F*S** The combined effect of fiber particle size and fiber source. The superscripts small letters (a-c), the difference in mean of the two fiber types in the rows and the capital letters (A-C) in the rows for particle size in the feed if considered significant if *($P \leq 0.05$)*.

Table 4. Influence of fiber source and particle size on classification fatty acid content (g/100g FA) in tail fat of Naimi lambs

FA[1]	Fiber Types				SEM	P value		
	Alfalfa hay		Wheat Straw					
	AH1	AH2	WS1	WS2		Fiber	Size	F*S
SFA	51.96	47.85	49.71	52.59	2.61	0.64	0.82	0.21
UFA	47.93	52.02	50.19	47.31	2.63	0.65	0.82	0.22
MUFA	43.11	46.39	45.64	42.65	2.71	0.83	0.95	0.28
PUFA	4.83	5.63	4.55	4.67	0.37	0.13	0.24	0.38
OCFA	9.15	11.26	11.55	11.23	1.37	0.41	0.53	0.39
n3	0.66^{a}	0.71^{a}	0.58^{b}	0.38^{c}	0.08	0.03	0.37	0.14
n6	3.33	4.01	3.27	3.62	0.31	0.49	0.14	0.61

n3/n6	5.08[c]	5.72[b]	6.02[b]	10.67[a]	1.15	0.03	0.05	0.12
AI	1.12	0.83	0.88	0.93	0.11	0.57	033	0.16
TI	1.27	1.03	1.08	1.08	0.10	0.55	0.28	0.31
hFA	42.94	45.62	44.77	43.56	1.62	0.84	0.58	0.22
HFA	33.75	29.73	30.1	29.79	1.81	0.34	0.28	0.32
hFA/ HFA	1.30	1.55	1.50	1.47	0.12	0.64	0.39	0.29

[1]AH1: long Alfalfa hay; [2]AH2: chopped alfalfa hay; [1]WS1: long Wheat Straw; [2]WS2: chopped Wheat Straw. **Fiber**; effect as Alfalfa hay and Wheat Straw - **Size**; effect of particle size - **F*S** The combined effect of forage particle size and fiber source. The superscripts small letters (a-c), the difference in mean of the two fiber types in the rows and the capital letters (A-C) in the rows for particle size in the feed if considered significant if ($P \leq 0.05$).

Table 5. Influence of fiber source and particle size on fatty acid content (g/100g FA) in omental fat of Naimi lambs

