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Impact of adding dietary different levels of protected methionine on wool characteristics, ultrastructure, and blood components of Barki sheep

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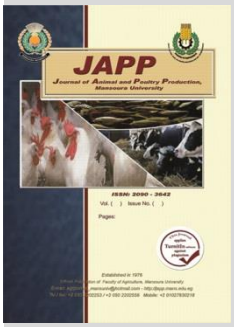

ABSTRACT

Twenty-five non-pregnant adult Barki ewes were used to investigate the effect of the addition of various levels of rumen-protected methionine as a feed additive on some wool characteristics, blood components, and wool ultrastructure. Animals were divided according to live body weight and wool measurements into 5 groups. The control group (G1) was fed a basal diet without any supplementations, while G2, G3, G4, and G5 were fed the basal diet supplemented with 6, 9, 12, and 15 g methionine /head/day, respectively, for six months. Wool samples were collected at the end of the experiment to record some wool measurements, such as fiber diameter, staple length, staple strength, elongation, point of break, and clean wool yield. Blood samples were collected to determine some blood biochemicals. Results showed that adding rumen-protected methionine for six months significantly enhanced the wool characteristics of Barki ewes, such as staple length, staple strength, elongation, point of break, fiber diameter, and clean wool yield. Supplementing dietary methionine affected coarse fiber wool scale properties of Barki ewes. The minimum value of scale width was recorded in G2, while the maximum value of scale width was recorded in G5. Also, G2 showed the highest value of scale distance to the edge. Wool of G4 recorded the lowest value of coarse fiber diameter. Methionine supplementation resulted in a slight effect on the values of some blood biochemical parameters. The a propriate dietary methionine supplementation was 12 g /head/day.

Keywords: Sheep, protected methionine, wool characteristics, wool ultrastructure, blood biochemicals.

INTRODUCTION

Nutrition is the most important environmental factor which affects wool characteristics. The most important limiting nutrient for ruminants fed with poor quality diets is protein. It becomes more important when ruminants achieve their optimum growth and peak production because their nutrient requirements vary according to their physiological statuses, such as growth, pregnancy, and lactation. For ruminants fed with low-quality diets with little protein, adding high-quality protein, or rumen-protected amino acids, particularly methionine, enhanced their performance (Younis and Abd-Elazem, 2019).

Ruminants can get their amino acid requirements from microbial protein synthesized in the rumen and dietary protein that escaped rumen degradation. The amount of amino acids and protein which escaped rumen degradation differ among different feeds, and this depends on their solubility and passage rate to the small intestine. The regulation of microbial protein synthesis depends on the amount of plant organic matter which was fermented in the rumen, but the concentration of ammonia and elements is not a limiting factor.

In addition, amino acid requirements for animals are not adequately met depending on the normal sources of dietary protein in some states. The rapid and extensive dietary protein degradation in the rumen created the necessity to develop the concept of protein protection fromthe rumen degradation to enhance the available essential amino acids for animal production and to reduce nitrogen loss, as urea in the urine.

Methionine is greatly important for metabolism and considered as a donor of active methyl groups by being converted into S-adenosylmethionine, releasing the active methyl group. Creating S-adenosylmethionine may undergo hydrolysis for homocysteine production (D'Mello 2003).

The synthesis of wool protein in animals depends on homocysteine. The most important essential amino acid for sheep is methionine. Dietary supplementation of methionine greatly enhanced the synthesis of body protein and increased weight gain.

Wool of Egyptian sheep is known for its coarse fibres, so it is not suited for industrial purposes. In Egypt, the demand for wool textile increased, and raw wool improved because of the wool industry expansion and increasing population (Youssef 2017). There was a significant increase in some wool traits of Barki ewes, such as clean wool yield, fiber length, staple strength, and fiber diameter, when protected methionine (7g Smartamin/animal daily) was added for six months (REF). It is concluded that studies must be made to improve the production of local wool suitable for the manufacturing purposes of woolen textiles to satisfy the taste of consumers and to decrease the expensive imported wool (Ramadan et al. 2017).

Regarding the effect on blood biocheicals, Liker et al.

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(2005) found a significant decrease in plasma glucose concentrations, and increase in urea, but no significant differences were found in the plasma concentrations of total protein, albumin, triacylglycerols, total cholesterol, and creatinine of cows that received 15 g rumen-protected DL-methionine per animal daily in the third term of gestation.

This study aimed to investigate the impact of dietary supplementation of rumen-protected methionine on some conventional wool characteristics, wool ultrastructural, and some blood biochemicals as indices of the metabolic status of Barki ewes.

MATERIALS AND METHODS

This experiment was carried out at Maryout Research Station, 35 km southwest of Alexandria, (Longitude 29.80°E, Latitude 31.02°N), belonging to Desert Research Center, Egypt, in cooperation with the Physiology and Biotechnology Lab, Department of Animal Production, Faculty of Agriculture, Mansoura University.

