EFFECT OF RUMEN-PROTECTED METHIONINE AND INORGANIC SULFUR SUPPLEMENTATION ON PRODUCTION AND QUALITY TRAITS OF WOOL IN LOHI SHEEP

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ABSTRACT

Since wool contains high-sulfur protein, the rumen needs enough inorganic sulfur to synthesize sulfur-containing amino acids (SAA) such as cysteine and methionine. Therefore, current investigation aimed to determine the effects of inorganic sulfur (as sodium sulfate, Na_2SO_4) and rumen-protected methionine (RPM) on production and quality of wool. Five different diets were given to sixty lactating ewes of Lohi breed under Randomized Complete Block Design (RCBD), where blocking was done by age *i.e.* younger (30 ± 5 months) and older (60 ± 5 months) having 30 animals in each block. The ewes were divided into five groups of 12 animals each, consisting of 6 ewes each from younger and older age cohorts. Five diets included C (control; no supplementation of Na_2SO_4 or RPM), S1 (Na_2SO_4 @ 0.18 % of dry matter), S2 (20 % additional Na_2SO_4 than that of S1), M1 (RPM @ 2 gm /head /day), M2 (20 % additional RPM than that of M1). *Ad libitum* grazing was allowed to all ewes on grazing field containing Jantar (*Sesbania sesban*) and local grasses along with 250 gm concentrate /head /day during six-month trial period. The study found that supplementation type, rate, and age of ewe significantly influenced plasma methionine concentration, staple length, fiber diameter, and clean wool yield. The highest plasma methionine concentration was observed in supplement group M2 (4.44 ± 0.04 µmol/dl) while the lowest plasma methionine concentration was observed in control group C (4.05 ± 0.04 µmol/dl). Rumen-protected methionine (RPM) supplementation had a more pronounced effect on these factors compared to Na_2SO_4 supplementation. Furthermore, the age-dependent responses were observed, wherein younger ewes showed greater responsiveness to both RPM and Na_2SO_4 supplementation compared to older ewes.

Key words: wool, rumen-protected methionine, inorganic sulfur, quality traits, Lohi sheep

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INTRODUCTION

One of the significant breeds of sheep that produces mutton and wool is the Lohi breed which has great adaptability with respect to temperature and humid climate. This breed encompasses approximately 40% of Punjab and 15% of nationwide sheep population (Ahmad *et al*., 2001; Sharif *et al*., 2022). The central districts of Punjab province are the home tract of this breed. Lohi breed is recognized for good quality coarse wool production, rapid growth rate and multiple births (Ahmad *et al*., 2001). Wool production holds an important place in Pakistan's economy as it serves as a key raw material for the warm clothing and carpet industries. According to official livestock statistics, Pakistan's total wool production was approximately 47.9 thousand metric tons during 2020–21 (Government of Pakistan, 2021; FAO, 2022). There are four major types of wool *i.e.* medium, fine, coarse and long wool. Pakistan is legendary in coarse wool production. Even though, quality of wool produced in Pakistan is not appropriate for making fine quality thread to be used in garments manufacturing however, it is a basic raw material for producing thread to be used in floor covering and carpet manufacturing. Lohi sheep breed has great adaptability with respect to temperature and humid climate (Jamil *et al*., 2023; Khoso *et al*., 2016; Ullah *et al*., 2020). Wool enjoys an important place in Pakistan's economy and is the basic input of warm cloth and carpet industries. Parameters (plasma methionine, staple length, fiber diameter and wool yield) of experimental ewes were taken before the start of the experiment (Ali *et al*., 2019; Kumar *et al*., 2017;. Rather *et al*., 2022). A direct relationship exists between fiber fineness and true fiber percentage i.e. the higher the true wool fiber percentage is, the finer will be fiber. Moreover, a marked positive correlation occurs between staple length and (Cao *et al*., 2021) mean fiber diameter, i.e. the finer the wool, shorter the staple (Khoso *et al*., 2016; Ullah *et al*., 2023). The average staple length of wool produced in Pakistani is 5-10 cm (Baba *et al*., 2020).

Sulfur is a major and integral constituent of several organic nutrients required by ruminants and is indispensable for rumen microbial synthesis of certain amino acids *viz.* methionine and cysteine, vitamins and enzymes (NRC, 1985). The supplementations of sulfur containing amino acids (cysteine and methionine) in sheep ration impressively increase the wool production (Sharif *et al*., 2022; Sherlock *et al*., 2001). Wool growth is chiefly a function of the quantity of amino acids reaching the intestine rather than energy supply (Ullah *et al*., 2021). The amount and pattern of amino acids of the proteins available in intestine, is very important in wool growth because they play a significant role in wool growth (Jamil *et al*., 2023; Sharif *et al*., 2022).