FA	Fiber Types				SEM	P value		
	Alfalfa hay		Wheat Straw					
	AH1	AH2	WS1	WS2		Fiber	Size	F*S
C8:0	0.01	0.01	0.01	0.02	0.002	0.65	0.58	0.23
C10:0	0.18[A]	0.13[B]	0.18[A]	0.12[B]	0.02	0.94	**0.03**	0.81
C12:0	0.53	0.60	0.93	0.67	0.19	0.26	0.64	0.43
C13:0	0.01	0.01	0.03	0.02	0.005	0.06	0.92	0.46
C14:0 iso	0.05	0.05	0.08	0.04	0.01	0.78	0.16	0.25
C14:0	5.54	5.62	7.21	6.01	0.59	0.12	0.36	0.31
C15:0 iso	0.30	0.31	0.40	0.38	0.05	0.12	0.93	0.66
C15:0 antiso	0.18	0.17	0.20	0.19	0.03	0.51	0.73	0.97
C14:1 cis9	0.08[b]	0.07[b]	0.11[a]	0.07[b]	0.005	0.01	0.001	0.03
C15:0	0.53	0.47	0.63	0.51	0.05	0.17	0.11	0.58
C16:0 iso	0.06[a]	0.05[a]	0.03[b]	0.03[b]	0.006	**0.005**	0.39	0.64
C16:1 cis7	0.24	0.19	0.24	0.21	0.02	0.81	0.13	0.64
C16:0	25.45	24.06	26.08	23.17	1.17	0.91	0.10	0.53
C17:0 antiso	0.03	0.04	0.08	0.05	0.01	0.19	0.87	0.55
C17:0 iso	0.43	0.41	0.44	0.47	0.04	0.52	0.95	0.62
C16:1 cis9	1.16[A]	0.97[B]	1.24[A]	0.95[B]	0.07	0.69	**0.01**	0.57
C17:0	2.35	2.37	2.39	2.56	0.08	0.20	0.26	0.40
C18:0 iso	0.13	0.10	0.12	0.10	0.02	0.86	0.26	0.84
C17:1 cis10	0.40	0.35	0.41	0.34	0.03	0.86	0.13	0.82
C18:0	25.78[B]	27.79[A]	23.50[B]	26.44[A]	1.07	0.13	**0.05**	0.67
C18:1 trans9	0.26[b]	0.34[b]	0.50[b]	0.64[a]	0.10	**0.052**	0.36	0.80
C19:0	3.38[c]	3.35[c]	4.64[b]	5.91[a]	0.66	**0.02**	0.37	0.35
C18:1 cis 9 (OA)	26.77	25.33	24.75	23.67	1.45	0.24	0.41	0.90
C18:1 cis 11	0.93	0.94	0.96	1.07	0.04	0.07	0.16	0.28
C18:1 cis 13	0.58	0.66	0.51	0.61	0.06	0.37	0.18	0.82
C19:0 iso	0.22[a]	0.23[a]	0.21[a]	0.10[b]	0.02	**0.01**	0.07	**0.02**
C18:2 trans9,12	0.29	0.16	0.15	0.15	0.02	0.29	0.53	0.63
C18:2 trans12,15	0.09	0.06	0.08	0.07	0.02	0.99	0.37	0.70
C18:2 (LA)	2.79[C]	3.86[B]	2.96[C]	4.32[A]	0.24	0.23	**0.001**	0.57
C20:2 cis11 cis14	0.04	0.04	0.03	0.02	0.01	0.30	0.42	0.71
C20:0	0.16	0.19	0.17	0.17	0.03	0.86	0.58	0.64
C18:3 cis6,9,12(n6)	0.01	0.01	0.02	0.03	0.006	0.06	0.65	0.34
C18:3 cis6,9,15(ALA)	0.46[a]	0.48[a]	0.31[b]	0.40[a]	0.04	**0.03**	0.29	0.49
C18:2 cis9, trans11(CLA)	0,47	0.43	0.37	0.44	0.05	0.42	0.82	0.33
C20:3cis5,8,11	0.03	0.02	0.02	0.03	0.005	0.72	0.57	-
C22:0	0.05	0.03	0.03	-	0.01	0.35	0.44	-
C20:3 cis8,11,140.03	0.03[a]	0.03[a]	0.01[c]	0.02[b]	0.001	**0.03**	0.22	0.54
C20:4 cis5,8,11,14	0.04	0.04	0.04	0.06	0.01	0.47	0.57	0.79
C22:4cis7,10,13,16	0.04	0.02	0.02	-	0.001	0.33	0.42	-

[1]AH1: long Alfalfa hay; [2]AH2: chopped alfalfa hay; [1]WS1: long Wheat Straw; [2]WS2: chopped Wheat Straw. **Fiber**; effect as Alfalfa hay and Wheat Straw - **Size**; effect of particle size - **F*S** The combined effect of forage particle size and fiber source. The superscripts small letters (a-c), the difference in mean of the two fiber types in the rows and the capital letters (A-C) in the rows for particle size in the feed if considered significant if ($P \leq 0.05$).

Table 6. Influence of fiber source and particle size on classification fatty acid content (g/100g FA) in omental fat of Naimi lambs