Animals:

Twenty-five non-pregnant adult Barki ewes were used in this study. Ewes were 3–4 years old, with an average live body weight of 28.95±1.37 kg. This study was conducted from September 2019 to March 2020. All animals were healthy and free of diseases. The animals were fed with Berseem hay (*Trifolium alexandrinum*) ad libitum, with a concentrate feed mixture of 60% TDN and 14% CP at a rate of ½ kg/head daily. This concentrate feed mixture consisted of 18% wheat bran, 50% cottonseed cake, 1% common salt, 15% yellow corn, 3% molasses, 2% limestone, and 11% rice polish. Animals drank water two times a day.

Experimental design:

Animals were divided into five groups (5 ewes/group) according to live body weight and nearly similar wool measurements. The control group was fed a basal diet without any supplementationsm while the second, third, fourth, and fifth groups were fed basal diet supplemented with rumen-protected methionine at levels of 12, 18, 24, and 30 g Smartamin /kg concentrate /day for six months respectively.

Wool characteristics:

Wool samples (approximately 20 g) were collected from the left mid-side position of each animal at the end of the experiment to record some wool measurements, such as fiber diameter, staple length, staple strength, elongation, point of break, and clean wool yield.

Staple length (STL):

Ten staples were obtained from every greasy wool sample randomly. The length of staples was recorded. A millimeter ruler on a black velvet-covered board was used to measure every staple. Just enough tension was applied to straighten the staple without stretching it. The length of wool staples was measured from the base of the staple up to the base of the triangle formed by the ends of the fibers at the tip of the staple. The length of each staple was recorded to the nearest 0.01 cm, and the average of every sample was calculated.

Fiber diameter (FD):

Short sections of 300 fibers were mounted in liquid paraffin oil, spread on a microscopic slide, and covered. FD was measured with Image Analyzer (Blue edition, Zen, 2012) with lens10/0.847.The mean FD and its standard deviation were calculated. During FD measurement, the number of medullated fibers was counted and its percentage was calculated and recorded for each sample.

Clean wool yield (CWY):

Representative samples from greasy fleece were taken and weighed in greasy condition to determine clean wool percentage according to the suggested method of ASTM (2014). The samples were scoured in a scouring train consisting of four electrically heated bowls. The liquid temperature was thermostatically controlled in each bowl and descended from the initial 50°C to 25-30°C. scouring liquor (2% soap and 1% Na_2CO_3) was squeezed out with rollers, and scoured samples were rinsed in water in the last bowl, dried in a basket centrifuge, and then dried in an electric oven at 105°C for 6 hours. The determination of clean wool percentage for each sample was carried out according to ASTM, (2014) using the following equation:

Yield (%) = (Weight of scoured and dried sample/weight of greasy sample) × 100.

Staple strength (STR):

A regular staple was obtained from every sample of wool randomly to estimate staple strength by measuring the force required to break it. Each staple was subjected to the Agritest Staple Breaker (Agritest Pty. Ltd.). The staple was broken into two parts: top and base. The measurement of staple strength was calculated and recorded for each sample in terms of Newton/Kilotex (Heuer, 1979; Caffin, 1980).

Point of break (POB) and elongation (ELO):

Three greasy staples of each sample were used to measure staple strength using Agritest Staple Breaker with the procedure displayed by El-Gabbas *et al*. (1999). Elongation, representing the increase in staple length as a proportion of the original length, was measured. Point of break, by weight, and by length (the weight and length of the top in proportion to the weight and length of both top and base) were calculated at the time of measuring staple strength.

Blood analyses:

Blood samples were obtained from the jugular vein of every ewe (five ewes /group) at the end of the study to record the concentration of some blood parameters, such as total proteins , albumin, globulin (calculated by total protein minus albumin), albumin to globulin ratio (A/G ratio), total cholesterol, triglycerides, urea, creatinine, and glucose as well as activity of alanine aminotransferase (ALT) and aspartate aminotransferase (ST).

Analysis of amino acids:

Amino acid analyzer (Sykam Clarity Amino Acid Analyzer SW, Central Laboratory of DRC) was used to determined plasma and wool contents of amino acids.

Scanning electron microscopy of wool:

The scanning electron microscope uses secondary electrons to "view" the surface characteristics of the fiber. Unlike optical microscopes the fiber interior is not viewed, indeed the surface is coated with a thin layer of gold to help speed displaying of the scanned surfac

Quanta FEG 250 scanning electron microscope (FEI Company, USA) was used for imaging coarse fiber wool scale properties of Barki ewes such as scale width, scale height (scale distance to the edge), scale regularity (means a slight difference between the scale width and the scale hight), coarse fiber diameter, coarse fiber regularity (means measuring coarse fiber diameter along with the same fiber). It

is available at a central lab of Desert Research Center, Cairo, Egypt. Samples were mounted onto scanning electron microscope stubs. Applied scanning electron microscope conditions were: a 10.1 mm working distance, with an in-lens detector with an excitation voltage of 20 kV.