The sulfur present in wool protein is mainly in the form of cysteine with smaller amounts present as cystine and methionine (Sharif *et al*., 2022). Cysteine and methionine are usually the first limiting amino acids for wool production (McCoard *et al*., 2016). Animal is able to get cysteine directly from diet or through the metabolism of methionine via trans-sulfuration pathway (Kohl *et al*., 2019) but reduced bioavailability of cysteine and methionine was reported from oral route due to their degradation through microbes in rumen of sheep (McCoard *et al*., 2016). Ruminally less degradable proteins with higher sulfur containing amino acids (SAA) concentration (*i.e.* canola meal) had a significant effect on wool growth rate and fiber diameter. Rumen-protected methionine is a protected amino acid which bypasses rumen without facing any degradation and is available in the intestine to be absorbed readily for the synthesis of SAA which in turn increases wool production. In earlier studies (Feng *et al*., 2013; Nezamidoust *et al*., 2012; Waterman *et al*., 2012) the researchers supplemented the sheep with RPM and Na_2SO_4 and detected significant levels of plasma methionine in the blood samples of supplemented sheep. To investigate whether grazing alone is sufficient for better wool production or it requires some wool promoting feed supplementation in Lohi sheep. We hypothesized that adding RPM and Na_2SO_4 would increase plasma methionine concentration and protein synthesis of ruminal bacteria which might be able to stimulate anabolism of ewe leading to increased wool production.

MATERIALS AND METHODS

Experimental design and selection of animals: Sixty lactating Lohi coarse wool producing ewes (N1 = 30 ewes aged 30 ± 5 months (age group 1), 35 ± 5 Kg B.W.; N2 = 30 ewes aged 60 ± 5 months (age group 2), 45 ± 5 kg body weight), were studied during six-month experimental period. Ewes were selected from Small Ruminant Training and Research Center (SRTRC), University of Veterinary and Animal Sciences, Pattoki, Pakistan. In this regard, the adaptation period of two weeks was given before the start of the experiments.

Supplement groups: Ewes were divided into five supplement groups: C, S1, S2, M1 and M2. Each group consisted of 12 ewes comprising six ewes from each age group *i.e.* younger age group 1 (30 ± 5 months) and older age group 2 (60 ± 5 months). The control group (C) was kept without supplementation. The ewes in group S1 were supplemented with recommended levels of inorganic sulfur (as Sodium Sulfate) @ 0.18 % of dry matter (DM) while the ewes in group S2 were supplemented with 20% additional inorganic sulfur than that of S1 on DM basis. The ewes in group M1 were supplemented with rumen-protected methionine (Mepron® Evonik Pakistan Pvt. Ltd.,) (RPM) @ 2 gm/ head/ day during six-month trial period, whereas the ewes in group M2 were supplemented with 20 % additional RPM than that of M1.

Housing and identification of experimental ewes: Ewes were kept separately in two different enclosures according to their age within the same locality. Each enclosure had 30 pens, and each pen had a separate feeding and watering trough. After grazing, each subgroup was allocated with a different color, which was painted along backbone of each ewe for prompt identification of experimental ewes.

Composition of experimental diet: *Ad libitum* grazing on grazing field containing Jantar (*Sesbania sesban*) and local grasses mainly comprising of *Cynodon dactylon*, *Cenchrus ciliarus*, *Bothriochloa pertursa*, *Agropyron cristatum* and *Lolium perenne* was allowed to all experimental ewes along with 250 gm concentrate per ewe per day. The ingredient and nutrient composition of concentrate such as corn grain, wheat bran, canola meal etc., is shown in Table 1.