FA	Fiber types				SEM	P value		
	Alfalfa hay		Wheat Straw					
	AH1	AH2	WS1	WS2		Fiber	Size	F*S
SFA	65.27	65.93	67.31	66.90	1.57	0.37	0.93	0.74
UFA	34.67	34.01	32.63	33.07	1.57	0.36	0.94	0.73
MUFA	30.44	28.01	28.55	27.56	1.51	0.32	0.41	0.84
PUFA	4.23^{B}	5.15^{A}	4.08^{B}	5.51^{A}	0.24	0.69	**0.001**	0.33
OCFA	7.83^{b}	7.75^{b}	9.44^{a}	10.51^{a}	0.66	**0.01**	0.47	0.41
n3	0.61	0.56	0.43	0.47	0.06	0.06	0.98	0.52
n6	3.10^{B}	4.13^{A}	3.24^{B}	4.59^{A}	0.24	0.25	**0.001**	0.53
n3/n6	5.17	7.59	8.96	9.80	1.66	0.11	0.35	0.64
AI	1.41	1.39	1.74	1.45	0.16	0.27	0.37	0.45
TI	1.36	1.32	1.22	1.21	0.07	0.15	0.79	0.82
hFA	32.77	32.41	30.63	31.50	1.50	0.34	0.86	0.69
HFA	31.52	30.28	34.23	29.84	1.52	0.48	0.10	0.33
hFA/ HFA	1.05	1.08	0.90	1.06	0.09	0.36	0.33	0.49

[1]AH1: long Alfalfa hay; [2]AH2: chopped alfalfa hay; [1]WS1: long Wheat Straw; [2]WS2: chopped Wheat Straw. **Fiber**; effect as Alfalfa hay and Wheat Straw - **Size**; effect of particle size - **F*S** The combined effect of forage particle size and fiber source. The superscripts small letters (a-c), the difference in mean of the two fiber types in the rows and the capital letters (A-C) in the rows for particle size in the feed if considered significant if ($P \leq 0.05$).

Table 7. Influence of fiber source and particle size on fatty acid profile content (g/100g FA) in kidney knob and channel fat (KKCF) of Naimi lambs

FA	Fiber Types				SEM	*P value*		
	Alfalfa hay		Wheat Straw					
	AH1	AH2	WS1	WS2		Fiber	Size	F*S
C10:0	0.14	0.11	0.12	0.10	0.01	0.56	0.10	0.66
C12:0	0.37	0.57	0.69	0.69	0.13	0.14	0.49	0.49
C14:0 iso	0.03	0.05	0.04	0.05	0.01	0.66	0.49	0.84
C14:0	4.37	5.32	6.10	5.82	0.67	0.13	0.64	0.40
C15:0 antiso	0.14	0.15	0.18	0.19	0.02	0.12	0.53	0.87
C15:0 iso	0.23	0.37	0.36	0.35	0.05	0.14	0.60	0.45
C14:1 cis9	0.09	0.05	0.10	0.06	0.02	0.70	0.14	0.96
C15:0	0.44	0.43	0.45	0.47	0.06	0.69	0.98	0.89
C16:0 iso	0.04^{b}	0.06^{a}	-	0.03^{c}	0.01	**0.03**	0.13	.
C16:1 cis7	0.19	0.13	0.19	0.20	0.03	0.38	0.43	0.31
C16:0	22.44	22.14	22.93	21.87	1.02	0.91	0.52	0.72
C17:0 antiso	0.05	0.03	0.04	0.04	0.02	0.83	0.61	0.62
C17:0 iso	0.38	0.37	0.38	0.44	0.04	0.33	0.47	0.40
C16:1 cis9	1.25	0.80	0.92	0.76	0.21	0.40	0.18	0.51
C17:0	2.21	2.17	2.14	2.46	0.14	0.45	0.32	0.22
C18:0 iso	0.13	0.12	0.12	0.12	0.01	0.81	0.77	0.54
C17:1 cis10	0.45	0.28	0.32	0.30	0.07	0.49	0.23	0.35
C18:0	26.11	30.65	27.99	30.53	2.79	0.76	0.24	0.73
C18:1 trans9	0.37^{C}	0.47^{B}	0.43^{B}	0.56^{A}	0.04	0.13	**0.02**	0.86
C19:0	3.18	3.62	4.15	4.59	0.81	0.26	0.60	0.99
C18:1 cis 9 (OA)	31.39^{A}	25.05^{C}	27.23^{B}	23.23^{C}	1.89	0.15	**0.02**	0.55
C18:1 cis 11	0.91	0.85	0.92	1.02	0.03	0.03	0.58	0.04
C18:1 cis 13	0.53^{B}	0.68^{A}	0.44^{C}	0.59^{B}	0.07	0.22	**0.05**	0.99
C19:0 iso	0.19	0.25	0.18	0.13	0.04	0.18	0.94	0.28
C18:2 trans9,12	0.21	0.13	0.13	0.11	0.03	0.15	0.16	0.37
C18:2 trans12,15	0.11	0.06	0.08	0.05	0.02	0.50	0.16	0.67
C18:2 cis9,12 LA	2.68^{C}	3.88^{B}	2.83^{C}	4.13^{A}	0.25	0.46	**0.001**	0.79
C20:2 cis11 cis14	0.07^{a}	0.03^{b}	0.02^{c}	0.03^{b}	0.01	**0.01**	0.19	0.02