Statistical analysis:

The **SAS (2008)** program, utilizing GLM procedure, was used to analyze the obtained data, and the differences among means were compared using Duncan Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Effect on wool characteristics:

The effect of adding various levels of rumen-protected methionine on some wool characteristics of Barki ewes is presented in Table (1). Animals supplemented with 12 g of methionine (G4) showed the highest staple length (STL) (11.13 ± 0.544 cm) compared to animals in other experimental groups. This was longer than the normal values stated by Nasr and Ramadan (2017) and Mohamed (2019), who found that the SL of Barki sheep was 7.50 ± 0.49 and 9.64 ± 0.227 cm, respectively. In accordance with our results, Ramadan et al. (2017) found that the addition of 7 g of methionine showed an insignificant increase in wool SL compared with the control. The wool SL increased with the increase in the duration of treatment. Methionine may be directed away from the fiber follicle (wool) and be used in the synthesis of inner root sheath protein. Methionine concentration of the inner root sheath protein was approximately four times that of the wool. This is in agreement with Sahlu and Fernandez (1992).

Various doses of rumen-protected methionine had no significant effects on staple strength values in all experimental animals (Table 1). Mohamed (2019) established that the staple strength of Barki sheep was 41.25 ± 2.740 N/kTex. In contrast to our results, staple strength increased significantly after adding 7 g of methionine for Barki sheep compared with control animals (Youssef, 2017). The staple strength of wool increased by increasing the duration of treatment. Also, Hynd et al. (2015) revealed that there was a reduction in staple strength of sheep treated with either amino acids found in Zein protein or amino acids minus methionine. There was a dramatic reduction in staple strength (10–12 N/kTex) relative to the controls (40–50 N/kTex). The omission of methionine from the mixture reduced staple strength. Methionine plays an important role in increasing the strength of wool by improving the tenacity of fiber. It may be related to the formation of S-adenosyl-L-methionine. This compound is a methyl donor for many important reactions which may affect wool properties.

Animals supplemented with 6 g of methionine (G2) had the highest wool elongation value (27.04 ± 4.427), while animals supplemented with 12 g of methionine (G4) showed the lowest wool elongation value (11.56 ± 4.427) compared to animals in other experimental groups (Table 1). Wool elongation of Barki sheep was found to be 23.45 ± 1.330 (Mohamed, 2019) and 51.34 ± 0.389 (Nasr and Ramadan, 2017). Although there was an insignificant increase in wool elongation of Barki sheep by supplementing methionine (Youssef, 2017), wool elongation increased in our study by increasing the duration of treatment. The cause of the increase in elongation appeared to be a consequence of a combination of the increase in FD and an increase in the intrinsic strength of the wool fibers.

Animals supplemented with 9 g of methionine (G3) had the highest wool point of break (POB) value (53.10 ± 1.563) compared to animals in other experimental groups, while G4 (12 g MET /head/day) had the lowest wool POB value (48.22± 1.563, Table 1). This is in agreement with previous studies conducted on Barki sheep (Youssef, 2017 and Mohamed, 2019). They showed that the wool POB in Barki sheep was 52.36 ± 1.033 and 48.29 ± 0.80 respectively. When diets of Barki sheep were supplemented by rumen-protected methionine, it caused an increase in POB of wool compared to control animals .However, the addition of lysine and methionine showed an insignificant increase in wool POB compared with the control. The POB of wool was not significantly influenced by the increase in the duration of treatment (Ramadan *et, al* .2017).

Table 1. Wool characteristics of Barki ewes as affected by the addition of dietary various levels of protected methionine.

Treatment	Wool character					
	Staple length (cm)	Staple strength (N/kTex)	Elongation (%)	Point of break (%)	Fiber diameter (µm)	Yield (%)
G1 (Control)	8.50^{b}	41.92	18.90^{ab}	18.50^{ab}	27.625	54.80^{b}
G2 (6 g MET /head/day)	9.90^{ab}	41.13	27.04^{a}	50.25^{ab}	31.75^{b}	63.62^{ab}
G3 (9 g MET /head/day)	9.10^{b}	44.80	25.80^{a}	53.10^{a}	32.06^{ab}	61.51^{b}
G4 (12 g MET /head/day)	11.13^{a}	48.80	11.56^{b}	48.22^{b}	35.42^{a}	74.11^{a}
G5 (15 g MET /head/day)	9.23^{b}	36.88	25.50^{a}	52.34^{a}	30.10^{bc}	57.45^{b}
±SEM	0.544	5.998	4.427	1.563	1.149	3.667

Mean values with similar superscripts within the same column did not differ significantly at P < 0.05.