Table 1: Ingredients and Nutrients Composition of Concentrate

Ingredient	Inclusion rate %	ME* (Mcal/kg DM)	DM %	CP %
Corn grain	40.00	1.27	35.60	3.80
Wheat Bran	14.00	0.35	12.74	1.96
Molasses	10.00	0.27	7.20	0.32
Cotton seed cake	10.00	0.25	8.90	2.30
Canola Meal	11.00	0.28	9.90	3.85
Corn Gluten 30%	10.50	0.30	9.45	2.63
Urea	1.00	-	0.99	2.88
Mineral Premix	0.50	-	0.42	-
Salt	1.00	-	-	-
Limestone	2.00	-	2.00	-
Total	**100.00**	**2.72**	**87.20**	**17.74**

*The metabolizable energy (ME) content of the concentrate was estimated from tabulated energy values of individual feed ingredients according to standard feeding systems (NRC, 2007). The total ME of the diet was calculated as the weighted sum of ME contributions from each ingredient: $ME=\sum(Pi \times MEi)$
where Pi represents the proportion of each ingredient in the diet and MEi denotes the corresponding metabolizable energy value (Mcal/kg dry matter). Based on this calculation, the ME content of the concentrate was estimated to be 2.72 Mcal/kg DM.

Feed samples analysis: The representative sample of grazing fodder was collected from field, cut into small pieces to facilitate easy handling and uniform sampling for analysis, rinsed in distilled water to remove dust and stored in a refrigerator to be freeze-dried as soon as possible after collection. The sample of concentrate was also taken. Samples were dried in the hot air oven at 65 °C for 24 h and ground to pass through 1 mm sieve before being stored in polythene bags at room temperature (25°C) (Khan *et al*., 2014). The crude protein content of the feed samples was determined through Kjeldahl method (Getachew & Mosneh 2024).

Blood plasma sample collection and storage: Blood samples were collected from all the individual animals included in the study at day zero (before the start of the experiment) and then on a fortnightly basis through jugular venipuncture into vacutainer tube containing EDTA immediately after collection and stored at 4 °C. Plasma from these samples was separated within one hour after blood collection by centrifugation at 3000 rpm for 3 minutes at 4 °C and stored at -85 °C until processing. Samples were processed at local pharmaceutical laboratory (Sousa *et al*., 2022).

Wool sample collection: The selected ewes were restrained properly for taking samples. At least 60 gm of wool was taken from each ewe at an area of 5x5 cm in mid side by using small animal clippers with a fine cutting blade while keeping the scissors as close to skin as possible. First sample was collected at day zero (before the start of the experiment) to measure the changes in fiber diameter, staple length and wool yield. All the samples were kept in an airtight polythene bag until further processing. The samples were processed in the National Veterinary Laboratory (NVL), National Agricultural Research Centre (NARC), Islamabad, Pakistan.

Scouring and determination of wool parameters: Scouring is a process of cleaning greasy wool. The first weight (W1) of sample was taken before scouring. In W1, the weight of impurities (vegetable matter, grease and dust) was also included. The wool sample was hand carded to remove dust and visible vegetable matter (Malik *et al*., 2021). While staple length was determined after getting W1 in scouring. Three staples were randomly selected and staple lengths were determined separately (Li *et al*., 2020).

Wool samples were processed for the determination of wool yield and changes in fiber diameter following standard laboratory procedures. Initially, the samples were machine carded to ensure proper separation and alignment of fibers. Subsequently, the prepared samples were conditioned in a controlled environment at a temperature of 20 ± 2 °C and relative humidity of 65 ± 5% for a period of 24 h to achieve moisture equilibrium prior to analysis. After conditioning, the samples were finely cut into small fiber snippets using a manicuring machine to facilitate slide preparation. The prepared samples were then used for the determination of fiber diameter using optical fiber diameter analyzer (OFDA 2000) (Memon *et al*., 2018).

Statistical evaluation: The collected data were analyzed using the General Linear Model (PROC GLM) procedure of SAS software (version 9.1). The analysis was performed following a Randomized Complete Block Design (RCBD). Treatment means were separated using Duncan's Multiple Range Test (DMRT) following an analysis of variance (ANOVA) at the 5% level of significance (Salinas *et al*., 2024).

RESULTS

Effect on Plasma Methionine concentration: The results of current study indicated that supplementation type and rate had a significant ($P \leq 0.05$) effect on plasma methionine concentration in all supplement groups (Table 2). Overall, higher plasma methionine concentration was observed in RPM supplemented groups whereas, lower plasma methionine concentration was observed in Na_2SO_4 supplemented groups. The highest plasma methionine concentration was observed in supplement group M2 (4.44 ± 0.04 µmol/dl) while the lowest plasma methionine concentration was observed in control group C (4.05 ± 0.04 µmol/dl). The supplement group M1 and S2 had non-significant ($P>0.05$) differences with each other but showed significant ($P \leq 0.05$) differences with all other groups while remaining supplement groups (C, S1 and M2) were found to be statistically significant ($P \leq 0.05$) with each other.