C20:0	0.19	0.23	0.21	0.23	0.03	0.79	0.34	0.79
C18:3 cis6,9,15(ALA)	0.39^{aB}	0.50^{aA}	0.15^{bC}	0.35^{bC}	0.04	**0.001**	**0.004**	0.29
C18:2 cis9, trans11(CLA)	0.53	0.35	0.32	0.35	0.07	0.24	0.37	0.21
C22:0	0.03	0.02	-	0.02	0.00	0.91	0.51	-
C20:4 cis5,8,11,14	0.02	0.04	0.02	0.05	0.01	0.68	0.07	0.57
C22:4 cis7,10,13,16	0.04	0.03	-	0.03	0.01	0.92	0.66	-

AH1: long Alfalfa hay; AH2: chopped alfalfa hay; WS1: long Wheat Straw; WS2: chopped Wheat Straw. **Fiber**; effect as Alfalfa hay and Wheat Straw - **Size**; effect of particle size - **F*S** The combined effect of forage particle size and fiber source. The superscripts small letters (a-c), the difference in mean of the two fiber types in the rows and the capital letters (A-C) in the rows for particle size in the feed if considered significant if ($P \leq 0.05$).

Table 8. Influence of fiber source and particle size on classification fatty acid content (g/100g FA) and kidney knob and channel fat (KKCF) of Naimi lambs

FA	Fiber Types				SEM	P value		
	Alfalfa hay		Wheat Straw					
	AH1	AH2	WS1	WS2		Fiber	Size	F*S
SFA	60.68	66.56	66.06	68.14	2.05	0.13	0.06	0.38
UFA	39.27	33.38	33.94	31.84	2.05	0.13	0.06	0.38
MUFA	35.22^{A}	28.37^{C}	30.53^{B}	26.74^{C}	2.03	0.16	**0.03**	0.47
PUFA	4.05^{B}	5.00^{A}	3.40^{C}	5.10^{A}	0.32	0.41	**0.003**	0.27
OCFA	7.32	7.66	8.21	8.99	0.80	0.20	0.50	0.79
n3	0.50^{a}	0.57^{a}	0.24^{c}	0.41^{b}	0.06	**0.001**	0.07	0.38
n6	2.99^{B}	4.06^{A}	2.94^{B}	4.31^{A}	0.25	0.70	**0.001**	0.57
n3/n6	6.11^{c}	7.373^{c}	12.23^{a}	10.41^{b}	1.22	**0.005**	0.82	0.24
AI	1.03	1.32	1.44	1.44	0.14	0.08	0.31	0.34
TI	1.85	2.11	1.74	1.96	0.14	0.38	0.12	0.90
hFA	37.27	32.08	32.43	30.52	1.52	0.36	0.86	0.49
HFA	27.19	28.01	29.72	28.37	1.52	0.36	0.86	0.49
hFA/ HFA	1.37	1.15	1.11	1.07	0.08	0.08	0.15	0.29

[1]AH1: long Alfalfa hay; [2]AH2: chopped alfalfa hay; [1]WS1: long Wheat Straw; [2]WS2: chopped Wheat Straw. **Fiber**; effect as Alfalfa hay and Wheat Straw - **Size**; effect of particle size - **F*S** The combined effect of forage particle size and fiber source. The superscripts small letters (a-c), the difference in mean of the two fiber types in the rows and the capital letters (A-C) in the rows for particle size in the feed if considered significant if ($P \leq 0.05$).