There were slight differences in wool FD values of the animals supplemented with 9 g of methionine (32.06 ± 1.149) and 15 g of methionine (30.10 ± 1.149) compared to other experimental animals, while animals supplemented with 12 g of methionine had highest wool FD value (35.42 ± 1.149). Ramadan *et. Al.* (2017) observed that adding methionine in a dose of 7 g for the diets of Barki ewes had no significant effect on wool fiber diameter. Likewise, the addition of lysine and methionine showed an insignificant increase in fiber diameter compared with control animals. Wool FD increased by increasing the duration of treatment (. Also, Galbraith (2000) stated that the increased diameter of Mohair fibers following methionine supplementation was due to the suggested role of the increased fraction of intermediate filament-associated protein in producing a greater volume of hair cortex cells. In addition, methionine performed some specific functions, perhaps in wool protein synthesis or cell division according to Reis (1991).

Animals supplemented with 12 g of methionine (G4) had the highest clean wool yield value (74.11 ± 3.667) compared to animals in other experimental groups. There was a slight difference in clean wool yield value in the animals supplemented with 6 g of methionine (63.62 ± 3.667) as compared to animals in other experimental groups (Table 1). Likewise, Sahlu and Fernandez (1992) revealed that grease and clean mohair yields of mature angora goat (average BW; 47.2 kg) were increased with methionine infusion, in agreement with previous studies conducted on sheep (Reis 1967; Williams et al. 1972; Reis et al. 1973). It was illustrated that adding methionine to the diets of Barki sheep in the form of rumen-protected tablets resulted in a significantly higher ($P < 0.05$) percentage of clean wool yield. The percentage of clean wool yield increased with the increase in the duration of treatment (Youssef, 2017).

Kidney and liver functions:

Table 2 illustrated that blood urea concentration was increased by increasing the level of rumen-protected methionine, while various levels of rumen-protected methionine had no significant effects on serum creatinine concentration in all experimental animals. Animals supplemented with 9 g of methionine had the highest ALT activity (24.11 ± 0.948), while G5 (15 g MET /head/day) had the lowest ALT activity (20.93 ± 0.948). There were no significant differences in activity of AST between both G3 and G4 and control (G1). Animals supplemented with 15 g of methionine (G5) had the highest AST activity (36.22 ± 0.884). Younis and Abd-Elazem (2019) confirmed insignificant differences in the entire blood constituent (urea, ALT, and AST) when Barki sheep received a diet supplemented with rumen-protected methionine (7 g /head daily). These results indicate that methionine and lysine supplementation decreased the plasma concentration of urea significantly. This was in accordance with Jackson et al. (2002). On the other hand, adding protected amino acids in the diets of high-yielding lactating Holstein cows had a significant effect ($P<0.05$) by decreasing creatinine in treatments compared with control. It was found that concentrations of determining blood plasma parameters, such as ALT and AST activities, were not affected by adding methionine or methionine and lysine. The plasma urea concentration in the methionine and lysine group was higher than in the methionine group (Trinacty *et al.* 2009). No significant differences were observed in serum ALT and AST activity of female blue foxes among methionine-supplemented groups ($P>0.05$) with different doses (2, 4, 6, and 8 g/kg) for 40 days (Guo et al., 2015). In this respect, Liker *et al.* (2006) stated that when Charolais growing beef cattle (average BW:249.6±43.8kg) received 10 g of rumen-protected DL-methionine daily for 94 days, plasma urea concentration tended to decrease at the end of the trial. The activity of ALT in these animals increased on the 68th and 94th days. The higher activities of ALT and AST could be a result of the increase in gluconeogenesis.

Table 2. Kidney and liver functions of Barki ewes as affected by the addition different dietary levels of protected methionine.

Treatment	Kidney function		Liver function	
	Urea (mg/dL)	Creatinine (mg/dL)	ALT (U/L)	AST (U/L)
G1 (Control)	50.08^{b}	0.781	21.19^{ab}	34.46^{ab}
G2 (6 g MET /head/day)	51.40^{ab}	0.766	23.10^{ab}	33.34^{b}
G3 (9 g MET /head/day)	53.47^{ab}	0.822	24.11^{a}	35.34^{ab}
G4 (12 g MET /head/day)	55.13^{a}	0.840	22.43^{ab}	34.90^{ab}
G5 (15 g MET /head/day)	55.28^{a}	0.856	20.93^{b}	36.22^{a}
±SEM	1.427	0.073	0.948	0.884

Mean values with similar superscripts within the same column did not differ significantly at $P < 0.05$.

Protein metabolites:

Table 3 showed that various doses of rumen-protected methionine had no significant effects on the values of albumin in all experimental groups. Animals supplemented with 9 and 12 g of methionine and the control group had the highest globulin and total protein values compared to animals in other experimental groups. Animals in G2 (6g MET /head/day) showed the highest value of albumin to globulin ratio (A/G ratio), G3 (9g MET /head/day) had the lowest A/G ratio. There were slight differences in the A/G ratio between the animals that had 12 and 15 g of methionine and control animals. Ribeiro *et al.* (2018) reported that the differences in globulin in goats of different breeds were related to physiological and genetic factors of animal adaptation. The higher globulin levels in a certain period resulted in a low A/G ratio in adult male normal and healthy Ibex weighing 40 – 50 kg (Al-Eissa *et al*. 2012). In addition, when lambs (Pelibuey East-Friesian weighing 22.7±3.2kg received oral doses of rumen-protected methionine (1.5 g/day) for 60 days, there was an increase in albumin and plasma protein (Rodríguez-Guerrero et al. 2018). On the other hand, Younge *et al.* (2001) established that there were no significant differences in some blood measurements of dairy cows offered grass-silage based diets like total protein, albumin, and globulin, between control, lysine, and methionine groups. In addition, Younis and Abd-Elazem (2019) confirmed that there were no significant differences concerning concentration of total protein when Barki sheep received a diet supplemented with rumen-protected methionine (7g/head daily).