Table 2: Effect of inorganic sulfur and rumen protected methionine (RPM) supplementation on plasma methionine concentration (µmol/dl), staple length (cm), monthly wool fiber incremental change (increase) in diameter (microns) and clean wool yield (%)

Supplement group*	Plasma methionine (µmol/dl) Mean ± SD	Staple length (cm) Mean ± SD	Incremental change (increase) in fiber diameter (microns) Mean ± SD	Clean wool yield (%) Mean ± SD
C	4.05 ± 0.16^a	0.90 ± 0.33^a	0.16 ± 0.12^a	58.89 ± 0.29^a
S1	4.13 ± 0.18^b	0.96 ± 0.30^a	0.16 ± 0.09^a	59.72 ± 1.26^b
S2	4.24 ± 0.20^c	1.18 ± 0.36^b	0.51 ± 0.32^b	60.91 ± 0.66^c
M1	4.24 ± 0.21^c	1.10 ± 0.33^b	0.54 ± 0.31^b	60.07 ± 0.94^d
M2	4.44 ± 0.31^d	1.34 ± 0.40^c	0.88 ± 0.42^c	61.82 ± 1.22^e

[a-e]Means denoted by different superscripts in a column were statistically significant ($P \leq 0.05$)
* C= control, S1= supplemented with Na_2SO_4 @ 0.18 % on DM basis, S2 = supplemented with 20% additional Na_2SO_4 than that of S1, M1= supplemented with RPM @ 2 gm/head/day, M2= supplemented with 20 % additional RPM than that of M1.

The results showed that plasma methionine concentration was significantly ($P \leq 0.05$) affected by the age of ewe (Table 3). The plasma methionine concentration in age group 1 (4.29 ± 0.27) was significantly higher ($P \leq 0.05$) than age group 2 (4.16 ± 0.22).

Table 3: Effect of ewe age on plasma methionine concentration (µmol/dl), monthly staple length, fiber diameter (microns) and clean wool yield (%)

Age group*	Plasma methionine (µmol/dl) Mean ± SD	Staple length (cm) Mean ± SD	Fiber diameter (microns) Mean ± SD	Clean wool yield (%) Mean ± SD
1	4.29 ± 0.27^a	1.20 ± 0.40^a	0.39 ± 0.37^a	60.81 ± 1.41^a
2	4.16 ± 0.22^b	0.99 ± 0.32^b	0.51 ± 0.39^b	59.75 ± 1.10^b

[a-b]Means denoted by different superscripts in a column were statistically significant ($P \leq 0.05$)
[*]Age group 1= ewes of 30 ± 5 months, Age group 2= ewes of 60 ± 5 months

The interaction model was tested, and it was found that the effect of age was different in different supplement groups *i.e.* the interaction of age with different supplementation type and rate was significant ($P \leq 0.05$) (Table 4). However, interaction model presented significant results, but supplementation rate and type influenced plasma methionine in both age groups. Therefore, the linear regression analysis was performed using supplementation level as an ordinal variable. In age group 1, plasma methionine concentration increased significantly with supplementation level ($Y = 4.01 + 0.13X$; $R^2 = 0.90$), indicating a strong linear relationship. In age group 2, a moderate linear increase was observed ($Y = 4.03 + 0.06X$; $R^2 = 0.78$). These results confirm that supplementation enhanced plasma methionine concentration in both age groups, with a greater response in younger ewes, supporting the significant interaction effect observed in the GLM analysis. In simple terms, plasma methionine was increasing linearly in response to supplementation in both age groups up to some extent, but major increase was observed in age group 1 (from 4.04 ± 0.18 in control to 4.60 ± 0.27 in M2 supplement group) as compared to age group 2 (from 4.06 ± 0.13 in control to 4.30 ± 0.28 in M2 supplement group).