DISCUSSION

Diet and genetics highly influence the rate of fat deposition in sheep both in visceral depots and overall carcass(Alfaia *et al.*, 2017). The type of feed as well as the rumen microbiota influence the volatile fatty-acid profiles such as butyric acid, propionic acid, and acetate (Alstrup *et al.*, 2016; Bharanidharan *et al.*, 2018). Although, most studies focused on different fiber and concentrate ratios, few addresses how fiber source or particle size alters visceral fatty-acid composition. Andres *et al.* (2019), found no significant shifts in fatty acid ratios overall; but reported that lambs fed with chopped alfalfa hay had higher saturated fatty acid content (Andres *et al.*, 2019).

This study evaluated four feed regimens with different particle size gradients in Naimi breed reared in Saudi Arabia. Lambs fed WS2 reduced the fat deposition percentage by 21.27% compared to other treated lambs. Overall, total fat in our Naimi lambs while the percentage of total fat was generally higher compared to other breeds in Saudi Arabia, such as the Harri was (22.41%), Najdi (19.5%) and Awassi (19.56%) (Suliman *et al*., 2021), as well as Barbarine breed (17.7 - 26.0%) (Atti and Mahouachi, 2011).

In addition to feed type, the fraction of fatty acids deposited in tissues depends on many different physiological factors. This study showed that both the fiber type and the particle size had an effect on these fatty acids and AI and TI in the tail fat, which may ultimately be associated with consumer health.

Wood *et al.* (2008), reported that adipose tissue contains higher fatty-acid levels than muscle tissue. Li et al. (2024) demonstrated that the balance of proteins and fats in a diet may influence the rate of fat deposition in the internal organs (Li *et al.*, 2024). Moreover, Pewan *et al.* (2021) found that ruminants fed a grain rich diet showed upregulate stearoyl-CoA desaturase activity, promoting fat deposition intramuscularly. Bharanidharan *et al.* (2018) reported that change in the size of feed particles and the amount of roughage to concentrate could reduce methane production in the rumen and increase propionate in the total VFA.

The results of this study showed that feeding alfalfa hay to lambs as fiber source had a significant

effect on omega-3 (n3) and ALA in tail fat, omental fat and KKCF. In contrast, lambs fed WS exhibited elevated levels of stearic acid and OCFA. Particle size of feed as AH2 and WS2 also had a significant influenced fatty-acid profiles, LA, PUFA and Omega 6 (n-6) in all tested fat depots. Reducing feed particles size can also increase rumen passage, reducing fermentation process. This has a detrimental effect on the structure of the rumen fluid and fatty acid synthesis (Castillo-Lopez *et al.*, 2020). Variations in fatty acid content are primarily attributed to fiber source and feed particle size. However, a large proportion of fatty acids remain unchanged; their structure and function may have changed due to biohydrogenation, but this is now dependent on other biological pathways. The short length of fibers and the low proportion in the feed may also alter in the biohydrogenation pathways of vaccenic acid (C18:1trans11) and rumen acid (CLA -18:2 cis9, trans11) which may potentially have adverse health effects for consumers (Bessa *et al.*, 2015; Santos-Silva *et al.*, 2019). In a study by Sun *et al.* (1994), found that differences in the characteristics feed influence the development of internal organs and the weight of the digestive system. Results showed that the use of chopped clover hay increased the fat formation.

In general, the tail fat is a marker of early maturity in lambs and is highly responsive to the type of diet depending on the energy level. This is because the tail fat serves as an energy reserve for sheep, particularly in poor nutrition conditions (Ettoumia *et al.*, 2022).

The amount of fiber consumed by animals affects the development of the rumen and may affect volatile fatty acids (VFA) concentration and thus rate at which long-chain fatty acids are deposition in the body. In sheep, fat percentage and body weight are related; each kilogram of body weight is 0.52% fat, with majority of fat being concentrated in the visceral parts. Acetic acid has been shown to play a crucial role in synthesis of fat, as the pathway for formation CoA is parallel the formation of fat (Liu *et al.*, 2024). Additionally, individual differences in the formation fatty acid can also lead to the formation of propionate. Propionate increases the production of methylated branched fatty acids by 1.7 percent and odd fatty acids (OCFA) by up to 0.7 percent in internal tissues (Berthelot *et al.*, 2001). On the other hand, Bas and Morand-Fehr (2000) reported that changes the fiber content of the diet and the use of grains may alter the fatty acids in lamb carcass.