Glucose, cholesterol, and triglycerides:

Table 4 illustrated that various doses of rumen-protected methionine had no significant effects on the values of glucose in all experimental animals. It was found that the increase in methionine dose caused a decrease in cholesterol values in treated groups . Animals in G4 (12 g of methionine) showed the highest triglycerides concentration, while G1 (control group) had the lowest value of triglyceride (136.018 ± 0.998). There were differences in triglycerides concentrations in animals that had 6, 9, and 15 g of methionine. Glucose and free fatty acids concentrations are good indicators of the energetic status. The supply of methionine enhanced the capacity of the liver in exporting triacylglycerols in the form of very-low-density lipoprotein and helping improve the negative effect of fatty acid collecting in the liver (Younis and Abd-Elazem 2019). However, Li et al. (2015) did not find any such effects in

blood cholesterol and triglycerides in lambs receiving an estimated dose of 37 to 116 mg/kg. In dairy cattle, it was reported that there was a decrease followed by an increase in plasma cholesterol level fed with methionine above NRC requirements (Bouyeh and Gevorgyan 2011). These differences may be because of the ability of complete oxidation of non-esterified fatty acids and synthesis of very-low-density lipoprotein, thereby increasing the incidence rates of fatty liver and ketosis (Grummer *et al.* 2004). Furthermore, it was found that methionine stimulated glucose and cholesterol synthesis (Rodriguez-Guerrero *et al.* 2018). However, Liker *et al.* (2005) found that there was a significant decrease in plasma glucose concentrations of cows that received 15 g rumen-protected DL-methionine per animal daily in the third term of gestation. No significant differences in plasma concentrations of triacylglycerols and total cholesterol were found. Rumen-protected methionine induced no significant effect on blood serum glucose and triglycerides concentration but had reduced blood serum cholesterol when compared with the methionine-limited group (Soltan *et al.* 2012).

Table 3. Blood proteins of Barki ewes as affected by the addition of different dietary levels of protected methionine.

Treatment	Parameter			
	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
G1 (Control)	7.28^{a}	3.06	4.22^{a}	0.42^{bc}
G2 (6 g MET /head/day)	6.32^{b}	3.12	3.20^{b}	0.50^{a}
G3 (9 g MET /head/day)	7.29^{a}	2.97	4.32^{a}	0.41^{c}
G4 (12 g MET /head/day)	7.44^{a}	3.12	4.32^{a}	0.42^{bc}
G5 (15 g MET /head/day)	6.48^{b}	3.03	3.45^{b}	0.47^{ab}
±SEM	0.232	0.051	0.235	0.016

Mean values with similar superscripts within the same column did not differ significantly at P < 0.05.

Table 4. Glucose, cholesterol, and triglycerides of Barki ewes as affected by the dietary addition of different levels of protected methionine.

Treatment	Parameter		
	Glucose (mg/dL)	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)
G1 (Control)	59.8	81.62^{c}	136.018^{c}
G2 (6 g MET /head/day)	58.86	87.47^{a}	136.81^{bc}
G3 (9 g MET /head/day)	60.2	85.12^{ab}	139.67^{ab}
G4 (12 g MET /head/day)	58.85	84.65^{b}	141.49^{a}
G5 (15 g MET /head/day)	62.72	84.41^{b}	136.32^{bc}
±SEM	1.351	0.86	0.998

Mean values with similar superscripts within the same column did not differ significantly at P < 0.05.

Wool amino acid profile:

Table (5) shows that the minimum value of wool Methionine (0.280% ±0.02) was recorded with animals that had 6 g MET/head/day. While the maximum value (0.380%± 0.02) was found in wool of control animals. These changes might be consistent with a slight increase in the wool proportion of high sulphur proteins after enrichment of sulphur compared to low sulphur proteins. Ramadan *et al.* (2017) found that adding protected amino acids Methionine or Lysine led to an increasing in Tyrosine, Serine, Glutamic acid, and Lysine significantly, but Valine, Leucine, Methionine, Glycine, and Proline decreased significantly compared to control animals.

Table 5. Effect of addition dietary various levels of protected methionine on wool content of amino acids in Barki ewes.