Table 4: Interaction of age with different supplements and its effect on plasma methionine concentration (µmol/dl), staple length (cm) and fiber diameter (microns)

Supplement groups[1]	Age groups[2]	Plasma methionine (µmol/dl) Mean ± SD	Staple length (cm) Mean ± SD	Fiber diameter (microns) Mean ± SD
C	1	4.04 ± 0.18[a]	1.00 ± 0.27	0.13 ± 0.05
	2	4.06 ± 0.13[a]	0.80 ± 0.35	0.19 ± 0.16
S1	1	4.21 ± 0.20[b]	1.11 ± 0.18	0.14 ± 0.08
	2	4.05 ± 0.18[a]	0.82 ± 0.33	0.19 ± 0.09
S2	1	4.28 ± 0.19[c]	1.29 ± 0.43	0.39 ± 0.22
	2	4.21 ± 0.21[b]	1.06 ± 0.23	0.60 ± 0.37
M1	1	4.27 ± 0.19[c]	1.18 ± 0.40	0.45 ± 0.27
	2	4.21 ± 0.23[b]	1.02 ± 0.21	0.62 ± 0.33
M2	1	4.60 ± 0.27[d]	1.42 ± 0.51	0.83 ± 0.51
	2	4.30 ± 0.28[c]	1.27 ± 0.23	0.93 ± 0.31

[a-c]Means denoted by different superscripts in a column were statistically significant ($P \leq 0.05$)
[1] C= control, S1= supplemented with Na_2SO_4 @ 0.18 % on DM basis, S2 = supplemented with 20 % additional Na_2SO_4 than that of S1, M1= supplemented with RPM @ 2 gm/head/day, M2= supplemented with 20 % additional RPM than that of M1
[2] Age group 1= ewes of 30 ± 5 months, Age group 2= ewes of 60 ± 5 months

Effect on monthly staple length: The results showed that supplementation type and rate had a significant ($P \leq 0.05$) effect on monthly staple length growth (Table 2). Higher monthly staple length growth was observed in RPM supplemented groups whereas, lower monthly staple length growth was observed in Na_2SO_4 supplemented groups. As for as, effect of dose rate was concerned, the supplement groups which were supplemented with higher dose of both supplements (RPM and Na_2SO_4) showed more monthly staple length growth as compared to those supplement groups which were supplemented with recommended dose of both supplements. The supplement group C and S1 had non-significant ($P>0.05$) difference with each other but had a significant ($P \leq 0.05$) difference with other (S2, M1 and M2) supplement groups. Supplement group S2 and M1 had non-significant ($P>0.05$) difference with each other but had a significant ($P \leq 0.05$) difference with remaining supplement groups (C, S1 and M2). Supplement group M2 had the greatest effect (1.34 ± 0.40 cm) over staple length growth while supplement group C had the lowest (0.90 ± 0.33 cm) one.

The results showed that ewe age had a significant ($P \leq 0.05$) effect on wool staple length (Table 3). The ewes of age group 1 (younger age cohort i.e., ewes of 30 ± 5 months)
performed better with 1.20 ±0.40 regarding increase in monthly wool fiber diameter as compared to ewes of age group 2 (older age cohort i.e., ewes of 60 ± 5 months) which showed lower performance 0.99 ± 0.32 for same parameters when they were supplemented with inorganic sulfur and RPM.

The results showed that age of ewe had a statistically non-significant ($P>0.05$) effect on staple length growth regarding interaction of age with different supplements and its effect on staple length (Table 4).

Effect of inorganic sulfur and rumen-protected methionine supplementation on monthly fiber diameter: The results indicated that supplementation type and rate had a significant ($P \leq 0.05$) effect on monthly wool fiber diameter growth as represented with incremental **change (increase) in fiber diameter** (Table 2). Higher monthly wool fiber diameter growth was observed in RPM supplemented groups whereas, lower monthly wool fiber diameter growth was observed in Na_2SO_4 supplemented groups. As far as the effect of dose rate was concerned, supplement groups, which were supplemented with higher dose of both supplements (RPM and Na_2SO_4) showed more monthly wool fiber diameter growth as compared to those groups which were supplemented with recommended dose of both supplements. The supplement group C and S1 had non-significant ($P>0.05$) difference with each other but showed significant ($P \leq 0.05$) variation with all other groups. All the remaining supplement groups (S2, M1 and M2) showed significant ($P \leq 0.05$) differences with each other. The maximum increase (0.88 ± 0.42 microns) in wool fiber diameter in a single month was confirmed in supplement group M2 and minimum increase (0.16 ± 0.12 microns) in fiber diameter was observed in control group C.

The results showed that ewe age had a significant ($P \leq 0.05$) effect on wool fiber diameter (Table 3). The ewes of age group 2 (older age cohort i.e., ewes of 60 ± 5 months) performed better with 0.51 ±0.39 regarding increase in monthly wool fiber diameter as compared to ewes of age group 1 (younger age cohort i.e., ewes of 30 ± 5 months) which showed lower performance 0.39 ± 0.37 for same parameters when they were supplemented with inorganic sulfur and RPM.