Another study by Meale *et al.* (2015) found that different sources of fiber and fat supplements in the diet can increase PUFA/SFA levels but do not alter N6/N3. A study, reconfirmed that the use of 40% alfalfa in the diet can reduce the conversion to C18:1 trans10, which affects the biohydrogenation of fatty acids, considering the size of feed particles when feeding lambs (Santos-Silva *et al.*, 2019). It should also be known that small feed particle sizes negatively affect rumen acidity by affecting the fermentation process, which can increase the concentration of polyunsaturated fatty acids (PUFA), and this is the effect of acidity on a type of bacterial communities in the rumen that promotes this conversion (De Nardi *et al.*, 2016). This study confirms that our results were identical when using alfalfa hay and wheat straw with small particles, resulting in increased PUFA. Further results show that the source of fiber in nutritionally comparable diets and the particle size of the fiber can influence the fatty acid profile of lamb meat (Macdonald *et al.*, 2023).

It is noted that all previous studies focused on the fat of the carcass and tail and did not take into account the fat of the viscera, despite (omental fat and KKCF), its consumption and its high proportion in the carcass. For this reason, this part is missing from our discussion due to the lack of studies on these parts.

The occurrence of coronary artery disease, which is mainly caused by atherosclerosis blocking the coronary arteries, is closely related to AI and TI (Wahle and Heys, 2002). The proposed these index aims to better understand the impact of food on human health. High value of AI and TI indicate an increased risk of cardiovascular disease due to lipid intake (Moussavi Javardi *et al.*, 2020). The AI values for dairy products are above 2.0, while those for meat are between 0.7 and 1.0 (Wahle and Heys, 2002). The result of the current study showed that AI and TI were within range requirements in tail fat but were higher in other organs. The value of AI was higher than in fat tail of fattening Chaal lambs (Ghafari *et al.*, 2016). This may be due to the nature of fat deposition in these organs (omental fat and KKCF), and the effect of type diets (de Castro *et al.*, 2023). In this study the greater ratio of hFA / HFA, the better and the average AI values for the tail fat (1.5), omental fat (1.01) and KKCF (1.26) lower. This investigation found no risk to human health and these values were in the range for meat, which is between 1.27 and 2.79 (Nudda *et al.*, 2019).

Conclusion: In conclusion, our data demonstrates that the source and size of the dietary fiber particle have a significant effect on fat deposition and fatty acid profile in tail fat, omental fat, and KKCF in Naimi sheep. The alfalfa hay (AH1) was particularly effective in enhancing the storage of fatty tissue with elevated levels of alpha-linolenic acid (ALA), and n3 fatty acids, whereas reduced the atherogenic index (AI). These findings indicate that fatty acids may be important indicators of the health of the fat in sheep meat. Therefore, targeted feeding strategies can be developed to modulate the rate of fat deposition by manipulating fiber sources, which in turn affect the rumen environment and microbial activity. This approach offers a promising way of optimizing the

nutritional quality and health characteristics of sheep meat.

Author Contributions: Conceptualization, methodology, validation, formal analysis, investigation, A.M.M; M.M.A and F.A.S writing—original draft preparation, writing—review and editing, A.M.M, M.M.A, F.A.S, R.S.A and S.A.A; visualization, R.A.A and A.M.M; supervision, R.S.A and M.M.A; project administration, M.M.A; funding acquisition, A.M.M.

Funding: This research was funded by the **Ongoing Research Funding Program, (ORF-2025-1036)**, King Saud University, Riyadh, Saudi Arabia.

Data Availability Statement: Informed consent was obtained from all subjects involved in the study.

Acknowledgments: The authors extend their appreciation to the **Ongoing Research Funding Program, (ORF-2025-1036)**, King Saud University, Riyadh, Saudi Arabia.

Conflict of Interest Declaration: The authors have declared that there is no conflict of interest.

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