Amino acid	G1 (Control)	G2 (6 g MET /head/day)	G3 (9 g MET /head/day)	G4 (12 g MET /head/day)	G5 (15 g MET /head/day)	±SEMSEMM
Asparagine	7.37	7.10	7.23	7.29	7.20	0.35
Threonine	5.75	5.60	5.82	6.15	5.70	0.26
Serine	9.09^{ab}	10.62^{a}	10.46^{ab}	8.99^{b}	10.12^{ab}	0.47
Glutamic	14.79^{b}	19.40^{a}	19.73^{a}	15.00^{b}	19.26^{a}	0.90
Glycine	5.60	4.80	5.10	5.53	4.82	0.26
Alanine	4.56	4.90	5.20	4.44	4.73	0.23
Cystine	5.79^{b}	6.14^{ab}	6.84^{a}	6.84^{a}	6.60^{ab}	0.30
Valine	4.19^{ab}	3.40^{c}	3.60^{bc}	4.73^{a}	3.60^{bc}	0.22
Methionine	0.380^{a}	0.280^{c}	0.340^{abc}	0.346^{ab}	0.300^{bc}	0.02
Isoleucine	2.57	2.70	2.90	2.95	3.12	0.18
Leucine	8.04	7.10	6.94	8.01	7.32	0.35
Tyrosine	4.89^{c}	6.60^{a}	6.10^{ab}	4.33^{c}	5.30^{bc}	0.38
Phenylalanine	3.16	2.90	2.86	2.95	3.20	0.18
Histidine	2.69	2.40	2.80	2.70	2.60	0.22
Lysine	3.35^{c}	4.80^{ab}	4.30^{b}	3.12^{c}	5.10^{a}	0.21
Arginine	6.90	5.30	5.20	5.11	5.10	0.88
Proline	6.21^{a}	4.21^{b}	3.30^{c}	6.79^{a}	3.88^{bc}	0.19

Mean values with different superscripts within the same row are significantly different at (P<0.05).

This modification seems to be due entirely to an alteration in the overall composition of the high-sulphur proteins and to an increase in their proportion in the fiber. These variations are not the result of a change in the composition of individual proteins but are due to alterations in their relative proportions and to the initiation of the synthesis of 'new' proteins, many of which are extremely rich in cystine.

The obtained values of wool content of methionine in this study agree with Youssef (2017), who measured wool content of methionine in Barki sheep and the maximum value was (0.37%±0.01). Also, it was illustrated that there was a

significant increase in wool content of amino acids as Lysine, Serine, and Tyrosine by supplementing dietary Lysine and Methionine. While, Methionine, Valine and Leucine reduced significantly compared to control animals. Responses could differ with methods of giving amino acids for the animal, the basal diets, doses of amino acids giving for animals, and environmental conditions. The relation between Lysine and methionine and wool content of amino acid needs further studies because lysine and methionine are the most important amino acids that affect wool structure.

Blood plasma amino acid profile:

Table (6) shows that adding different doses of methionine for Barki ewes caused an increase in plasma content of methionine, arginine, and glutamic acid compared to control animals. The minimum value of methionine (0.14% ±0.05) was recorded with control animals. While the maximum value (0.43%± 0.05) was found in the plasma of animals that had 12 g MET /head/day.

The obtained values of plasma content of methionine in this study agree with Youssef (2017), who measured plasma content of methionine in Barki sheep and the maximum value was (0.40%±0.02). Also, Younis and Abd-Elazem (2019) found that adding protected amino acids lysine or/and methionine caused a significant increase in lysine, glutamic, and arginine concentrations, while it caused a significant decrease in proline and ammonia concentrations.

Ramadan *et al.,* (2017) found that concentrations of arginine, glutamic, and lysine in Barki sheep increased significantly when protected amino acids methionine and lysine were supplemented, for diets ewes but ammonia and proline concentrations decreased significantly.

Also, Wakeling *et al.,* (1970) established that infusing sheep with DL or L methionine into the abomasum or duodenum in a substantial increase in methionine concentration in plasma above a certain level infusion.

Amos *et al.,* (1974) found that infusing angora goat with methionine (1.06 grams per day) in two equal levels, caused an increase in molar proportions of methionine and lysine in blood plasma, while plasma concentration of threonine decreased. Cottle and Velle, (1989) established that variation in average amino acid concentrations in plasma with the dose administered intraluminally in Norwegian sheep weighing 87.5+3.5kg recommended that methionine requirement in the diets was found to be 10-15 grams per day.

Table 6. Effect of addition dietary various levels of protected methionine on blood plasma content of amino acids in Barki ewes.