The interaction model was tested, and it was found that the effect of age was explicitly not different in different supplement groups i.e. the interaction of age with different supplement type and rate was statistically non-significantly different (P>0.05) (Table 4).

Effect on clean wool yield: The results indicated that supplementation type and rate had a significant (P≤0.05) effect on clean wool yield (Table 2). Higher clean wool yield was observed in RPM supplemented groups whereas, lower clean wool yield was observed in Na_2SO_4 supplemented groups. As far as the effect of dose rate was concerned, supplement groups, which were supplemented with higher dose of both supplements (RPM and Na_2SO_4) showed more clean wool yield as compared to those groups which were supplemented with recommended dose of both supplements. The highest clean wool yield (61.82 ± 1.22 %) was witnessed in supplement group M2 while the lowest (58.89 ± 0.29 %) clean wool yield was observed in control group C.

The results indicated that age had a significant (P≤0.05) effect on clean wool yield (Table 3). Younger age group (ewes of 30 ± 5 months) produced the maximum clean wool (60.81 ± 1.41) whereas; older age group (ewes of 60 ± 5 months) produced minimum clean wool (59.75 ± 1.10). Furthermore, the interaction analysis indicated that non-significant effect (P>0.05) of age with different supplement type and rate.

DISCUSSION

Sulfur is a major and very basic constituent of several organic nutrients required by ruminants and is indispensable for the synthesis of sulfur containing amino acids by rumen microbial flora *viz*. methionine, and cysteine, as well as vitamins and enzymes (Ayyat *et al*., 2021; Cao *et al*., 2021). Many researchers described mechanisms by which ruminal microorganisms produce sulfur containing amino acid (SAA) by using inorganic sulfur (Stapay *et al*., 2021; Sharif *et al*., 2022). It was reported that ruminal microorganisms first reduce sulfate to sulfide, which is then used for the synthesis of sulfur-containing amino acids (Cilek, 2015; Rogers *et al*., 2012). Rumen-protected methionine is basically a protected methionine which bypasses rumen without any degradation and becomes available in intestine to be absorbed readily for the synthesis of sulfur containing amino acids (Stapay *et al*., 2021; Türkoğlu *et al*., 2023). This mechanism explains the observed increase in plasma methionine concentration following RPM supplementation in the present study. The increase may also be associated with improved amino acid balance and reduced catabolism of methionine for maintenance functions, allowing greater availability for protein synthesis and metabolic activities.

The findings of the present study are consistent with earlier reports demonstrating significant increases in plasma methionine concentration following RPM supplementation. For example, grazing Merino weaners supplemented with RPM at 2 g/head/day exhibited significantly (P≤0.05) elevated plasma methionine concentrations 14–16 h after feeding (Stapay *et al*., 2021; Wang *et al*., 2017). Similarly, an 80 µmol/L plasma methionine concentration was achieved through four-week abomasal methionine infusion (Sharif *et al*., 2022). Other researchers have reported significantly (P≤0.05) elevated plasma methionine upon supplementation with rumen-protected sources (Cao *et al*., 2021; McCoard et al., 2016; Wang *et al*., 2017; Sharif *et al*., 2022). Specifically, RPM increased plasma methionine and lysine in Targhee lambs (Cao *et al*., 2021), attributed to intestinal release and absorption of amino acids (Stapay *et al*., 2021). Furthermore, RPM supplementation over 44 days significantly (P≤0.05) enhanced plasma methionine in heifers, confirming successful intestinal delivery of rumen-protected methionine (Waterman *et al*., 2012). The positive response observed in the current study may be explained by the increased bioavailability of methionine escaping ruminal degradation. Methionine is frequently considered the first limiting amino acid in ruminants; therefore, supplementation with RPM improves amino acid balance, supports hepatic protein synthesis, and enhances overall metabolic efficiency. Moreover, sulfur supplementation may stimulate ruminal microbial growth, resulting in increased microbial protein synthesis and subsequent amino acid availability to the host animal. The linear relationship reported between dietary RPM level and plasma methionine concentration (Stapay *et al.*, 2022; Wang *et al*., 2017) further supports the dose-dependent improvement in methionine availability.

In Holstein cows, a higher plasma methionine concentration was observed with 60 g/day RPM compared to controls, while 30 g/day showed no such effect (Bach & Stern, 2000). Several authors (Cao *et al*., 2021; Wang *et al*., 2017; Sharif *et al*., 2022) reported that concentrate containing 550 mg/kg RPM did not alter plasma methionine, whereas 3300 mg/kg RPM significantly increased it. These discrepancies among studies may be attributed to differences in RPM dosage, rumen protection efficiency, dietary composition, animal species, physiological stage, feeding system, and duration of supplementation. Variations in ruminal fermentation characteristics and microbial adaptation may also influence methionine metabolism and absorption efficiency. Therefore, the magnitude of response to sulfur or RPM supplementation appears to depend not only on the quantity supplemented but also on the overall nutritional and physiological status of the animals.

As far as the effect of inorganic sulfur supplementation was concerned, (Feng *et al*., 2013) reported that the plasma methionine concentration was higher in zinc sulfate ($ZnSO_4$) supplemented groups of Cashmere goats as compared to control group. The results of the current study were also in agreement with the findings concluding that sulfate treatment along with betaine (a naturally occurring compound with methyl donor properties) amplified ($P \leq 0.05$) plasma methionine concentration in ewes (Nezamidoust *et al.,* 2012). The increase in plasma methionine observed in sulfur-supplemented animals may be explained by enhanced sulfur availability for ruminal microbial synthesis of sulfur-containing amino acids. Sulfur acts as a precursor for microbial protein synthesis, and its adequate supply improves microbial growth and efficiency of nitrogen utilization. In addition, betaine may spare methionine utilization by serving as an alternative methyl donor, thereby increasing circulating methionine availability for metabolic and productive functions.

Cysteine, an important component of wool, can be synthesized inside the body of animal when supplemented with inorganic sulfur in the form of sodium sulfate and animal utilizes it for wool production (Cao *et al*., 2021; McCoard *et al*., 2016). In our trial, monthly growth of 1.18 cm of staple length was achieved when ewes were supplemented with 20% additional inorganic sulfur (S2). Our findings were in close alignment with the results of (Cao *et al*., 2021; Qi *et al*., 1992) who stated that the sheep supplemented with sodium sulfate up to 25% of dietary sulfur (DM basis) produced 17% more wool than un-supplemented sheep. The results of our investigation were in compliance with findings of other researchers (Cao *et al*., 2021; Sharif *et al*., 2022) who reported that sulfate and betaine (inorganic sulfur) supplementation resulted into better staple length growth. (Li *et al*., 2013; Sharif *et al*., 2022) also reported that adding inorganic sulfur to the basal diet of sheep improved the wool growth rate. These consistent findings suggest that inorganic sulfur supplementation enhances wool production primarily through increased synthesis of sulfur-containing amino acids, improved keratin deposition, and stimulation of wool follicle metabolism.

The results of the present study are consistent with the findings of previous study (Sharif *et al*., 2022), which described that staple length was increased by 20-30 %, when sheep were supplemented with RPM @ 2gm/ head/ day and this effect was independent of the level of grain supplement. In another study, a 31% increase in staple length observed (Stapay *et al*., 2021; Wang *et al*., 2023) when sheep were supplemented with abomasal infusion of methionine. The improvement in staple length observed in these studies may be attributed to the increased post-ruminal availability of methionine, which bypasses ruminal degradation and becomes directly available for intestinal absorption and utilization.

Our findings were in understanding to the earlier results of (Komolong *et al*., 2001; Stephenson *et al*., 1991; Uys, 2020) describing that there was 30-60% increase in wool growth when methionine was administered post-ruminal to bypass rumen degradation (sheep kept on maintenance diets under pen settings). It has been reported that infusion of methionine (2.46 gm/day) into abomasum of sheep increased wool follicle bulb cell mitotic rate by 35 % which resulted into an increased fiber growth (Rogers *et al*., 2012; Wang *et al*., 2023). However, the findings of current study did not match with findings of Sahlu & Fernandez, (1992) who stated that fiber length was not affected by intra-peritoneal infusion of methionine in Angora goats. This difference might be attributed to differences in species and route of administration in animals under that study.

Regarding the age of the animals, our findings were in agreement with the outcome of the studies conducted by (Abasi-Mousa *et al*., 2023; Bukhari *et al*., 2022; Wang *et al*., 2023) suggesting that the age had a significant ($P \leq 0.05$) influence on staple length. They stated that younger (18 months old) ewes produced longest staple length as compared to older (66 months) ewes, which produced shortest staple length. Low productivity in elder ewes might be attributed due to low feed intake, which resulted in decreased wool follicle efficiency or might be due to reproduction as conceded earlier. Feed conversion ration may have an important role in this regard since this character is somewhat downregulated with increase in age after puberty. Similar trends in staple length growth were observed by (Bhateshwar *et al*., 2022; Kaleri *et al*., 2023; Sharif *et al*., 2022; Wang *et al*., 2023) in Arabi sheep.