Amino acid	T1 (Control)	T2 (6 g MET /head/day)	T3 (9 g MET /head/day)	T4 (12 g MET /head/day)	T5 (15 g MET /head/day)	±SE
Asparagine	7.62	9.60	9.61	8.62	9.50	0.64
Threonine	5.66	6.60	6.58	5.84	6.60	0.35
Serine	5.37	5.20	5.30	5.35	5.40	0.29
Glutamic	12.23^{b}	14.62^{a}	14.50^{a}	12.79^{ab}	14.80^{a}	0.67
Glycine	3.84	4.12	3.96	3.41	4.10	0.28
Alanine	4.99	5.10	4.90	4.97	5.20	0.24
Cystine	2.18	2.30	2.40	2.55	2.20	0.12
Valine	7.25	7.10	7.90	6.90	7.40	0.34
Methionine	0.14^{b}	0.33^{a}	0.32^{a}	0.43^{a}	0.40^{a}	0.05
Isoleucine	3.21	3.10	2.96	2.98	3.00	0.15
Leucine	9.65	8.60	8.46	9.38	8.20	0.44
Tyrosine	4.58	4.70	4.60	4.49	4.80	0.26
Phenylalanine	5.43	5.40	5.50	5.30	5.10	0.27
Histidine	3.73	3.60	3.92	3.53	3.70	0.19
Lysine	8.93	10.40	10.20	8.91	10.60	0.52
Arginine	2.81^{b}	4.10^{a}	4.20^{a}	2.82^{b}	4.00^{a}	0.19
Proline	6.17^{a}	3.84^{b}	3.40^{b}	5.90^{a}	3.70^{b}	0.17

Mean values with different superscripts within the same row are significantly different at (P<0.05).

Oke *et al* (1986) found that feeding rumen-protected lysine or rumen-protected methionine and abomasal infusion of lysine caused a similar increase in plasma concentration of these amino acids, but feeding unprotected lysine and methionine did not increase plasma levels of these amino acids. Other researchers have demonstrated that the blood concentration of limiting amino acids remains relatively constant until tissue requirements are met (Bergen, 1979).

Scanning electron microscopy (SEM) of wool:

Images of the scanning electron microscope revealed that wool in all groups has a unique structure consisting of two major morphological parts, the cuticle and the cortex and a scale-like particle on the cortex along the fibre length (Fig. 1).

Similar findings were reported by Zimmerman *et al.* (2011). Scales of fibers play an important role in reflecting sunlight and type of luster as well as in some industrial properties such as friction, smoothness, and felting (Helal *et al.,* (2015).

Table (7) shows the surface features of the cuticle scales of Barki wool fibers as influenced by adding different levels of rumen-protected methionine. The surface structure of Barki wool fibers is composed of many visible scales along with the fiber. The minimum value of scale width (14.07µm ± 1.44) was recorded with animals that had 6 g MET /head/day (G2), while the maximum value of scale width (21.34µm ± 1.44) was recorded with animals that had 15 g MET /head/day (G5, P<0.05, Table 7).

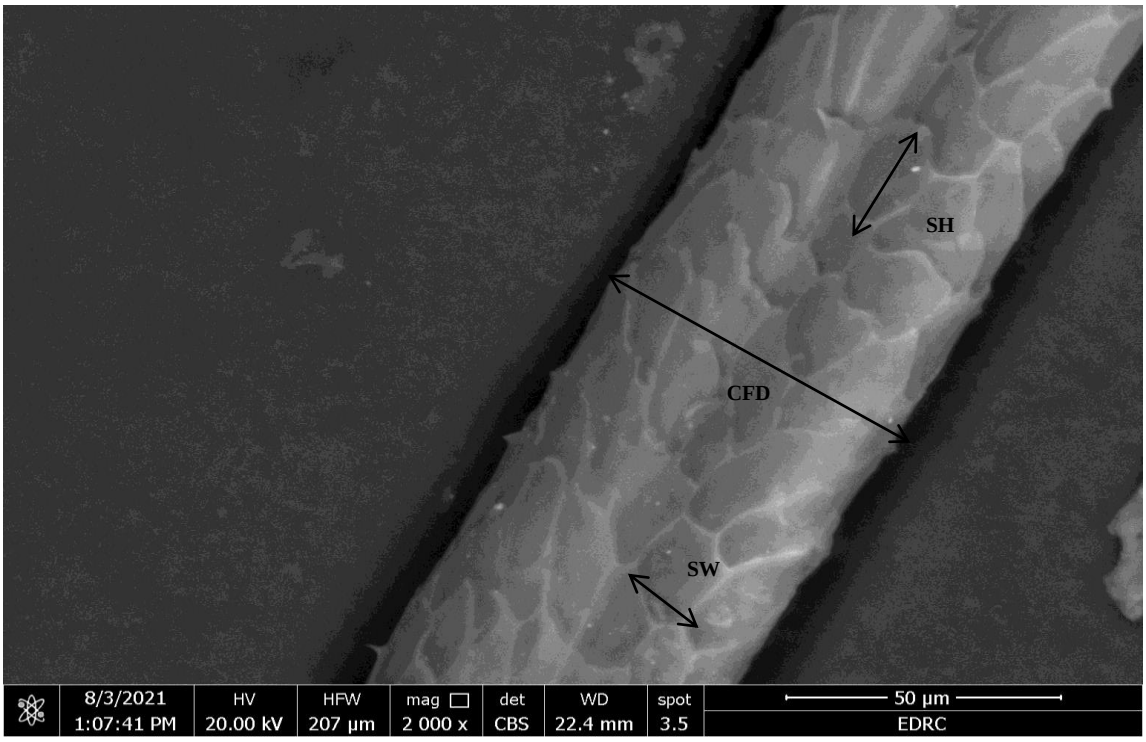

Fig 1. Scanning electron microscopy photo of coarse fiber wool scale properties of Barki ewe at magnification level 2000X. SH: scale height (scale distance to edge), SW: scale width, CFD: coarse fiber diameter.

Also, it was observed that the height of the scale is not well defined at some points since the scale is not exactly in the shape of a rectangle. Wool of animals in G2 (6 g MET/head/day) showed the highest value of scale distance to the edge (22.69 µm ± 2.19) compared to animals in other experimental groups. Animals supplemented with 12 g methionine/head/day (G4) recorded the lowest value of coarse fiber diameter (47.73 µm ± 0.61, P<0.05), this may be because these animals had the lowest values of scale width and scale distance to the edge.

Table (7) Effect of addition dietary various levels of protected methionine on wool ultrastructural (coarse fiber wool scale properties) of Barki ewes.

Treatment	Parameter				
	Scale width (µm)	Scale height (µm)	Coarse fiber diameter (µm)	Scale regularity	Coarse Fiber diameter regularity
G1 (control)	17.37^{ab}	17.14^{ab}	52.65^{c}	Regular	Regular
G2 (6 g MET/head/day)	14.07^{b}	22.69^{a}	56.53^{b}	Non-Regular	Regular
G3 (9 g MET/head/day)	16.69^{ab}	15.97^{ab}	58.62^{a}	Regular	Regular
G4 (12 g MET/head/day)	16.78^{ab}	15.04^{b}	47.73^{e}	Regular	Regular
G5 (15 g MET/head/day)	21.34^{a}	21.31^{ab}	49.94^{d}	Regular	Regular
±SEM	1.44	2.19	0.61		

Mean values with different superscripts within the same column are significantly different at (P<0.05).

All treatments showed regular wool fibers scales, except for G2 because of the high difference between values of scale distance to the edge and scale width. There was a regularity in coarse fiber diameter along with the same fiber in all experimental animals. In addition, the wool fiber scale edges of all experimental ewes were found to be smooth.

CONCLUSION

This study indicated that adding dietary rumen-protected methionine at a level of 12 g/head/day for six months significantly enhanced the wool quality of Barki ewes, in terms of conventional and ultrastructural wool characteristics without affecting the health status.

Conflict of Interest: The authors declare no conflict of interest.

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تأثير إضافة مستويات غذائية مختلفة من الميثونين المحمي على صفات الصوف، التركيب الخارجي ومكونات الدم في الأغنام البرقي

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تم استخدام 25من النعاج البرقي البالغة (غير حوامل) لدراسة تأثير إضافة مستويات مختلفة من الميثونين المحمي كاضافة علفية على بعض صفات الصوف , مكونات الدم, والتركيب الخارجي لالياف الصوف . تم تقسيم الحيوانات طبقا لوزن الجسم الحي وقياسات الصوف الي خمس مجاميع المجموعة المقارنة: تم تغذيتها عل العليقة القياسية بدون أي إضافات. بينما تغذت المجموعة الثانية والثالثة والرابعة والخامسة علي العليقة القياسية مضافا اليها الميثونين المحمي بمعدل 6 ,9, 12, 15جم/الرأس/اليوم علي التوالي لمدة ستة اشهر . تم جمع عينات الصوف في نهاية التجربة لتسجيل بعض قياسات الصوف مثل قطر الليفة، طول الخصلة، قوة شد الخصلة، الاستطالة، نقطة القطع ونسبة الصوف النظيف . تم الحصول على عينات الدم لتقدير بعض قياسات الدم البيوكيميائية. اوضحت النتائج أن إضافة الميثونين المحمي لمدة ستة أشهر أدت الي تحسين صفات الصوف الناتج من النعاج البرقي معنويا مثل طول الخصلة، قوة شد الخصلة، الاستطالة، نقطة القطع، قطر الليفة ونسبة الصوف النظيف. أثرت إضافة الميثونين الغذائي على خصائص حراشيف الياف الصوف الخشنة لدي نعاج البرقي حيث سجلت المعاملة الثانية اقل قيمة لعرض الحرشفة بينما سجلت المعاملة الخامسة أكبر قيمة لعرض الحرشفة. كما سجلت المعاملة الثانية اعلي قيمة لطول الحرشفة وسجلت المجموعة الرابعة اقل قيمة لقطر ليفة الصوف الخشنة . أدت إضافة الميثونين إلى اختلافات طفيفة في قيم بعض قياسات الدم البيوكيميائية. أفضل إضافة غذائية للميثونين هي (12 جم/الرأس/اليوم).