The findings of our study were similar with results of previous studies (Sharif *et al*., 2022; Staple *et al*., 1993) which described that when weaner lambs were supplemented with RPM @ 2gm/ head/ day, the effect on fiber diameter was significantly ($P \leq 0.05$) better since fiber diameter was increased by 0.83-1.56 microns. The mechanism involved for increase in fiber diameter observed in the current study may be attributed to improved availability of sulfur-containing amino acids, particularly methionine and cysteine, which are essential for keratin synthesis and wool follicle development. Thicker fibers contain greater keratin mass, thereby increasing total wool production. Moreover, the findings of another prior study (Rather *et al*., 2019) were also in line with our study which reported that appreciable increase in fleece weight along with increase in fiber diameter in Angora goats during the first three 6-monthly shearing system. From production point of view, a greater wool diameter was preferred since it would boost the yield of clean wool, which would ultimately enhance the farmer's income (Das *et al*., 2012; Kumar *et al*., 2022; M. Rather *et al*., 2022). However, an excessive increase in fiber diameter may reduce wool fineness and lower its commercial value despite increasing fleece weight. Therefore, the desirability of increased fiber diameter depends largely on breed characteristics, intended wool use, and market demands. In the present study, the increase in fiber diameter was relatively moderate and accompanied by improved fleece growth, suggesting that sulfur supplementation enhanced wool production without causing excessive coarseness. Thus, the response may represent a favorable balance between increased wool yield and acceptable fiber quality.

The findings of our study agreed with results of earlier studies (Cao *et al*., 2021; Cilek, 2015) describing that there was 30–60 % increase in clean wool yield when sheep were offered maintenance diets under pen conditions along with rumen-protected methionine supplementation. Furthermore, one more study (Rogers *et al*., 2012) stated that ruminal administration of unprotected methionine did not increase clean wool yield although wool growth rate was increased by 3% in that study. Our results were also in agreement with the findings of another previous study (Wang *et al*., 2017) which demonstrated that unprotected methionine undergoes sever degradation by ruminal microbes. Cao *et al*., (2021) reproted that the supplementation of most limiting amino acid (e.g. methionine) made second limiting amino acid (e.g. lysine) check. In addition, Rogers *et al*., (2012) stated that clean wool yield of adult Lohi was 66.17 ± 10.94% and the effect of age and breed was significant on clean wool yield. Hence, present study findings were in line with Staikova & Stancheva, (2009) who reported that 56 months older ewes produced less wool yield as compared to 30 months older ewes. Thus, supplying methionine enhanced clean wool yield in the study, likely due to its role as a sulfur-containing amino acid essential for keratin synthesis in wool fibers. With wool protein rich in sulfur amino acids, increased post-ruminal methionine improved follicular protein synthesis and fiber development. Methionine is often a limiting amino acid for ruminants due to degradation by ruminal microorganisms. The use of rumen-protected methionine likely minimized microbial loss and increased intestinal availability, leading to better nutrient utilization and wool growth. This improved availability may also foster essential biochemical reactions that support clean wool production.

Conclusion: In conclusion, supplementation with rumen-protected methionine demonstrated comparatively greater effects than Na_2SO_4 on the evaluated parameters, including plasma methionine concentration and selected wool characteristics. Responses were generally more pronounced when supplements were provided at 20% above the recommended level. Age-related variation in responsiveness was also observed, with younger ewes tending to respond more favorably than older ewes, except for fiber diameter. However, although several responses were statistically significant, the magnitude of improvement was relatively modest, and therefore the physiological and commercial relevance of these changes under practical production conditions should be interpreted with caution.

Compliance with ethical standards: The study protocols and the welfare of the animals used for the research were approved by the Animal Care and Use Committee of University of Veterinary and Animal Sciences, Lahore, Pakistan.

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Author contribution: SAC and MQS conceived and designed the study while SAC and ZMI extracted the data. ARA, IK, MQS and ZMI analyzed the data. MA, ARA, MZF and SAC wrote the initial draft of the manuscript. SAC, MZF, ARA, IK, MA, ZMI and MQS revised the final draft of the manuscript.

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